Received: 23 September 2021 | Accepted: 28 November 2021

DOI: 10.1002/jmv.27486

SHORT COMMUNICATION

JOURNAL OF MEDICAL VIROLOGY WILEY

SARS-CoV-2 antibody kinetics in blood donors with a previously positive SARS-CoV-2 antibody test within a seroprevalence survey

Mette B. Levring[1] | Dorte K. Holm[1] | Anna C. Nilsson[1] | Joschka M. Bauer[1] | Iben S. Jensen[1] | Jesper R. Davidsen[2,3] | Line D. Rasmussen[4] | Ulrik Sprogøe[1] | Søren T. Lillevang[1]

[1]Department of Clinical Immunology, Odense University Hospital, Odense, Denmark

[2]Department of Clinical Research, University of Southern Denmark, Odense, Denmark

[3]Department of Respiratory Medicine, Odense University Hospital, Odense, Denmark

[4]Department of Infectious Diseases, Odense University Hospital, Odense, Denmark

Correspondence
Mette B. Levring, Department of Clinical Immunology, Odense University Hospital, Odense, Denmark.
Email: mette.boegh.levring@rsyd.dk

Abstract

The persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies is a matter of importance regarding the coronavirus disease 19 (COVID-19) pandemic. To observe antibody dynamics, 105 blood donors, positive for SARS-CoV-2 antibodies by a lateral flow test within a seroprevalence study, were included in this study. Thirty-nine (37%) of 105 the donors were confirmed positive by a total Ig Wantai enzyme-linked immunosorbent assay (ELISA). Three (8%) in this group of 39 reported severe and 26/39 (67%) mild to moderate COVID-19 symptoms. By further ELISA-testing, 33/39 (85%) donors were initially positive for IgG antibodies, 31/39 (79%) for IgA, and 32/39 (82%) for IgM, while 27/39 (69%) were positive for all three isotypes. Persistence of IgG, IgA, and IgM was observed in 73%, 79%, and 32% of donors, respectively, after 6–9 months of observation. For IgM antibodies, the decline in the proportion of positive donors was statistically significant ($p = 0.002$) during 12 months observation, for IgG only the decline at 3 months was statistically significant ($p = 0.042$). Four donors exhibited notable increases in antibody levels. In conclusion, persistent SARS-CoV-2 IgA antibodies and IgG antibodies at 6–9 months are present in approximately three of four individuals with previous mild to moderate COVID-19.

KEYWORDS
antibody, blood donors, COVID-19, lateral flow test, SARS-CoV-2

1 | INTRODUCTION

In 2019, a new infectious disease, coronavirus disease 19 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged. Serious cases of COVID-19 may lead to pneumonia and respiratory distress. However, many patients are asymptomatic and unaware of the infection.[1] Several countries have utilized seroprevalence of SARS-CoV-2 antibodies in healthy blood donors to include asymptomatic cases in prevalence estimates.[2] Since COVID-19 became endemic, many SARS-CoV-2 immunoassays have been marketed. One of the first assays available in Denmark was a lateral flow test (LFT) from Livzon for Immunoglobulin G (IgG) and M (IgM) SARS-CoV-2 antibodies. Many SARS-CoV-2 antibody test kits have since become available. Antibody kits measure either functionally neutralizing antibodies or binding antibodies, which to some degree correlate with neutralizing antibodies.[3] The COVID-19 antibody response has been shown to be lesser in mild cases [4,5] and antibody levels to wane over time.[6] Furthermore, prior SARS-CoV-2 infection only partially protects against reinfection,[7] and loss of antibodies has been suggested as a

risk for reinfection.[8] The aim of this prospective observational study was to investigate the dynamics of binding antibody levels over time and to observe reinfections after mild to moderate COVID-19 in a cohort of Danish blood donors.

