# High avidity of vaccine-induced immunoglobulin G against SARS-CoV-2: potential relevance for protective humoral immunity

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## Abstract

Avidity of immunoglobulin G (IgG) is defined as its binding strength to its target antigen. As a consequence of affinity maturation of the IgG response, avidity is maturing as well. Therefore, acute infections are characterized by low-avidity IgG, whereas past infections are usually associated with high-avidity IgG. Avidity maturation is also observed as a consequence of optimal vaccination. Avidity has been shown to play a significant role in protective humoral immunity in many microbial systems. After severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the situation is different compared to other viral infections, as the moderate degree of avidity reached in most cases of infection is similar to that reached after only one vaccination step. In contrast, two vaccination steps lead to a much higher avidity of IgG directed towards viral spike protein S1 (S1) in the majority of vaccinated individuals. Therefore, it seems that two vaccination steps allow for a more extended affinity/avidity maturation than natural infection. The degree of avidity maturation after two vaccination steps is heterogeneous. It can be further enhanced by a third vaccination step. Complete avidity maturation seems to depend on sustained availability of antigen during the maturation process. Variants of concern seem to increase the affinity of their receptor-binding domain (RBD) to angiotensin-converting enzyme-2 (ACE2) and/or to decrease the susceptibility for neutralizing antibodies. Classical neutralization tests do not necessarily reflect the avidity of neutralizing IgG, as they operationally dissect the binding reaction between S1 and IgG from the binding of the S1 to ACE2. This approach fades out critical competition reactions between IgG and ACE for RBD of the S1. Quantitative avidity determination might be an essential tool to define individuals that only possess suboptimal protective immunity after vaccination and therefore might benefit from an additional booster immunization.

## Keywords

Humoral immune response, avidity, severe acute respiratory syndrome coronavirus 2, protective immunity

# Introduction

Avidity is defined as the average binding strength of an antibody population towards an antigen [1, 2]. It is determined by the strength of immunoglobulin G (IgG) binding to its epitopes. Therefore, avidity is directly correlated to the off-rates of the underlying affinity of IgG. The maturation of IgG avidity occurs in parallel to the maturation of affinity, which is a regular and essential immunological process after microbial infections and vaccinations [1–4]. Avidity determination of IgG allows the differentiation between acute and past infections. This is of particular importance in cases where the differential determination of IgM and IgG does not lead to unequivocal diagnostic conclusions due to irregular IgM responses, such as missing, delayed, or persistent IgM [4]. The role of IgG avidity for protective humoral immunity has been recently discussed [5]. Despite its direct relationship to the establishment of protective immunity, avidity determination has been neglected so far 1) in the context of the serological diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, 2) the evaluation of vaccination efforts directed towards SARS-CoV-2, 3) the establishment of mathematical models related to the break of the present SARS-CoV-2 pandemics and 4) the discussions by the various medical and political authorities that are responsible for optimal health management.

After natural infection with SARS-CoV-2, the avidity of IgG directed towards viral nucleoprotein (NP), spike protein S1 (S1) and its receptor-binding domain (RBD) has been shown to not exceed a plateau of low or intermediate avidity in most cases. High avidity [avidity indices (AIs) > 0.6, determined through application of 7 mol/L urea] was only found in rare cases [6, 7]. The same picture is overt for avidity maturation after infection with seasonal coronaviruses [8]. In contrast to natural infection, two steps of vaccination reach an IgG response towards S1 and RBD of high avidity in most vaccinated individuals [7]. These findings might have relevance for the understanding of the repeated waves of coronavirus infections and for the establishment of protective immunity through adequate vaccination strategies directed towards SARS-CoV-2.

# Avidity determination in serological diagnosis

During the present SARS-CoV-2 pandemic, the direct, specific, and sensitive detection of viral RNA through polymerase chain reaction (PCR) is the primary tool for the detection of clinically inapparent and apparent infections with SARS-CoV-2. It was also recognized that serological analysis is required for the retrospective detection of infection, for epidemiological studies, and for efforts to determine potential protective humoral immunity. Serological analysis has gained additional importance as soon as vaccination trials had been implemented and vaccination programs started in many countries. Surprisingly, the diagnostic potential of avidity determination has not been fully utilized until now.

### Definition and determination of IgG avidity

Avidity determination is based on affinity maturation, a central and regularly occurring mechanism of the humoral response. Affinity maturation of IgG is due to hypermutation of the genes coding the variable parts of IgG in B cells and clonal selection of B cells with a higher affinity of their IgG [9–11]. Therefore, within 2–3 weeks after classical viral infection or vaccination, detectable IgG is of low affinity. Through the continuous and irreversible process of affinity maturation, a gradual increase in avidity is then observed. It results in the appearance of high-affinity IgG a few months after infection or completed prime-boost vaccination.

Affinity defines a biophysical process that depends on the interplay between the kinetics of binding of IgG to its epitope, the strength of binding, and the kinetics of a possible release reaction. Affinity determination can be precisely performed with defined, individual IgG. This complexity of affinity, particularly in polyclonal IgG responses, cannot be easily determined in routine serology. However, it is possible to quantitatively determine the strength of binding of the IgG population to its epitopes—a process that has been termed "avidity" in the context of microbial diagnostics.

Avidity determination is used in serological analysis for several decades. As avidity depends on the degree of fit between IgG and its epitope to the same extent as the overall process of affinity, avidity can be used as a valid surrogate marker for affinity in the context of practical application. Therefore, low

avidity IgG indicates a very early interaction between an infectious agent and the immune system, whereas IgG of high avidity, occurring usually several weeks/a few months after primary infection, indicates past infection. As memory cells that express IgG of high avidity are generated after completed affinity/avidity maturation, anamnestic responses are characterized by high avidity and can thus be clearly differentiated from primary infections.

Avidity of IgG is quantified by determining to which degree IgG that is specifically bound to its target can be released by defined concentrations of chaotropic agents such as urea, guanidine hydrochloride, thiocyanide, or diethylamine [1, 12–15]. Thereby the incubation time with the agent has to be precisely defined. The comparison of the result obtained from the assay treated with the chaotropic agent to that of a control assay allows determining the AI. This approach requires precise treatment conditions and a strictly quantitative serological assay. Enzyme-linked immunosorbent assay (ELISA), immunoblot (line) assays, quantitative immunofluorescence assays, and other quantitative serological assays are suitable for avidity determination [1, 14, 16, 17].

In a recent publication from Hedman's group, the present state of the quantitative analysis of avidity maturation is explicitly and impressively outlined [2].

