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.

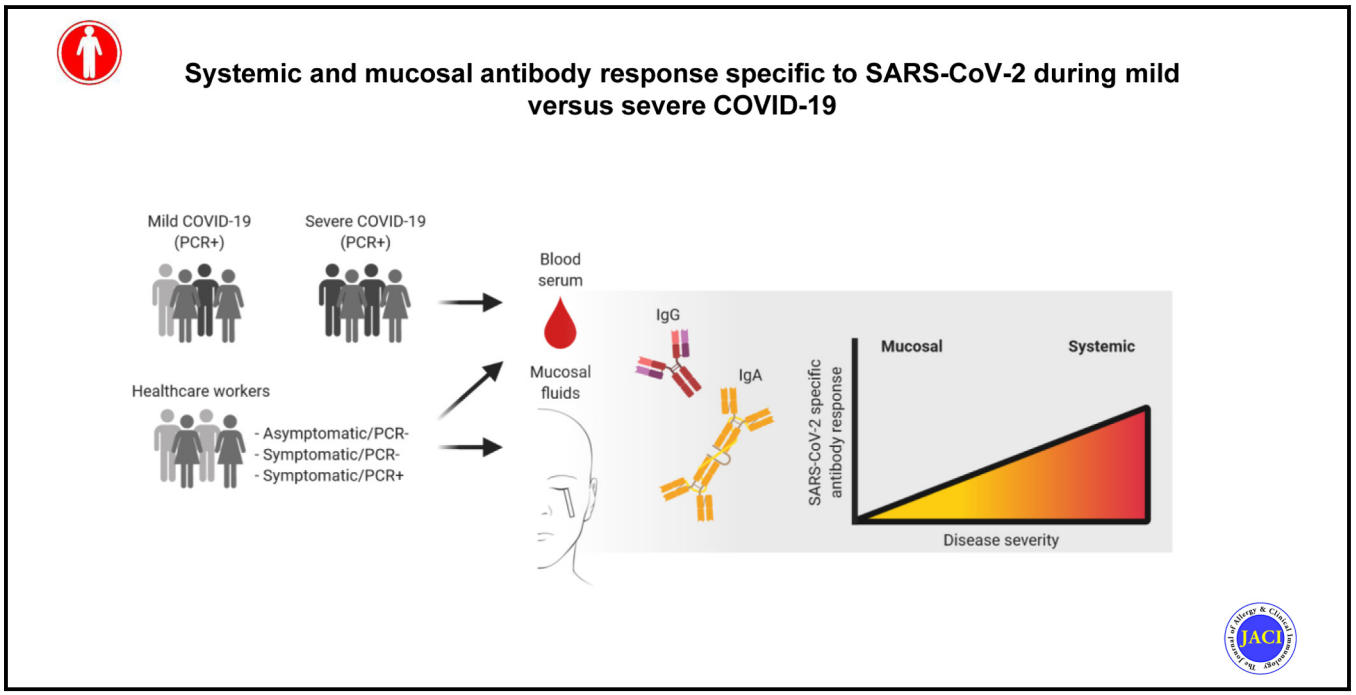
Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted 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.

Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19

Carlo Cervia, MMed,[a]* Jakob Nilsson, MD, PhD,[a]* Yves Zurbuchen, MMed,[a] Alan Valaperti, PhD,[a] Jens Schreiner, PhD,[a] Aline Wolfensberger, MD,[b] Miro E. Raeber, MMed,[a] Sarah Adamo, MMed,[a] Sebastian Weigang, MSc,[c] Marc Emmenegger, MSc,[d] Sara Hasler,[a] Philipp P. Bosshard, PhD,[e] Elena De Cecco, PhD,[d] Esther Bächli, MD,[f] Alain Rudiger, MD,[g] Melina Stüssi-Helbling, MD,[h] Lars C. Huber, MD,[h] Annelies S. Zinkernagel, MD, PhD,[b] Dominik J. Schaer, MD,[i] Adriano Aguzzi, MD,[d] Georg Kochs, PhD,[c,j] Ulrike Held, PhD,[k] Elsbeth Probst-Müller, MD, PhD,[a] Silvana K. Rampini, MD,[i] and Onur Boyman, MD[a,l]

Zurich, Schlieren, and Uster, Switzerland, and Freiburg, Germany

GRAPHICAL ABSTRACT



Background: Whereas severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antibody tests are increasingly being used to estimate the prevalence of SARS-CoV-2 infection, the determinants of these antibody responses remain unclear.

Objectives: Our aim was to evaluate systemic and mucosal antibody responses toward SARS-CoV-2 in mild versus severe coronavirus disease 2019 (COVID-19) cases.

From [a]the Department of Immunology, [b]the Department of Infectious Diseases and Hospital Epidemiology, [d]the Institute of Neuropathology, [e]the Department of Dermatology, and [i]the Department of Internal Medicine, University Hospital Zurich; [c]the Institute of Virology, Medical Center and [j]the Faculty of Medicine, University of Freiburg; [f]the Clinic for Internal Medicine, Uster Hospital; [g]the Department of Medicine, Limmattal Hospital, Schlieren; [h]the Clinic for Internal Medicine, City Hospital Triemli Zurich; [k]the Department of Biostatistics, at Epidemiology, Biostatistics and Prevention Institute and [l]the Faculty of Medicine, University of Zurich.

*These authors contributed equally to this work.

This work was funded by the Swiss National Science Foundation (grant 4078P0-198431 [to O.B. and J.N.] and grant 310030-172978 [to O.B.]), Swiss Academy of Medical Sciences fellowships (No. 323530-191220 [to C.C.], No. 323530-191230 [to Y.Z.], and No. 323530-177975 [to S.A.]), a Young Talents in Clinical Research Fellowship by the Swiss Academy of Medical Sciences and Bangerter Foundation (No. YTCR 32/18 [to M.R.]), the Bundesministerium für Bildung und Forschung through Deutsches Zentrum für Luft- und Raumfahrt (No. 01KI2077 [to G.K.]), the Clinical Research Priority Program of University of Zurich for CRPP CYTIMM-Z (to O.B.), the Pandemic Fund of University of Zurich (to O.B.), and an Innovation Grant of University Hospital Zurich (to O.B.).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication June 4, 2020; revised October 5, 2020; accepted for publication October 20, 2020.

