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Research Article

Evaluation of the SARS-CoV-2-IgG response in outpatients by five commercial immunoassays

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Abstract

Commercially available immunoassays have been developed for sensitive and specific detection of antibodies against SARS-CoV-2. While high sensitivity has been reported in hospitalized COVID-19 patients, little is known about the performance of the assays in ambulatory patients. Therefore, we evaluated the SARS-CoV-2-IgG response in 51 SASR-CoV-2-PCR-confirmed outpatients with five commercial immunoassays. The sensitivity in serum samples, collected at a median of 24 days after onset of symptoms, detected by the Anti-SARS-CoV-2-ELISA IgG (Euroimmun), EDI™ Novel Coronavirus COVID-19 IgG ELISA (Epitope Diagnostics), Liaison® SARS-CoV-2 S1/S2 IgG (Diasorin), SARS-CoV-2 IgG on the Architect™ i2000 (Abbott), and Elecsys® Anti-SARS-CoV-2 (IgM/IgA/IgG) on the cobas™ e801 (Roche) was 84.3%, 78.4%, 74.5%, 86.3%, and 88.2%, respectively. The sensitivity in serum samples, collected >20 days after onset of symptoms, varied between 75.0% and 90.0%, and in samples, collected at least 28 days after onset of symptoms, did not increase, except in the Anti-SARS-CoV-2-ELISA IgG by Euroimmun (90.0%). There was not an obvious association between the type of the antigen (N versus S protein) and the overall sensitivity of the assays. Our results show significant individual differences of the IgG response against SARS-CoV-2, additionally confirmed in three patients with follow-up serum samples and seven asymptomatic but PCR-positive contact persons. In conclusion, our study shows that commercially available immunoassays detect SARS-CoV-2-IgG or total antibodies in outpatients with a satisfying sensitivity, but lower than that reported for hospitalized patients. In asymptomatic persons the SARS-CoV-2-IgG response may even be absent in a relevant percentage of persons.

Keywords: SARS-CoV-2-IgG, serology, antibody response, COVID-19, immunoassay

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a new coronavirus which causes an acute respiratory disease, named COVID-19. It emerged in China in December 2019 and led to a worldwide pandemic, declared by the World Health Organization (WHO) on March 11th 2020. As of August 25th, more than 23 million cases have been recorded worldwide.

While diagnosis of acute infection with SARS-CoV-2 is done by real-time polymerase chain reaction (RT-PCR) in respiratory samples there is still an increasing demand on serological testing for both epidemiological studies and the assessment of infection status in individuals. Recent studies have confirmed the suitability of various commercial immunoassays including high-throughput random access assays for the determination of SARS-CoV-2-IgG in COVID-19 patients [1], [2], [3], [4]. Available assays detect antibodies either against the spike (S) protein or against the nucleocapsid (N) protein. Antibodies against the N protein are mounted early in disease while the S protein has been shown to be the target for neutralizing antibodies [5], [6].

SARS-CoV-2-IgG were detected second to third weeks after onset of symptoms in up to 100% of hospitalized patients by use of various commercial immunoassays [2], [7], [8], [9], [10], [11], [12]. It has been shown that SARS-CoV-2-IgG titers were higher in critical cases compared to less critical patients and that severe ill patients seroconverted earlier than mild cases [7], [13], [14]. Therefore, it might be assumed that the serological response in outpatients with a less severe clinical status differs from that of hospitalized patients. Outpatients, mildly infected or even asymptomatic contact persons are, however, the main target population for a serological screening in order to evaluate the disease epidemiology. Consequently, this group represents the vast majority of patients, requesting SARS-CoV-2-IgG testing in our laboratory. So, we evaluated the SARS-CoV-2-IgG response (and SARS-CoV-2 total antibody response, respectively, in one immunoassay) in 51 outpatients with past SARS-CoV-2 infection confirmed by RT-PCR as well as

7 asymptomatic contact persons with past positive SARS-CoV-2-PCR.

Materials and methods

Serum samples

All serum samples were sent to our laboratory for SARS-CoV-2-IgG determination between March 24th and May 6th 2020 from outpatients. All patients had a positive result of SARS-CoV-2-RT-PCR in a nasopharyngeal swab (at least 7 days before serum collection) in our laboratory information system (LIS). Information about clinical symptoms, the day of onset of symptoms and past hospital treatments for COVID-19 was obtained. Altogether, 60 serum samples were obtained from 51 patients, with clinical symptoms and confirmed-PCR, ambulatory treated SARS-COV-2 infection, fulfilling the clinical diagnostic criteria of the Robert-Koch-Institut (https://www.rki.de/). All patients recovered at the time point of the blood collection. In addition, 7 serum samples from 7 asymptomatic persons with a positive SARS-CoV-2-PCR in the past (before 9 to 56 days) were investigated. All these seven persons were contact persons to PCR-confirmed COVID-19 patients.

