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SARS-CoV-2-IgG response is different in COVID-19 outpatients and asymptomatic contact persons

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ABSTRACT

Commercially available immunoassays have been developed for sensitive and specific detection of antibodies against SARS-CoV-2. While a fast and reliable IgG response has been reported for samples from hospitalized COVID-19 patients, less is known about ambulatory patients. We evaluated the SARS-CoV-2-IgG response by the Anti-SARS-CoV-2-ELISA IgG (Euroimmun) in a defined cohort of SARS-CoV-2-PCR-confirmed outpatients and asymptomatic contact persons including 137 serum samples from PCR-confirmed outpatients (n = 111) and asymptomatic but PCR-positive contact persons (n = 26) sent to our laboratory as part of routine diagnostics for determination of SARS-CoV-2-IgG. Overall positivity rate for SARS-CoV-2-IgG was 81.1 % in outpatients (irrespective of sampling before or after day 21 after onset of symptoms) but significantly lower in asymptomatic contact persons (15.4 %, $p < 0.0001$). In contact persons without symptoms the ct values of the PCR assays were significantly higher (5–7 threshold cycles) than in outpatients, and ct values were significantly negative correlated to the SARS-CoV-2-IgG ratio, suggesting a lower viral load as a possible explanation for lower rate of seropositivity. In summary, our study shows that serological response to SARS-CoV-2 in outpatients including asymptomatic persons is less pronounced than in hospitalized patients. Further controlled studies are urgently needed to determine serological response in outpatients and asymptomatic persons since this is the main target population for seroepidemiological investigations.

1. Introduction

SARS-CoV-2 is a new coronavirus which causes an acute respiratory disease named COVID-19. It emerged in China in December 2019 and has led to a worldwide pandemic declared by the World Health Organization (WHO) on March 11th 2020. As of June 23rd, more than 9 million cases have been recorded worldwide.

While diagnosis of acute infection with SARS-CoV-2 is done by RT-PCR in respiratory samples, there is an increasing demand on serological testing for both epidemiological studies and assessment of infection status in individuals. Recent studies have confirmed the suitability of various commercial immunoassays including high-throughput random access platforms for determination of SARS-CoV-2-IgG in COVID-19 patients [1–4].

Within the third week after onset of symptoms SARS-CoV-2-IgG were detected in up to 100 % of hospitalized patients by use of various commercial immunoassays [2,5–10]. It has been shown that SARS-CoV-2-IgG titers were higher in critically ill compared to less critically ill patients and that severely ill patients seroconverted earlier than those with mild disease [5,11,12]. Therefore, it might be assumed that serological response in outpatients with a less severe clinical course of COVID-19 differs from that of hospitalized patients. Outpatients and milder infected or even asymptomatic contact persons are, however, the main target population for screening for SARS-CoV-2 antibodies in order to evaluate disease epidemiology. Moreover, this group represents the vast majority of patients requesting SARS-CoV-2-IgG testing in our laboratory. Therefore, we evaluated the SARS-CoV-2-IgG response in outpatients and asymptomatic contact persons with past SARS-CoV-2 infection confirmed by RT-PCR.

2. Materials and methods

2.1. Serum samples

Serum samples were sent to our laboratory from ambulatory patients for determination of SARS-CoV-2-IgG. The MVZ Labor

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Ravensburg is private laboratory serving a large number of private practices and hospitals in Southwest Germany as well as most coronavirus test center in the region. All serum samples sent to our laboratory for SARS-CoV-2-IgG determination between March 24th and May 6th 2020 from outpatients with a positive result of SARS − COV-2-RT-PCR in a nasopharyngeal swab (at least 7 days before serum collection) were considered for analysis (n = 158). Information about clinical symptoms, day of onset of symptoms, and past hospital treatment for COVID-19 was obtained. Patients with past hospital treatment for COVID-19 (n = 11) and patients in whom clinical information could not be obtained (n = 10) have been excluded from analysis. Out of the remaining 137 patients, 111 patients had clinically and PCR − COnfirmed, ambulatory treated SARS − COV-2 infection and fulfilled the clinical diagnostic criteria of the Robert-Koch-Institut (www.rki.de). All had recovered at the time point of blood collection. 26 persons had no clinical symptoms but were PCR-positive due to contact with PCR-confirmed COVID-19 patients.