Nurmi et al. [2] also explain the determination of the optimal concentration of the chaotropic agent for best differentiation. Basically, it is necessary to determine the concentration of chaotropic agent that causes less than a 40% decrease of signal when the avidity assay is run with sera that are proven to be taken several months past infection. In this case, an AI of 0.6 would be the mark to distinguish between high and low avidity. In some cases, a further step of intermediate avidity is included in the calculation and presentation of data [6, 7].

The optimal concentration of chaotropic agent is variable between test systems using different antigens, as certain proteins may be denatured by high concentrations of the chaotropic agent. Therefore, the assay has to be carefully optimized with regard to the maximal concentration of the chaotropic agent that can reasonably be applied [2, 18].

The scientific readership should be aware that the term "avidity" is not only used in the connotation as outlined above—defining the strength of binding between IgG and corresponding epitopes, as determined by dissociation of this binding. Sometimes it is also used to specifically define the difference in the strength of IgG binding to its target with one binding site or using both binding sites of the Y-shaped molecule for binding to two entities of its target [19, 20]. Finally, readers should be aware that the differences in the strength of binding reactions that establish agglutination between bacteria and IgG, based on different complexity of the IgG and target population, have also been termed "avidity" in the past.

## The significance of IgG avidity determination for the serology of microbial infections

Classical microbiological serology was initially based on the differential determination of IgM and IgG responses towards the agent in focus. Thereby, IgM responses were taken as a strong indication for acute infection, whereas IgG responses in the absence of detectable IgM were considered to indicate past infection. This simple pattern has been proven to be misleading in a significant number of cases, due to the high variability of the measurable IgM response after viral infections [4]. It became obvious that the strict sequence of IgM followed by IgG, as it is immanent to humoral immune responses on the B cell level, is not necessarily reflected in the IgM and IgG titers that are finally detected in serum. Several reasons for this apparent discrepancy are outlined in the relevant study [4].

Particularly the pioneering work of Klaus Hedman and colleagues has opened a perspective to cope with this problem. They promoted the introduction of avidity determination as essential addition to routine infection serology [1–44]. Since then, avidity determination has been established and confirmed as an extremely useful tool in the serology of infections with rubella virus [14, 21], parvovirus B 19 [22], cytomegalovirus (CMV) [23–26], Epstein-Barr virus (EBV) [16, 17, 27–33], human herpesvirus-6 (HHV-6) and HHV-7 [34], tick-borne encephalitis virus [35], measles virus [36–38], mumps virus [39], west

nile virus [40, 41], hepatitis A virus [42], hepatitis C virus [43–45], dengue virus [46], borrelia [47] and toxoplasmosis [48].

## Natural infections with SARS-CoV-2: high variability of the IgM/IgG response and only moderate IgG avidity maturation

Many qualified serological studies related to SARS-CoV-2 have been published since the beginning of the pandemics. A comprehensive analysis of the early studies revealed a high degree of variability of the IgM and IgG response towards SARS-CoV-2 [4]. This analysis indicated that the sole determination of the IgM and IgG responses did not allow an unequivocal differentiation between acute and past infections. Therefore, it was suggested that avidity determination of IgG towards SARS-CoV-2 antigens might be the adequate tool to resolve the serological problems related to SARS-CoV-2 infection [4], in analogy to the analytical strength of avidity determination as shown before for rubella, toxoplasmosis, EBV, and other agents [14, 16–18, 21–48]. In these classical applications, avidity determination had allowed to clearly differentiate between acute and past infections, even in the presence of atypical IgM results (IgM persistence or reactivation in past infections and missing IgM in acute infections).

We used a commercial line assay for establishing avidity testing of IgG directed towards SARS-CoV-2 NP, S1, and the RBD of S1 [6, 7]. This assay also contained the NPs of four seasonal coronaviruses 229E, NL63, OC43, HKU1 for control purposes. To our great surprise, the evaluation of sera from a large number of nonhospitalized coronavirus disease 2019 (COVID-19) patients, taken at different times after the onset of PCR-confirmed COVID-19, showed that avidity maturation of IgG directed towards NP, S1, and RBD of SARS-CoV-2 was only initially increasing. It then usually remained at a plateau of low or intermediate avidity, when 7 mol/L urea was applied. Only in very rare cases, high avidity was reached under these conditions to an extent that is known from other viral infections [6, 7]. Therefore, it seemed that in most cases of natural infection, IgG affinity/avidity maturation had not been extended to a maximal level, as it was expected, based on the experience with other infections [14, 16–18, 21–48]. This phenomenon was operationally termed "incomplete avidity maturation of IgG". This finding seemed to be unique, as it is generally not paralleled by similar findings for the serological response towards other microbial agents. The only exception found after intensive literature search is a restriction to low avidity after natural infection and establishment of high avidity after optimal vaccination in the respective immune responses directed towards human papillomaviruses (HPVs) 16 and 18 [49].

As a few sera showed IgG of high avidity at the urea concentration of 7 mol/L in our study [6, 7], it was excluded that our conclusion on incomplete avidity maturation of IgG after SARS-CoV-2 infection was a misinterpretation, caused by partial or complete denaturation of the antigens by the high urea concentration. These controls assured that high avidity IgG was indeed established in a few sera and can be precisely quantified. However, most sera derived from individuals with natural infection only showed intermediate or low avidity. The correctness of our conclusions, based on the (few) positive controls, was in line with those by other authors that also stated that only a moderate level of avidity maturation of IgG was observed after SARS-CoV-2 infection [50–54]. Benner et al. [55] did not explicitly point to incomplete avidity maturation. However, their data showed that IgG directed towards S1 reached a plateau of avidity after 20 days that was characterized by an AI of 0.5 or lower in most sera when 6 mol/L urea had been applied. Luo et al. [56] and Löfstrom et al. [57] reported on complete avidity maturation after SARS-CoV-2 infection, and thus seemed to contradict our findings. However, a closer look at the technical details of their work [56, 57], showed that they had applied 3 mol/L urea [56] or 4 mol/L urea [57], compared to 7 mol/L urea that had been used in our studies [6, 7]. The use of 3 or 4 mol/L urea allowed to nicely demonstrate that IgG avidity maturation was indeed operating during the first three months after infection. However, the endpoint of assumed high avidity IgG (AI > 0.6) that was based on dissociations with 3 or 4 mol/L urea would appear as intermediate or low avidity when 7 mol/L urea would have been applied. This aspect can be directly deduced from reference [6], where avidity determination has been performed with variable urea concentrations. There it is demonstrated that serum samples that seem to exhibit high avidity IgG at

4 mol/L urea (AI > 0.6) can be differentiated into samples with AI < 0.6 and few samples with truly high avidity (completed avidity maturation) with an AI > 0.6.