Immunoassays for SARS-CoV-2 antibody testing

The Anti-SARS-CoV-2-ELISA IgG (Euroimmun, Luebeck, Germany, antigen S1 spike protein) and the EDI™ Novel Coronavirus COVID-19 IgG ELISA (Epitope Diagnostics, San Diego (CA), USA, antigen N protein) were performed fully automated on the Euroimmun Workstation ELISA and the DSX processor system (Dynex Technologies, Denkendorf, Germany), according to the manufacturer's instructions and within three days after receiving of samples. The results of EDI™ ELISA were calculated as a ratio of the optical density (OD) of the serum sample divided by the OD of negative control plus 0.18 according to the manufacturer's instructions (negative <0.9, equivocal 0.9–1.09, positive ≥1.1). In addition, the following fully automated random access assays were evaluated: Liaison® SARS-CoV-2 S1/S2 IgG (antigen S1 and S2 spike protein) on the Liaison®XL (Diasorin, Dietzenbach, Germany), SARS-CoV-2 IgG (antigen N protein) on the Architect™ i2000 (Abbott, Wetzlar, Germany), and Elecsys® Anti-SARS-CoV-2 (antigen N protein, no differentiation of IgM, IgA and IgG) on the cobas™ e801 (Roche Diagnostics, Mannheim, Germany). On the random access platforms, the samples were measured within one day in batches. Samples were stored at 4–8°C for up to one week, then frozen at –20°C and thawed only once.

SARS-CoV-2 RT-PCR testing

SARS-CoV-2-RNA detection by real-time RT-PCR from nasopharyngeal swabs was performed within routine diagnostics according to the manufacturers' instructions with the cobas® SARS-CoV-2 assay on the cobas® 6800 analyzer (Roche Diagnostics, target genes envelope (E) gene and open reading frame (orf) 1 region), the AmpliGnost SARS-CoV-2 E-Gen qPCR (Privates Institut für Immunologie und Molekulargenetik (PIIM), Karlsruhe, Germany) with the cobas® omni channel reagent kit (Roche) on the cobas® 6800 analyzer and the AmpliGnost SARS-CoV-2 E-Gen PCR (PIIM) and AmpliGnost SARS-CoV-2 N-Gen PCR (PIIM) on the LightCycler®480 II (Roche).

Results

Comparison of sensitivity of five commercial immunoassays in outpatients

A collection of 60 serum samples from 51 PCR-confirmed outpatients was used for the comparison of sensitivity of the five commercial immunoassays. These sera were sampled 10 to 54 days (median 24 days) after onset of symptoms. A calculation of sensitivity was done firstly irrespective of the time point of serum sampling after onset of symptoms. Equivocal results were counted as negative (n=2 in the Anti-SARS-CoV-2-ELISA IgG (Euroimmun) and the Liaison® SARS-CoV-2 S1/S2 IgG, n=4 in the EDI™ IgG ELISA). The sensitivity ranged from 74.5% to 88.2% (Table 1). The highest sensitivity was achieved with the assays by Abbott and Roche followed closely by the Euroimmun. In samples collected >20 days after onset of symptoms, the sensitivity varied between 75.0% and 90.0% and was higher than in samples collected within the first three weeks in all assays apart from the EDI™ IgG ELISA (Table 1). In a subgroup of samples collected at least 28 days after onset of symptoms (n=20) the sensitivity did not increase further apart from the measurement in the Anti-SARS-CoV-2-ELISA IgG (Euroimmun) (Table 1).

In each of three patients three follow-up serum samples were availalbe. Patients 1 and 2 were a couple with mild illness, anosomia and without fever. Patient 3 had fever and cough, followed by anosmia and dysgeusia. All five assays showed positive results already in the first serum sample in patient 3. Even though there were marked differences between the assays in the time point of seroconversion in the patients 1 and 2 (Table 2).

Seven serum samples from seven asymptomatic contact persons with PCR-confirmed SARS-CoV-2 infection were available and tested with all five immunoassays in addition to the above samples from outpatients. All samples were negative in the Anti-SARS-CoV-2-ELISA IgG (Euroimmun), one of each sample was positive in the other immunoassays (Table 3).