Available online November 20, 2020.

Corresponding author: Onur Boyman, MD, Department of Immunology, University Hospital Zurich, Gloriastrasse 23, 8091 Zurich. E-mail: onur.boyman@uzh.ch.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749

© 2020 The Authors. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

https://doi.org/10.1016/j.jaci.2020.10.040

Methods: Using immunoassays specific for SARS-CoV-2 spike proteins, we determined SARS-CoV-2–specific IgA and IgG in sera and mucosal fluids of 2 cohorts, including SARS-CoV-2 PCR-positive patients (n = 64) and PCR-positive and PCR-negtive health care workers (n = 109).

Results: SARS-CoV-2–specific serum IgA titers in patients with mild COVID-19 were often transiently positive, whereas serum IgG titers remained negative or became positive 12 to 14 days after symptom onset. Conversely, patients with severe COVID-19 showed a highly significant increase of SARS-CoV-2–specific serum IgA and IgG titers after symptom onset. Very high titers of SARS-CoV-2–specific serum IgA were correlated with severe acute respiratory distress syndrome. Interestingly, some health care workers with negative SARS-CoV-2–specific serum antibody titers showed SARS-CoV-2–specific IgA in mucosal fluids with virus-neutralizing capacity in some cases. SARS-CoV-2–specific IgA titers in nasal fluids were inversely correlated with age.

Conclusions: Systemic antibody production against SARS-CoV-2 develops mainly in patients with severe COVID-19, with very high IgA titers seen in patients with severe acute respiratory distress syndrome, whereas mild disease may be associated with transient production of SARS-CoV-2–specific antibodies but may stimulate mucosal SARS-CoV-2–specific IgA secretion. (J Allergy Clin Immunol 2021;147:545-57.)

Key words: *COVID-19, SARS-CoV-2, SARS-CoV-2–specific antibodies, SARS-CoV-2–specific IgA, SARS-CoV-2–specific IgG, humoral immune response, mucosal immune response, COVID-19 severity, COVID-19 seroprevalence*

Abbreviations used

ACE2:	Angiotensin-converting enzyme 2
ARDS:	Acute respiratory distress syndrome
COVID-19:	Coronavirus disease 2019
Ct:	Cycle threshold
ECD:	Extracellular domain
HCW:	Health care worker
IQR:	Interquartile range
RBD:	Receptor-binding domain
RT-qPCR:	Reverse-transcriptase quantitative PCR
S:	Spike
SARS-CoV:	Severe acute respiratory syndrome coronavirus
SARS-CoV-2:	Severe acute respiratory syndrome coronavirus 2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), is a Betacoronavirus related to severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus.[1-4] The zoonotic introduction of Middle East respiratory syndrome coronavirus and SARS-CoV into the human population resulted in limited outbreaks, whereas the appearance of SARS-CoV-2 has led to a rapidly spreading pandemic. As of October 05, 2020, COVID-19 had been confirmed to have affected about 35.2 million individuals worldwide and caused an estimated 1.04 million deaths.[5] Several characteristics of SARS-CoV-2 have likely contributed to its rapid spread. These include the ability of SARS-CoV-2 to efficiently replicate in the upper respiratory tract mucosa of humans,[6] its variable incubation time of about 3 to 14 days, and the presence of many asymptomatic and presymptomatic SARS-CoV-2–infected individuals producing sufficient amounts of virus for human-to-human transmission.[7-9] Thus, SARS-CoV-2 infection is frequently unrecognized.

When symptomatic, COVID-19 can range from a mild flu-like illness in about 81% of affected patients to a severe and critical disease in about 14% and 5% of affected patients, respectively.[10-12] Mild COVID-19 is characterized by fatigue, fever, sore throat, cough, and mild pneumonia. Severe disease features dyspnea, hypoxia, and radiographic evidence of lung involvement of 50% or more, and critical COVID-19 results in acute respiratory distress syndrome (ARDS) with respiratory failure, multiorgan dysfunction, and shock. The World Health Organization proposed a classification of symptomatic COVID-19 into (1) mild illness, (2) mild pneumonia, (3) severe pneumonia, (4) ARDS (based on the Berlin definition of ARDS),[13] and (5) sepsis and septic shock.[14]

Human angiotensin-converting enzyme 2 (ACE2) serves as a cell entry receptor for SARS-CoV-2. Pneumocytes and other host cells expressing ACE2 are therefore particularly susceptible to infection by SARS-CoV-2. Mechanistically, SARS-CoV-2 binds to ACE2 via the receptor-binding domain (RBD) of the S1 subunit of its surface spike (S) glycoprotein.[3,15] Thus, humoral immunity targeting the S protein could interfere with SARS-CoV-2 infection, as evidenced from serologic studies.[16,17]

As with other coronaviruses, symptomatic SARS-CoV-2 disease causes an acute infection with activation of the innate and adaptive immune systems. The former leads to the release of several proinflammatory cytokines, including IL-6. Conversely, other antiviral cytokines, such as the type I and III interferon pathways, are hampered by coronaviruses, including SARS-CoV and SARS-CoV-2.[18-20] Subsequently, B cells and T cells become activated, resulting in the production of SARS-CoV-2–specific antibodies, comprising IgM, IgA, and IgG.[21] Whereas coronavirus-specific IgM production is transient and leads to isotype switch to IgA and IgG,[22] these latter antibody subtypes can persist for extended periods in the serum and in nasal fluids.[23] Whether SARS-CoV-2–specific IgG antibodies are correlated with virus control is a matter of intense discussion.[16,17,24,25]

