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Assay dependence of long-term kinetics of SARS-CoV-2 antibodies

Anneleen Schallier*, Sarah De Baets, Dirk De Bruyne, Kenny Dauwe, Margaux Herpol, Pedro Couck

Cerba Healthcare Belgium, CRI, Zwijnaarde, Belgium

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ABSTRACT

Since the worldwide outbreak of the novel coronavirus (SARS-CoV-2), the question raised whether infected patients would elicit long-lasting protective immunity. Several companies developed serological assays for the detection of SARS-CoV-2 antibodies. In this study, we compared 4 different serology assays in convalescents up to 7 months post-infection. Both Abbott assays showed a significative decrease of IgG antibodies over time. Whereas the Elecsys Anti-SARS-CoV-2 N assay (Roche) initially showed a significant increase, antibody titers significantly decreased at the latest timepoint. Although not significant, the Elecsys Anti-SARS-CoV-2 S assay (Roche) showed tendency towards increasing titers overtime. Our data showed that results of SARS-CoV-2 serology should be interpreted with caution.

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread rapidly around the world. First cases of COVID-19 appeared in Belgium in February 2020. As seen worldwide, testing capacity for the diagnosis of acute infection by molecular techniques was initially very low. Consequently, indications for testing was limited (e.g., severely ill patients, physicians) (Sciensano). By the end of April 2020, several companies developed serological assays for the detection of SARS-CoV-2 antibodies. These assays are utile for epidemiological purposes and follow-up of the immune status of patients (post-infection and/or vaccination). Few publications were published on the longitudinal follow-up of these antibodies. Furthermore, these publications showed discordant results (Muecksch et al., 2020). In this study, we compared 4 different serology assays in convalescents up to 7 months post-infection.

2. Materials and methods

2.1. Study design

This study was approved by the standing Committee on Ethics of the University Hospital Gent. Experiments were performed in accordance with the guidelines and regulations. All participants signed an informed consent.

2.2. Patients and blood sampling

Serum samples were collected from a cohort of 54 patients. Infection has been confirmed by polymerase chain reaction assay in 43 of the 54 participants. The other 11 patients had symptoms compatible with COVID-19 disease, lived in close contact with proven COVID-19 patients, but were not tested because of limited test availability. A questionnaire, mentioning the symptoms confer the National Guidelines, was completed. None of the patients was hospitalized.

Serum samples were collected at 4 different time points: Timepoint 0 (median 34 days after positive PCR/symptoms; 95 CI 34.7 to 41.3; maximum 72 days; minimum 13 days; n = 54) - Timepoint 1 (median 78 days; 95 CI 77.1 to 84.1; maximum 116 days; minimum 59 days; n = 50) - Timepoint 2 (median 145 days; 95 CI 142 to 149; maximum 179 days; minimum 122 days; n = 48) - Timepoint 3 (median 223 days; 95 CI 225 to 233; maximum 261 days; minimum 200 days; n = 49). Patients were enrolled in the study when a sample was obtained for a minimum of 3 timepoints.

2.3. SARS-CoV-2 immunoassays

Four different immunoassays were performed according to manufacturer's instructions (Table 1). The first assay is the SARS-CoV-2 IgG assay (Abbott, 6R86-32, Sligo, Ireland). The second assay is the SARS-CoV-2 IgG II Quant assay (Abbott, 6S60-22, Sligo, Ireland). Both assays are chemiluminescent microparticle immunoassays. The third and fourth assay are respectively the Elecsys Anti-SARS-CoV-2 (Roche, 09203079190, Mannheim, Germany) and Elecsys Anti-SARS-CoV-2 S (Roche, 09289275190, Mannheim, Germany) assay. Both assays are based on electrochemiluminescence immunoassay "ECLIA" technique.

* Corresponding author. Tel.: +32 9 329 23 00; fax: +32 9 329 23 01 2.
E-mail address: aschallier@cri.be (A. Schallier).

