Nine Cases of SARS-CoV-2-PCR-positive Samples Showed No Increase of Antibodies Against SARS-CoV-2

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Abstract. Background/Aim: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been affecting Hokkaido, Japan since late February 2020 until present. The aim of this study was to report the relationship between anti-SARS-CoV-2 antibody-positive and SARS-CoV-2 PCR-positive cases by analyzing anti-SARS-CoV-2 antibodies (IgG and total-Ig). Patients and Methods: Serum samples were collected from care workers and nurses in two nursing homes and two hospitals which underwent virus outbreak. All people were confirmed to be SARS-CoV-2-positive by RT-qPCR and their sera was analyzed for anti-SARS-CoV-2 antibodies (IgG and total-Ig). Results: Although 34 out of 43 samples (79.1%) showed enough amount of anti-SARS-CoV-2 antibodies, 9 RT-qPCR-positive samples (20.9%) showed absence of anti-SARS-CoV-2 antibodies in their sera. Conclusion: The results that 20.9% of RT-qPCR-positive samples with SARS-CoV-2 showed absence of anti-SARS-CoV-2 antibodies provides a possibility that the innate immune reaction could eliminate the virus without activating adaptive immune reaction.

From December 2019, the entire world has been affected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that originated in Wuhan, China (1), and the World Health Organization (WHO) declared that coronavirus disease 2019 (COVID-19) a global pandemic on March 11th 2020 (2). Hokkaido, Japan, has been also affected by SARS-CoV-2 beginning late February 2020 (3). In April, May and November, two nursing homes and two hospitals had outbreak and facilities had more than 10 PCR-positive people who are care workers and nurses. All were confirmed to have COVID-19 infection by RT-qPCR for SARS-CoV-2 according to the nationally recommended protocol (4). The PCR-positive samples were analyzed for anti-SARS-CoV-2 antibodies (IgG and total-Ig) to analyze the relationship between antibody-positive and PCR-positive cases.

Patients and Methods

Samples. The study protocol was approved by the Institutional Review Board for Human Use of the Health Sciences University of Hokkaido and the other four facilities (I) Nursing Home Barato Akashia-Heights (II) Chitose Daiichi Hospital (III) Kin-ikyo Chuo Hospital (IV) Nursing home Dream House. Written informed consent was obtained from all patients before study. The PCR-positive samples collected more than 30 days from the date of PCR positivity from these 4 facilities.

Measurement of antibody. The measurement of anti-SARS-CoV-2 antibodies (IgG and total-Ig) in sera were done by using the Vitros Immunodiagnostic Products anti-SARS-CoV-2 total Ig test and the anti-SARS-CoV-2 IgG test (Ortho Clinical Diagnostics) (5). The sensitivity of the anti-SARS-CoV-2 total Ig test was reported to be 100% in samples collected more than 6 days from the date of PCR positivity (5). The sensitivity of the anti-SARS-CoV-2 IgG test was also reported to be 100% in samples collected at least 15 days following initial disease manifestation (6).

Results

Thirty-four out of 43 PCR-positive samples (79.1%) showed enough amounts of IgG and total-Ig against SARS-CoV-2. However, nine PCR-positive samples (20.9%) (3 out of 11; Nursing Home Barato Akashia-Heights, 3 out of 10; Chitose Daiichi Hospital and 1 out of 8; Kin-ikyo Chuo Hospital; 2 out of 14; Nursing home Dream House) showed absence of anti-SARS-CoV-2 antibodies in their sera (Table I). The
The anti-SARS-CoV-2 IgG and total-Ig assays were performed by VITROS XT 7600 immunoassay system (Ortho-Clinical Diagnostics, Rochester, NY, USA). The antibody values were adjusted by the calibrator and control reagents and estimated by the signal to cutoff (S/C) values of <1.00 and ≥1.00 corresponding to non-reactive and reactive results, respectively. PCR: Polymerase chain reaction; Ab: antibody; Ig: immunoglobulin.

Table I. Results of RT-PCR and antibody test against SARS-CoV-2.

