Opinion Paper

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Commercial immunoassays for detection of anti-SARS-CoV-2 spike and RBD antibodies: urgent call for validation against new and highly mutated variants

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Abstract: Measuring the level of protection conferred by anti-SARS-CoV-2 (trimeric) spike or RBD (receptor binding domain) antibodies (especially total and IgG) is a suitable and reliable approach for predicting biological protection against the risk of infection and severe coronavirus disease 2019 (COVID-19) illness. Nonetheless, SARS-CoV-2 has undergone a broad process of recombination since the identification of the prototype lineage in 2019, introducing a huge number of mutations in its genome and generating a vast array of variants of interest (VoI) and concern (VoC). Many of such variants developed several mutations in spike protein and RBD, with the new Omicron (B.1.1.529) clade displaying over 30 changes, 15 of which concentrated in the RBD. Besides their impact on virus biology, as well as on the risk of detection failure with some molecular techniques (i.e., S gene dropout), recent evidence suggests that these mutations may also jeopardize the reliability of currently available commercial immunoassays for detecting anti-SARS-CoV-2 antibodies. The antigen (either spike or RBD) and epitopes of the prototype SARS-CoV-2 coated in some immunoassays may no longer reflect the sequence of circulating variants. On the other hand, anti-SARS-CoV-2 antibodies elicited by highly mutated SARS-CoV-2 variants

may no longer be efficiently recognized by the currently available commercial immunoassays. Therefore, beside the compelling need to regularly re-evaluate and revalidate all commercially available immunoassays against live virus neutralization assays based on emerging VoCs or VoIs, diagnostic companies may also consider to redevelop their methods, replacing former SARS-CoV-2 antigens and epitopes with those of the new variants.

Keywords: COVID-19; immunoassays; SARS-CoV-2; variants.

Introduction

Deciphering the level and duration of immunity developing after SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) natural infection or COVID-19 (coronavirus disease 2019) vaccination remains a crucial issue for planning public health, social and healthcare policies worldwide, since the overall number of primary or breakthrough (i.e., post-vaccination) infections, especially those accompanied by severe or critical illness, is a major determinant of virus circulation and healthcare pressure [1]. Although the efficacy that a former SARS-CoV-2 infection or a primary vaccination cycle may both confer against COVID-19 varies widely according to several important factors (i.e., age, sex, body mass index, immunocompetence, co-morbidities, SARS-CoV-2 variants, type of vaccine, period passed from infection or vaccination, and so forth), population studies suggest that natural immunity may be associated with short-term (i.e., <6 months) 85–95% protection against both asymptomatic and symptomatic SARS-CoV-2 infection, whilst primary COVID-19 vaccination may be associated with similar or even higher protection (i.e., 90–100%) against these same endpoints, during the same period of time [2]. Such optimal level of protection (Influenza vaccines have much lower efficacy, typically between 50 and 60%) [3], tends to gradually decline over time, reaching an almost critical point at 5–6 months after infection or vaccination,

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when adaptative immunity seems no longer effective to prevent – at best – the infection or, in the worst scenario, to attenuate the risk of developing severe or critical forms of COVID-19 [4, 5].

Measuring natural and vaccine-induced immunity against SARS-CoV-2

It is now abundantly clear that adaptative response against a given exogenous pathogen is based on at least two major pathways, encompassing humoral (i.e., antibodies-mediated) and cellular (i.e., cell-mediate) immunity. For respiratory viruses like SARS-CoV-2, humoral immunity entails the generation of several types of immunoglobulins (Ig) against the pathogen (especially IgA, IgG and IgM), which have the primary function to protect the body mucosae from virus penetration (i.e., secretory IgG and dimeric IgA), as well as to neutralize the virus once it penetrates the body (especially IgG), thus fostering its clearance by immune cells or limiting/abolishing its binding to host cell receptors (i.e., neutralization) [6]. Measuring the levels of protection conferred by anti-SARS-CoV-2 antibodies, especially total Ig and IgG, is increasingly regarded as suitable and reliable approach for predicting biological protection [7]. To this end, several lines of evidence now attest clearly that the risk of breakthrough infections is almost linearly dependent (i.e., inversely correlated) with the serum levels of both anti-SARS-CoV-2 spike (trimeric) protein and receptor binding domain (RBD) IgG [8]. A close correlation between neutralizing antibodies measured by Plaque Reduction Neutralization Test (PRNT) and some available IgG Trimeric and RBD immunoassays has been described [9]. Not surprisingly, however, SARS-CoV-2 reinfection more often occurs in people with absent or low levels of neutralising antibodies measured immediately before recurrent infections [10]. Such evidence would hence support the conclusion that measuring either anti-SARS-CoV-2 spike or RBD antibodies (especially IgG) could reliably predict the overall level of natural or vaccine-induced protection, thus allowing to plan public health measures aimed at more efficiently curbing virus circulation and transmission (i.e., mandatory vaccination, lockdowns, school closures, social distancing in public places, face masking and so forth) [11].

