Letter to the editor

Could anti-ACE2 antibodies alter the results of SARS-CoV-2 Ab neutralization assays?

To the Editor,

Arthur et al. [1]. published in September 2021 clear data proving the existence of specific anti-ACE2 antibodies in a high percentage of patients with history of SARS-CoV-2 infection: (i) 0% in outpatients with negative SARS-CoV-2 PCR virus test, (ii) 5% in outpatients with positive SARS-CoV-2 PCR virus test, (iii) 81% in patients that had a known positive virus test by PCR and had been symptom free for at least two weeks prior to donation of plasma and (iv) 93% in inpatients with positive SARS-CoV-2 PCR virus test.

Later, Murphy and Longo [2] discussed a possible role of anti-idiotype antibodies in SARS-CoV-2 infection and vaccination and, recently, some comments in NEJM [3–5] also suggest the possible involvement of anti-idiotype antibodies induced after SARS-CoV-2 infection and/or vaccination in the pathogenesis of severe adverse reactions such as myocarditis, immune-mediated thrombosis, thrombocytopenia or vaccine-related neurologic sequelae [3] and other protracted adverse events after vaccination [5]. Through the lens of the anti-idiotype network hypothesis, formulated in 1971 by Jerne [6], every induced antibody specific for an antigen (Ab1) is capable of inducing specific antibodies against itself as a form of down-regulation [7]. These anti-idiotype antibodies (Ab2) could be structurally similar to the original antigens and, hence, be able to bind to the same receptors that the original antigen binds to. Considering SARS-CoV-2 spike protein as the original antigen, it could be possible to have Ab1 mirroring ACE2 and Ab2 mirroring spike RBD. In that case, those anti-idiotype antibodies (Ab2) could be able to bind to the ACE2 receptor.

Independently of the origin of anti-ACE2 antibodies we want to focus on the fact that it is experimentally proved that many patients with previous SARS-CoV-2 infection have antibodies specific for ACE2 [1]. Considering that fact, we wonder if these anti-ACE2 antibodies could alter the results of some Ab neutralization assays causing some kind of misinterpretation in the neutralization capacity. This possible interference in the assay should be especially considered in those assays based on the binding to ACE2, given that both spike and anti-ACE2 antibodies would compete for ACE2 binding.