The significance of this finding is further substantiated in the next paragraph, where it is shown that two vaccination steps were usually leading to high avidity IgG with AIs above 0.6 when 7 mol/L urea had been applied [7]. Therefore, in summary, the work by Luo et al. [56] and Löfström et al. [57], is not contradicting our conclusions, rather it is supporting them. Finally, the work by Liu et al. [58], using 6 mol/L urea, reported on low avidity IgG during the first 45 days after SARS-CoV-2 infection, in line with our findings, but showed no data for later time points.

As the infection with seasonal coronaviruses also leads to incomplete avidity maturation in a high percentage of cases [8], the restriction of avidity maturation might be considered as a general biological strategy of coronaviruses. It might ensure the repeated waves of reinfection that are seen for seasonal coronaviruses [59, 60]. Such a scenario needs to be prevented in the case of SARS-CoV-2.

## Two vaccination steps with SARS-CoV-2 vaccine establish high avidity IgG in most cases

Rather unexpectedly, two vaccination steps with the BioNTech/Pfizer messenger RNA (mRNA) vaccine established IgG avidity that was significantly higher than that reached after natural infection [7]. High avidity of IgG directed towards S1 and RBD was usually paralleled by high IgG concentrations [7]. Only 1/71 cases completely failed to establish detectable avidity maturation, and 4/71 cases showed low avidity after the second vaccination in our study [7]. These serological findings after prime-boosting vaccination are paralleled by the corresponding findings on the level of the involved immune cells [61].

Interestingly, in previously uninfected COVID-19 outpatients, the degree of avidity maturation after one vaccination step, as well as the median of the IgG concentration resembled the values obtained for individuals that had been infected with SARS-CoV-2 [7]. The significance of this analogy was underlined by our finding that one step of vaccination of previously SARS-CoV-2-infected individuals resulted in the immediate onset of highly efficient avidity maturation, reaching the same level or more as found for immunologically naive individuals with two vaccination steps [7]. These findings illustrate that despite incomplete avidity maturation after natural infection, the resultant immunological memory contributed to avidity maturation after one vaccination step in an analogous and equivalent mode as a preceding vaccination step.

Our finding indicates that one step of vaccination is sufficient for previously infected individuals for the establishment of complete avidity maturation, at a similar level as achieved in immunologically naive individuals with two vaccination steps. It also shows that infection without subsequent immunization leads to a state of humoral immunity that is of lower quality than complete immunization [7]. Therefore, it seems to be absolutely necessary to vaccinate individuals despite their previous infection, in order to achieve maximal avidity maturation of IgG directed towards SARS-CoV-2.

The finding of complete avidity maturation after two vaccination steps towards SARS-CoV-2 has been confirmed by other groups [53, 62]. The work by Neumann et al. [53] also included the mRNA vaccine from Moderna and the adenoviral vector vaccine from AstraZeneca, which caused analogous effects as the BioNTech/Pfizer vaccine [7, 62].

Based on the mechanism of avidity maturation and the players involved in the induction of the humoral immune response towards SARS-CoV-2, it was expected that heterologous immunization schemes, combining the AstraZeneca and the BioNTech vaccine, should result in a similar outcome as priming and boosting with BioNTech vaccine alone. As reported by Hillus et al. [63], the IgG responses 3 weeks after boosting were comparable in the homologous and heterologous immunization groups. The avidity of IgG directed towards S1 was significantly higher in the heterologous group, compared to the homologous group (median AI 0.93 *versus* 0.73). The authors suggested that this difference in avidity might have been due to a longer dosing interval in the heterologous group. Rose et al. [64] confirmed that induction of high avidity by the BioNTech vaccine requires priming and boosting. In their study, priming by the vector vaccine AZD1222 caused higher

avidity than priming by the mRNA vaccine from BioNTech. Heterologous boosting with the BioNTech vaccine then caused a further increase in avidity.

In conclusion, priming and boosting seem to be absolutely required for the induction of high avidity IgG. It seems that priming can be achieved either by SARS-CoV-2 infection or vaccination with various established vaccines [7]. To our knowledge, a potential boosting effect by viral infection after priming by one vaccination step has not yet been reported, whereas boosting by vaccination after preceding infection with SARS-CoV-2 is very effective in induction of high-avidity IgG [7].

Two additional take-home messages should be considered in addition:

1) The responses to two doses of vaccination showed a certain degree of heterogeneity [7, 65] and therefore a minority of vaccinated individuals did not reach high avidity through classical prime-boosting. This group of individuals could be determined by specific avidity testing and is advised to preferentially encounter a second booster step. Recent work [65] shows that a second booster step (i.e., third vaccination) causes a further increase in avidity, resulting in the transition to high-avidity IgG for the complete group of individuals that had only reached low or intermediate avidity after two vaccination steps.

2) High avidity of IgG directed towards S1 and its RBD after prime-boosting is reached for the virus on which the vaccine is based, in our case this is the originally isolated SARS-CoV-2 wildtype strain (Wuhan-hu-1) [7]. The induction of high avidity IgG towards upcoming variants of concern (VOC) requires additional avidity maturation triggered by a second booster step (third vaccination step).

## Mechanisms that potentially determine incomplete or complete avidity maturation

It is well established that affinity/avidity maturation depends on antigen persistence, i.e., an extended antigen availability throughout the maturation process [66–68]. As priming and boosting by two SARS-CoV-2 vaccination steps, either with mRNA vaccine or with a combination of adenoviral vector vaccine and mRNA vaccine, are sufficient to induce high avidity IgG [7, 53, 62–64], these regimes seem to provide an optimal antigen concentration to the immune system for a sufficiently long-time interval for completion of affinity maturation.

Natural infection with SARS-CoV-2 seems to be sufficient for priming, but not for the maintenance of the complete process of avidity/affinity maturation [7]. This finding might indicate that SARS-CoV-2 replication does not provide sufficiently high concentrations of antigen for the time required to provoke a sustained avidity maturation process [66–68]. Alternatively, the site of SARS-CoV-2 replication might not be optimal for stimulation of the immunological processes that drive affinity/avidity maturation.

