Received: 30 April 2020 | Revised: 9 May 2020 | Accepted: 10 May 2020

DOI: 10.1111/pai.13278

ORIGINAL ARTICLE

WILEY

Symptoms and immunoglobulin development in hospital staff exposed to a SARS-CoV-2 outbreak

Susanne Brandstetter[1,2] | Samra Roth[1] | Susanne Harner[1] | Heike Buntrock-Döpke[1,2] | Antoaneta A. Toncheva[2] | Natascha Borchers[2] | Rudolf Gruber[3] | Andreas Ambrosch[3] | Michael Kabesch[1,2]

[1]Research and Development Campus Regensburg (WECARE) at the Hospital St. Hedwig of the Order of St. John, University of Regensburg, Regensburg, Germany

[2]University Children's Hospital Regensburg (KUNO) at the Hospital St. Hedwig of the Order of St. John, University of Regensburg, Regensburg, Germany

[3]Institute of Laboratory Medicine, Microbiology and Hygiene, Hospital of the Order of St. John, Regensburg, Germany

Correspondence
Michael Kabesch, University Children's Hospital Regensburg (KUNO), At St. Hedwig 's Hospital of the order of St. John, Steinmetzstr. 1-3, D-93049 Regensburg, Germany.
Email: michael.kabesch@barmherzige-regensburg.de

Editor: Ömer Kalaycı

Abstract

Background: Worldwide, the number of SARS-CoV-2 infections is increasing. Serological immunoglobulin tests may help to better understand the development of immune mechanisms against SARS-CoV-2 in COVID-19 cases and exposed but asymptomatic individuals. The aim of this study was to investigate exposure to SARS-CoV-2, symptoms, and antibody responses in a large sample of healthcare workers following a COVID-19 outbreak.

Methods: A COVID-19 outbreak among staff members of a major German children's and women's hospital was followed by massive RT-PCR SARS-CoV-2 tests and provided the opportunity to study symptoms, chains of infection, and SARS-CoV-2–specific antibody responses (IgG and IgA) by ELISA. Study participants were classified as COVID-19 cases, and persons with close, moderate, or no exposure to SARS-CoV-2 in the clinical setting, respectively.

Results: Out of 201 study participants, 31 were COVID-19 cases. While most study participants experienced many symptoms indicative for SARS-CoV-2 infection, anosmia and coughing were remarkably more frequent in COVID-19 cases. Approximately 80% of COVID-19 cases developed some specific antibody response (IgA and IgG) approximately 3 weeks after onset of symptoms. Subjects in the non-COVID-19 groups had also elevated IgG (1.8%) and IgA values (7.6%) irrespective of contact history with cases.

Conclusion: We found that a significant number of diseased did not develop relevant antibody responses three weeks after symptom onset. Our data also suggest that exposure to COVID-19 positive co-workers in a hospital setting is not leading to the development of measurable immune responses in a significant proportion of asymptomatic contact persons.

The peer review history for this article is available at https://publons.com/publon/10.1111/pai.13278

KEYWORDS
antibody response, COVID-19 outbreak, COVID-19 outbreak, COVID-19 pandemic, healthcare workers, immunoglobulin development, infection chain, SARS-CoV-2

1 | INTRODUCTION

The novel coronavirus SARS-CoV-2 has infected 2.9 million people worldwide (29th April, WHO).[1] Cases are defined by direct virus detection tests via RT-PCR from upper airways. However, due to a worldwide shortage in test capacities, persons who experience symptoms indicative for SARS-CoV-2 infection are much more likely to get tested. Thus, it is unclear how many individuals have really been exposed to the virus and may have developed an immune reaction without symptoms. Also, it is not clear how many individuals develop an immune response after COVID-19. Serological tests for immunoglobulin-based immune responses have just become available and may help to answer these questions.[2-4]

In our large university children's and maternity hospital in Regensburg, Germany, an outbreak of COVID-19 occurred in March 2020, affecting a total of 36 hospital staff members. Another 50 employees had close contact to the infected individuals from that outbreak. Massive RT-PCR testing for SARS-CoV-2 was performed in affected and unaffected personnel. Through the implementation of social distancing, protection, and quarantine measures, the outbreak was successfully contained within two weeks.[5] This outbreak gave us the opportunity to study exposure and antibody responses (IgA and IgG) in relation to previously performed RT-PCR tests over time in a highly informative population of healthcare workers.