2.1.1. Immunoassay for SARS-CoV-2 antibody testing

SARS-CoV-2-IgG antibodies were determined within three days after receipt of samples by Anti-SARS-CoV-2-ELISA IgG (Euroimmun, Luebeck, Germany, antigen S1 spike protein) on the Euroimmun Workstation ELISA according to the manufacturer's instructions.

2.1.2. 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, target gene nucleocapsid gene) on the LightCycler®480 II (Roche). Ct values were recorded if available.

2.1.3. Statistical analysis

For statistical analysis Analyse-it (version 5.65) for Microsoft Excel was used. Calculation of significant differences between groups were done by the Wilcoxon-Mann-Whitney test. Correlation analysis was based on calculation of Pearson correlation coefficient r including the significance level. p-values < 0.05 were regarded as significant.

3. Results

Serum samples of the 111 outpatients were collected between day 10 and 68 (median day 32) after onset of symptoms. Positive SARS-CoV-2-IgG was detected in 81.1 % (90/111) (Table 1). Seropositivity rates of outpatients obtained up to day 20 and after day 20 after onset of symptoms were similar (81.8 % versus 81.0 %, Table 1).

Serum samples of 26 asymptomatic PCR-confirmed contact persons were sampled between day 9 and 56 (median day 29) after the day of the PCR-positive swab. The positivity rate of SARS-CoV-2-IgG amounted to 15.4 % (4/26), which was significantly lower than that of the outpatients ($p < 0.001$). The positivity rate tended to be higher at a later time point of serum sampling (18.8 % after day 20 versus 10.0 % before day 20 of PCR-positive swab, overall 15.4 %, Table 1), but the difference was not significant ($p = 0.547$). The day of the first PCR-positive swab was also recorded in all outpatients and the median was identical to that of the asymptomatic contact persons (median day 29).

Since the SARS-CoV-2-IgG response may be dependent on the magnitude of viral exposure we obtained the Ct values of the RT-PCR runs of the nasopharyngeal swab. Ct values were available in 94 patients (84.7 %) and 23 asymptomatic contact persons (88.5 %), including amplification of the E gene (n = 82 patients and n = 19 contact persons), orf gene (n = 50 patients and n = 8 contact persons), and N gene (n = 20 patients and n = 2 contact persons). Subgroup analysis of N gene PCR results was not done due to the small number of swabs investigated in the contact persons group.

For comparison of ct values between the two groups we evaluated RT-PCR results of different targets and PCR assays, i.e. all E gene PCR assays irrespective of the PCR analyzer used (cobas® 6800 system and LightCycler™ 480 II), the E gene amplified on the cobas® 6800 system, and the orf gene amplified on the cobas® 6800 system. In addition, we compared the mean ct values obtained in each patient between the two groups. In the group of asymptomatic PCR-positive contact persons the mean ct values were significantly higher than in the group of outpatients in all investigated subgroups (Table 2). The difference in ct values was between 5 and 7 threshold cycles.

Ct values of the RT-PCR runs in relation to the SARS-CoV-2-IgG ratio in the patients and asymptomatic contact persons are depicted in Fig. 1. Mean Ct values and SARS-CoV-2-IgG ratio values showed a significant negative correlation in the overall group ($r = -0.360$, $n = 117$, $p < 0.0001$) as well as in the subgroups of outpatients ($r = -0.226$, $n = 94$, $p = 0.028$) and asymptomatic contact persons ($r = -0.776$, $n = 23$, $p < 0.0001$). In addition, a ROC analysis was performed to determine diagnostic performance of the ct value with respect to the detection of SARs-CoV-2-IgG positivity/negativity. The optimal ct threshold for discrimination of SARS-CoV-2-IgG-positive and -negative persons was determined as ct 34 with a sensitivity and specificity of 93.7% and and 82.4%, respectively. The AUC of the ROC curve amounted to 0.938 (95 % CI = 0.814 – 0.954, p < 00,001).

4. Discussion

Determination of SARS-CoV-2-IgG antibodies is the method of choice for evaluation of SARS-CoV-2 seroprevalence. Measurement of SARS-CoV-2-IgG by automated immunoassays allow rapid investigation of large numbers of samples. Although immunoassays cannot determine the neutralizing ability of SARS-CoV-2-IgG they facilitate evaluation of seroprevalence and results of some tests have been shown to correlate

Table 1
SARS-CoV-2-IgG results in outpatients and asymptomatic contact persons.