Analogous findings have been reported for avidity maturation after vaccination towards or natural infection with HPVs [49]. Similar to the situation found for SARS-CoV-2, two vaccination steps with HPV antigens lead to neutralizing IgG with high avidity, whereas natural infection only causes a marginal humoral response characterized by low avidity. Therefore, the vaccination regime towards HPV seems to provide viral antigen to the immune system in an optimal and sufficiently extended mode, whereas the natural infection that is restricted to epithelial cells and takes places without classical viremia, does not seem to foster antigen-driven avidity maturation [49, 69]. A look at other infection systems confirms the significance of these conclusions related to SARS-CoV-2 and HPV, as complete avidity maturation can be experimentally prevented when the replication of the infectious agents is limited through therapeutic treatment [70, 71]. Taken together, the negative effect of suboptimal availability of antigen on the process of affinity/avidity maturation seems to be a rational explanation for the mechanism that is underlying incomplete avidity maturation after natural SARS-CoV-2 infection.

This conclusion is further fostered by the finding that COVID-19 patients with more severe disease frequently show higher avidity of their IgG directed towards SARS-CoV-2 than patients with the milder disease [7, 54–56]. This scenario might be explained by a higher initial viral load that 1) leads to more severe disease [72, 73], and 2) also causes a higher degree of availability of viral antigens for the immune system.

This would then allow triggering avidity maturation to a somewhat higher degree, according to established mechanisms [66–68]. It is rational to speculate that the increased avidity might provide a potential benefit for combating the virus at least in some of the patients.

An alternative explanation for the negative effect of SARS-CoV-2 infection on avidity maturation of IgG induced by the infection might be deduced from the reported destruction of germinal centers (GCs) in lymph nodes after SARS-CoV-2 infections [74]. As GCs are the site of affinity maturation [66], this concept is intriguing at first glance. However, this concept is in direct contradiction to the discrete enhancement of IgG avidity in patients with more severe disease, as discussed above [7, 54–56], and therefore cannot be taken as a valid explanation for incomplete avidity maturation after SARS-CoV-2 infection in general. As the findings by Kaneko et al. [74] had been established by the analysis of lymph nodes of COVID-19 patients that had died from the disease, they might describe an extreme situation. In this context, SARS-CoV-2 infection might have interfered with the function of GCs and in this way had prevented avidity maturation. As a consequence of blocking avidity maturation, the failure to complete clearance of infection might then have contributed to the fatal outcome. This view is supported by the finding that patients that did not recover from more severe COVID-19 frequently showed extremely low AIs, whereas surviving patients showed a relative increase in avidity [54, 75–77]. These findings strongly point to the role of high avidity for protective immunity, as will be further discussed in the next chapter.

# Avidity maturation and protective immunity

## Established causal relationships between avidity maturation and protective immunity in various infections and vaccinations

The regular occurrence of avidity/affinity maturation after microbial infection allows concluding that a sufficiently high concentration of antibodies that bind to their target specifically and with high affinity/ avidity, might be a central and essential element of the successful antimicrobial defense. The aspect of avidity maturation in relation to protective immunity has been discussed by den Hartog et al. [78], while the role of memory cells with the potential to rapidly mount a protective high avidity IgG response after secondary infections have been discussed by Inoue et al. [79]. These important concepts have been particularly substantiated by numerous findings on the connection between 1) failing avidity maturation and lack of protective humoral immunity and 2) increased avidity and increased protective immunity. Failures to generate high avidity IgG towards CMV seem to play a role in the intrauterine transmission of CMV [25, 80–82]. It was also shown that low avidity of IgG towards varicella-zoster virus (VZV) was associated with the risk of repeated chickenpox [83, 84]. Waning antibody levels and low avidity were discussed as the cause for reinfections with the measles virus [85], in line with the findings by Paunio et al. [36]. IgG avidity seemed to correlate with the neutralizing capacity of IgG directed towards the dengue virus [86]. The failure of a formalin-treated vaccine towards respiratory syncytial virus was shown to be due to the failure of the vaccine to induce high avidity IgG [87]. Immunization of macaques with a simian human immunodeficiency virus (SHIDV) DNA vaccine demonstrated a strong correlation between the avidity of IgG directed towards the viral envelope protein and protection towards viral infection [88]. Pegu et al. [89] confirmed these findings and demonstrated that the avidity of IgG towards the envelope protein was higher in protected immunized animals compared to nonprotected animals. In line with these findings, high-affinity IgG was shown to be responsible for the protection of cattle towards the foot-and-mouth disease virus [90]. Protective immunity directed towards influenza virus was shown to be causally related to the induction of IgG of high avidity, both in model experiments using ferrets [91, 92] and in a double-blind, randomized, placebo-controlled trial in humans [93]. Favorable disease outcomes after Zika virus [94] or Ebola virus infection [95] also seem to be determined by the avidity of IgG directed towards the respective causative agents. Furthermore, IgG of high avidity seems to determine protective immunity directed towards infections with pneumococci [96, 97] and *Neisseria meningitidis* [98]. Children with vaccine failure related to *Haemophilus* influenza type B showed higher antibody concentrations induced by the vaccine, compared to protected children, but were characterized by vaccine-induced IgG of

low avidity [99]. Finally, protection against malaria has been shown to be associated with high-affinity IgG directed towards the merozoite antigens of *Plasmodium falciparum* [100].

## The relationship between avidity maturation and protective immunity in SARS-CoV-2 infections, vaccinations, and COVID-19

Ravichandran et al. [101] showed that antibody responses towards SARS-CoV-2 infections in PCR-positive asymptomatic individuals as well as symptomatic patients were characterized by differential IgM/IgG/IgA epitope repertoire at the mucosal site compared with serum. Importantly, they also provided evidence that the avidity of IgG directed towards SARS-CoV-2 S1 was significantly lower in symptomatic patients compared to asymptomatic individuals. Furthermore, in both groups of patients/individuals, the IgG avidity at the mucosal site was always lower than that in the corresponding sera. These data are in agreement with a protective function of IgG avidity for protective immunity directed towards SARS-CoV-2-induced disease. They also illustrate site-specific differences in the humoral immune response.

In a subsequent longitudinal study, Ravichandran et al. [75] analyzed immune markers associated with disease severity and resolution. Thereby the focus was on COVID-19 patients with "mild" or "severe" disease. This study impressively demonstrates that IgG directed towards SARS-CoV-2 S1 and exhibiting higher affinity was associated with disease resolution, whereas neutralizing IgG titers did not correlate with disease outcome. Ravichandran et al. [75] conclude "that the ability of virus-specific B cells to enter GCs in lymph nodes and to undergo affinity maturation may play an important determinant in the ultimate effectiveness of viral control and disease resolution. Thus, vaccines that can elicit high-affinity antibodies may have a substantial advantage for *in vivo* clinical outcomes of SARS-CoV-2 infection and contribute to the amelioration of disease in infected individuals. It seems that in patients with more severe COVID-19, deficiency in the cluster of differentiation 4 (CD4) cells and especially T follicular helper cells subsets causes a block to affinity maturation, as these immune cells are required for the entry of B cells into GCs". The concept related to the block of affinity maturation is in good agreement with the findings published by Kaneko et al. [74].