Key Message

Investigating SARS-CoV-2–specific antibody responses in COVID-19 cases and their co-workers, we found that only 80% of cases had elevated IgG and IgA values, respectively, while antibody response in individuals without contact was rare. These findings are important as they show that a relevant antibody response in asymptomatic individuals after contact with infected persons does not seem to be a major mechanism that one should rely on too much in achieving herd immunity.

2 | METHODS

2.1 | Design and recruitment

This study has a cross-sectional design. In the course of the outbreak, we had tested numerous employees of the hospital repeatedly as previously outlined[5] for the presence of SARS-CoV-2 by RT-PCR. All adult staff members who underwent standard RT-PCR testing and gave informed consent were invited to participate in this study which was approved by the Ethics Committee of the University of Regensburg (file number: 20-1767-101).

2.2 | Data assessment and storage

Exposure to SARS-CoV-2 was categorized into four groups: (a) COVID-19: individuals with at least one positive PCR SARS-CoV-2 test. (b) Close contact: individuals with a negative PCR SARS-CoV-2 test who had close contact to a co-worker with COVID-19. Close contact was defined as unprotected contact with a distance of less than 2 m for 15 minutes or longer (as defined by the Robert-Koch-Institute (RKI)[6]). (c) Moderate contact: individuals with a negative PCR SARS-CoV-2 test who had moderate contact to a co-worker with COVID-19 or who had recently returned from a risk area (as defined by RKI). Moderate contact was defined as contact with a distance of less than 2 m while using personal protective equipment or unprotected contact with a distance of more than 2 m (as defined by RKI). (d) No contact: individuals with a negative PCR SARS-CoV-2 test who were not aware of any contact to a COVID-19 patient. Sociodemographic information and COVID-19–related self-reported symptoms were collected in a structured interview performed by study nurses and securely documented in a qnome database (www.qnome.eu).

2.3 | SARS-CoV-2 RT-PCR

The accredited laboratory at the University of Regensburg performed the published Drosten protocol using primers and probes within the E-gene.[7] The lower limit of detection was 300 copies/ml, which compares favorably with commercial assays, for example the Roche und Cepheid test. The clinical samples were spiked with an RNA phage as internal control to exclude inhibitory effects. For these reasons, there is great confidence that results were truly positive or negative.

2.4 | SARS-CoV-2 Antibody ELISA test

Outcome of the study was the SARS-CoV-2–specific antibody response as defined by ELISA. A total volume of 2.7 mL of blood was collected by trained study personnel, and anti-SARS-CoV-2 IgG and IgA ELISA using the recently CE validated beta-version of the commercial kit (EUROIMMUN AG, Lübeck, Germany; https://www.euroimmun.com) was performed according to the manufacturer's protocol.