Group	PCR + outpatients (n = 111) Positivity rate (%)	Group	Asymptomatic PCR + contact persons (n = 26) Positivity rate (%)
Overall	81.1 (90/111)[a]	Overall	15.4 (4/26)
Day* 10–20 (median day 17)	81.8 (9/11)[b]	Day# 9–20 (median day 14)	10.0 (1/10)
Day* 21–68 (median day 34)	81.0 (81/100)[c]	Day# 21–56 (median day 31)	18.8 (3/16)
		Day# 28–56 (median day 34)	14.3 (2/14)

* Day after onset of symptoms.
\# day after positive RT-PCR result in nasopharyngeal swab.
[a] Four equivocal results counted as negative.
[b] One equivocal result counted as negative.
[c] Three equivocal results counted as negative.

Table 2
Comparison of ct values of the PCR results in outpatients and asymptomatic contact persons.

	Outpatient	Asymptomatic contact person	p-value
E gene, all assays	N = 82	N = 19	0.0002
Mean ct value	28.96	34.44	
Mean SE (SD)	0.629 (5.70)	1.105 (4.81)	
E gene, cobas® 6800	N = 58	N = 10	0.011
Mean ct value	28.98	34.05	
Mean SE (SD)	0.798 (6.06)	1.588 (5.02)	
orf gene, cobas® 6800	N = 50	N = 8	0.012
Mean ct value	26.62	31.53	
Mean SE (SD)	0.685 (4.84)	1.646 (4.66)	
All genes, all assays	N = 94	N = 23	< 0.0001
Mean ct value	28.60	34.40	
Mean SE (SD)	0.564 (5.47)	0.930 (4.46)	

positively with the results of neutralization tests [1,13].

We investigated the SARS-CoV-2-IgG response in a defined cohort of samples obtained within our routine diagnostics. This cohort reflects the typical population of patients in which determination of SARS-CoV-2 serostatus is requested, i.e. outpatients with past COVID-19 disease and asymptomatic people who were in contact to a COVID-19 case wishing to know whether they have mounted a SARS-CoV-2-IgG response. Testing was done by the Anti-SARS-CoV-2-ELISA IgG by Euroimmun since this test was used continuously in our laboratory since March 2020. The positivity rate obtained in the cohort of outpatients with past SARS-CoV-2 infection (81.1 %) was lower than that (up to 100 %) reported previously for hospitalized patients [2,5,7–10]. In our opinion, it reflects an overall sensitivity which can be assumed for investigation of larger cohorts, taking into account individual differences in the humoral and cellular immune response and heterogeneity of the testing population. A very recent study published as preprint found comparable results to our findings but investigated a smaller number of patients and asymptomatic persons [14].

Interestingly, the overall seropositivity rate determined in the group of 26 asymptomatic but PCR-positive contact persons was significantly lower (15.4 %) than in outpatients. Regarding this low rate of seropositivity there are different possible explanations. (a) Infected persons who do not develop clinical disease may possibly combat the coronavirus on the mucosa of their upper respiratory tract preventing a systemic humoral immune response. According to a very recent study SARS-CoV-2-S-protein-specific IgA in nasal and tear fluid may play a role in primary defense of SARS-CoV-2 and has been found in mucosal samples even in seronegative asymptomatic health care workers [11]. (b) Previous publications have demonstrated that the humoral immune response towards SARS-CoV-2 is dependent on the duration and magnitude of viral antigen exposure [15,16]. Therefore, it may be postulated that the group of asymptomatic contact persons have been exposed to a lower amount of viral antigen. For this reason, we analysed the ct values of the RT-PCR runs and, indeed, found significantly higher