2 | MATERIALS AND METHODS

2.1 | Inclusion

For surveillance purposes, a Danish national SARS-CoV-2 seroprevalence project was initiated in April 2020. Blood donors were screened for SARS-CoV-2 antibodies each time they attended a blood collection facility. The screening comprised SARS-CoV-2 IgG and IgM in EDTA plasma by a commercial LFT (Diagnostic Kit for IgM/IgG Antibody to Corona Virus, Zhuhai Livzon Diagnostics, Inc.). Samples yielding a positive reaction in IgM and/or IgG bands were considered positive. From April 6 to May 28, 2020, the project identified 137 (133 individual donors) out of 9851 samples as positive for SARS-CoV-2 antibodies. These 133 donors were invited to enter this study, initiated in October 2020. The Regional Committees on Health Research Ethics for Southern Denmark approved this study (S-20200146).

2.2 | SARS-CoV-2 antibody testing

A blood sample was collected from each donor at inclusion and at each subsequent donation. All samples were tested/re-tested for SARS-CoV-2 antibodies using four semi-quantitative CE-IVD approved tests: SARS-CoV-2 Total Ig enzyme-linked immunosorbent assay (ELISA) and a SARS-CoV-2 IgM ELISA (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.) and IgG and IgA ELISA (EUROIMMUN Medizinische Labordiagnostika AG). Initial testing was performed with the total Ig ELISA. Samples yielding a positive reaction were considered truly positive and subsequently tested with the SARS-CoV-2 IgM ELISA and the SARS-CoV-2 IgG and IgA ELISAs. Results >1.1 arbitrary units (AU) were considered positive. SARS-CoV-2 IgG and IgA tests were categorized as weakly positive ≥1.1–3.0 AU, intermediate >3–6 AU, or strong >6 AU. The SARS-CoV-2 IgM tests were categorized as weakly positive ≥1.1–10 AU, intermediate >10–20, or strong >20 AU. An increment of >2 AU was considered significant. The LFT test was not repeated on any samples.

2.3 | Questionnaires on COVID-19

At inclusion and sequentially at each donation, donors were issued questionnaires on COVID-19 regarding duration, self-reported severity, nature of symptoms, hospital stays, exposure, and preventive measures. At study termination, donors answered a final questionnaire on SARS-CoV-2 vaccination status.

3 | RESULTS

3.1 | Donor demographics

Among the 133 donors with a positive LFT, 105 (79%) consented to enter the study. Donors had a median age of 33 (interquartile range [IQR]: 23–43) and 48/105 (46%) were males. Participants yielded a total of 626 blood samples (median 4 samples/donor, IQR: 4–6). The median observation time was 9.3 months (IQR: 8.3–10.4). On the initial sample, 39/105 (37%) tested positive for SARS-CoV-2 total Ig.

3.2 | COVID-19 symptoms

Within the group of total Ig-positive donors, 29/39 (74%) indicated previous symptoms of COVID-19 in the first questionnaire. Of these, 3/29 (10%) reported severe or very severe, 17/29 (59%) moderate, and 9/29 (31%) mild symptoms. Only 2/39 (5%) donors had been hospitalized. The most common symptoms were fever (67%), fatigue (64%), dry cough (46%), reduced sense of smell (38%), and joint pain (33%) (Figure 1). Nine of 39 (23%) were aware of SARS-CoV-2 exposure.

In the complementary group of 66/105 (63%) total Ig negative donors, 21/66 (32%) indicated COVID-19 symptoms in the first questionnaire. Within this subgroup 2/21 (10%) reported severe or very severe symptoms, 9/21 (43%) moderate, and 10/21 (48%) mild. Common symptoms were fatigue (62%), dry cough (62%), fever (20%), and a sore throat (20%). Three of 66 (5%) were aware of SARS-CoV-2 exposure and none had been hospitalized.

Detection of SARS-CoV-2 Total Ig antibodies was associated with the presence of fever ($p < 0.001$), dry cough ($p < 0.01$), shivers ($p = 0.02$), fatigue ($p < 0.001$), reduced sense of smell ($p < 0.001$), or reduced sense of taste ($p < 0.001$) (χ^2 test). There was no correlation between symptom severity and IgG ($p = 0.63$) or IgA ($p = 0.79$) antibodies (χ^2 test).

