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Not all SARS-CoV-2 IgG and neutralizing antibody assays are created equal

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We read with interest the recent article of Malipiero et al. [1], who used a commercial anti-RBD (receptor binding domain) IgG quantitative chemiluminescent immunoassay and a surrogate virus neutralization test (sVNT) to investigate the kinetics of anti-SARS-CoV-2 (neutralizing) antibodies up to 6 months after administration of a primary cycle of Pfizer/BioNTech BNT162b2 vaccine in 57 local healthcare workers. Notably, these authors found that despite high neutralizing bioactivity was retained throughout the study period, the anti-RBD IgG antibodies markedly declined 6 months after primary vaccination. Such discrepancy is quite surprising and prompts us to make some biological and analytical considerations.

First, although it is conceivable that a standard primary vaccination cycle has been administered to the population of healthcare workers studied by Malipiero et al. (i.e., two 0.3 mL intramuscular injections of 30 μg of vaccine at 3-week interval), the specific protocol that was used is not comprehensively specified in their published work, so that the trajectory of anti-SARS-CoV-2 antibodies levels would not be readily interpretable, neither straightforwardly comparable with that found in other studies.

The use of a sVNT (ACE2-RBD Neutralization assay; Dia.Pro Diagnotic Bioprobes, Milano, Italy) is another important aspect that deserves further scrutiny. It is now widely acknowledged that the reference technique for studying the neutralizing potential of serum or plasma entails the use of live virus neutralization assays, which basically include focus-reduction neutralization tests, plaque reduction neutralization tests and live virus micro-neutralization assays [2]. In a recent study, Meyer et al. found a relative modest correlation between a sVNT and live virus or pseudovirus neutralization tests (correlations of 0.656 and 0.494, respectively), underpinning also that the sensitivity of the sVNT technique was only around 80% (e.g., only samples with $\geq$160 titre were found to be always positive with sVNT) [3]. In another preliminary report, Sholukh et al. reported that the correlation between a sVNT and a reference cell-based neutralization assay (50% neutralizing dilution; ND50) was only around 0.40 [4]. These analytical drawbacks would hence lead us to conclude that sVNTs are not suitable replacement of cell-based neutralization assays for being used as the reference technique for testing the performance of commercial anti-SARS-CoV-2 (neutralizing) antibodies. Importantly, several other reports, such as that published by Bayart et al. for example [5], have clearly shown that neutralizing antibodies assessed with pseudo-virus neutralization test (pVNT), which is a more reliable technique for this purpose than the sVNT [2], undergo a dramatic decline over time (i.e., over 90%) in Pfizer/BioNTech BNT162b2 vaccine recipients, with nearly half of vaccinated subjects turning negative after 6 months.

As then concerns the commercial anti-S-RBD IgG antibodies method used in the study of Malipiero et al. (sCOVG, Siemens Healthineers, Erlangen, Germany), a recent clinical and analytical evaluation of this quantitative chemiluminescence immunoassay published by Irsara et al. has revealed excellent performance compared to VNTs assayed on Vero 76 clone E6 cells, displaying a correlation of 0.843, an overall qualitative agreement of 98.5% and diagnostic sensitivity as high as 91% [6].

We are hence persuaded that concluding that the value of anti-RBD IgG is a (relatively) poor marker of neutralizing bioactivity seems unwarranted at this point in time, since this could only be reliably defined by using live virus neutralization assays. It shall also be clearly acknowledged that the current performance of the many commercial immunoassays that measure neutralizing anti-SARS-CoV-2 antibodies vary significantly when compared with the gold standard technique, as recently shown in a study that compared five anti-SARS-CoV-2 IgG chemiluminescent techniques with a plaque reduction neutralization test (PRNT) (i.e., correlations between 0.799 and 0.872) [7]. Finally, the clinical significance of the many currently available anti-SARS-CoV-2 IgG immunoassays needs to be tested and validated in patients infected by highly mutated variants, such as the recently emerged Omicron (B.1.1.529) strain [8].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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