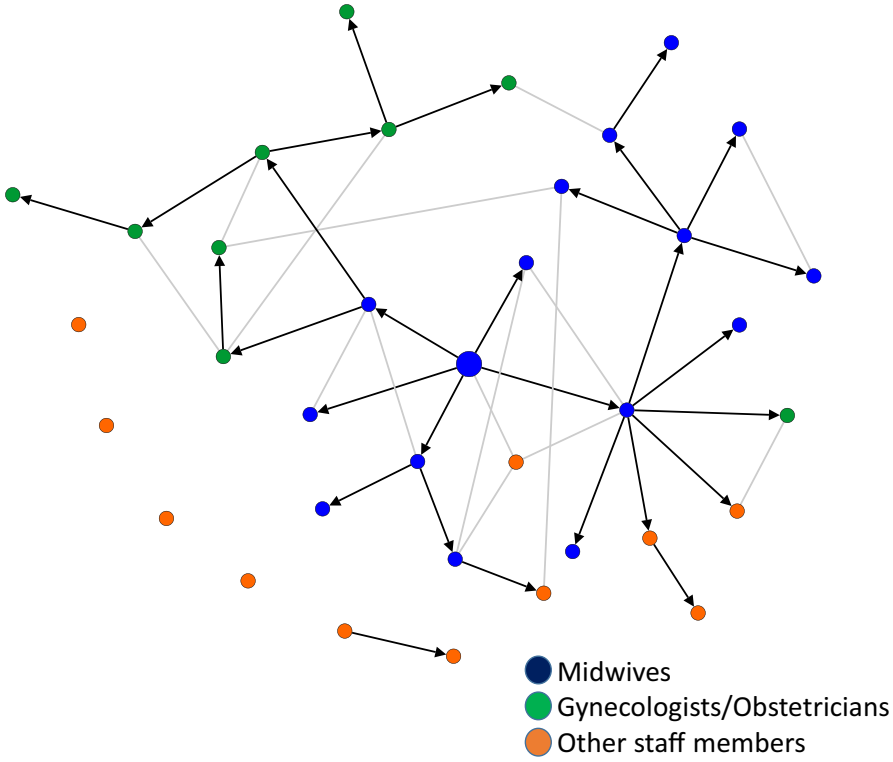

FIGURE 1 Transmission chains for all COVID-19 cases (N = 36) identified during the outbreak in the hospital. Black arrows indicate the most plausible chain of infection, and gray lines indicate alternative chains of infection

TABLE 1 General characteristics and self-reported symptoms of study participants stratified for exposure status

	All (N = 201)	COVID-19 (n = 31)	Close contact (n = 50)	Moderate contact (n = 63)	No contact (n = 57)	Difference between COVID-19 and all other groups[a] *p*
Female, N (%)	171 (85.1)	26 (83.3)	37 (73.4)	56 (88.9)	52 (91.2)	n.s.
Age						
18-35 years, N (%)	72 (35.8)	14 (46.7)	23 (44.9)	16 (25.4)	19 (33.3)	
36-50 years, N (%)	72 (35.8)	9 (26.7)	17 (34.7)	28 (44.4)	18 (31.6)	
51-65 years, N (%)	57 (28.4)	8 (28.4)	10 (21.3)	19 (30.1)	20 (35.1)	n.s.
Self-reported symptoms						
Runny nose, N (%)		11 (35.5)	28 (56.0)	38 (60.3)	32 (56.1)	0.025
Sore throat, N (%)		13 (41.9)	7 (14.0)	7 (11.1)	15 (26.3)	0.002
Headache, N (%)		15 (48.4)	14 (28.0)	16 (25.4)	16 (28.1)	0.017
Exhaustion/fatigue, N (%)		13 (41.9)	8 (16.0)	10 (15.9)	6 (10.5)	<0.001
Muscle aches, N (%)		15 (48.4)	10 (20.0)	7 (11.1)	6 (10.5)	<0.001
Anosmia, N (%)		16 (51.6)	2 (4.0)	1 (1.6)	1 (1.7)	<0.001
Shortness of breath, N (%)		7 (22.6)	0 (0.0)	2 (3.2)	1 (1.7)	<0.001
Coughing, N (%)		16 (51.6)	2 (4.0)	1 (1.6)	1 (1.7)	<0.001
Fever, N (%)		16 (51.6)	11 (22.0)	6 (9.5)	14 (24.6)	0.001
Diarrhea, N (%)		6 (19.3)	6 (12.0)	3 (4.8)	1 (1.7)	0.010
Other, N (%)		6 (19.3)	0 (0.0)	2 (3.2)	0 (0.0)	<0.001
Any symptom, N (%)		30 (96.8)	50 (100.0)	62 (98.4)	57 (100.0)	n.s.