3.3 | SARS-CoV-2 antibody persistence

Of the 39 donors initially SARS-CoV-2 total Ig positive, none became total Ig negative. In this group, 33/39 (84%) donors were initially SARS-CoV-2 IgG positive, 31/39 (79%) were IgA positive, and 32/39 (82%) were IgM positive. Twenty-seven of 39 (69%) donors were initially positive for all three antibody isotypes (Table 1). Nine of 33 (27%) donors initially IgG positive became IgG negative. Seven of 31 (23%) donors initially IgA positive became IgA negative, and 23/32 (72%) of donors initially IgM positive became negative. The persistence of IgG, IgA, and IgM antibodies in donors with ≥2 positive samples was a median of 8 months (IQR: 8–10), 9 months (IQR: 8–10), and 8 months (IQR: 4.5–10), respectively. We observed large interindividual differences in IgG, IgA, and IgM levels, but generally, antibodies had a waning tendency (Figure 2). In the 34 total Ig

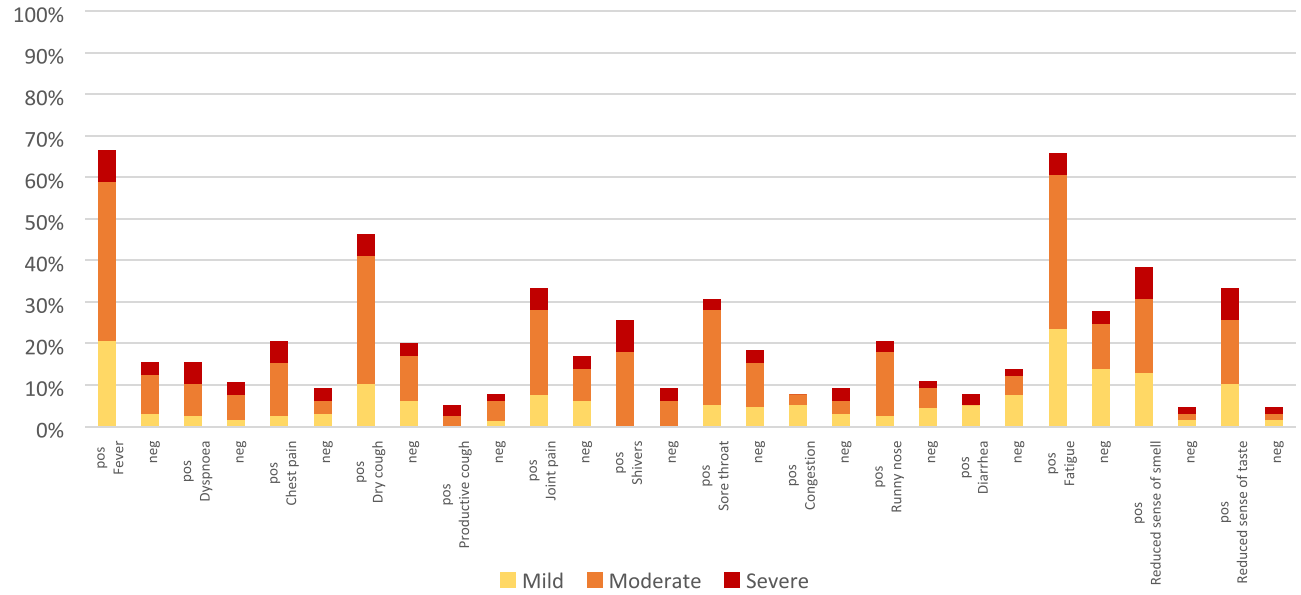

FIGURE 1 The percentages of donors reporting each symptom in the first questionnaires in the group of donors positive (pos) and negative (neg) for SARS-CoV-2 antibodies, respectively, on the Wantai total Ig ELISA on the sample from April and May 2020. The proportion of self-reported severity of symptoms of the donors is depicted for each individual symptom as mild (yellow), moderate (orange), and severe/very severe (red).