Similar strong circumstantial evidence for the role of avidity for protective immunity towards SARS-CoV-2 infection and COVID-19 was presented in the work by Moura et al. [54]. They also showed that the avidity of IgG towards SARS-CoV-2 antigens was increased in patients with more severe COVID-19 compared to less affected patients. As discussed in a previous chapter, this increase might be the result of the extended availability of viral antigens to drive avidity maturation. However, some patients with severe disease analyzed in the study by Moura et al. [54] remained at quite low levels of avidity throughout the study. This group of patients showed a strong overlap with those patients that eventually died, as all patients that died showed very low AIs (< 0.3). This finding seems to indicate a correlation between low avidity and the most severe outcome of disease, including death. Mechanistically, in this group, avidity maturation might have been prevented through the SARS-CoV-2-mediated loss of GCs in secondary lymphatic organs [74]. The study by Moura et al. [54] is strongly supporting the work by Ravichandra et al. [75] and vice versa.

Further confirmation for the role of IgG avidity for protective immunity towards SARS-CoV-2 infection and COVID-19 is derived from the work presented by Tang et al. [76].

They showed that minimal affinity maturation against the SARS-CoV-2 S1 protein was the key immunological parameter of intensive care patients that died from COVID-19, whereas disease resolution in COVID-19 patients was associated with a stronger antibody affinity maturation to SARS-CoV-2 S1 protein. The essential role of affinity/avidity maturation for prevention of severe disease and death was contrasted by discordance between neutralization titers and disease severity and outcome.

In an additional study, the same group gave further confirmation of the association of higher antibody affinity to the SARS-CoV-2 S1 protein with survival outcome, as most survivors showed IgG of higher affinity, whereas there was no correlation with the level of neutralizing antibodies [77].

These conclusive findings on the role of high IgG avidity for protective immunity towards SARS-CoV-2-mediated disease are complemented by studies that show (at least partial) protective immunity even under conditions of intermediate or lower avidity. In a large prospective cohort study on

40,000 health care workers, Hall et al. [102] showed that 21 days after only one vaccination step with the BioNTech/Pfizer vaccine, a protective effect of already 72% was achieved [102]. The protective effect increased to 86% seven days after the second vaccination. The rise in the rate of protection between the two vaccination steps was much less impressive than the rise in avidity determined for IgG towards RBD/S1 in several studies [7]. These findings, therefore, indicate that avidity is not the only factor that modulated protective immunity under these conditions.

The regular clearance of SARS-CoV-2 several days after natural infection, despite the reported incomplete avidity of the involved IgG [6, 7, 50–54], also seems to provide evidence for protective immunity at lower or intermediate avidity of IgG directed towards the viral surface.

These findings might be explained by the biophysical law of mass action. It is predictable that high concentrations of specific IgG that are directed towards binding-relevant epitopes of RBD, but are only of low avidity, might establish a protective effect based on their concentration, despite low avidity. This effect might be as efficient as the effect of a lower concentration of analogous IgG with high avidity.

Indeed, the equivalence of functionality of high concentrations of low avidity IgG compared to lower concentrations of high avidity IgG has been experimentally confirmed in competition experiments among patient IgG, SARS-CoV-2 RBD, and the cellular receptor angiotensin-converting enzyme-2 (ACE2) [20]. The authors concluded that patients with high anti-RBD signals have either higher-affinity antibody clones or sufficiently high concentrations of anti-RBD antibodies to outcompete wildtype ACE2.

What might then be the overall benefit derived from the generation of immune cells that allow generating high avidity IgG directed towards critical viral surface structures? As the IgG responses towards SARS-CoV-2 are waning after natural infection, as well as after vaccination [103, 104], reinfection might be facilitated by low concentrations of low avidity IgG. In contrast, preceding avidity maturation would open the chance to prevent reinfection and/or disease through the action of memory B cells and long-lived plasma cells that lead to the rapid establishment of a high avidity IgG response [78, 79, 105].

A rational mechanistic explanation for the need for high avidity IgG for protective immunity directed towards SARS-CoV-2 is derived from the finding that the interaction between RBD of SARS-CoV-2 S1 and its cellular receptor ACE2 [106–108] is driven by high affinity [109–112]. Therefore, neutralizing IgG that has the potential to interfere with this interaction, can be predicted to require high affinity/avidity towards RBD or neighboring sequences that affect RBD [109] in order to efficiently compete with ACE2 for binding to RBD of SARS-CoV-2 S1. This aspect seems to be one of the key issues for the understanding of infection control and present vaccination strategies, as new SARS-CoV-2 VOC seem to bind to ACE2 with higher affinity than the original isolate of SARS-CoV-2 [113–115].

## SARS-CoV-2 variants: competition between avidity-determined binding reactions between IgG and S1/RBD *versus* S1/RBD and ACE2

The constant generation of mutations within replicating SARS-CoV-2 populations, in combination with selection processes, leads to the emergence of new viral variants. These seem to dominate the dynamics of the present pandemics [116–122]. In a simplified picture, the mutations in the new variants either enhance the affinity of the S1 for the cellular receptor ACE2 and in this way enhance the efficiency of viral entry and subsequent spread of the virus, or decrease the binding of neutralizing antibodies to epitopes on the S1 protein [116–122], and thus allow (partial) escape from established protective humoral immunity.

The D614G mutation increased the affinity of the S1 protein for ACE2 [116, 119, 123] though it is not located directly in the RBD region, but adjacent to it [119]. As a result, viral entry into cells, infectivity, and human-to-human transmission were increased [123, 124]. This seemed to contribute to a rapid SARS-CoV-2 spread around the world, as seen by the steep increase in global frequency of the D614G mutation starting in March 2020, reaching a value of 70% already in June 2000 [124]. As summarized by Chakraborty et al. [116], the D614G mutation is found in the VOC P.1, B.1.427, B.1.1.7, B.1.429, and B.1.351, and in the variants of interest B.1.525, B.1.526 (sublineage B.1.526.1), B.1.617 (all sublineages like B.1.617.1, B.1.617.2 and B.1.617.3),

P.2, P.3, B.1.616 and B.1.427. Importantly, the D614G mutation-maintained neutralization susceptibility, as measured by classical neutralization tests [123, 124].