The challenge of SARS-CoV-2 mutations and variants

Since the identification of the prototype strain in Wuhan at the end of the year 2019, SARS-CoV-2 has undergone a

broad process of recombination, introducing a huge number of mutations in its genome, generating nearly 4,000 descendants according to GISAID (Global Initiative on Sharing All Influenza Data) [12], with several clades finally defined as variants of concern (VoC) or variants of interest (VoI) by the World Health Organization (WHO) [13]. This is not really surprising, since coronaviruses tend to mutate over time, though to lower extent compared to Influenza virus, for example [14]. This is the typical consequence of a biological (natural) pressure to which all viruses are subjected to for increasing their virulence (i.e., transmissibility), for better adaptation to host and/or environment (i.e., invasiveness) or for “escaping” the host immune response (i.e., immune escape) [15]. Mutations developing within the sequence of viral receptor moieties tend to accumulate at higher frequency, since they would contribute to confer all the three aforementioned advantages. SARS-CoV-2 makes no exception to this rule, as the many variants that have been identified during the past 2 years have developed a broad number of mutations in the spike protein and in its RBD, as summarized in Table 1 [13, 16]. Among these large number of new clades, the most recently emerged, called with the Greek letter “Omicron” (B.1.1.529) by the WHO, is causing major concern. According to a recent article, still only available as preprint, Sarkar et al. identified the presence of as many as 37 dominant mutations in the spike protein of this SARS-CoV-2 variant (31 mutations in the S1 domain – 15 of which within the RBD and 10 related to the receptor binding motif (RBM), and six mutations in the S2 subunit) [17], along with a subspecies lacking four of these mutations (i.e., Group 2, less represented, lacking the K417N, N440K, G446S and N764K mutations). Preliminary experiments suggest that the large number of mutations in the RBD of the Omicron variant may enable greater binding efficiency to host cells receptors (i.e., angiotensin converting enzyme 2; ACE2) [18], thus providing a reliable explanation to the higher infectivity that this highly mutated virus is seemingly displaying.

Analytical consequences of SARS-CoV-2 mutations

Besides their impact on virus biology, as well as on SARS-CoV-2 detection by means of some molecular biology techniques targeting specific gene sequences where mutations may have occurred (i.e., *S* gene dropout or *S* gene target failure seen in SARS-CoV-2 variants bearing the Δ 69-70 deletion, such as Omicron BA.1), another major technical problem connected to emergence of such a huge number of mutations in the spike protein and in the RBD has been highlighted by many studies. Wang et al. revealed that

Table 1: SARS-CoV-2 variants and mutations over time in the spike protein and in its receptor binding domain (RBD).

Variant	Name	Emergence	Date	Mutations in the spike protein (in the RBD in bold)
B.1.1.7 ^a	Alpha (α)	UK	Dec, 2020	Δ 69-70, Δ Y144, N501Y , A570D, P681H, T716I, S982A, D1118H
B.1.351 ^a	Beta (β)	South Africa	Dec, 2020	L18F, D80A, D215G, R246I, K417N , E484K , N501Y , D614G, A701V
B.1.1.128 (P.1) ^a	Gamma (γ)	Brazil	Jan, 2021	L18F, T20N, P26S, D138Y, R190S, K417T , E484K , N501Y , H655Y, T1027I
B.1.617.2 ^a	Delta (δ)	India	May, 2021	T19R, G142D, Δ E156, Δ F157, R158G, L452R , T478K , D614G, P681R, D950N
B.1.427/9	Epsilon (ϵ)	US (California)	Mar, 2021	S13I, W152C, L452R
B.1.1.128 (P.2)	Zeta (ζ)	Brazil	Mar, 2021	E484K , D614G, V1176F
B.1.525	Eta (η)	Multiple (Nigeria)	Mar, 2021	Q52R, A67V, Δ 69-70, Δ 144, E484K , D614G, D677H, F888L
B.1.526	Iota (ι)	US (New York)	Nov, 2020	L5F, T95I, D253G, E484K , D614G and A701V
P3	Theta (θ)	Philippine	Apr, 2021	Δ 141-143, E484K , N501Y , D614G, P681H
B.1.617.1	Kappa (κ)	India	Apr, 2021	E154K, L452R , E484Q , D614G, P681R, Q1071H
C.47 ^b	Lamda (λ)	Peru	Dec, 2020	G75V, T76I, Δ 246-252, L452Q , F490S , D614G, T859N
B.1.621 ^b	Mu (μ)	Columbia	Jan, 2021	T95I, Y144S, Y145N, R346K , E484K , N501Y , D614G, P681H, D950N
B.1.1.529 ^a	Omicron ($\omicron$)	South Africa	Nov, 2021	A67V, Δ 69-70, T95I, Δ 142-144, Y145D, Δ 211, L212I, ins214EPE, G339D , S371L , S373P , S375F , K417N , N440K , G446S , S477N , T478K , E484A , Q493R , G496S , Q498R , N501Y , Y505H , T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F

^aSARS-CoV-2 Variants of Concern (VoC) as of December 9, 2021; ^bSARS-CoV-2 Variants of Interest (Vol) as of December 9, 2021.