TABLE 1 Patterns of SARS-CoV-2 antibody isotype positivity in the initial sample of the 39 donors confirmed antibody positive by the Wantai SARS-CoV-2 total Ig test

Antibody pattern	No.	Percentage
IgG + IgA + IgM	27/39	69
IgG + IgA or IgM	5/39	13
IgG only	2/39	5
IgM and/or IgA	4/39	10
Total Ig only	1/39	2.6

Note: SARS-CoV-2 IgG and IgA antibodies were measured by the SARS-CoV-2 IgG and IgA ELISA by EUROIMMUN, respectively. SARS-CoV-2 IgM was measured by SARS-CoV-2 IgM ELISA from Wantai.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

positive donors with an observation period >6 months IgG, IgA, and IgM antibodies persisted above cutoff in 73%, 79%, and 32%, respectively (Figure 3). In the 20 donors observed >9 months IgG, IgA, and IgM persisted in 55%, 75%, and 30%, respectively. With regard to patterns of antibody persistence (Figure 3) statistical analysis of the proportions of strong and intermediate antibodies across time showed no statistical difference for IgA (χ^2 test, $p = 0.270$). For IgG, the proportion of strong and intermediate antibodies at 0–3 months differed statistically significantly from the proportions at >3–6 months, >6–9 months, and >9–12 months (χ^2 test, $p = 0.042$). The proportions of the three latter IgG groups did not differ statistically significantly (χ^2 test, $p = 0.457$). For IgM, proportions at all time-intervals differed significantly (χ^2 test, $p = 0.002$) indicating a significant decline in these antibodies over time.

3.4 | Emergence of SARS-CoV-2 antibodies and cases of possible reinfection

During the study, 7/66 (11%) donors initially total Ig negative, turned total Ig positive, due to vaccination (6/7) or COVID-19 (1/7). Ten of 105 participants (10%) received at least one SARS-CoV-2 vaccine dose during the study. Four of the 10 (40%) were initially total Ig positive and experienced a rise in IgG levels post-vaccination.

Of the total Ig-positive donors, 6/39 (15%) were initially negative for IgG (Table 1). Two of six donors were negative for all three antibody isotypes. One of the two donors became IgA and IgG antibody positive during the second wave of COVID-19 in Denmark but indicated no new symptoms of COVID-19. The remaining 4/6 (67%) donors had IgA and/or IgM antibodies. One of four was IgA only, 2/4 were IgM only, of which one became negative of all isotypes and the other became IgG and IgA positive. The final donor was IgM and IgA positive with a borderline IgG antibody level (1.1 AU).

Five of 39 (39%) total Ig-positive donors had notable rises in IgA and/or IgG antibody levels without a concurrent rise in IgM antibodies. None had received a SARS-CoV-2 vaccine and only one indicated onset of COVID-19 symptoms. Four of five indicated no exposure to SARS-CoV-2, while one was uncertain.

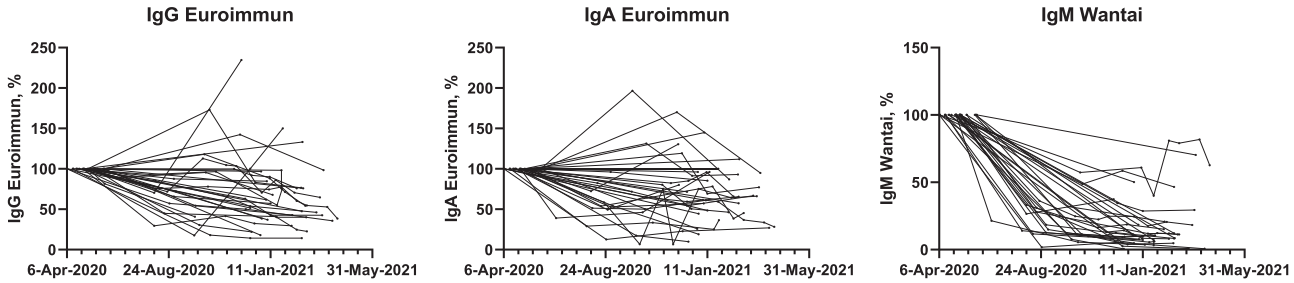

FIGURE 2 SARS-CoV-2 antibody dynamics over time. Each line represents measurements over time from an individual blood donor. Antibody levels from the samples donated from April and May 2020 are indexed to 100% for each donor. SARS-CoV-2 IgG as measured by SARS-CoV-2 IgG ELISA from EUROIMMUN, IgA levels as measured by SARS-CoV-2 IgA ELISA from EUROIMMUN, and IgM levels as measured by SARS-CoV-2 IgM ELISA from Wantai are shown in individual plots. ELISA, enzyme-linked immunosorbent assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