Unlike the internal nucleocapsid protein of SARS-CoV-2, which shares about 90% amino acid sequence homology with the nucleocapsid protein of SARS-CoV, the S1 subunit shares only 64% amino acid sequence homology and shows limited homology with other human coronaviruses, such as 229E, NL63, OC43, and HKU1, which use different viral entry receptors.[3,26] Thus, antibodies generated to previous coronavirus infections are unlikely to cross-react with the S1 protein of SARS-CoV-2 and should therefore not significantly account for any seroreactivity toward the S1 subunit.[26]

Despite intensive research efforts, several determinants of SARS-CoV-2–specific antibody production remain ill-defined, such as its relation to COVID-19 severity, disease duration, patient age, and comorbidities. There is also a paucity of knowledge on SARS-CoV-2–specific IgA and IgG antibodies at mucosal sites and how their titers are correlated with COVID-19 parameters. And finally, it is unclear whether tissue-associated IgA and IgG secretion, rather than their systemic production,

TABLE I. Demographic and clinical characteristics of the patient cohort

Characteristic	Patients with mild COVID-19 (n = 26)	Patients with severe COVID-19 (n = 38)	Total (n = 64)	*P* value
Age (y), median (IQR)	46.0 (31.50-48.50)	67.5 (59.0-79.0)	59.5 (42.75-75.25)	<.0001
Sex (male/female)	11/15	24/14	35/29	.1282
COVID-19 disease severity, no. (%)*				
Mild illness	17 (65.4)	—	17 (26.6)	—
Mild pneumonia	9 (34.6)	—	9 (14.1)	—
Severe pneumonia	—	20 (52.6)	20 (31.3)	—
Mild ARDS	—	7 (18.4)	7 (10.9)	—
Moderate ARDS	—	7 (18.4)	7 (10.9)	—
Severe ARDS	—	4 (10.5)	4 (6.3)	—
Level of care at blood sampling time point, no. (%)				
Outpatient	14 (53.8)	—	14 (21.9)	<.0001
Hospitalized	12 (46.2)	38 (100)	50 (78.1)	<.0001
Comorbidity, no. (%)				
Hypertension	6 (23.1)	22 (57.9)	28 (43.8)	.0098
Diabetes	4 (15.4)	12 (31.6)	16 (25)	.2393
Heart disease	2 (7.7)	17 (44.7)	19 (29.7)	.0018
Cerebrovascular disease	1 (3.8)	4 (10.5)	5 (7.8)	.6404
Lung disease	3 (11.5)	6 (15.8)	9 (14.1)	.7275
Kidney disease	6 (23.1)	10 (26.3)	16 (25)	>.9999
Malignancy	—	4 (10.5)	4 (6.3)	.1397
Systemic immunosuppression	2 (7.7)	4 (10.5)	6 (9.4)	>.9999
Immunosuppression, no. (%)				
Glucocorticoids	2 (7.7)	4 (10.5)	6 (9.4)	>.9999
Mycophenolate mofetil	1 (3.8)	—	1 (1.6)	.4062
Calcineurin inhibitors	1 (3.8)	1 (2.6)	2 (3.1)	>.9999
Azathioprin	1 (3.8)	2 (5.3)	3 (4.7)	>.9999
Leflunomide	1 (3.8)	—	1 (1.6)	.4062
Mesalazine	1 (3.8)	—	1 (1.6)	.4062

IQR, Interquartile range.
Patients were divided into those with mild versus those with severe COVID-19. Disease severity was defined according to the World Health Organization classification.[14] Categoric values between mild and severe COVID-19 were compared by using the Fisher exact test, and continuous variables were compared by using the nonparametric Wilcoxon test.
*COVID-19 severity at blood sampling according to World Health Organization guidelines.[14]

might be evident in SARS-CoV-2–exposed individuals experiencing mild disease.

METHODS

Human subjects and patient characteristics

Following written informed consent, patients and health care workers (HCWs) were recruited for sampling of blood and mucosal secretions. We studied 2 cohorts: (1) patients with reverse-transcriptase quantitative PCR (RT-qPCR)-confirmed SARS-CoV-2 infection (n = 64; median age 59.5 years) with mild versus severe COVID-19 and (2) HCWs (referred to as the HCW cohort; n = 109; median age 36 years) with or without symptoms, who tested negative or positive for SARS-CoV-2 by RT-qPCR. HCWs included employees of University Hospital Zurich belonging to all professional groups, both with and without patient contact. Exposure was defined as contact with a patient with RT-qPCR–confirmed COVID-19 without adequate safety measures.[27] Because of preexisting comorbidities, 6 patients were under stable immunosuppressive treatment at the time of inclusion (Table I); conversely, patients receiving B-cell–depleting agents, such as rituximab,[28] were excluded from our study. For longitudinal analyses of serum and mucosal SARS-CoV-2–specific antibody responses, 2 subjects with mild COVID-19 were sampled repeatedly during the course of their disease. Our patients with COVID-19 were classified according to the World Health Organization criteria[14] as (1) those with mild COVID-19, comprising mild illness and mild pneumonia or (b) those with severe COVID-19, including severe pneumonia and ARDS. Our cohort did not contain any patients with sepsis or septic shock. The study was approved by the Cantonal Ethics Committee of Zurich (BASEC 2016-01440 and 2020-00363).

Collection of serum, tears, nasal fluid, and saliva

A subgroup of members of the HCW cohort (referred to as the HCW mucosal subgroup [n = 33]) volunteered to be sampled for blood, as well as (simultaneously) for tears, nasal fluid, and saliva. Venous blood samples were collected in BD Vacutainer CAT serum tubes (Becton Dickinson, Franklin Lakes, NJ). Tears were sampled by using filter paper produced for Schirmer tear tests (HS Clement Clarke Ophtalmic, Harlow, United Kingdom). Nasal fluids were collected by inserting a dry soft tissue into the nasal cavities for 5 minutes (Vostra, Aachen, Germany). Unstimulated saliva was collected for 5 minutes.

