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Answer to the letter of Lippi & Plebani entitled "Not all SARS-CoV-2 IgG and neutralizing antibody assays are created equal"

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Dear Editor,

We really appreciate the interest of Lippi and Plebani [1] for our recent paper, which offers us the opportunity to provide some additional clues. The primary aim of our study was to collect data on the long-term kinetics of binding and functional anti-SARS-COV-2 antibodies in a cohort of BNT162b2-vaccinated health care workers (HCW) over an observation period of 6 months post-vaccination. The main findings were the not unexpected decline of binding anti-RBD antibodies and the lack of a consistent and time-independent correlation between anti-RBD IgG levels and neutralizing bioactivity, which settled at high level from T2 (50 days post-1st dose administration) thereafter with a non significant declining trend between T2 and T3 (6 months post-1st dose administration). Lippi and Plebani pointed out that a systematic lack of accuracy in surrogate virus neutralization assay (sVNT) platforms compared to conventional ones should prevent us from concluding that the absolute anti-RBD level is not a reliable proxy of neutralizing bioactivity in BNT126b2-vaccinated individuals.

We certainly agree that sVNT platforms should not replace live virus neutralization assays as the gold standard for identifying and titering neutralizing antibodies to SARS-CoV-2 wild-type and variant strains, as we also admitted as a limitation of our study. However, we hint that the accuracy of these tests and their correlations relies on the clinical setting to which they are applied. Indeed, despite the existence of a WHO International Standard for both binding and neutralizing assays [2], it is not yet clear how the clinical setting (COVID-19 vaccinated vs. experienced patients) and the emergence of variants of concern could undermine the harmonization process between these different assay platforms under real-world conditions [3,4]. sVNT platforms, by investigating the wild-type RBD-induced neutralizing activity only, are likely to underestimate the neutralizing potency of serum from COVID-19 experienced individuals, especially when VOC are involved [5,6]. On the other hand, as repeatedly shown by comparative analyses in vaccinated cohorts [7–9], the diagnostic reliability of sVNT seems to be retained when assessing vaccine-induced immune-responses, provided that the mRNA sequence of the vaccine RBD matches that of the recombinant RBD in the binding and competition assays. The cross-sectional studies cited by Lippi and Plebani [5,6] quantified humoral immunogenicity markers in two cohorts of convalescent COVID-19 patients while the cohort studied by Bayart et al. [10] is as well affected by a large number (31%) of individuals with a positive serological scrutiny for SARS-CoV-2 infection before vaccination. In fact, the primary aim of the study by Meyer et al. [5] was the diagnostic value and comparability of different immune assay platforms for a diagnosis of SARS-CoV-2 infection rather than the long-term monitoring of the immune response to a spike-based vaccine. Notably, the very same study of Sholukh et al. [6] showed that the correlations between the sVNT, on the one side, and 4 live virus/pseudovirus neutralization assays, on the other, are much higher ($r = 0.73–0.8$) when the percentage of neutralization ($ND_{\%}$ in the original manuscript) is considered instead of the ND_{50} titer reported by Lippi and Plebani. Further on, all cited studies did not assess whether patients were infected by the Wuhan original strain or a variant strain, which, for the above reasons, could affect inter-assays harmonization. As to the study by Bayart et al. [10], which showed a much faster decline in neutralizing antibody titers using a live VNT than we assessed with our sVNT, available literature has so far provided contrasting results about long-term decay of circulating antibodies after mRNA vaccination, with some studies providing evidence for persistent and sustained neutralizing bioactivity of serum up to 6 months from vaccination [11,12]. It's our opinion that such discrepancies are resulting from multiple factors, including but not limited to inter-assays inconsistencies, such as previous exposure to cross-reacting common cold coronaviruses, age, history of previous COVID-19, sex, and other comorbidities in vaccinated cohorts.

We would also like to remark that in our study we did not disclaim the existence of a correlation between anti-RBD IgG levels and neutralizing bioactivity in vaccinated individuals nor that the high neutralizing activity we assessed at 6 months is necessarily protective, since the 1 month-apart swab-based monitoring protocol we use might have missed asymptomatic carriers. Actually, we observed an increasing strength of correlations from T2 and T3 despite a declining titer of anti-RBD IgG, likely as a result of affinity maturation of IgG by somatic hypermutation of spike/RBD-specific B cells [13–15]. This temporal trend has already been tracked after SARS-CoV-2 vaccination [12] and implies that an absolute conversion factor between anti-RBD IgG levels

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and neutralizing titers would be unreliable. As opposite, the studies cited by Lippi and Plebani [16,17] were not designed to provide data on anti-RBD IgG and neutralizing activity and their correlation on the long-term, when divergent kinetics may become apparent. Should a neutralizing antibodies absolute threshold of protection ever become available (hopefully using the WHO International Standard), implementation of the anti-RBD IgG level as a time-independent standalone correlate of protection may confuse risk stratification [14].

We are grateful to Lippi and Plebani for their appropriate remarks, which gave us the opportunity to better explain and contextualize our findings, but, for the above reasons, we believe the arguments and studies they brought about would not impair our findings. On the contrary, we believe that sVNT are user-friendly, high-throughput, standardized [18] immune-assays that may represent invaluable tools when implemented in the appropriate clinical and epidemiological context.

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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