[a]Group differences were determined using chi-square test.

Reagent wells were coated with recombinant structural protein (S1 domain) of SARS-CoV-2 for the IgA and IgG assay. Serum or plasma samples were diluted 1:100. The optical density (OD) was detected at 450 nm. An OD ratio of the measurement of each sample to the supplied calibrator was calculated. According to the manufacturer's recommendations, for the IgA assay an OD ratio of < 0.8 was considered

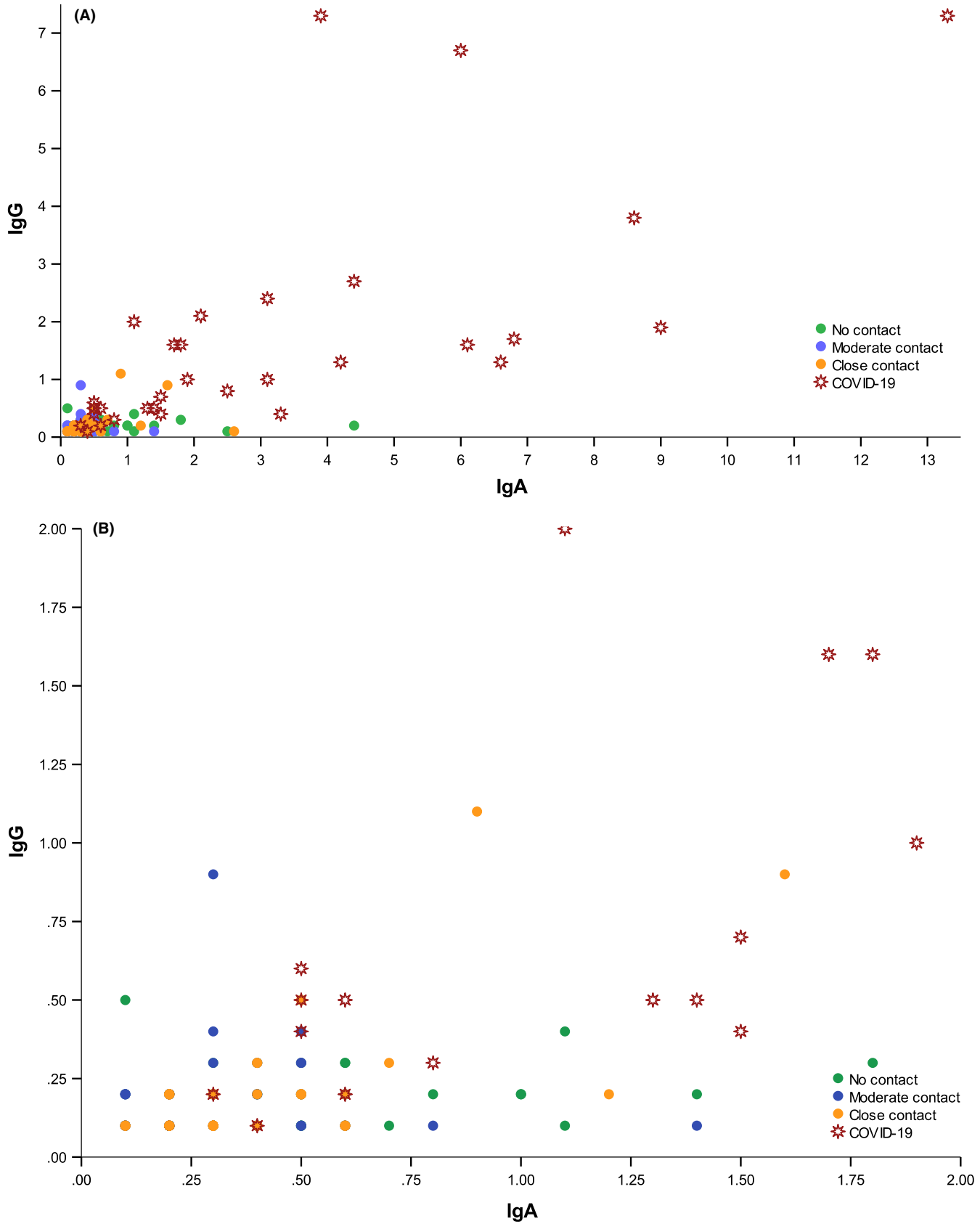

FIGURE 2 Scatterplot of IgA and IgG values. IgG and IgA are expressed as OD ratio. Different exposure groups are indicated by different types of scatter (see caption). (A) Full range of IgG and IgA values in the study population. (B) Enlargement for IgG and IgA values ranging from 0 to 2

negative, 0.8 to 1.0 borderline and > 1.0 positive, while for IgG, an OD ratio < 0.5 was considered negative, 0.5 to 1.0 borderline and > 1.0 positive, respectively.

2.5 | Statistics

Characteristics of study participants are presented using descriptive statistics. Differences between the exposure groups were analyzed using chi-square tests. All analyses were performed using SPSS.23. For the depiction of infection chains (Figure 1), the open-source software Gephi was used.

3 | RESULTS

In March 2020, a SARS-CoV-2 outbreak occurred in our hospital as previously reported.[5] In brief, a total of 36 hospital staff members were tested positive for the virus and of these, 34 acquired mild to moderate forms of COVID-19 while two individuals remained asymptomatic. A great majority of infections could be traced back to one index individual who returned from vacation in the ski resort of Ischgl, Austria. Respective infection chains are depicted in Figure 1. A total of 379 individuals underwent SARS-CoV-2 RT-PCR testing in that outbreak.