IgA and IgG immunoassays

A commercial ELISA specific for the S1 protein of SARS-CoV-2 was used according to manufacturer's instructions (SARS-CoV-2 IgA and IgG immunoassay, Euroimmun, Lübeck, Germany) and validated by using serum samples from hospitalized patients with confirmed COVID-19 as positive controls and serum samples collected before the COVID-19 pandemic as negative controls. The results showed a specificity for anti-S1 IgA greater than 95% and for anti-S1 IgG greater than 99%, which is in accordance with recently published data.[29] Serum samples were analyzed at a 1:100 dilution, and mucosal samples were analyzed at a 1:5 dilution (with 0.9% NaCl). For serum IgA, OD ratios of 1.1 to 2.0 were considered borderline positive and values higher than 2.0 were considered positive. For serum IgG, OD ratios of 0.8 to 1.1 were considered borderline positive and values greater than 1.1 were considered positive.

Furthermore, we assessed the samples from the HCW mucosal subgroup by using an in-house immunoassay for IgA and IgG against S protein extracellular domain (ECD), RBD, and nucleocapsid protein.[30] Mucosal

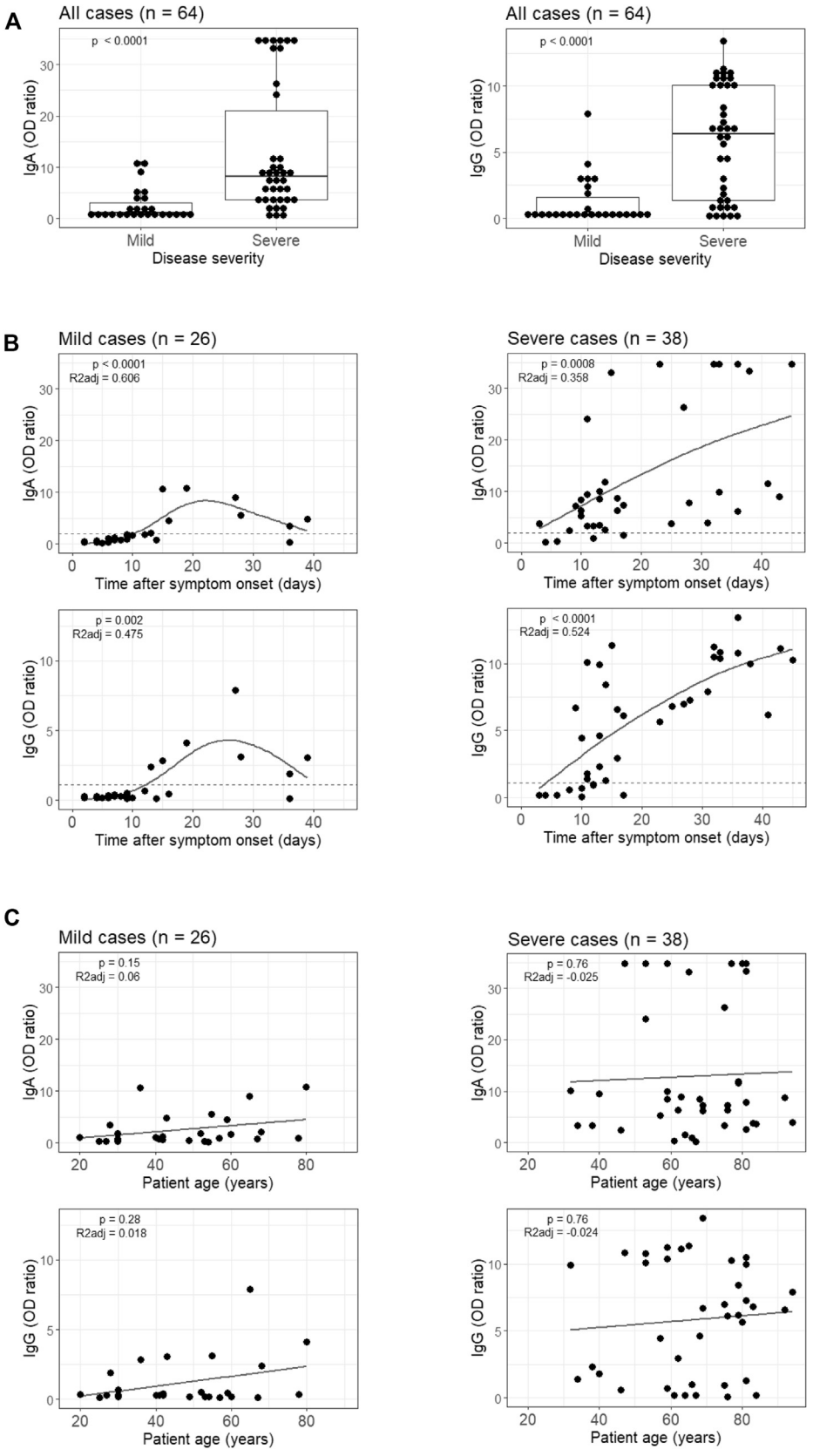


FIG 1. Influence of COVID-19 severity, disease duration, and patient age on SARS-CoV-2–specific serum IgA and IgG titers. **A,** Comparison of SARS-CoV-2 S protein subunit S1-specific serum IgA and IgG titers (OD ratio) in patients with mild (n = 26) versus severe COVID-19 (n = 38). The average times between reported symptom onset and sample collection were 13.5 days (median 9 days) in patients with mild cases and 20.2 days (median 15.5 days) in patients with severe cases. **B,** Generalized additive modeling of S1-specific IgA and IgG serum titers as a function of days between reported symptom onset and sample collection in patients with mild (n = 26) versus severe COVID-19 (n = 38). Dashed lines indicate borders between positive and borderline or negative serum values of S1-specific IgA (*top*) and IgG (*bottom*). **C,** Linear modeling of S1-specific IgA and IgG serum titers as a function of patient age in patients with mild (n = 26) versus severe cases (n = 38). *P* values and adjusted R^2 (R2adj) of linear and generalized additive models were computed by using logarithmized IgA/IgG titers.

