Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Short communication

SARS-CoV-2 antibody response dynamics and heterogeneous diagnostic performance of four serological tests and a neutralization test in symptomatic healthcare workers with non-severe COVID-19

David S.Y. Ong,Frans Keuren,Marijke van der Vliet,Bianca M. Boxma – de Klerk, Johannes G.M. Koeleman

A R T I C L E   I N F O

Keywords:
COVID-19
SARS-CoV-2
Neutralization
Healthcare workers
ELISA
Serology
Antibody

A B S T R A C T

Background: Most COVID-19 patients experience non-severe illness. The presence of SARS-CoV-2 antibodies suggest possible protection against re-infections in prior SARS-CoV-2 infected individuals.

Objectives: The aims of this prospective observational study were to longitudinally assess the antibody response during the first 4–6 months after polymerase chain reaction (PCR) confirmed SARS-CoV-2 infection, and to study the diagnostic performance of four different enzyme-linked immunosorbent assays (ELISAs) and a surrogate virus neutralization test (sVNT) in symptomatic healthcare workers (HCWs) with non-severe COVID-19.

Study design: HCWs in a teaching hospital were included between March 8 and June 15, 2020, when they had a PCR-confirmed SARS-CoV-2 infection in the past 3 months. The performances of four ELISAs (Wantai, Bio-Rad Platelia, BioTrading Immy clarus, and Euroimmun) were evaluated in serum samples obtained at the moment of study inclusion and subsequently at 1, 2 and 3 months thereafter. Furthermore, in the last available serum sample sVNT by GenScript was performed.

Results: 309 samples from 80 positive HCWs were included of whom 70 (88%) were SARS-CoV-2 seropositive. The detection rates of SARS-CoV-2 antibodies by the different ELISAs were heterogenous ranging from 64% for the Euroimmun ELISA to 88% for the Wantai ELISA. The Wantai ELISA had the highest and almost perfect agreement with sVNT (96%, Cohen’s kappa 0.83).

Conclusion: SARS-CoV-2 (neutralizing) antibodies were detectable in most symptomatic individuals with non-severe COVID-19. The presence of antibodies remained stable up to six months after initial infection. There is large variability in diagnostic test performance between ELISA tests.

1. Introduction

The emergence of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a large pandemic. The first SARS-CoV-2 infected patient in the Netherlands was detected on February 27, 2020. To what extent immunity develops after primary infection is still a matter of investigation. Moreover, the question which markers could be used to assess immunity has become relevant. Most COVID-19 patients are only mild symptomatic [1], and some studies have suggested that weaker immune responses may be found in these patients in comparison to the minority of patients with severe disease [2-4]. Therefore, considerable uncertainty remains regarding the possible protection against re-infections in most SARS-CoV-2 infected individuals. The humoral immunity is a key component of protective immunity, which is mainly characterized by antibodies formation [5-7]. Specific enzyme-linked immunosorbent assays (ELISAs) can detect the presence of IgM, IgA, IgG or total antibodies against SARS-CoV-2. This study aimed to assess the antibody response in the first four to six months after SARS-CoV-2 infection, and to compare the diagnostic performance of different ELISAs and an antibody neutralization test in symptomatic healthcare workers (HCWs) with non-severe COVID-19.

* Corresponding author at: Kleiweg 500, 3045 PM Rotterdam, The Netherlands.
E-mail address: davidsyong@gmail.com (D.S.Y. Ong).

https://doi.org/10.1016/j.jcv.2021.104904
Received 8 April 2021; Received in revised form 15 June 2021; Accepted 22 June 2021
Available online 27 June 2021
1386-6532/© 2021 Elsevier B.V. All rights reserved.
2. Methods

HCWs in a teaching hospital in the Netherlands were eligible between March 8 and June 15, 2020, when they had a reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) confirmed SARS-CoV-2 infection in the past three months. During this period there was a strict local hospital policy that all HCWs with symptoms of a viral respiratory illness (including fever, cough, shortness of breath, myalgias, sore throat, dysgeusia, or anosmia) should be immediately tested for SARS-CoV-2 infection. These HCWs were tested on nasopharyngeal swabs by either a validated in-house SARS-CoV-2 RT-qPCR assay according to the national reference method [8], or by the CE-IVD kit GeneFinderTM COVID-19 Plus RealAMP Kit using the Sample to Result Platform ELITE InGenius® [9].