the neutralizing potential (expressed as Focus Reduction Neutralization Test with 50% neutralization; FRNT50) of serum or plasma in mRNA-based COVID-19 vaccines recipients was diminished by several folds, especially for some variants like the South-African β (B.1.351) or the Columbian μ (B.1.621) [19]. Another recent preprint article reported an over 40-fold decline in FRNT50 vs. the new Omicron variant [20], which is not only worrying in a healthcare perspective (i.e., magnified risk of recurrent or breakthrough infections), but may also jeopardize the reliability of the currently available commercial immunoassays for detecting anti-SARS-CoV-2 spike and RBD total or IgG antibodies. It must be clearly underpinned that the data presented by Cele et al. in their preprint article must be interpreted with great caution for several drawbacks, including that (i) figures show that a pseudotype lentivirus assay was used for detecting neutralization, which is not as good as a live virus assay [21]; (ii) a very limited set of samples ($n=6$) was collected in naïve recipients of Pfizer/BioNTech mRNA-based vaccine; and (iii) 12 days post-vaccination (range 10–39 days) for collecting samples is perhaps a too early period for testing neutralizing activity, since the peak of such activity is reached between 14 and 28 days after completing a primary vaccination cycle. Nonetheless, another article, published immediately afterwards, confirmed the evidence that the B.1.1.529 Omicron variant may be more resistant than any other known SARS-CoV-2 variants to neutralization (i.e., between 20 and 23 folds decline in neutralization capacity compared to the Delta variant), whilst sufficient degree of neutralization could only be reached after administration of a third booster dose of

mRNA vaccines [22]. Not surprisingly, though, Pfizer/BioNTech has confirmed with in-house experiments that sera from recipients of a complete primary vaccination cycle with two doses of BNT162b2 display a nearly 25-fold reduction in neutralizing antibodies activity against the Omicron variant, whilst a more robust protection could be reached by administering a third vaccine dose (i.e., producing an over 20-fold increase of antibody titers) [23]. Even more importantly, the anti-SARS-CoV-2 monoclonal antibodies casirivimab and imdevimab failed to retain neutralizing activity against this highly mutated virus [22], thus confirming that anti-SARS-CoV-2 antibodies triggered by vaccination or previous infections do not efficiently target new epitopes present on the B.1.1.529 Omicron variant.

An important question shall hence engage the minds of laboratory professionals, clinicians, diagnostic companies and even policymakers and patients. Are immunoassays in current clinical use for detection of anti-SARS-Cov-2 antibodies (either anti-spike trimeric or anti-RBD) still valid for detecting (and monitoring) the neutralizing activity against highly mutated SARS-CoV-2 variants? It is virtually unquestionable that some of these variants have introduced such a huge number of mutations in the spike protein (i.e., >30 for Omicron), so that the antigen (either spike or RBD) and its relative epitopes coated in the immunoassays may no longer reflect the original sequence and structure of the prototype SARS-CoV-2 lineage first detected in Wuhan, in 2019 (Table 1). The ensuing questions that will arise are, therefore: Which types of antibodies are we actually measuring? Do they still retain clinical significance, in terms of being capable to really mirror the neutralizing potential against

Figure 1: Impact of new SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) mutations on commercially available immunoassays for detecting anti-spike and anti-RBD (receptor binding domain) antibodies.

new SARS-CoV-2 variants, considering that these new strains display a decay of antibody-related neutralization *in vivo* (Figure 1)? And, on the other hand, will anti-SARS-CoV-2 antibodies elicited by highly mutated SARS-CoV-2 variants (such as the Omicron) be efficiently recognized by the currently available commercial immunoassays?

The road ahead

Beside the compelling need to regularly re-evaluate and revalidate all commercially available immunoassays for detecting anti-SARS-CoV-2 spike and RBD antibodies against live virus neutralization (LVN) assays (e.g., focus-reduction neutralization tests, plaque reduction neutralization tests and live virus micro-neutralization assays) based on new VoC or VoI, two strategies may be identified for overcoming this challenge. The first is drastic and almost unrealizable, since it entails that immunoassays be replaced by LVN tests, which are very time-consuming, expensive, have low throughput and can only be carried out in laboratories with high levels of biosafety. The second strategy, which seems more globally feasible, encompasses that diagnostic companies will need to periodically review and redevelop their assays, replacing former SARS-CoV-2 antigens and epitopes

(in the spike protein and/or RBD), with those of the new variants, like some pharmaceutical companies are already planning for updating the formulation of their vaccines [24]. This seems essential for preventing to generate (i) the false sense of reassurance that an elevated anti-SARS-CoV-2 antibodies level that has been developed versus former viral antigens will be thoughtfully protective against new variants and (ii) the false impression of having a low anti-SARS-CoV-2 antibodies response elicited by new variants when these antibodies cannot be reliably recognized by the currently available immunoassays.

As we learn more about the new variants and their impact on serological assays, we encourage diagnostic companies and clinical laboratories to initiate accurate validation studies to ensure that the currently available commercial immunoassays for detecting anti-SARS-CoV-2 spike and RBD antibodies are still capable of predicting neutralizing activity against emerging and highly-mutated SARS-CoV-2 variants.

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