SARS-CoV-2 Ab neutralization assays represent a standard for assessing antibody-mediated protection. There are many SARS-CoV-2 Ab neutralization assays that have been designed using these three components: (i) SARS-CoV-2 antigen (usually RBD of SARS-CoV-2 spike protein), (ii) human ACE2 (human receptor for SARS-CoV-2 spike) and (iii) detection reagent coupled to one of them. All the assays in which ACE2 is in contact with the sample at any step of the detection process could be affected by the presence of anti-ACE2 antibodies in the sample. These anti-ACE2 antibodies could cause an overestimation of the quantification of actual neutralizing antibodies in the sample. After analyzing a representative set of currently available SARS-CoV-2 Ab neutralization assays we have classified them in 4 types, based on their basic components and their design. The Fig. 1 displays the design foundations for these 4 types of assays and how the presence of anti-ACE2 could alter or not the assay. All of them are competitive assays and the final signal is inversely proportional to the quantity of neutralizing antibodies.
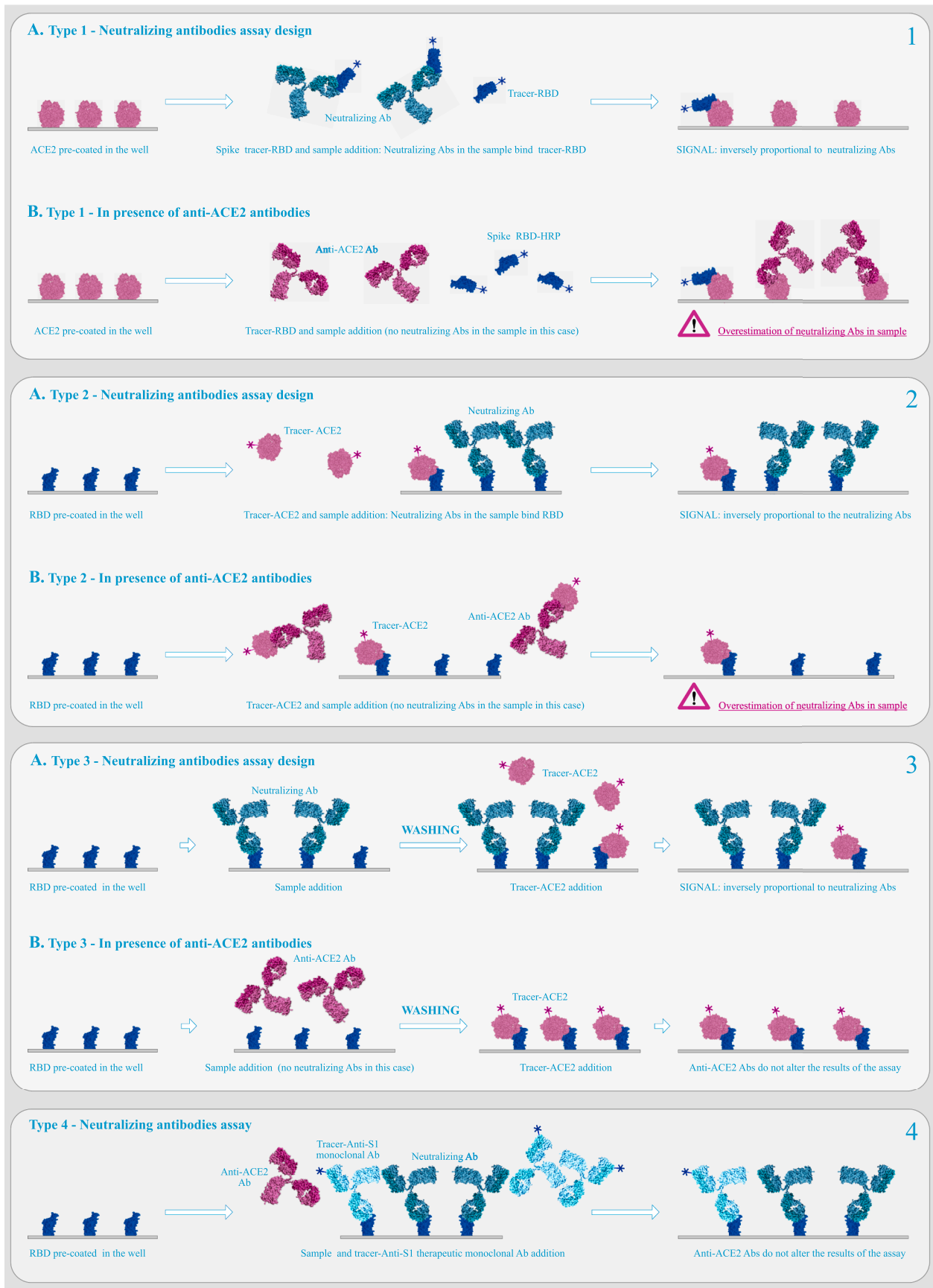
In conclusion, the possibility of the existence of anti-ACE2 antibodies in individuals with previous SARS-CoV-2 infection should be taken into account in the design of SARS-CoV-2 Ab neutralization assays.