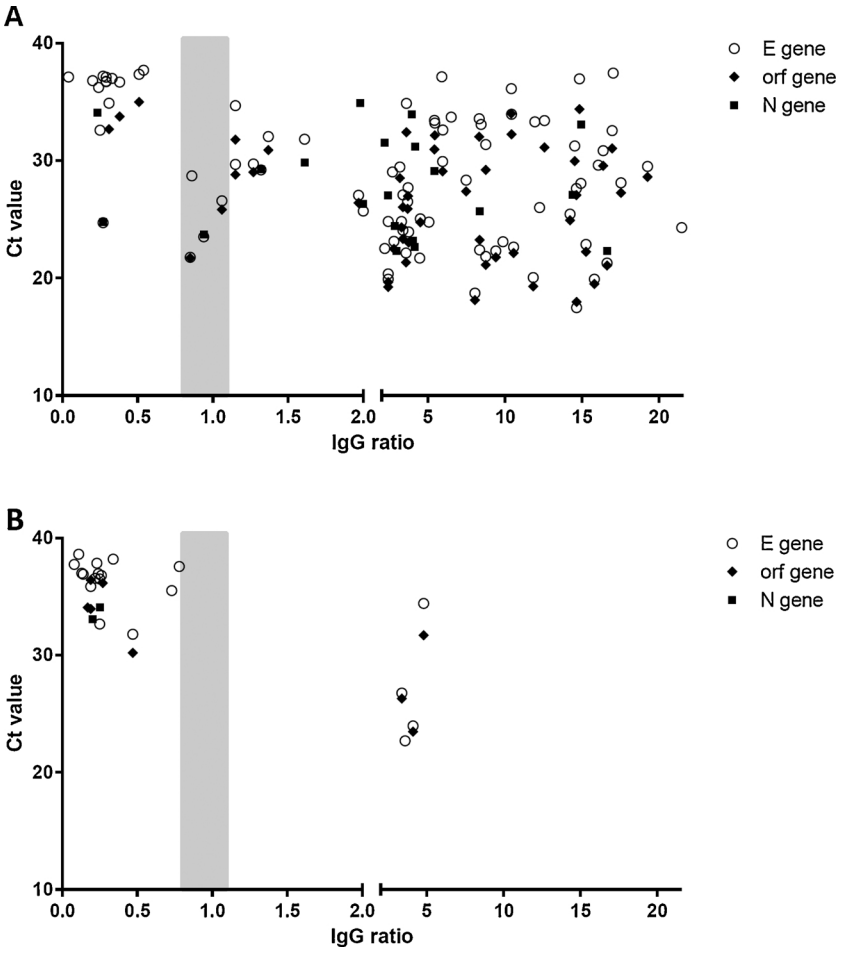

Fig. 1. Relation of Ct values of the RT-PCR runs and Anti-SARS-CoV-2-ELISA IgG ratio in symptomatic outpatients (A) and asymptomatic contact persons (B).
Figure legend: Ct values of 94 outpatients and 23 asymptomatic contact persons were plotted against SARS-CoV-2-IgG ratios determined by SARS-CoV-2-ELISA IgG (Euroimmun). Data included ct values for the E gene (n = 82 patients and n = 19 contact persons), orf gene (n = 50 patients and n = 8 contact persons), and N gene (n = 20 patients and N = 2 contact persons).

ct values, i.e. significantly lower viral loads, in the swabs obtained from asymptomatic contact persons compared to outpatients. In addition, ct values and the SARS-CoV-2-IgG ratio were significantly negative correlated in both groups. Due to the correlation between the ct value and SARS-CoV-2 IgG, the antibody status could be determined with a certain sensitivity and specificity using a ct threshold (Ct = 34) value determined by ROC analysis. Below the threshold value the IgG result is positive (sensitivity 94 %) and above the threshold value it is negative (specificity 72 %). (c) 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 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, the majority of swab samples was positive for two SARS-CoV-2 gene targets. Nevertheless, our study has some limitations: The cohort included in our study represented a well defined but not prospectively acquired collection of serum samples, and single patients may have been missed that putatively had PCR-tests in another laboratory. The time point of swab sampling in asymptomatic persons and outpatients were in median comparable but individual differences in swab sampling, use of different swab and transport systems cannot be excluded.

In summary, our study shows that SARS-CoV-2-IgG antibodies are found significantly less frequently in PCR-positive asymptomatic contact persons compared to PCR-positive outpatients. Significantly higher ct values in contact persons and the negative correlation of the ct values with the SARS-CoV-2-IgG ratio suggests a lower viral load as a possible explanation for lower rate of seropositivity in asymptomatic contact persons. Further studies are needed to determine serological response in mildly infected and asymptomatic persons since this is the main target population for seroepidemiological studies.