A more complex scenario has been elucidated for the S1 mutation N501Y, which is found in the variants B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma) and P.3 (theta).

This mutation is directly located in the RBD of S1 and causes an increased transmissibility due to enhanced binding affinity for ACE2. The enhanced affinity seems to be due to increased electrostatic interactions between RBD and ACE2 [116, 122, 125]. As determined by Fratev [126], not only the S1 RBD-ACE2 interaction was increased for this mutant, but in parallel the affinity of a neutralizing antibody directed towards RBD was simultaneously decreased about 160-fold. The latter finding is in perfect agreement with the findings and conclusions by Zhou et al. [122], which explain the successful and rapid spread of SARS-CoV-2 variants carrying the mutation N507Y, based on the doubled effect of an RBD mutation on 1) higher affinity for ACE2 and 2) lower susceptibility for neutralizing IgG. They also allow illustrating the crucial role of IgG affinity/avidity for protective humoral immunity: it is obvious that protective IgG that efficiently counteracts RBD containing the N507Y mutation, would require higher affinity for RBD than IgG that neutralizes wild-type RBD, in order to compensate the higher affinity of the mutant RBD for ACE2. This can be reached by the strategy of third immunizations towards SARS-CoV-2, as the second booster effect triggered by the third immunization with the BioNTech vaccine not only increased the concentration of neutralizing IgG, but also increased its avidity [65].

The significance of these mechanistic considerations related to the RBD mutation N507Y for the situation of vaccinated human populations can be deduced from the findings of Kustin et al. [127]. They hypothesized that if vaccine effectiveness against a VOC was reduced, its proportion among breakthrough cases would be higher than in unvaccinated controls. Their findings on disproportional infection of vaccinees compared to unvaccinated control individuals indicated reduced vaccine effectiveness against VOCs within particular time windows.

In line with the findings for the mutation N507Y, the mutation L452R carried by the SARS-CoV-2 variants B.1.427/B.1.429 (epsilon) and B.1.617.1 (kappa) [128] caused enhanced affinity between ACE2 and the spike RBD. The mutation L452R caused increased infectivity and reduced neutralization by sera from vaccinated individuals [129].

The RBD mutations L452R, P681R, and T478K in the variant B1.617.2 (delta) cause high binding affinity to ACE2 [117, 130] and thus explain the high transmission rate of this variant. The improved transmissibility of the variant C.37 (lambda) has been attributed to the mutation L452Q, which seems to cause enhanced virus-cell interactions through ACE2 [131]. Finally, the mutation K417N/T represents an exception, as it causes a decrease in the affinity of RBD towards ACE2 [132].

The study of SARS-CoV-2 variants is teaching us that enhanced affinity of variant RBD for ACE2, as well as decreased susceptibility of S1 for neutralizing antibodies, favor the escape of variant SARS-CoV-2 from humoral control. Counteraction towards these combined actions can be eventually achieved through the increase in avidity/affinity of IgG induced by adequate vaccination strategies. These must include optimal conditions for sustained affinity/avidity maturation.

The overall picture of mutations related to VOC shows that enhanced affinity for ACE2 and decreased susceptibility for neutralizing antibodies is linked to the same locus in some cases or represents separate loci [119, 121, 132–135]. Also, loci not located directly in the RBD region may influence the affinity of RBD [119]. As enhanced infectivity and viral transmission are the result of all these mutations, efficient control and protective immunity seem to require an enhanced avidity of IgG [136].

The recently isolated omicron variant is characterized by enhanced transmissibility and an astonishing degree of antibody escape. It, therefore, raises major concerns and triggers the establishment of adequate countermeasures. The omicron variant contains several deletions and more than 30 mutations, many of them concentrated in the RBD of S1 [137–139]. Despite the presence of several mutations that are known to increase the affinity of RBD to ACE2, the affinity of omicron RBD towards ACE2 was much lower, when compared to RBD of beta and delta variants. It was even significantly lower than wild-type RBD [139]. It seems that

various mutations in the RBD region counteracted the affinity-enhancing effects of some of the mutations. These astonishing experimental data [139] are in line with conclusions based on mathematical modeling studies [140]. They indicate that the affinity of omicron RBD is not the likely cause for the rapid spread of this virus. Rather, the rapid spread might be explained by the near-complete lack of neutralizing activity against omicron in polyclonal sera after two doses of the BioNTech/Pfizer vaccine, in convalescent individuals, as well as resistance to different monoclonal antibodies in clinical use, as reported by Gruell et al. [138]. The complete lack of neutralizing activity by some monoclonal antibodies seems to indicate that omicron RBD lacks several epitopes that are relevant for protection. The neutralizing effect of other monoclonal antibodies points to the expression of certain neutralization-relevant epitopes. The lack of neutralizing activity of convalescent sera and sera from double vaccinated individuals then might either indicate that these sera do not contain IgG directed towards these epitopes or alternatively, that the avidity/affinity of such IgG in these sera is no longer sufficiently high for causing a neutralizing effect. This would imply that the epitopes had been changed by the mutation in a way that changes the affinity/avidity towards IgG subpopulations that had high affinity/avidity towards the unmutated form of the epitopes. The finding that boosting through a third immunization step (boosting step) leads to considerable neutralizing potential [138] is best explained by an increase of avidity of potentially neutralizing IgG through the booster immunization. The significance of this finding for protective immunity *in vivo* remains open, as Schubert et al. [139] showed that a third vaccination step did not seem to enhance the efficiency of binding between IgG and RBD. The discrepancy between the data obtained by Gruell et al. [138] and Schubert et al. [139] seems to be based on the use of different methods—neutralization test [138] *versus* direct binding assay [139]. It is not unlikely that new, omicron-specific vaccines need to be developed for the establishment of desirable protective immunity.

## Neutralization and avidity tests: complementary approaches

Neutralization tests are frequently termed the "gold standard" of SARS-CoV-2 serology, as can be easily seen from a Google Scholar survey. It is often implicated that a strong neutralizing potential *in vitro* is a clear indicator for strong protection against SARS-CoV-2 virus infection or COVID-19 *in vivo*.

However, there is also a strong basis for contradiction to this view, based on recent studies: Ravichandran et al. [75], Tang et al. [76], and Tang et al. [77] provided clear evidence for a discordance between serum neutralization titers and recovery from COVID-19, whereas a higher antibody affinity of IgG directed towards S1/RBD was associated with disease resolution and fortunate clinical outcome of the patients.