4 | DISCUSSION

The study demonstrates that only 37% of donors positive for SARS-CoV-2 antibodies by a Livzon LFT in April and May 2020 were SARS-CoV-2 total Ig positive by ELISA. As qPCR-tests are only reactive during the first weeks after symptom onset, and since donors were included retrospectively, it was not possible to ascertain true SARS-CoV-2 infection by qPCR. Consequently, only the combination of antibody test results may confirm previous SARS-CoV-2 infection, making antibody test assay performance crucial. The low reproducibility of LFT results by other assays in our study indicates a low specificity of this test, thus underlining that the Livzon LFT is not suitable for testing individuals, as previously shown by this group.[5] LFT was not repeated on any of the donors, nor were any LFT negative donors included, which could have contributed to an estimation of the sensitivity and specificity of the LFT. The total Ig ELISA from Wantai has been shown to have a high sensitivity and specificity[9] but, whether individuals positive by LFT and negative by ELISA total Ig can be considered *truly false-positive* remains unknown. The two donors negative of all three antibody isotypes in their initial sample, could be cases of false-positive total Ig ELISAs, a perspective corroborating the lack of a gold standard SARS-CoV-2 antibody test.

As part of the national seroprevalence project, LFT results were made available to each individual donor. Donors accessing their results in April/May 2020 were not aware of the high probability of receiving a false-positive result. The consequences of receiving a false-positive antibody result are unknown, but in the worst case, donors could become noncompliant to COVID-19 preventive recommendations or decline vaccination. Interestingly, in comparison with LFT positive/total Ig negative donors, those who were total Ig positive more frequently reported "classic" COVID-19 symptoms, that is, fever, fatigue, and loss of the sense of smell and taste. The high percentage of donors indicating COVID-19 symptoms (31%) in the group of LFT positive/total Ig negative may be due to recall bias, i.e. giving significance to unspecific symptoms as COVID-19. Since a control group comprising LFT negative donors was not included, it is not possible to estimate the extent of recall bias. Furthermore, the initial questionnaire was issued several months after initial LFTs, another possible cause of donor recall bias.

There were high (79%–84%) rates of confirmed IgG, IgA, and/or IgM antibodies in the initial sample from donors who were total Ig positive. The persistence of antibodies differed according to isotype. Not surprisingly, IgM antibodies were the least persistent, falling from 79% to 26% in the observation period, while IgG and IgA were more persistent and still measurable in 73% and 79% of donors after 6–9 months. In light of the mild to moderate symptoms of these donors, this appears quite persistent. Focusing on strong and intermediate antibody levels, both IgG and IgM exhibited significant decreases in proportions of antibody-positive donors over time, which for IgG antibodies stabilized beyond 3 months with lower levels of IgG antibody appearing more persistent. Despite measurable low-level antibodies for up to a year, we cannot conclude whether individuals are protected against reinfection as the antibody levels determined by the applied assays only partly correspond to neutralizing antibody levels[3] and as exact antibody levels correlated to protection are unknown. Furthermore, the immune system's defense against SARS-CoV-2 is not only dependent on neutralizing antibodies, but also partly on a T-cell response.[10]

The few donors having notable rises in antibody levels could be cases of reinfection, however, only one indicated new symptoms of COVID-19. As methods to retrospectively confirm true infection are imperfect, it may only be speculated, as to whether the increases in antibodies were in fact a result of reinfection or of reproducibility issues of semi-quantitative assays.

A strength of this study is that each donated sample was tested with a variety of SARS-CoV-2 antibody assays, thereby enabling assay comparison. Among discussed limitations, the number of included donors is small, mainly due to the low COVID-19 prevalence in Denmark and a short inclusion period. Furthermore, many donors only yielded 2-3 samples, probably due to less frequent donations during the pandemic.