Authors ‘contributions

NW designed the study and wrote the manuscript. RF analyzed the RT-PCR assays. SD, MV, RI, and NW obtained clinical information and analyzed data. DP did statistical analysis. All authors were involved in scientific discussion.

Declaration of Competing Interest

There are no conflicts of interest by all authors.

References

[1] N. Kohmer, S. Westhaus, C. Ruehl, S. Ciesek, H.F. Rabenau, Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays, J. Clin. Virol. 1 (June 129) (2020) 104480.

[2] R. Lassaunière, A. Frische, Z.B. Harboe, A.C.Y. Nielsen, A. Fomsgaard, K.A. Krogfelt, et al., Evaluation of nine commercial SARS-CoV-2 immunoassays, medrxiv (2020) doi:https://doiorg/101101/2020 04 0920056325.

[3] I. Montesinos, D. Gruson, B. Kabamba, H. Dahma, S. Van den Wijngaert, S. Reza, et al., Evaluation of two automated and three rapid lateral flow immunoassays for the detection of anti-SARS-CoV-2 antibodies, J. Clin. Virol. 128 (July) (2020) 104413.

[4] M. Tre-Hardy, A. Wilmet, I. Beukinga, J.M. Dogne, J. Douxfils, L. Blairon, Validation of a chemiluminescent assay for specific SARS-CoV-2 antibody, Clin. Chem. Lab. Med. (May 25) (2020).

[5] P. Kellam, W. Barclay, The dynamics of humoral immune responses following SARS-CoV-2 infection and the potential for reinfection, J. Gen. Virol. (May 20) (2020).

[6] G. Lippi, G.L. Salvagno, M. Pegoraro, V. Militello, C. Caloi, A. Peretti, et al., Assessment of immune response to SARS-CoV-2 with fully automated MAGLUMI 2019-nCoV IgG and IgM chemiluminescence immunoassays, Clin. Chem. Lab. Med. (April 16) (2020).

[7] Q.X. Long, B.Z. Liu, H.J. Deng, G.C. Wu, K. Deng, Y.K. Chen, et al., Antibody responses to SARS-CoV-2 in patients with COVID-19, Nat. Med. (April 29) (2020).

[8] R. Woelfel, V.M. Corman, W. Guggemos, M. Seilmaier, S. Zange, M.A. Mueller, et al., Virological assessment of hospitalized patients with COVID-2019, Nature 581 (May 7809) (2020) 465–469.

[9] M. Egger, C. Bundschuh, K. Wiesinger, C. Gabriel, M. Clodi, T. Mueller, et al., Comparison of the Elecsys® Anti-SARS-CoV-2 immunoassay with the EDI enzyme linked immunosorbent assays for the detection of SARS-CoV-2 antibodies in human plasma, Clin. Chim. Acta 30 (May 509) (2020) 18–21.

[10] J.D. Whitman, J. Hiatt, C.T. Mowery, B.R. Shy, R. Yu, T.N. Yamamoto, et al., Test performance evaluation of SARS-CoV-2 serological assays, medRxiv (May 17) (2020).

[11] Cervia C. Njzyvasjwa, et al., Systemic and Mucosal Antibody Secretion Specific to SARS-CoV-2 During Mild Versus Severe COVID-19, doi:https://doiorg/101101/2020 05 21 108308 (2020).

[12] J. Zhao, Q. Yuan, H. Wang, W. Liu, X. Liao, Y. Su, et al., Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019, Clin. Infect. Dis. (March 28) (2020).

[13] A.J. Jääskeläinen, S. Kuivanen, S. Kekäläomem, M.J. Ahava, R. Liinov, H. Kallio-Kokko, et al., Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation, J. Clin. Virol. (2020) 129, https://doi.org/10.1016/j.jcv.2020.104512.

[14] W. Solbach, J. Schiffner, I. Backhaus, D. Burger, R. Staiger, B. Tiemer, et al., Antibody profiling of COVID-19 patients in an urban low-incidence region in Northern Germany, doi:https://doiorg/101101/2020 05 30 20111393, medRxiv (2020).

[15] Y. Liu, L.M. Yan, L. Wan, T.X. Xiang, A. Le, J.M. Liu, et al., Viral dynamics in mild and severe cases of COVID-19, Lancet Infect. Dis. 20 (June 6) (2020) 656–657.

[16] F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, et al., Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study, Lancet 395 (10229) (2020) 1054–1062 March 28.