Subsequently, 201 staff members were asked to participate in this study by giving blood and providing information on symptoms and exposure. General characteristics and self-reported symptoms of study participants are shown in Table 1. Gender distribution in the study sample reflects the overall gender distribution in our children's and women's hospital. Out of all study participants, 31 (15.4%) were COVID-19 cases, 50 (24.9%) reported close contact, 63 (31.3%) moderate contact, and 57 (28.4%) had no contact to any COVID-19 case, respectively. In COVID-19 cases, the most frequently reported symptoms were coughing, fever, headache, and anosmia (each symptom was reported by about 50% of cases), followed by sore throat and exhaustion/fatigue (about 40%). When comparing study participants with and without a positive test for SARS-CoV-2, the differences in self-reported symptoms were particularly remarkable for anosmia and coughing. These two symptoms occurred only in less than 3% of study participants without COVID-19.

Figure 2A,B displays IgG and IgA values for all study participants. The time span between the onset of symptoms and the antibody test ranged between 15 and 28 days (median: 22 days; IQR (interquartile range): 20-24). Among the COVID-19 cases, the variability of values is large: IgG values range from 0.2 to 7.3, IgA values from 0.3 to 13.3. In Table 2, we show the relationship between IgA and IgG values in all four groups related to the cutoff levels from the manufacturer. Within the COVID-19 cases, 22.6% showed no antibody response, neither IgG nor IgA. IgG was elevated in 48.8%, and 74.2% had elevated IgA values. When comparing COVID-19 cases with and without antibody response, we found no significant differences in the collected characteristics of these groups (data not shown). Overall, there were 14 individuals (8.2%) in the non-COVID-19 groups who showed some kind of elevated IgG or IgA values. IgG was borderline or elevated in three individuals only (two of whom were from the close contact group) while borderline or elevated IgA was measured in 13 individuals. Interestingly, elevated antibody levels did not correlate with clinical exposure in the workplace as assessed during the outbreak. [Correction Statement: Correction added on 22 July 2020 after first online publication: Result section has been updated in this version.]