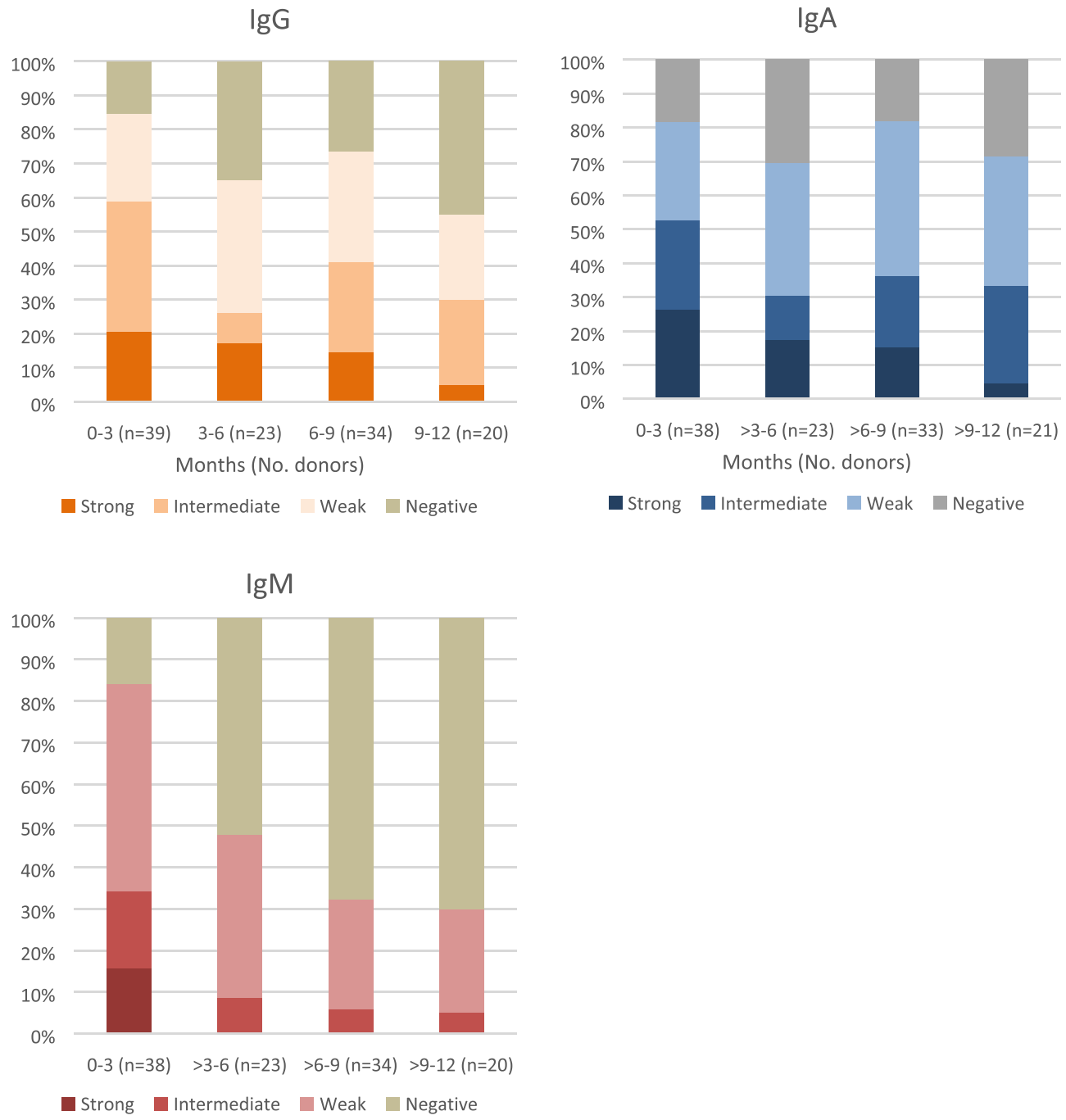

FIGURE 3 Percentage of blood donors positive for IgG, IgA, and IgM SARS-CoV-2 antibodies, respectively, in each time period of 3 months from the first donated sample in April/May 2020. SARS-CoV-2 IgG and IgA antibodies were measured by the SARS-CoV-2 IgG and IgA ELISA by EUROIMMUN, respectively. SARS-CoV-2 IgM was measured by SARS-CoV-2 IgM ELISA from Wantai. SARS CoV-2 IgG, IgA, and IgM antibody measurements of >1.1 arbitrary units (AU) are considered positive on all three assays. SARS-Cov-2 IgG and IgA antibody levels are categorized as weak ≥1.1–3.0 AU, intermediate >3–6 AU, and strong >6 AU. SARS-CoV-2 IgM antibody levels are categorized as weak ≥1.1–10 AU, intermediate >10–20, and strong >20 AU. ELISA, enzyme-linked immunosorbent assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