TABLE II. Linear models for prediction of IgA and IgG serum titers

Serum titer	Coefficient	95% CI	*P* value
SARS-CoV-2–specific IgA serum titer			
Intercept	–0.90	–1.92 to 0.12	.083
Severe disease	1.35	0.74 to 1.96	<.0001
Days	0.053	0.03 to 0.08	<.0001
Age	0.011	–0.01 to 0.03	.31
Hypertension	0.27	–0.48 to 1.02	.47
Diabetes	–0.23	–0.89 to 0.42	.48
Heart disease	–0.18	–0.87 to 0.52	.61
Lung disease	–0.18	–0.95 to 0.59	.63
Malignancy	–1.77	–2.87 to –0.67	.002
Cerebrovascular disease	0.24	–0.81 to 1.30	.64
Kidney disease	–0.17	–0.88 to 0.53	.63
Immunosuppression	–0.44	–1.46 to 0.57	.39
SARS-CoV-2–specific IgG serum titer			
Intercept	–1.66	–2.81 to –0.51	.005
Severe disease	1.42	0.73 to 2.11	.0001
Days	0.07	0.04 to 0.10	<.0001
Age	0.0012	–0.02 to 0.03	.92
Hypertension	0.41	–0.43 to 1.26	.33
Diabetes	–0.19	–0.92 to 0.55	.62
Heart disease	–0.13	–0.91 to 0.65	.73
Lung disease	–0.31	–1.18 to 0.55	.47
Malignancy	–0.89	–2.13 to 0.34	.15
Cerebrovascular disease	–0.44	–1.63 to 0.75	.46
Kidney disease	0.21	–0.58 to 1.01	.59
Immunosuppression	–0.76	–1.90 to 0.39	.19

Multiple linear model of S1 protein–specific IgA serum titers (logarithmized) and IgG serum titers (logarithmized) as a function of disease severity (mild versus severe), days since onset of symptoms, patient age, presence of comorbidities (hypertension, diabetes mellitus, heart disease, cerebrovascular disease, lung disease, kidney disease, and malignancy), and immunosuppressive treatment (n = 64).

samples were prediluted 1:2 in sample buffer (PBS Tween-20, 0.1%, and 1% milk), and serum was prediluted 1:20 in sample buffer and transferred to antigen-coated 1536-well assay plates by using acoustic dispensing technology (ECHO 555, Labcyte, San Jose, Calif) with serial dilutions ranging from 1:5 to 1:640 (mucosal samples) and from 1:50 to 1:6400 (serum samples). ODs were measured at 450 nm in a multimode plate reader (Elmer EnVision, Perkin, Rodgau, Germany), followed by fitting with a logistic regression and determination of the inflection point of the sigmoidal curve (–log(EC50)). Negative values were depicted as 0.

RT-qPCR

Nasopharyngeal swabs were subjected to RT-qPCR by using the TaqMan SARS-CoV-2 Assay Kit v2 (Thermo Fischer, Waltham, Mass), the 2019-nCoV CDC qPCR Probe Assay (2019-nCov CDC EUA Kit; Integrated DNA Technologies, Inc, Coralville, Iowa), or the Roche cobas SARS-CoV-2 Test CE-IVD (Roche, Basel, Switzerland) according to the manufacturers' instructions. The cycle threshold (Ct) values for the different SARS-CoV-2 PCR targets were compounded and reported as averages.

SARS-CoV-2 microneutralization assay

Serum, nasal fluid, and tear fluid samples were diluted in Dulbecco modified Eagle medium plus 2% FCS, 20 mM HEPES, and 0.05% $NaHCO_3$. Twofold serial dilutions were mixed with an equal volume of SARS-CoV-2 viral solution (350 plaque-forming units/50 μL), resulting in final sample dilutions ranging from 1:10 to 1:40 for sera and 1:5 to 1:20 for mucosal fluid samples. The serum-virus mix was incubated for 1 hour at room temperature and dispersed on 96-well plates containing a semiconfluent VeroE6 monolayer. The plates were incubated for 20 hours at 37°C in a humidified atmosphere with 5% CO_2 before fixation and fluorescent staining with a SARS-CoV nucleocapsid protein–specific antibody (catalog no. 200-401-A50, Biomol, Hamburg, Germany). The assay was evaluated by fluorescence microscopy, with plaque reduction less than 50% interpreted as absent, from 50% to 80% interpreted as partial, and from 80% to 100% interpreted as full neutralization; all results were compared with a control without patient serum or mucosal fluid.

Statistics

Descriptive statistics for the cohort of patients (stratified according to mild versus severe disease) and the HCW cohort are presented as median and interquartile ranges for continuous variables, and as numbers and percentages of the total for categoric variables. For the comparison of location parameters in 2 independent groups, the Wilcoxon rank sum test was used in a version accounting for ties.[31] For comparison of more than 2 independent groups, the nonparametric Kruskal-Wallis test was used. Multiple linear regression models were used to quantify the association between log-transformed IgA and IgG titers as outcomes as well as a set of predefined independent variables. These included disease severity, age, duration of symptoms (days), and patient comorbidities. Generalized additive models were used to evaluate potential nonlinear relationships of disease duration with the 2 outcomes, as already described. The Wilcoxon test was used to test for differences between 2 continuous variables in the tables, and *P* values were adjusted by using the Holm method. The Fisher exact test was used for comparing 2 categoric variables, and the chi-square test was used for comparing 3 categoric variables. Nonparametric Spearman correlations were used for the comparison between different immunoassays.

Statistical analyses were performed with R software (version 3.6.1) and by using the packages coin and mgcv. GraphPad Prism software (GraphPad Software, Inc, La Jolla, Calif) was used for visualization. *P* values in the patient cohort were adjusted for multiple testing by using the method proposed by Benjamini-Hochberg.[32] Adjusted *P* values were considered statistically significant if smaller than the significance level of $\alpha = 0.05$. In the HCW mucosal subgroup, evidence was quantified on a continuous scale, and the results were considered exploratory.