The study protocol was approved by both the Medical Research Ethics Committee United (protocol number R20.030) and our Hospital Board of Directors (protocol number 2020–066). Written informed consent was obtained before study participation.

We evaluated the presence of anti-SARS-CoV-2 antibodies in symptomatic HCWs at baseline (i.e., study inclusion) and after 1, 2 and 3 months using the following ELISAs: (1) Wantai SARS-CoV-2 Ab ELISA; (2) Bio-Rad Platelia SARS-CoV-2 Total Ab; (3) BioTrading Immy clars SARS-CoV-2 Total Antibody Enzyme Immunoassay; and (4) Euroimmun Anti-SARS-CoV-2 S1 IgG ELISA. The Wantai, Bio-Rad, and BioTrading ELISAs detect total antibodies, whereas the Euroimmun ELISA only detects IgG. The Wantai and BioTrading ELISAs target the receptor binding domain of the spike protein (S-RBD), whereas the Bio-Rad ELISA targets the nucleocapsid protein and the Euroimmun ELISA the S1 of the spike protein. From the last available serum sample of each patient, we used the GenScript SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit to detect all types of neutralizing antibodies (nAb) against SARS-CoV-2 herein. Results of assays were interpreted positive or negative according to the manufacturer’s instructions, and borderline results were also interpreted positive for our analysis. SARS-CoV-2 seropositivity was defined as the presence of SARS-CoV-2 antibodies according to at least one of the four ELISAs.

All analyses were performed using R version 3.3.2 (R Foundation for Statistical Computing). We compared groups using Chi-square test for categorical variables. Cohen’s kappa was used to assess in between assay agreement. P-values <0.05 were considered to be statistically significant.

3. Results

In total, 80 SARS-CoV-2 positive HCWs were included after a median of 54 days (interquartile range (IQR) 44–65) following a positive SARS-CoV-2 RT-qPCR result. The median age was 41 (IQR 28–54, range 20–64) years, and 66 (82.5%) were female.

Ideally, 320 blood samples from 80 HCWs over multiple time points should have been obtained. However, due to loss of follow-up for some HCWs, 309 (96.6%) samples were collected in total.

Seventy (87.5%) HCWs were SARS-CoV-2 seropositive at study inclusion, and remained seropositive during follow-up afterwards. This stability was mainly observed for the Wantai ELISA. In contrast, the BioTrading and Euroimmun ELISAs showed most heterogeneity in results within a patient including different combinations of consecutive positive and negative results over time.

The remaining 10 (12.5%) seronegative HCWs were negative at all time points and according to the four ELISAs, but one of these samples was (weak) positive according to the sVNT. Among the seronegative, the corresponding cycle threshold (Ct) values of the SARS-CoV-2 positive respiratory samples were above 35 (i.e., very low virus load corresponding to <100 copies per mL and close to the limit of detection) in 9 (90%) cases. In contrast, among 68 seropositive HCWs (i.e., excluding 2 HCWs with missing Ct values but RT-qPCR-confirmed positive tests), only 4 (5.9%) had Ct values above 35 (p<0.01).

The performance of the different ELISAs in detecting antibodies were heterogenous ranging from 63.8% to 87.5% at study inclusion, and from 58.9% to 87.7% at the final sampling moment (Table 1). sVNT was performed in 74 (92.5%) HCWs, of whom 63 (85.1%) were positive. Of note, among the seropositive HCWs, 62 (96.9%) had positive nAb. The Wantai ELISA had the highest agreement with sVNT (95.9%, Cohen’s kappa 0.83), followed by Bio-Rad ELISA (91.9%, Cohen’s kappa 0.74), and the BioTrading and Euroimmun ELISAs (both 71.6% agreement, Cohen’s kappa 0.37) (Table 2).