Table 1: Sensitivity of five commercial SARS-CoV-2 assays in outpatients (n=51)

	Sensitivity (%) (overall)	Sensitivity (%) in subgroups of samples obtained at different time points after onset of symptoms		
Assay	All samples, median day 24 (n=51)	day 10 to 20*, median day 17 (n=11)	day 21 to 54*, median day 27 (n=40)	day 28 to 54*, median day 32 (n=20)
Anti-SARS-CoV-2-ELISA IgG (Euroimmun), S1 protein	84.3 (43/51)	81.8 (9/11)	85.0 (34/40)	90.0% (18/20)
EDI™ IgG ELISA (Epitope Diagnostics), N protein	78.4 (40/51)	90.9 (10/11)	75.0 (30/40)	70.0% (14/20)
Liaison® SARS-CoV-2 S1/S2 IgG (Diasorin), S1 and S2 protein	74.5 (38/51)	63.6 (7/11)	77.5 (31/40)	80.0% (16/20)
SARS-CoV-2 IgG (Abbott), N protein	86.3 (44/51)	81.8 (9/11)	87.5 (35/40)	85.0% (17/20)
Elecsys® Anti-SARS-CoV-2 (Roche), N protein, IgM/IgA/IgG	88.2 (45/51)	81.8 (9/11)	90.0 (36/40)	85.0% (17/20)

* day after onset of symptoms

Table 2: Results of five commercial SARS-CoV-2 assays in follow-up sera from three outpatients

Case/ No.	Day after onset of symptoms	Day after last positive PCR	Anti-SARS-CoV-2 IgG (Euroimmun) Ratio*	EDI™ IgG ELISA (Epitope Diagn.) Ratio**	Liaison® SARS-CoV-2 S1/S2 IgG (Diasorin) AU/ml$	SARS-CoV-2 IgG (Abbott) COI#	Elecsys® Anti-SARS-CoV-2 IgM/IgA/IgG (Roche) COI##
1--1	20	–2	0,69	0,28	5,71	1,19	0,23
1--2	27	5	0,86	0,57	8,26	**3,49**	**3,05**
1--3	40	18	**2,18**	0,75	13,7	**3,81**	**6,33**
1--4	68	46	**1,24**	0,48	<12	n.d.	**10,1**
2--1	18	16	1,07	0,49	6,59	0,26	**2,67**
2--2	25	23	**1,37**	0,82	10,3	1,06	**1,71**
2--3	39	37	**1,75**	**1,23**	**18,6**	**1,90**	**10,5**
2--4	67	65	1,07	0,64	**23,2**	n.d.	**7,14**
3--1	19	3	**1,8**	**1,51**	**18,7**	**7,05**	**10,7**
3--2	24	7	**2,8**	**1,38**	**20,7**	**6,78**	**13,2**
3--3	31	14	**2,22**	1,09	**17,6**	**5,72**	**10**
3--4	81	64	**1,51**	0,66	**18,7**	n.d.	**24,8**

* Ratio <0.8 negative, 0.8–1.09 equivocal, ≥1.1 positive; ** Ratio <0.9 negative, 0.9–1.09 equivocal, ≥1.1 positive;
$ <12 AU/ml negative, 12.0–14.5 AU/ml equivocal, ≥15 AU/ml positive; # COI <1,4 negative, COI ≥1.4 positive; n.d. not determined;
cutoff index (COI) <1.0 negative, COI ≥1.0 positive

Discussion

The determination of SARS-CoV-2-IgG antibodies is the method of choice for the evaluation of SARS-CoV-2 seroprevalence. Measurements of SARS-CoV-2-IgG by automated immunoassays preferably run on high-throughput platforms allows a rapid investigation of large sample numbers. Although immunoassays cannot determine the neutralizing ability of SARS-CoV-2-IgG, they facilitate an evaluation of seroprevalence. The results of some tests have been shown to correlate positively with the results of neutralization tests [1], [15].

A comparison of five commercial immunoassays in serum samples taken at least ten days after onset of symptoms from 51 PCR-confirmed COVID-19 outpatients revealed an overall sensitivity of the assays from 74.5% to 88.2%. Highest sensitivities were reached by the SARS-CoV-2-IgG test by Abbott, the Elecsys® Anti-SARS-CoV-2 test by Roche (both N-protein-based tests) and the Anti-SARS-CoV-2-ELISA IgG by Euroimmun (S1-protein-based) while the EDI™ Novel Coronavirus COVID-19 IgG ELISA showed a lower sensitivity. The maximal sensitivity was achieved in samples taken after the third week (using the tests by Roche, Abbott and Epitope Diagnostics) or fourth week after onset of symptoms (using the tests by Euroimmun and Diasorin). It has to be noted that the Elecsys® Anti-SARS-CoV-2 test by Roche detects not only IgG antibodies but total antibodies. An early antibody response may, therefore, in part be due to IgM and/or IgA antibodies. In comparison to data obtained in severely ill and hospitalized patients, where sensitivities up to 100% were found, in samples taken two to three weeks after onset of symptoms, sensitivities of all assays were lower in our cohort of outpatients. SARS-CoV-2-IgG titers were higher