RESULTS

COVID-19 severity, disease duration, and patient age influence SARS-CoV-2–specific serum IgA and IgG secretion

Serum samples from 64 patients with RT-qPCR–confirmed mild (n = 26) and severe (n = 38) cases of COVID-19 (Table I) were assessed for IgA and IgG antibodies toward the SARS-CoV-2 S1 protein by using highly specific immunoassays. The mean periods between reported symptom onset and serum collection were 13.5 days (median 9 days) in the group of patients with mild COVID-19 and 20.2 days (median 15.5 days) in the group with severe COVID-19, respectively (see Fig E1, *A* in this article's Online Repository at www.jacionline.org). On average, patients with severe disease had higher serum titers of S1-specific IgA ($P < .0001$) and IgG ($P < .0001$) than did patients with mild COVID-19 (Fig 1, *A*). In patients with mild COVID-19, serum titers of S1-specific IgA increased slightly ($P < .0001$) as a function of time from serum sampling to symptom onset (Fig 1, *B*). Likewise, serum titers of S1-specific IgG increased moderately ($P = .002$) in patients with mild COVID-19 (Fig 1, *B*). These antibody responses revealed no significant pattern associated with patient age ($P = .15$ for IgA and $P = .28$ for IgG) (Fig 1, *C*); sex (see Fig E1, *B* and *C*); preexisting comorbidities, including hypertension, diabetes mellitus, heart disease, cerebrovascular disease, lung disease, and kidney disease; or immunosuppressive treatment. In patients with a history of solid cancer, lower S1-specific IgA titers were detectable (Table II).

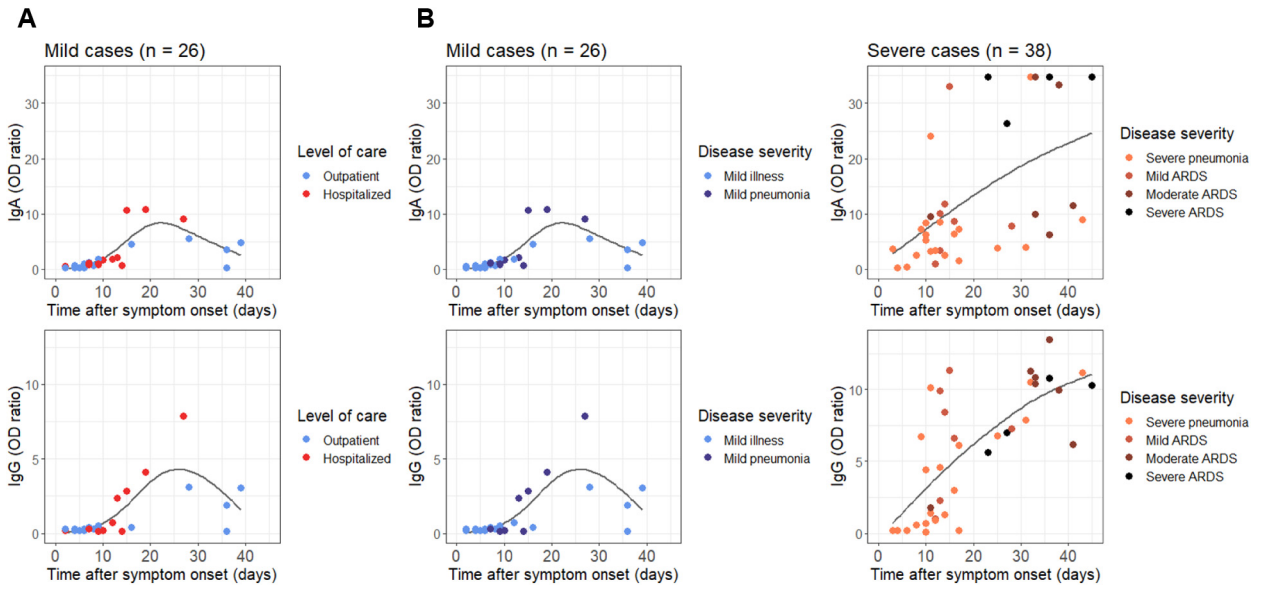


FIG 2. S protein–specific serum antibodies compared with level of care and disease severity. **A**, Level of patient care at the time of blood sampling, visualized in the generalized additive models of S1-specific IgA and IgG serum titers as a function of days between sampling and reported symptom onset in patients with mild cases of COVID-19 (n = 26). Patients with severe cases were all hospitalized and are thus not depicted. **B**, Disease severity at the time of blood sampling, visualized in the generalized additive models of S1-specific IgA and IgG serum titers as a function of days between sampling and reported symptom onset. Comparison of patients with mild (n = 26) versus severe cases (n = 38).

On average, positive S1-specific serum IgA titers became evident in patients with mild COVID-19 10 days after symptom onset (Fig 1, *B*). S1-specific serum IgA titers peaked in samples drawn at around 3 weeks from symptom onset, whereas in subjects tested later S1-specific serum IgA tended to be lower. As for S1-specific serum IgG concentrations, they remained negative or reached positive values in patients with mild COVID-19 around 12 to 14 days after symptom onset (Fig 1, *B*).

In stark contrast to those with mild cases, patients with severe COVID-19 showed a strong correlation of serum titers of S1-specific IgA with disease duration ($P = .0008$), with the correlation being even more pronounced for serum titers of S1-specific IgG ($P < .0001$) (Fig 1, *B*). On average, these antibody responses became positive in samples obtained on day 3 or 4 for IgA and day 4 or 5 for IgG, and they appeared to be independent of patient age ($P = .76$ for IgA and $P = .76$ for IgG), sex, and comorbidities (Fig 1, *B* and *C*, Table II, and see Fig E1, *B* and *C*).