4. Discussion

About 85% of HCWs developed nAb after non-severe SARS-CoV-2 infection. This prospective observational study also shows that there is large heterogeneity between four different ELISAs, which were CE-IVD approved and authorized by FDA for emergency use except for the BioTrading ELISA. The Wantai ELISA performed best and had almost perfect agreement with the sVNT. SARS-CoV-2 infection elicited antibodies seem to target both S-RBD and nucleocapsid protein, but it remains difficult to conclude regarding the association between antigen target sites and qualitative performance of the different ELISAs used.

Our study findings are in line with previous reports showing SARS-CoV-2 antibody responses present at 3 to 6 months after infection, and which remained stable over time [10-12]. The proportion of 12.5% seronegative cases in our study is in between the observed proportions in asymptomatic and hospitalized symptomatic patients in previous studies, in which antibody titers remained negative in 5% of symptomatic RT-qPCR-positive patients [13], and 15% to 40% of asymptomatic RT-qPCR-positive patients were seronegative [2,14].

Interestingly, almost all seronegative HCWs had a very low virus load upon diagnosis (i.e., RT-qPCR Ct-value >35), which was in contrast to seropositive HCWs who had higher virus loads. In any case, the association between virus load in nasopharynx and likelihood of SARS-CoV-2 seroconversion as observed in our study requires further investigation.

The presence of neutralizing antibodies is considered a functional

Table 1

| Sampling moment | 1 – at inclusion | 2 – one month after inclusion | 3 – two months after inclusion | 4 – three months after inclusion |
|-----------------|-----------------|-----------------------------|-----------------------------|-------------------------------|
| Days between positive PCR result and serology sampling moment | 54 (44–65) | 81 (72–94) | 113 (102–131) | 140 (133–159) |
| ELISA | Presence of SARS-CoV-2 antibodies (a) | |
| Wantai SARS-CoV-2 Ab ELISA | 70/80 (87.5) | 69/79 (87.3) | 67/77 (87.0) | 64/73 (87.7) |
| Bio-Rad Platelia SARS-CoV-2 Total Ab ELISA | 65/80 (81.3) | 61/78 (78.2) | 60/77 (77.9) | 59/73 (80.8) |
| BioTrading Immy clars SARS-CoV-2 Total Antibody Enzyme Immunoassay | 50/80 (72.5) | 59/78 (75.6) | 56/77 (72.7) | 44/73 (60.3) |
| Euroimmun Anti-SARS-CoV-2 S1 IgG ELISA | 51/80 (63.8) | 53/78 (67.9) | 47/76 (61.8) | 43/73 (58.9) |

The numbers are presented in median (interquartile range) or absolute numbers (percentage).

(a) Borderline test results were considered positive for the analysis.
(b) One sample of one patient was tested on Wantai SARS-CoV-2 Ab ELISA only due to sample availability.
(c) One sample of one patient was tested on all ELISAs except on Euroimmun because of insufficient material available.
Table 2

Agreement and Cohen’s kappa between four different ELISAs and a surrogate virus neutralization kit.

|          | Bio-Rad | BioTrading | Euroimmun | GenScript SARS-CoV-2 Surrogate Neutralization Test Kit |
|----------|---------|------------|-----------|------------------------------------------------------|
|          | SARS-CoV-2 | SARS-CoV-2 | Total Ab   | Anti-SARS-CoV-2 S1 IgG ELISA                         |
|          | Total Ab | Enzyme Immunoassay |            |                                                     |
| Wantaï   | 92.2%   | 83.1%       | 75.2%     | 95.9%                                                 |
|          | k = 0.72| k = 0.51    | k = 0.38  | k = 0.83                                              |
| SARS-CoV-2|         |             |           |                                                      |
| Ab ELISA |         |             |           |                                                      |
| Bio-Trading Immunoassay | 80.5%   | 71.6%       | k = 0.56  | k = 0.37                                              |
| SARS-CoV-2 Total Antibody Enzyme |         |             |           |                                                      |
| Euroimmun Anti-SARS-CoV-2 S1 IgG ELISA | 71.6%   |             | k = 0.37  |                                                      |

Data are presented in percentage agreement and Cohen’s kappa.

correlate of immunity and provides at least partial resistance to subsequent infections by virus antigen binding to prevent interaction with host cells [15,16]. In that light, it is reassuring that 85% of HCWs developed neutralizing antibodies after infection.