| Nursing Home Barato Akashia-Heights | ID   | PCR Test date | Ab Test date | Total Ig value | IgG value |
|--------------------------------------|------|---------------|--------------|----------------|-----------|
| N0067A8B                             | 4/28/2020 | 6/17/2020 | 0.07 | 0 |
| N009919B                             | 4/29/2020 | 6/17/2020 | 0.04 | 0.01 |
| N001738B                             | 4/28/2020 | 6/17/2020 | 0.08 | 13.40 |
| N00604B                              | 5/4/2020  | 6/17/2020 | 295.00 | 7.8 |
| N001646B                             | 5/11/2020 | 6/17/2020 | 236.00 | 8.07 |
| N003894B                             | 4/29/2020 | 6/17/2020 | 45.20 | 10.7 |
| N005108B                             | 5/5/2020  | 6/17/2020 | 138.00 | 1.30 |
| N002078B                             | 5/15/2020 | 6/17/2020 | 110.00 | 5.17 |
| N003849B                             | 4/22/2020 | 6/17/2020 | 89.80 | 0.01 |
| N008549A                             | 5/29/2020 | 6/30/2020 | 295.00 | 13.40 |
| N003894B                             | 4/22/2020 | 6/17/2020 | 82.00 | 0.01 |
| Nursing Home Dream House             |
| ID   | PCR Test date | Ab Test date | Total Ig value | IgG value |
| M08511                             | 4/30/2020 | 6/30/2020 | 0.01 | 0 |
| M06310                             | 4/30/2020 | 6/30/2020 | 0.02 | 0 |
| M01070                             | 5/9/2020  | 6/30/2020 | 0.02 | 0 |
| M08117                             | 4/30/2020 | 6/30/2020 | 3.03 | 0.16 |
| M03574                             | 4/19/2020 | 6/30/2020 | 65.30 | 2.31 |
| M09297                             | 4/27/2020 | 6/30/2020 | 46.70 | 2.06 |
| M04585                             | 4/23/2020 | 6/30/2020 | 245.00 | 5.92 |
| M01693                             | 4/23/2020 | 6/30/2020 | 79.00 | 2.00 |
| M01226                             | 4/28/2020 | 6/30/2020 | 87.20 | 5.00 |
| M04427                             | 4/13/2020 | 6/30/2020 | 154.00 | 5.31 |
| Kin-ikyo Chuo Hospital               |
| ID   | PCR Test date | Ab Test date | Total Ig value | IgG value |
| F02184                             | 11/8/2020 | 1/14/2021 | 134.00 | 20.20 |
| M03574                             | 4/19/2020 | 6/30/2020 | 138.00 | 9.59 |
| M09297                             | 4/27/2020 | 6/30/2020 | 89.80 | 1.30 |
| M04585                             | 4/23/2020 | 6/30/2020 | 82.00 | 0.01 |
| M01693                             | 4/23/2020 | 6/30/2020 | 89.80 | 1.30 |

three anti-SARS-CoV-2 antibody-absent cases in the nursing home Barato Akashia-Heights showed PCR-positive with high Ct values (32.19, 33.94, and 36.68) and calculated viral copies were less than 100 copies. These three people showed negative results of IgG values and Total Ig values again from sera collected 14 days later.

Discussion

The results of the present study showed that 9 cases out of 43 SARS-CoV-2-PCR-positive samples showed no increase of antibodies against SARS-CoV-2. These results show a possibility of innate immune reaction that could eliminate the virus without activating adaptive immune reaction involving B lymphocytes, helper T cells and plasma cells. Many reports showed an increase of NK cells in SARS-CoV-2-infected people with severe symptoms. (7, 8). In the present study, all cases whose antibodies against SARS-CoV-2 were measured were asymptomatic. It has been reported that the sensitivity of the anti-SARS-CoV-2 total Ig test and the anti-SARS-CoV-2 IgG test is greater than 95% (5, 6, 9), and that antibodies are positive in more than 95% of PCR-positive patients. For the first time, our study examined the presence of antibody production in PCR-positive patients, and we found that innate immune response might eliminate SARS-CoV-2 in more than 20% of SARS-CoV-2 PCR-positive patients before adaptive immune system start up. Smetana et al. reported the role of interleukin-6 (IL6) on lung complications in patients with COVID-19 and they mentioned that inhibitors of IL6 signaling represent a promising approach that can be employed for attenuation of a cytokine storm and might be beneficial for patients with COVID-19 (10). IL-6 is involved in the regulation of B cell response into antibody producing cells (11). Presumably asymptomatic SARS-CoV-2-infected people
whose antibodies against SARS-CoV-2 were measured in the present study might produce very low levels of IL6 and might not induce enough B cell differentiation to antibody-producing cells. Further studies are needed to clarify the role of innate immunity and IL6 during production of antibodies against SARS-CoV-2 in asymptomatic SARS-CoV-2-infected people.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors’ Contributions

All Authors contributed to the study conception and design. Materials preparation, data collection, and analysis were performed by TK, YK, TO, MT, YT, OU, KN and RI. The first draft of the manuscript was written by TK, YK, YT and MK and all authors commented on previous versions of the manuscript. All Authors read and approved the final manuscript.