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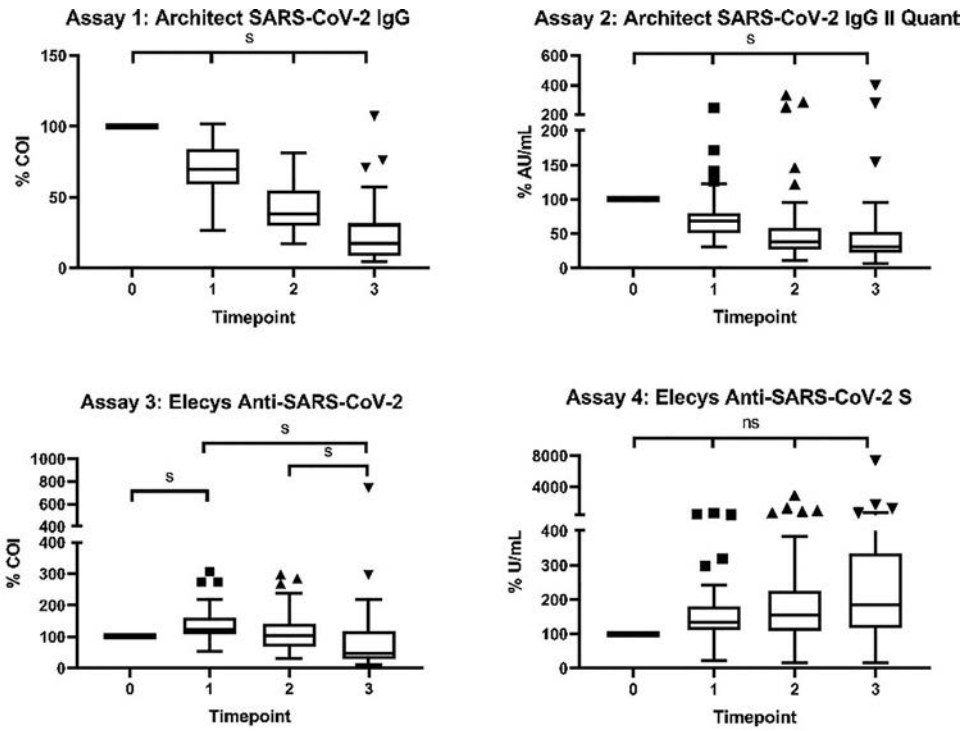

Fig. 1. Results of antibody responses against SARS-CoV-2. First timepoint (0: median 34 days after positive PCR or symptoms) is used as starting point (=100%). Other timepoints are normalized to this first point (T1: median 78 days; T2: median 179 days; T3: median 223 days). Assay 1 is the SARS-CoV-2 IgG assay (Abbott). Assay 2 is the SARS-CoV-2 IgG II Quant assay (Abbott). Assay 3 and 4 are respectively the Elecsys Anti-SARS-CoV-2 (Roche) and Elecsys Anti-SARS-CoV-2 S (Roche) assay. Statistics were performed by means of Wilks' lambda MANOVA test. Significant difference is shown by "s" symbol on the graph, whereas non-significance is shown by "ns"-symbol. Boxplots show a horizontal line as mean value, together with upper 75 and lower 25 percentiles.

2.4. Statistical analysis

Wilks' lambda MANOVA test was performed to test whether there are differences between the means of the antibody response in function of time. *P*-values <0,05 were considered statistically significant.