Our study has some limitations. First, this study included merely a follow-up period of four to six months after the first positive RT-qPCR result. Second, we used a SVNT and not the conventional plaque reduction test, which is considered the gold standard for the assessment of nAb. Nevertheless, the SVNT used in our study was extensively validated and showed a very high correlation with conventional viral neutralization tests [17]. Third, we did not assess T-cell responses against SARS-CoV-2, which could also contribute to protective immunity against re-infections in recovered COVID-19 patients with no detectable antibodies [18,19].

In conclusion, it is crucial to be aware of large performance differences among SARS-CoV-2 serological tests. Most persons will develop neutralizing antibodies against SARS-CoV-2 after non-severe COVID-19 infection that will persist up to at least 6 months.

Funding

This research was funded by the Franciscus Gasthuis & Vlietland Hospital, Rotterdam, the Netherlands and did not receive any additional funding from agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

We would like to thank our laboratory technicians and team managers for their assistance in performing the serological tests.

Contribution

DSYO, BMBK and JGHR contributed to the conception and design of the study. MdV and FK acquired the data. DSYO and FK analyzed the data. All authors contributed to the interpretation of the data. DSYO drafted the first manuscript and all other authors revised it critically for important intellectual content. All authors approved this manuscript version to be submitted.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Z. Wu, J.M. McGoogan, Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: summary of a report of 72 314 Cases From the Chinese Center for Disease Control and Prevention, JAMA 323 (2020) 1239–1242, https://doi.org/10.1001/jama.2020.2641.
[2] Q.-X. Long, X.-J. Tang, Q.-L. Shi, Q. Li, H.-J. Deng, J. Yuan, et al., Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections, Nat. Med. 26 (2020) 1200–1204, https://doi.org/10.1038/s41591-020-0965-6.
[3] J. Zhao, Q. Yuan, H. Wang, W. Liu, X. Liao, Y. Su, et al., Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. Clin. Infect. Dis. 71 (2020) 2027–2034, https://doi.org/10.1093/cid/ciaa344.
[4] L. Ren, L. Zhang, D. Chang, J. Wang, Y. Hu, H. Chen, et al., The kinetics of humoral response and its relationship with the disease severity in COVID-19, Commun Biol 3 (2020) 790–797, https://doi.org/10.1038/s42003-020-01526-8.
[5] S.P. Lumley, D. O’Donnell, N.E. Stoesser, P.C. Matthews, A. Howarth, S.B. Hatch, et al., Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers, N. Engl. J. Med. 384 (2021) 353–540, https://doi.org/10.1056/NEJMoa204545.
[6] A. Jeffery-Smith, N. Iyanger, S.V. Williams, J.Y. Chow, F. Alano, K. Hoschler, et al., Antibodies to SARS-CoV-2 protect against re-infection during outbreaks in care homes, September and October 2020, Euro Surveill. 26 (2021) (2005), https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.
[7] A. Addetia, K.H.D. Crawford, A. Dingens, H. Zhu, P. Roychoudhury, M.-L. Huang, et al., Neutralizing Antibodies Correlate with Protection from SARS-CoV-2 in Humans during a Fishery Vessel Outbreak with a High Attack Rate, J. Clin. Microbiol. 58 (2020) 77, https://doi.org/10.1128/JCM.02107-20.
[8] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K.W. Chu, et al., Detection of 2019 novel coronavirus (2019-ncov) by real-time RT-PCR, Euro Surveill. 25 (2020) 2431, https://doi.org/10.2807/1560-7917.ES.2020.25.24.2001901.
[9] P. de Candia, F. Prattichizzo, S. Garavelli, G. Matarese, T. Cells, Warriors of SARS-CoV-2 Infection, Trends Immunol. 42 (2021) 18–24, https://doi.org/10.1016/j.tiim.2020.11.002.