Also, the increased affinity of S1 protein for ACE2 by certain VOCs with the D614G mutation [116, 119, 123], with the resultant increased viral entry into cells, and subsequent increased human-to-human transmission were not reflected in the outcome of neutralization tests, when sera from patients infected with wild-type virus were compared to those infected by mutant viruses [123, 124].

Finally, the strong discrepancy of the results obtained with sera taken from individuals with threefold vaccination in the studies by Gruell et al. [138] and Schubert et al. [139] deserves attention, as the neutralization test [138] seems to indicate a certain degree of protective immunity of IgG from individuals with three vaccinations, whereas a direct binding assay between RBD and IgG did not lead to the same conclusion [139].

A closer look at the technique of classical neutralization tests [141] allows to define their inherent problems: classical neutralization tests with SARS-CoV-2 or pseudoviruses carrying SARS-CoV-2 S1 are performed in two steps [141], i.e., usually 1 h binding reaction between serum and virus/pseudovirus, followed by infection of suitable cells with this mixture. This dissection of the overall process into two separate entities leads to the neglecting of the role of IgG avidity towards S1/RBD, as well as the affinity between S1/RBD for ACE2 which is in competition to IgG affinity/avidity to S1/RBD during prevention of infection by IgG. When, during the first reaction step, serum dilutions are preincubated with virus or pseudovirus for 1 h, in order to allow loading of the viral (pseudoviral) particle with IgG that specifically targets the S1, due to the length of time, also IgG of lower affinity/avidity has a chance to bind to the virus. When, during the second step, the preincubated mixture is added to the cellular detection system, even the loading of viral particles

with low avidity IgG may prevent infection and is then taken as an indication of neutralizing activity. Under conditions where cellular ACE2 and IgG compete directly for S1/RBD, IgG of low avidity can be predicted to have no chance to compete with the high affinity between ACE2 and S1/RBD. Neutralization would not be possible with low avidity IgG. Therefore, classical neutralization tests certainly determine antibodies that specifically bind to the viral S1, but cannot predict prevention of infection/disease *in vivo*, as can be seen from the reports on the discordance between neutralization titers and disease severity and outcome [75–77].

The same problem is inherent to competition tests that have been established to substitute for classical neutralization tests [142]. These substitution tests use a preincubation between crystallizable fragment (Fc)-labeled RBD and serum, followed by addition to a microwell plate with fixed ACE2. Again, this test format determines the titer of antibodies that bind specifically to the binding-relevant sites of RBD, but they do not allow to determine the impact of the avidity of the specifically binding antibodies, as the relevant and competing processes are also performed separately.

One exciting exception from the classical principle of neutralization tests has been applied in the work published by Pratesi et al. [62]. The competition/neutralization test used in their study utilizes a set-up that seems to allow direct competition between the relevant reaction partners. The test system is based on RBD coated to the bottom of a microwell plate. The buffer in the well receives serum dilutions and ACE2 labeled with Fc. The assay is then incubated. In this assay, the competition reaction between ACE2 and IgG for binding to RBD occurs directly at the RBD-coated surface, establishing a chance to actually determine the power of IgG to outcompete ACE2 from binding to RBD. It will be important to clarify whether this assay can determine the protective effect of neutralizing IgG *in vivo*. So far, it was demonstrated that vaccination with the BioNTech vaccine, caused a rise in neutralizating activity in parallel to the rise of avidity after the second step of vaccination [62].

Neumann et al. [53] as well as Pratesi et al. [62] demonstrated a correlation between avidity maturation of IgG directed towards S1/RBD and neutralizing potential. These correlation studies are interesting and important, but not yet sufficient to distinguish between coincidence and causal relationship. Furthermore, a detailed look at the data presented by Benner et al. [55] shows that despite the discussed correlation between the results of the neutralization and the avidity tests, several sera with low avidity showed high activity in the neutralization test.

Due to the test problems discussed in this chapter and recent findings [75–77], we suggest complementing neutralization tests with parallel avidity tests for the estimation of successful vaccination with protective immunity. If double testing is not practicable, the determination of individuals with low avidity IgG towards RBD/S1 might be useful to determine people that might have a potential higher risk for SARS-CoV-2 infection/COVID-19. These individuals should definitely receive an additional vaccination step. These analytical approaches should be especially beneficial for the elderly, as this group of individuals 1) has additional risks due to a higher frequency of comorbidities [143], 2) has been shown to frequently possess a more limited immunological repertoire [144], and 3) seems to establish high avidity of IgG after two vaccination steps less frequently than younger individuals [145]. The finding of lower avidity, despite completed immunization of the elderly, has been confirmed by the demonstration of a reduced number of hypermutations in the B cell population in this group [146].

Due to the complexity and significance of emerging virus variants, the test systems for IgG avidity should allow parallel determination of the concentrations and avidities of IgG directed towards RBD/S1 from the vaccine strain of SARS-CoV-2 and RBD/S1 from selected VOCs, such as the delta and omicron variant of SARS-CoV-2. This goal might be achieved with several available test platforms. Such test systems can be expected to clarify within one determination 1) whether the vaccine has established high avidity IgG towards the vaccine strain at sufficiently high concentration and 2) whether this response shows analogous or deviating activity towards S1/RBD of actual virus variants. Such information might be required for rational health management and eventually also for the development of new vaccines.

## Conclusions

Infection with SARS-CoV-2 leads to incomplete avidity maturation in most cases, characterized by low or intermediate avidity. In contrast, two vaccination steps with either mRNA or vector-based vaccine, or a combination thereof, allow complete avidity maturation, characterized by high avidity in the majority of cases. The combination of infection followed by at least one subsequent vaccination step allows complete avidity maturation as well. Therefore, optimal vaccination rather than natural vaccination alone seems to be necessary for the control of the present pandemic, as the protective role of high avidity IgG has been shown to be essential for the control of microbial infections and vaccinations.

Incomplete avidity maturation may be caused by insufficient availability of antigen throughout the process of avidity maturation. In addition, avidity maturation can be prevented by SARS-CoV-2-mediated interference with the function of GCs.

Classical neutralization tests fade out the role of IgG avidity for S1/RBD in the competition reaction between ACE2 and S1/RBD, which is driven by high affinity. They define IgG populations that bind to S1/RBD with high specificity, but are not necessarily predictive for protective immunity. New results indicate that high avidity IgG is predictive of fortunate disease outcomes from COVID-19, whereas IgG titers determined in classical neutralization tests can be discordant with disease outcome.