Table 3: Sensitivity of five commercial SARS-CoV-2 assays in PCR-confirmed asymptomatic contact persons (n=7)

No.	Day after positive PCR	Anti-SARS-CoV-2 IgG (Euroimmun) Ratio*	EDI™ IgG ELISA (Epitope Diagn.) Ratio**	Liaison® SARS-CoV-2 S1/S2 IgG (Diasorin) AU/ml[$]	SARS-CoV-2 IgG (Abbott) COI[#]	Elecsys® Anti-SARS-CoV-2 IgM/IgA/IgG (Roche) COI[##]
1	9	0.30	0.37	<3.8	0.06	0.06
2	13	0.19	0.62	8	0.02	0.06
3	14	0.17	0.6	**58.3**	0.03	0.07
4	14	0.47	0.65	5.21	**3.81**	**2.01**
5	14	0.11	0.63	<3.8	0.02	0.08
6	14	0.08	**1.31**	<3.8	0.06	0.07
7	56	<0.8	0.19	<3.8	0.03	0.06

* Ratio <0.8 negative, 0.8–1.09 equivocal, ≥1.1 positive; ** Ratio <0.9 negative, 0.9–1.09 equivocal, ≥1.1 positive;
[$] <12 AU/ml negative, 12.0–14.5 AU/ml equivocal, ≥15 AU/ml positive; ; [#] COI <1,4 negative, COI ≥1.4 positive; n.d. not determined;
[##] cutoff index (COI) <1.0 negative, COI ≥1.0 positive; n.d. not determined

in severely ill compared to lesscritical patients [7], [13], [14]. Wajnberg et al., who investigated SARS-CoV-2-IgG by an ELISA test in PCR-confirmed outpatients in New York, reported a positivity rate of 82% at a median of 23 days after onset of symptoms [16]. In contrast, Meyer et al. detected SARS-CoV-2-IgG in 97.7% of serum samples in 44 patients from an outpatient clinic in Switzerland by use of the Anti-SARS-CoV-2-ELISA IgG (Euroimmun) [17]. Regarding the different viral antigens used in the tests, there was no obvious association between the type of the antigen (N versus S protein) and the overall sensitivity of the assays. In addition, there was no association between the type of the antigen and the dynamics of IgG response in the three follow-up patients. Our results show significant individual differences of the IgG response against SARS-CoV-2 as reported by others before [1], [6].

Actually, the positivity rate determined in the seven asymptomatic but PCR-positive contact persons was much lower (0 to 1 out of 7 positive). Regarding this low rate of seropositivity, there are different possible explanations:

- Infected persons, who do not develop a clinical disease may possibly combat the coronavirus on the mucosa of their upper respiratory tract, preventing a systemic humoral immune response. According to a recent study SARS-CoV-2-S-protein-specific IgA in nasal and tear fluids may play a role in the primary defense of SARS-CoV-2 and has been found in mucosal samples even in seronegative asymptomatic health care workers [13].
- Previous publications have demonstrated that the humoral immune response towards SARS-CoV-2 depends on the duration of viral antigen exposure [18], [19]. Therefore, it may be postulated that the group of asymptomatic contact persons have been exposed to a lower amount of viral antigen.
- The possibility of false positive RT-PCR results has to be taken into account. Contamination of samples can never completely be excluded, but the following reasons make this explanation unlikely: First, samples that were investigated on the cobas® 6800 analyzer were mainly directly put into the analyzer without a prior opening in the laboratory; second, we retested a large collection of swabs with a weak positive result in an E-gene-specific PCR with another different PCR assay and revealed consistent results (data not shown) and third, many swab samples were positive for two SARS-CoV-2 gene targets.

In summary, our study shows that commercial immunoassays detect SARS-CoV-2-IgG or total antibodies in outpatients with a sensitivity of 75% to 88%, which is lower than the reported one for severe ill and hospitalized patients. Regarding the overall sensitivity, the fully automated assays by Abbott and Roche as well as the ELISA by Euroimmun were superior to the other assays. In asymptomatic persons with past SARS-CoV-2 infection the SARS-CoV-2-IgG response may be absent in a relevant percentage of persons. For the detection of a seroconversion in mild cases and asymptomatic patients, serum should be collected not earlier than three weeks after onset of symptoms or approximately four weeks after contact to a COVID-19 patient.

Notes

Competing interests

The authors declare that they have no competing interests.

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