TABLE 2 Frequency tables of SARS-CoV-2–specific IgA vs. IgG values in COVID-19 cases and exposure groups

IgG	Normal: ≤0.8	Borderline: [>0.8; ≤1.0]	Elevated: >1.0	∑
(a) COVID-19	**IgA**			
Normal: ≤0.5	7	0	4	11
Borderline: [>0.5; ≤1.0]	1	0	4	5
elevated: >1.0	0	0	15	15
∑	8	0	23	31
(b) close contact	**IgA**			
Normal: ≤0.5	46	0	2	48
Borderline: [>0.5; ≤1.0]	0	0	1	1
Elevated: >1.0	0	1	0	1
∑	46	1	3	50
(c) mod. contact	**IgA**			
Normal: ≤0.5	61	0	1	62
Borderline: [>0.5; ≤1.0]	1	0	0	1
Elevated: >1.0	0	0	0	0
∑	62	0	1	63
(d) no contact	**IgA**			
Normal: ≤0.5	49	2	6	57
Borderline: [>0.5; ≤1.0]	0	0	0	0
Elevated: >1.0	0	0	0	0
∑	49	2	6	57

4 | DISCUSSION

During the outbreak of COVID-19 in our hospital, the majority of infections occurred when affected individuals were not yet symptomatic, and symptoms were grossly unspecific with the exception of anosmia and coughing. Any seroconversion was observed in 77% of our SARS-CoV-2–infected individuals with mild to moderate disease and only in a very limited number of individuals without disease but exposure only. Antibody responses neither related to the degree of exposure to SARS-CoV-2 nor to the duration in which SARS-CoV-2 was still observable in the throat by RT-PCR testing after convalescence.

This is a relatively small but very peculiar study population as it consists only of closely monitored and repeatedly tested hospital employees. Young people and especially women are highly overrepresented in our study sample, reflecting the typical composition of staff members in our and other mother-child hospitals. These characteristics may contribute to the fact that all COVID-19 cases experienced mild and moderate courses of the disease. However, with regard to our results on exposure status and antibody responses the composition of our study population is not likely to have biased our findings.

When comparing the symptoms of SARS-CoV-2–infected study participants with mild and moderate courses of the disease to the SARS-CoV-2 negative staff of the hospital, it is striking but not unexpected how many non-affected individuals also reported symptoms, as the novel coronavirus hit Europe exactly at the height of a major influenza wave and the onset of the pollen allergy season. It also became evident from our accurate reconstruction of the outbreak that almost all spreading of disease occurred during the prodromal, asymptomatic phase of the disease. Almost all infections linked to the outbreak occurred in the first week. Once testing and the general order to wear face protection masks at all times in the hospital were issued, the chain of infection was broken.[5]

We tested for the development of IgA and IgG antibody responses in our mild to moderate COVID-19 cases at least 15 days after the onset of symptoms (median 22 days) as previous reports suggested that antibodies can be measured reliably only around day 10 of the disease.[8] This was replicated also in our laboratory for the test used here, when IgG was detected in 100% of severe COVID cases with ARDS (n = 25) within 10 days after start of symptoms/admission to the hospital (mean 6.4 days, data available on request). In our population, at least 77% of cases had developed some kind of antibody response when tested.

Indeed, measurements for IgM would have added to the interpretation of the antigen response, but these were not available at this early stage in a validated and certified form of test. While IgA indicates front line mucosal defense against viral invasion, IgG is a good indicator for the long-time immune memory after contact with the virus and IgM is a more immediate built antibody after first time contact with an immune-active agent. It has been speculated by some authors that in those patients not affected severely by a SARS-CoV-2 infection, IgA plays a more prominent role but data on the topic are still inconclusive. It remains to be seen in follow-up tests if immunoglobulins will be detectable in these individuals at a later stage.

In our study population of mild to moderate cases, the level of IgG against coronavirus was not related to the severity of the disease. In severely affected COVID-19 patients, rather high levels of antibodies were previously reported[2,8] but even in one third of severe cases, IgG could not be detected.[4] In addition, IgG production did not coincide with the duration of direct virus RNA detection in the throat samples after symptoms had ceased (data not shown). At least five of the 36 diseased (13.8%) had virus RNA detectable with RT-PCR beyond day 21 after the start of symptoms, with a maximum of 45 days.