Many VOCs exhibit increased affinity for ACE2 and have lost susceptibility for the attack by neutralizing antibodies. The omicron variant has not increased its affinity for ACE2, but seems to escape the humoral immune system due to the substantial modification of neutralization-relevant epitopes. The determination of IgG avidity for S1/RBD might help to define those individuals that have suboptimal avidity maturation despite prime-boosting. These individuals should get additional boosting preferentially.

The spread of VOC requires establishing test systems that monitor in parallel the IgG concentrations and avidities directed towards S1/RBD of the vaccine strain and actual VOC. Immunoblot or luminex multiplex assays are feasible platforms to achieve this goal. Avidity determination seems to be particularly necessary to determine and optimize the avidity status of the elderly, as this group often shows lower avidity values after basis immunization, in addition to a reduced B cell repertoire.

## Notes added in proof

Lustig et al. [65] recently presented data on 12,413 individuals that had received three vaccinations with the BioNTech/Pfizer vaccine BNT162b2. Their study confirmed that the second vaccination step was necessary to achieve high-avidity IgG directed towards RBD in the majority of individuals [7]. However, the response after second vaccination was also characterized by considerable heterogeneity, as a substantial percentage of individuals had developed IgG of low or intermediate avidity. Lustig et al. [65] impressively demonstrated that the third vaccination step caused a further increase in avidity in practically all of these individuals. It thus shifted the IgG towards RBD to high avidity in all cases that had reached only low or intermediate avidity after the second vaccination step, and further increased avidity in those individuals which had already reached high avidity after second vaccination. This uniform increase of IgG avidity might be seen as the most important benefit of third vaccination, in addition to the increase in IgG concentration.

Gargouri et al. [147] reported on reinfection of four previously infected health care workers. They showed that the variants' features of first and second infections were different in all four cases, providing irrefutable evidence for reinfection. All four patients were reinfected by a variant that carries the spike mutation S477N, which enhances affinity to ACE2 [132]. This finding is pointing to the crucial importance of the competition between IgG and ACE2 for RBD during protection and the central role of IgG avidity in this process. Their data are in line with the findings by van Binnendijk et al. [148], who demonstrated that 5/8 cases of reinfection occurred with a different lineage of SARS-CoV-2. Van Binnendijk et al. [148] also reported on a rapid increase in avidity after reinfection. This confirms that one round of infection with SARS-CoV-2 is not sufficient to induce complete avidity maturation [7] and also shows for the first time that SARS-CoV-2 infection may exert boosting the potential as well.

Importantly, Kuhlmann et al. [149] showed that even three doses of mRNA vaccines may not be sufficient to prevent infection and symptomatic disease after infection with the omicron variant. Their finding of breakthrough infections is in line with the immune escape characteristics of the omicron variant. It is thus crucial for our understanding of the present phase of the SARS-CoV-2 pandemic. The work by Kuhlmann et al. [149] is in perfect agreement with the weekly report of the Robert Koch Institute (Germany), dated, January 27th, 2022, where it is reported that even three vaccination steps only have a marginal effect on SARS-CoV-2 omicron infection, though they established a partial protective effect towards more severe disease.

The aspect of immune evasion by the omicron variant is addressed in a very straightforward approach by Cameroni et al. [150], who showed that 26/29 receptor binding motif antibodies had lost their neutralizing activity when tested on the omicron variant. This finding gives an impressive quantitative estimation of the degree of immune escape of the omicron variant. It allows to predict that only a small subpopulation of IgG, induced by mRNA vaccines based on wild type SARS-CoV-2, has the potential to bind to epitopes on omicron RBD that are relevant for neutralization. Due to this rather small overlap between epitopes on the original strains of SARS-CoV-2 and the omicron strain, additional booster vaccinations with the present vaccines therefore might have a certain limitation to further improve protection towards the omicron variant. This is particularly relevant for individuals of higher age, due to their potentially reduced B cell repertoire [144]. However, two additional vaccination steps with an omicron-specific vaccine might establish an IgG response of high avidity, matching to the epitopes of omicron RBD and thus preventing COVID-19. In individuals that have been recently infected with the omicron variant, at least one additional vaccination step with an omicron-specific vaccine can be predicted to be necessary to achieve high-avidity IgG directed towards RBD of this variant. This degree of protection can be predicted to be necessary for the prevention of the spread of the virus within the population and the subsequent induction of COVID-19.

Wratil et al. [151] recently reported on neutralizing antibody dynamics in a longitudinal cohort of COVID-19 convalescent and infection-naive individuals vaccinated with mRNA BNT162b2. They concluded that an increase in antibody avidity may be critical for a highly potent infection-neutralization. Three consecutive spike antigen exposures were paralleled by stepwise increases in antibody avidity. The data by Wratil et al. [151] confirmed a step-increase in antibody avidity after a single vaccine dose in previously infected individuals, as originally reported by Struck et al. [7] and confirmed the findings by Lustig et al. [65], who had shown that three vaccination steps were necessary to induce high avidity IgG directed towards SARS-CoV-2 RBD in all individuals within a group of infection-naive subjects. Finally, the data presented by Wratil et al. [151] are confirming the findings by van Binnendijk et al. [148], who have shown that secondary infection with SARS-CoV-2 has boosting potential for IgG avidity.

It is obvious that the degree of complexity of SARS-CoV-2 variants and their interaction with IgG of varying avidity requires a rational approach to determine the state of protection 1) on an individual basis and 2) focused on the actually endangering variant(s). Such a serological approach is technically feasible and should include the determination of avidity. It may be beneficial for individuals engaged in medical or social professions, associated with a high risk of infection, as well as individuals that are more vulnerable than others.

## Abbreviations

ACE2: angiotensin-converting enzyme-2

AIs: avidity indices

CMV: cytomegalovirus

COVID-19: coronavirus disease 2019

GCs: germinal centers

HPVs: human papillomaviruses

IgG: immunoglobulin G

mRNA: messenger RNA

NP: nucleoprotein

PCR: polymerase chain reaction

RBD: receptor-binding domain

S1: spike protein S1

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

VOC: variants of concern

# Declarations

### Conflicts of interest

Georg Bauer is the inventor of two patents related to avidity determination (WO 00/54055; PCT/EP00/01883) and the coinventor of a patent application related to avidity determination and SARS-CoV-2 serology (EP 2019/2550).

### Ethical approval

Not applicable.

### Consent to participate

Not applicable.

### Consent to publication

Not applicable.

### Availability of data and materials

Not applicable.

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