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References

1 Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H and Cao B: Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 395(10229): 1054-1062, 2020. PMID: 32171076. DOI: 10.1016/S0140-6736(20)30566-3

2 Bedford J, Enria D, Giesecke J, Heymann DL, Ihekweazu C, Kobinger G, Lane HC, Memish Z, Oberste MS, Pedersen L, Nielsen L, Hansson O, Korsholm TL, Møller BK, Hansen AT, Iversen KK, Nielsen KK, Nielsen PB, Hasselbalch RB, Fogh K, Norsk JB, Kristensen JH, Schönning K, Kirchhoff F, Nielsen AC, Landys LH, Lüftiger M, Holm DK, Nielsen AC, Szekeres SE, Grum-Schwensen B, Aagaard B, Jensen TG, Nielsen DM, Ulhm U and Dessau RB: Comparison of 16 Serological SARS-CoV-2 Immunoassays in 16 Clinical Laboratories. J Clin Microbiol 59(5): e02596-20, 2021. PMID: 33574119. DOI: 10.1128/JCM.02596-20

3 Hisada S, Murayama T, Tsubouchi K, Fujita S, Yada S, Wakamiya S and Aramaki E: Surveillance of early stage COVID-19 clusters using search query logs and mobile device-based location information. Sci Rep 10(1): 18680, 2020. PMID: 33122686. DOI: 10.1038/s41598-020-75771-6

4 Shirato K, Nao N, Kato H, Takayama I, Saito S, Kato F, Kato H, Sakata M, Nakatsu Y, Mori Y, Kageyama T, Matsuyama S and Takeda M: Development of genetic diagnostic methods for detection for novel coronavirus 2019(nCoV-2019) in Japan. Jpn J Infect Dis 73(4): 304-307, 2020. PMID: 32074516. DOI: 10.7883/yoken.JJID.2020.061

5 Mullins KE, Merrill V, Ward M, King B, Rock P, Caswell M, Ahlman M, Harris AD and Christenson R: Validation of COVID-19 serologic tests and large scale screening of asymptomatic healthcare workers. Clin Biochem 90: 23-27, 2021. PMID: 33472036. DOI: 10.1016/j.clinbiochem.2021.01.004

6 Theel ES, Harrington J, Hilgert H and Granger D: Performance Characteristics of Four High-Throughput Immunoassays for Detection of IgG Antibodies against SARS-CoV-2. J Clin Microbiol 58(8): e01243-20, 2020. PMID: 32513859. DOI: 10.1128/JCM.01243-20

7 Carsetti R, Zaffina S, Piano Mortari E, Terreri S, Corrente F, Capponi C, Palomba P, Mirabella M, Cascioli S, Palange P, Cuccaro I, Milito C, Zumla A, Mauerer M, Camisa V, Vincenzi M, Santoro A, Cimini E, Marchioni L, Nicastri E, Palmieri F, Agrati C, Ippolito G, Porzio O, Concato C, Onetti Mada A, Raponi M, Quintarelli C, Quinti I and Locatelli F: Different innate and adaptive immune responses to SARS-CoV-2 infection of asymptomatic, mild, and severe cases. Front Immunol 11: 610300, 2020. PMID: 33991280. DOI: 10.3389/fimmu.2020.610300

8 Yan L, Cai B, Li Y, Wang MJ, An YF, Deng R, Li DD, Wang LC, Xu H, Gao XD and Wang LL: Dynamics of NK, CD8 and Tfh cell mediated the production of cytokines and antiviral antibodies in Chinese patients with moderate COVID-19. J Cell Mol Med 24(24): 14270-14279, 2020. PMID: 33145962. DOI: 10.1111/jcmm.16044

9 Harritshøj LH, Gybel-Brask M, Afzal S, Kamstrup PR, Jørgensen CS, Thomsen MK, Hilsted L, Friis-Hansen L, Szeczi PB, Pedersen L, Nielsen L, Hansen CB, Garred P, Korsholm TL, Mikkelsen S, Nielsen KO, Møller BK, Hansen AT, Iversen KK, Nielsen PB, Hasselbalch RB, Fogh K, Norsk JB, Kristensen JH, Schönning K, Kirkby NS, Nielsen ACY, Landys LH, Lüftiger M, Holm DK, Nilsson AC, Saekmose SG, Grum-Schwensen B, Aagaard B, Jensen TG, Nielsen DM, Ulhm U and Dessau RB: Comparison of 16 Serological SARS-CoV-2 Immunoassays in 16 Clinical Laboratories. J Clin Microbiol 59(5): e02596-20, 2021. PMID: 33574119. DOI: 10.1128/JCM.02596-20

10 Smetana K Jr and Brábek J: Role of interleukin-6 in lung complications in patients with COVID-19: Therapeutic implications. In Vivo 34(3 Suppl): 1589-1592, 2020. PMID: 32503815. DOI: 10.21873/inivo.11947

11 Matsuda T, Yamasaki K, Taga T, Hirano T and Kishimoto T: Current concepts of B cell modulation. Int Rev Immunol 5(2): 97-109, 1989. PMID: 8691054. DOI: 10.3109/08830188900961976

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