3. Results

As shown in Figs. 1 and 2, antibody responses for both Abbott assays showed a significant decrease in function of time. For assay 1, *P*-values were <0,001 between all timepoints. Furthermore, 30/49

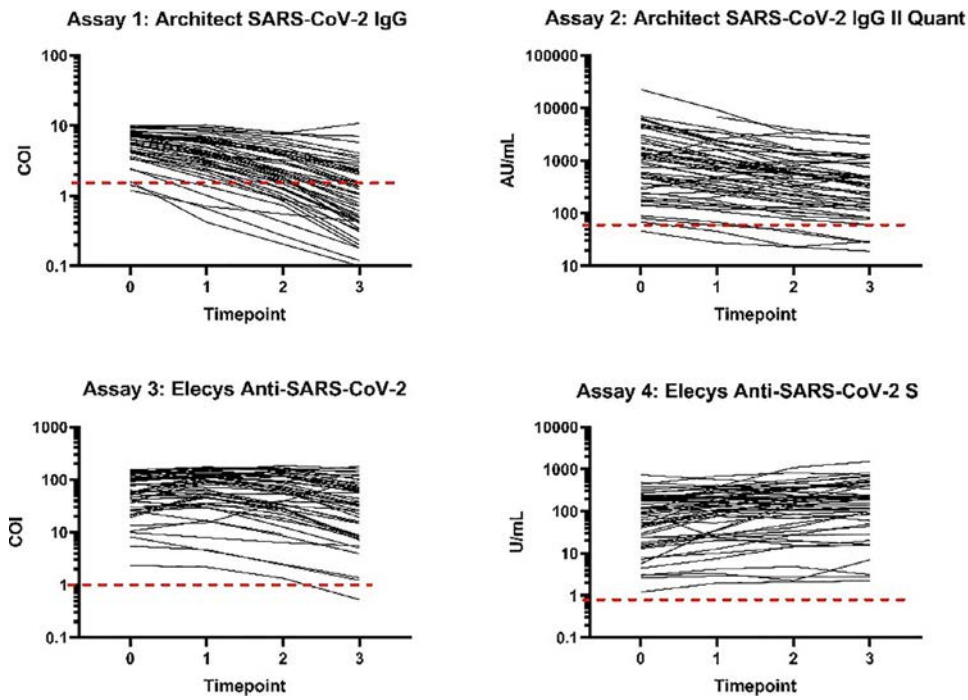

Fig. 2. Individual results of antibody responses against SARS-CoV-2. Timepoints: T0: median 34 days after positive PCR / first symptoms; T1: median 78 days; T2: median 179 days; T3: median 223 days. The dotted line represents the cut off proposed by the manufacturer.

Table 1
Characteristics of the different assays.

Assay	Company	Target	Cut off: not reactive	Cut off: reactive	Units		Measurement range
1 SARS-CoV-2 IgG assay	Abbott	N	<1,4	≥1,4	COI	qualitative	N.A.
2 SARS-CoV-2 IgG II Quant assay	Abbott	S	<50,0	≥50,0	AU/mL	quantitative	21,0–40 000,0
3 Elecsys Anti-SARS-CoV-2	Roche	N	<1,0	≥1,0	COI	qualitative	N.A.
4 Elecsys Anti-SARS-CoV-2 S	Roche	S	<0,8	≥0,8	U/mL	quantitative	0,4–250

COI = cut off index; N.A. = not available.

patients showed false negative results on timepoint 3. Indeed, antibody titers of these patients dropped below the cut-off. For assay 2, *P*-values were <0,001, except between timepoints 1 and 2 and between timepoints 2 and 3. *P*-values were <0,01 for the latter timepoints. At the latest timepoint measured, 4/49 patients showed false negative results. Significant difference ($P < 0{,}001$) was observed for different timepoints for the Elecsys Anti-SARS-CoV-2 assay (Roche). As shown in Figs. 1 and 2, an initial significant increase of antibody titers could be detected for this third assay. This increase is followed by a significant decrease towards timepoint 3. Only 1/49 patients showed false negative result at the latest timepoint. Although not significant, a tendence towards increasing titers was observed overtime with the Elecsys Anti-SARS-CoV-2 S assay (Roche).

4. Discussion

Since the start of the worldwide spread of COVID-19 virus, serology assays were developed to measure circulating antibody levels. These assays are useful for epidemiological surveys, vaccination strategy and prediction of immunity (Muecksch et al., 2020). Although several studies showed initially promising sensitivity and specificity of the available serology assays, other publications announced rapid decay of anti-SARS-CoV-2 antibodies (Ibarrondo et al., 2020; Tanis et al., 2021). Studies investigating the long-term kinetics of antibody titers are crucial for the COVID-19 strategy.