When patients were grouped according to level of care, in those with mild cases of COVID-19 we observed that S1-specific serum IgA titers did not show any discernible pattern, whereas S1-specific serum IgG titers were higher in hospitalized patients than in patients treated as outpatients (Fig 2, *A*). Thus, we next assessed disease severity, and as expected, younger patients tended to have milder disease, whereas older patients had more severe manifestations (see Fig E2 in this article's Online Repository at www.jacionline.org). There was no time-dependent pattern visible for S1-specific serum IgA titer, whereas S1-specific serum IgG titers showed a stronger increase over time in patients with mild pneumonia versus in those with mild illness (Fig 2, *B*). Strikingly, very high titers (>25 OD ratio) of SARS-CoV-2–specific serum IgA, but not serum IgG, were correlated with severe ARDS (Fig 2, *B* and see Fig E3 in this article's Online Repository at www.jacionline.org). In a multiple linear model on all patients, there was strong evidence for an association between severe disease, days after symptom onset, and increased S1-specific serum IgA and IgG responses. Immunosuppressive therapy was not associated with decreased S1-specific serum IgG titers (Table II).

In summary, disease severity appeared to influence S1-specific serum IgA and IgG titers, and S1-specific IgA responses might occur transiently in patients with mild disease. To evaluate this latter hypothesis, we conducted a longitudinal study in 2 selected patients with mild COVID-19, as presented in the next section.

S1-specific antibody responses can be transient and delayed in patients with mild COVID-19

We followed up 2 adults (a 42-year-old woman and a 42-year old man living together as a couple) with mild, RT-qPCR–confirmed SARS-CoV-2 infection. He (patient COV2-A0013) developed fatigue and cough from day 0 onward, followed by fever on day 1 and dysosmia on days 9 to 16. She (patient COV2-A0014) showed signs of fatigue and sore throat from day 0 onward, fever between days 2 and 5, and cough on day 3 (Fig 3, *A*).

The RT-qPCR Ct values at detection were low on days 1 to 20 for patient COV2-A0013 and on day 7 for patient COV2-A0014, indicating the presence of high amounts of SARS-CoV-2 RNA in their nasal swabs (Fig 3, *B*). On day 30 for patient COV2-A0013 and from day 17 onward for patient COV2-A0014, the Ct values increased to 40 and higher, thus indicating that the amount of virus RNA had dropped below the detection limit (Fig 3, *B*).

Patient COV2-A0013 showed S1-specific serum IgA titers that were negative on day 7; rose to borderline values on day 10; became positive on day 14 at a titer of 3.8 OD ratio, where they