In conclusion, this study shows that persistent SARS-CoV-2 IgA antibodies and IgG antibodies at 6–9 months are present in approximately three of four individuals with previous mild to moderate COVID-19.

ACKNOWLEDGMENTS

We thank the Department of Clinical Immunology for finances and staff making this study possible. We thank the volunteer blood donors participating. The Open Patient Exploratory

Network at the University of Southern Denmark provided data management.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Søren T. Lillevang and Dorte K. Holm are responsible for the conception and design. Mette B. Levring, Dorte K. Holm, Anna C. Nilsson, Iben S. Jensen, Joschka M. Bauer, Jesper R. Davidsen, and Line D. Rasmussen collected the data. Mette B. Levring, Iben S. Jensen, Anna C. Nilsson, and Dorte K. Holm are responsible for project administration. Mette B. Levring and Ulrik Sprogøe are responsible for the statistical analysis. Mette B. Levring, Ulrik Sprogøe, Dorte K. Holm, and Anna C. Nilsson are responsible for the interpretation and writing. All authors are responsible for the review and revision of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Mette B. Levring https://orcid.org/0000-0002-4366-382X

REFERENCES

1. van den Hurk K, Merz EM, Prinsze FJ, et al. Low awareness of past SARS-CoV-2 infection in healthy plasma donors. *Cell Rep Med*. 2021;2(3):100222.
2. Byambasuren O, Dobler CC, Bell K, et al. Comparison of seroprevalence of SARS-CoV-2 infections with cumulative and imputed COVID-19 cases: Systematic review. *PLoS One*. 2021;16(4):e0248946.
3. Tang MS, Case JB, Franks CE, et al. Association between SARS-CoV-2 neutralizing antibodies and commercial serological assays. *Clin Chem*. 2020;66(12):1538-1547.
4. Yuan Y, Wang H, Zhao J, et al. Severe acute respiratory syndrome coronavirus 2 viral RNA load status and antibody distribution among patients and asymptomatic carriers in Central China. *Front Cell Infect Microbiol*. 2021;11:559447.
5. Nilsson AC, Holm DK, Justesen US, et al. Comparison of six commercially available SARS-CoV-2 antibody assays-choice of assay depends on intended use. *Int J Infect Dis*. 2021;103:381-388.
6. Chia WN, Zhu F, Ong SWX, et al. Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. *Lancet Microbe*. 2021;2(6):e240-e249.
7. Hansen CH, Michlmayr D, Gubbels SM, Molbak K, Ethelberg S. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. *Lancet*. 2021;397(10280):1204-1212.
8. Piri SM, Edalatfar M, Shool S, Jalalian MN, Tavakolpour S. A systematic review on the recurrence of SARS-CoV-2 virus: frequency, risk factors, and possible explanations. *Infect Dis (Lond)*. 2021;53(5):315-324.
9. Charlton CL, Kanji JN, Johal K, et al. Evaluation of six commercial mid- to high-volume antibody and six point-of-care lateral flow assays for detection of SARS-CoV-2 antibodies. *J Clin Microbiol*. 2020;58(10):1-12.
10. Toor SM, Saleh R, Sasidharan Nair V, Taha RZ, Elkord E. T-cell responses and therapies against SARS-CoV-2 infection. *Immunology*. 2021;162(1):30-43.

How to cite this article: Levring MB, Holm DK, Nilsson AC, et al. SARS-CoV-2 antibody kinetics in blood donors with a previously positive SARS-CoV-2 antibody test within a seroprevalence survey. *J Med Virol*. 2022;94:1711-1716. doi:10.1002/jmv.27486