Serology assays for SARS-CoV-2 employ viral nucleoside (N) or spike surface protein (S) antigen. The viral spike protein is considered to be the preferred antigen, because it shows high specificity and shows to be the main antigen provoking neutralizing antibodies (Petherick, 2020). Therefore, S-based may be preferred to N-based assays (Muecksch et al., 2020).

In this study, we compared 4 different serology assays from 2 different companies. Both Abbott and Roche launched their first serology assay in April/May 2020. Both kits, the SARS-CoV-2 IgG assay (assay 1, Abbott) and Elecsys Anti-SARS-CoV-2 assay (assay 3, Roche) utilize N antigen. With the start of vaccination, both companies developed assays directed against the S protein: SARS-CoV-2 IgG II Quant assay (assay 2, Abbott) and SARS-CoV-2 S assay (assay 4, Roche).

As demonstrated by Muecksch et al., 2020, the Abbott SARS-CoV-2 assay shows decreasing antibody levels in function of time, making this test not useful for epidemiological purposes. In concordance with Muecksch et al., 2020 and Gudbjartsson et al.,2020, we describe an initial increase in antibodies until 145 days (min 122 - max 179 days) for Elecsys Anti-SARS-CoV-2 assay (assay 3). But, at the latest timepoint in this study (about 7 months after the initial infection), the antibody titers of the latter assay decrease. This observation is in discordance with Gaebler et al.,2021 and Favresse et al., 2020, who showed increased levels up until respectively 6.2 months and 32 weeks. Overall, our results indicate that both N-protein based assays show decreasing antibody levels.

Although not significant, a tendency towards increasing antibody titers was observed for the S-based Roche assay (assay 4). These data are in concordance with reports that describe increasing total antibodies, using pan-immunoglobulin assays (Gaebler et al., 2021; Schaffner et al., 2020). To our knowledge, no other publication evaluated the S-based Abbott assay (assay 2). Although the decline was slower in time, compared to the N-based assay, we also observed the risk of false negative results at the latest timepoint by using this test.

It has been described that most antibody responses in COVID-19 patients target in particular the S1 subunit and RBD region of the S viral protein. These regions are thought to elicit the most potent neutralizing effect (Kim et al., 2020). Furthermore pan-immunoglobulin assays show better performance than isotype-specific assays (Schaffner et al., 2020). In combination with the long-lasting detection of antibody titers observed, one could state that the SARS-CoV-2 S assay (Roche) is the superior assay for epidemiologic purposes in this study. Unfortunately, up until now, there is no proof that these persistent levels of antibody titers will induce protection against a second COVID-19 infection. Promising data were published by Deng et al., 2020. Re-exposure to SARS-CoV-2 virus in convalescent monkeys showed no recurrence of COVID-19 disease. Further studies are needed to investigate which serology assays show the best prediction towards protective antibodies against subsequent exposures to SARS-CoV-2.

5. Conclusions

Several companies urgently marketed COVID-19 serology assays. In this longitudinal study, we explored the kinetics of SARS-CoV-2 antibodies until 223 days post positive PCR or presence of symptoms by means of 4 different assays. We showed that results of these assays should be interpreted with caution. Although this study lacks information on correlation of antibodies with neutralizing activity, we could show that the pan-immunoglobulin SARS-CoV-2 S assay of Roche showed the best performance for epidemiological purposes.

Author contribution

Anneleen Schallier: conceptualization, supervision, writing– original draft; Sarah De Baets: formal analysis, investigation, writing – review and editing; Dirk De Bruyne: formal analysis, investigation; Kenny Dauwe: formal analysis, writing – review and editing; Margaux Herpol: conceptualization, writing – review and editing; Pedro Couck: writing – review and editing.

Declaration of competing interest

Authors have no competing interests to declare

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