We had speculated that in those that had contact with infected individuals, antibody responses may have occurred dependent on the degree of exposure and probably without causing symptoms (silent infections). At least in our study population, this is not a convincingly so. Overall, only 14 individuals with negative PCR test results for SARS-CoV-2 had some measurable IGA or IgG responses (mostly IgA). It cannot be ruled out that especially these IgA responses were directed against common cold coronaviruses, as results from the manufacturer indicate that approximately 10% of sera from the era before SARS-CoV-2 showed unspecific IgA measurements. Our results are within that margin of error and thus must be interpreted with caution.

We conclude from our data that approximately 22% of COVID-19 patients do not mount a measurable immune response within 20 days after symptoms have occurred. The hypothesis that SARS-CoV-2 infection leads to immunity is still not proven, and our study raises doubt that this is a given. A relevant antibody response in asymptomatic individuals after contact with infected persons does not seem to be a major mechanism that one should rely on too much in achieving herd immunity.

ACKNOWLEDGMENTS

We are grateful to all staff members of our hospital who participated in the study and supported this research project. Moreover, we would like to thank Elke Fischer, Sarah Geitner, Silvia Gran, Patricia Schöberl, Elisa Valletta, and Christine Wolff for the collection and management of data for this study. We thank Jakob Niggel and Gabriel Zink from Datadesk.de for the donation of IT support in data managing and Barbara Schmidt from the virology laboratory of the University of Regensburg to provide the RT-PCR data from the outbreak phase.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

Susanne Brandstetter: Data curation; Formal analysis (lead); Investigation (equal); Methodology (lead); Writing-original draft (lead). **Samra Roth:** Data curation (equal); Investigation (equal). **Susanne Harner:** Data curation (equal); Investigation (equal). **Heike Buntrock-Doepke:** Investigation (equal); Project administration (lead). **Antoaneta A. Toncheva:** Methodology (lead). **Natascha Borchers:** Methodology (supporting). **Rudolf Gruber:** Formal analysis (equal); Methodology (equal). **Andreas Ambrosch:** Investigation (equal); Methodology (equal); Validation (equal). **Michael Kabesch:** Conceptualization (lead); Project administration (supporting); Resources (lead); Supervision (lead); Validation (equal); Writing-original draft (equal); Writing-review & editing (lead).

ETHICAL APPROVAL

The study was approved by the Ethics Committee of the University of Regensburg (file number: 20-1767-101).

ORCID

Michael Kabesch https://orcid.org/0000-0003-0697-1871

REFERENCES

1. World Health Organization (WHO). Corona virus disease (COVID-19) Pandemic. https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Accessed April 29, 2020.
2. Okba NMA, Muller MA, Li W, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. *Emerg Infect Dis*. 2020;26(7). https://doi.org/10.3201/eid2607.200841
3. Jin Y, Wang M, Zuo Z, et al. Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019. *Int J Infect Dis*. 2020;94:49-52.
4. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clin Infect Dis*. 2020. https://doi.org/10.1093/cid/ciaa344
5. Kabesch M, Roth S, Brandstetter S, et al. Successful containment of COVID-19 outbreack in a large maternity and perinatal center while continuing clinical service. *Ped Allergy Immunol*. 2020. https://doi.org/10.1111/pai.13265
6. Robert-Koch-Institute, Berlin, Germany. Strategies for managing contact persons in health care workers. https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/HCW.html. Accessed April 16, 2020.
7. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3). https://doi.org/10.2807/1560-7917.ES.2020.25.3.200004
8. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature*. 2020. https://doi.org/10.1038/s41586-020-2196-x

How to cite this article: Brandstetter S, Roth S, Harner S, et al. Symptoms and immunoglobulin development in hospital staff exposed to a SARS-CoV-2 outbreak. *Pediatr Allergy Immunol*. 2020;31:841–847. https://doi.org/10.1111/pai.13278