A. Type 1 - Neutralizing antibodies assay design

1

ACE2 pre-coated in the well

Neutralizing Ab

Tracer-RBD

Spike tracer-RBD and sample addition: Neutralizing Abs in the sample bind tracer-RBD

SIGNAL: inversely proportional to neutralizing Abs

B. Type 1 - In presence of anti-ACE2 antibodies

ACE2 pre-coated in the well

Anti-ACE2 Ab

Spike RBD-HRP

Tracer-RBD and sample addition (no neutralizing Abs in the sample in this case)

⚠ Overestimation of neutralizing Abs in sample

A. Type 2 - Neutralizing antibodies assay design

2

RBD pre-coated in the well

Tracer- ACE2

Neutralizing Ab

Tracer-ACE2 and sample addition: Neutralizing Abs in the sample bind RBD

SIGNAL: inversely proportional to the neutralizing Abs

B. Type 2 - In presence of anti-ACE2 antibodies

RBD pre-coated in the well

Tracer-ACE2

Anti-ACE2 Ab

Tracer-ACE2 and sample addition (no neutralizing Abs in the sample in this case)

⚠ Overestimation of neutralizing Abs in sample

A. Type 3 - Neutralizing antibodies assay design

3

RBD pre-coated in the well

Neutralizing Ab

Tracer-ACE2

Sample addition

WASHING

Tracer-ACE2 addition

SIGNAL: inversely proportional to neutralizing Abs

B. Type 3 - In presence of anti-ACE2 antibodies

RBD pre-coated in the well

Anti-ACE2 Ab

Sample addition (no neutralizing Abs in this case)

WASHING

Tracer-ACE2

Tracer-ACE2 addition

Anti-ACE2 Abs do not alter the results of the assay

Type 4 - Neutralizing antibodies assay

4

RBD pre-coated in the well

Anti-ACE2 Ab

Tracer-Anti-S1 monoclonal Ab

Neutralizing Ab

Sample and tracer-Anti-S1 therapeutic monoclonal Ab addition

Anti-ACE2 Abs do not alter the results of the assay

(caption on next page)

Fig. 1. Impact of the presence of anti-ACE2 Ab in some types of SARS-CoV-2 Ab neutralizing assays. 1. A shows the design of type 1 assay that uses microplates pre-coated with ACE2. If neutralizing antibodies exist in the sample they will bind to the SARS-CoV-2 spike RBD complexed with the tracer (HRS, biotin, others). In this competitive assay the final signal is inversely proportional to the quantity of neutralizing antibodies in the sample. Only the RBD molecules that did not join to neutralizing Abs can bind to the ACE2 fixed in the microplate whose signal will be detected. B displays how the assay could work in presence of anti-ACE2 Abs and absence of neutralizing Abs (for easier evaluation of the impact of anti-ACE2 Abs). In this case anti-ACE2 Abs could bind to the ACE2 fixed in the wells reducing the final signal causing an overestimation of neutralizing Abs in the sample. 2. A. displays how the type 2 assays works. RBD are pre-coated in the wells (or in the beads) and sample and ACE-2 complexed with a tracer (HRS, biotin, others) are added. If neutralizing antibodies exist in the sample they will bind to the RBD in the wells. The signal finally detected is inversely proportional to the number of neutralizing Abs in the sample. B displays the potential impact of the presence of anti-ACE2 Abs. Anti-ACE2 Abs could bind to the tracer-ACE2 producing a reduction in the final signal. It would imply an overestimation of the quantity of neutralizing Abs in the sample. 3. A displays the type 3 assay steps. The microplate has pre-coated RBD. The sample is added and, when there are neutralizing Abs in the sample, they bind to the RBD in the wells. Then, after a washing step, only the molecules bound to RBD remain in the wells. In the next step ACE2 complexed with a tracer is added. ACE2 only can bind to the free RBD in the wells. As it is showed in B the presence of anti-ACE2 Abs in the sample would not alter the final results of this type of assay because anti-ACE2 Abs would be removed in the washing step. 4. It schematically displays the assays based on anti-spike monoclonal antibodies. Given that microplates would be RBD pre-coated and ACE2 does not participate in this type of assay, the presence of anti-ACE2 Abs would not have any expected impact.

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References

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