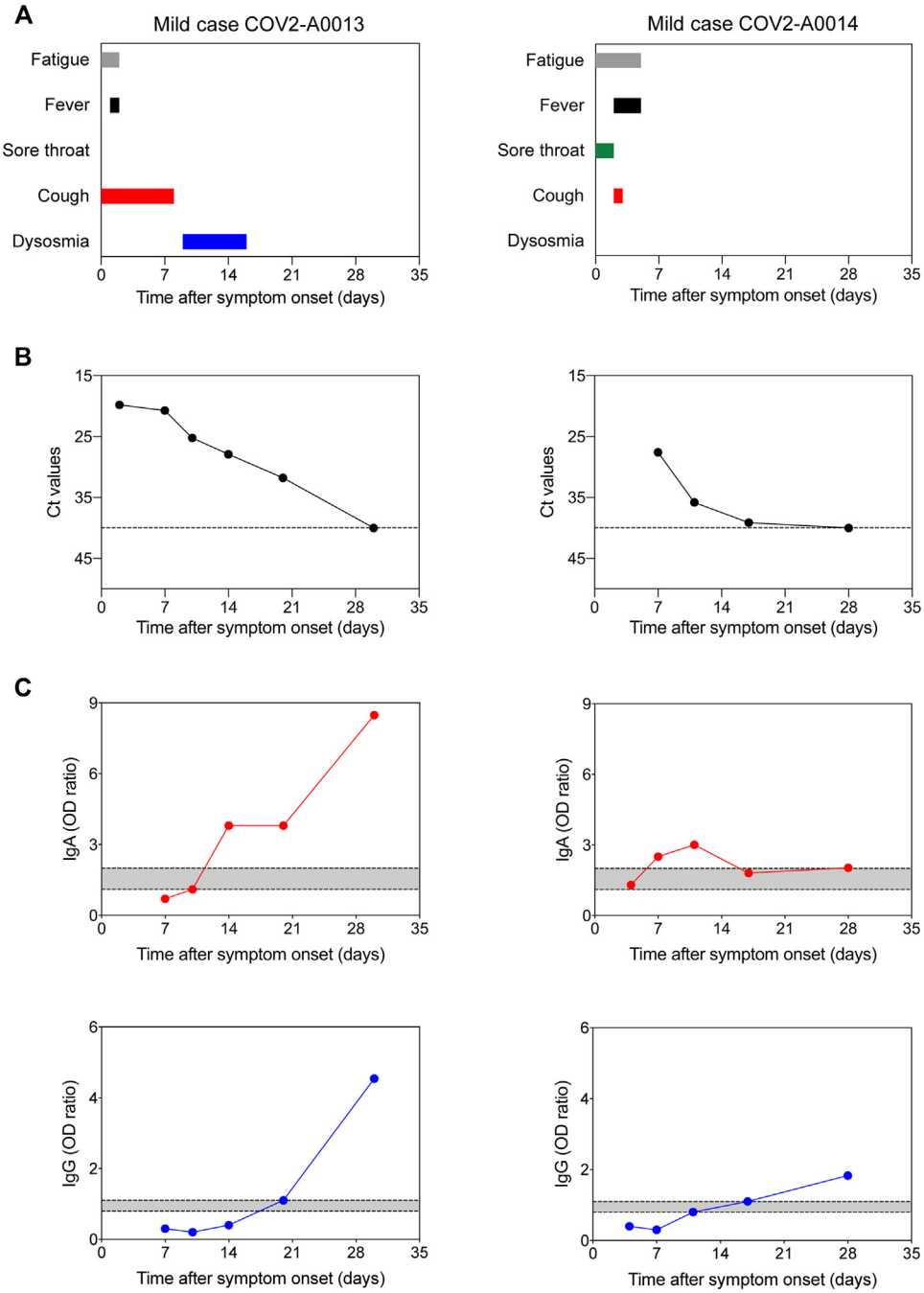


FIG 3. Longitudinal study of 2 mild cases of COVID-19. **A**, Time of reporting of indicated symptoms in 2 patients with mild COVID-19, including a 42-year old male (COV2-A0013 [*left panels*]) and a 42-year old female (COV2-A0014 [*right panels*]). **B**, Ct values of SARS-CoV-2 RT-qPCR assay performed on nasopharyngeal swabs. **C,** S1-specific IgA and IgG serum titers analyzed on different days after symptom onset. Data are shown on a longitudinal axis. Dashed lines indicate cutoffs for positive, borderline, and negative serum values of S1-specific IgA (*top*) and IgG (*bottom*), with the gray shaded area highlighting the borderline values.

remained on day 20; and further increased to a titer of 8.5 OD ratio on day 30. His S1-specific serum IgG titers remained negative on days 7 to 14, became borderline positive on day 20, and became clearly positive at an OD ratio of 4.5 on day 30 (Fig 3, *C*). Conversely, the S1-specific serum IgA titers in patient COV2-A0014 were borderline on day 4 and became positive on days 7 and 11, followed by a drop to borderline values on days 17 and 28. Her S1-specific serum IgG titers were negative on days 4 to 7, became borderline on day 11 and weakly positive at an OD ratio of 1.1 on day 17, and remained weakly positive at an OD ratio of 1.8 on day 28 (Fig 3, *C*). We compared these results with those of longitudinal analyses of S1-specific serum IgA and IgG values in

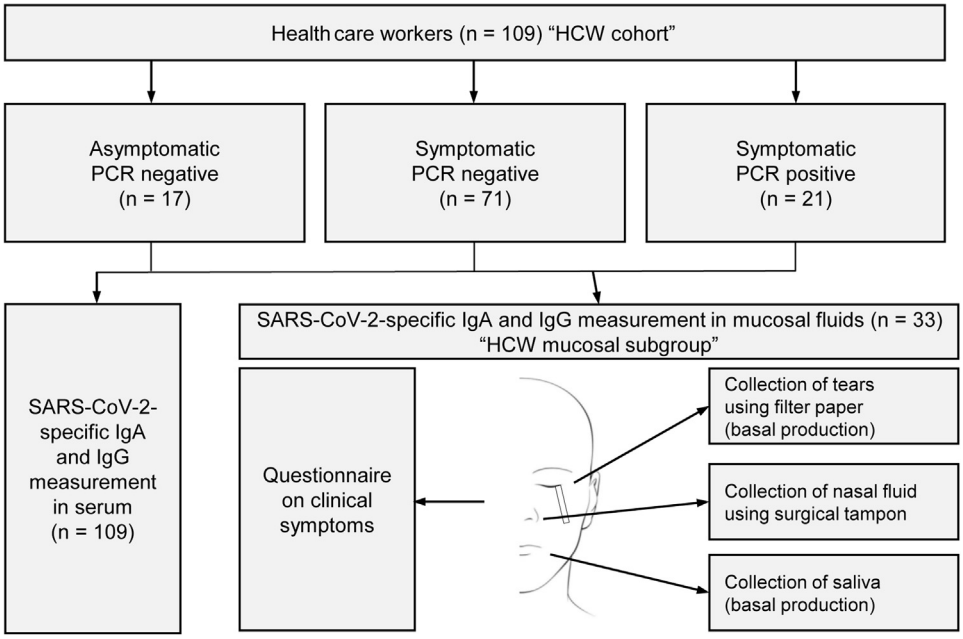


FIG 4. Flowchart showing characterization of the HCW cohort. We grouped our HCW cohort (n = 109) as follows: (1) asymptomatic, PCR-negative (n = 17) reporting exposure (see the Methods section) to a patient with COVID-19 11 to 24 days before sampling; (2) symptomatic, PCR-negative (n = 71); and (3) symptomatic, PCR-positive (n = 21). All HCWs were analyzed for SARS-CoV-2 S1–specific serum IgA and IgG values. In a subgroup (the HCW mucosal subgroup), tears, nasal fluid, saliva, and serum were collected simultaneously. Self-reported symptoms of each participant of the HCW mucosal subgroup were recorded. The 33 HCWs in the HCW mucosal subgroup were grouped in the same way as the HCW cohort: (1) asymptomatic, PCR-negative (n = 9); (2) symptomatic, PCR-negative (n = 13), sampled on average 26.5 days after symptom onset; and (3) symptomatic, PCR-positive (n = 11), sampled on average 26.5 days after symptom onset.

2 different situations. In asymptomatic controls, S1-specific serum IgA and IgG titers remained negative throughout the period of assessment, whereas in patients with severe COVID-19 both antibody responses increased after day 4 to 5 and were markedly elevated on day 14 to 15 (see Fig E4 in this article's Online Repository at www.jacionline.org).

These longitudinal data in patients with mild COVID-19 demonstrate that S1-specific serum IgA production can be transient, whereas S1-specific serum IgG production occurs late and is correlated with the severity of clinical symptoms.

Some seronegative HCWs show SARS-CoV-2–specific IgA at mucosal sites

Having observed that in patients with mild COVID-19, S1-specific serum IgA and IgG production can be transient, delayed, or even absent, we assessed serum S1-specific antibody responses in a well-defined cohort of HCWs (n = 109; the HCW cohort). These HCWs either did or did not have clinical symptoms suggestive of COVID-19, and on the basis of respiratory secretions tested by RT-qPCR, they were either negative or positive for SARS-CoV-2. We grouped them as follows (Fig 4): (1) asymptomatic, RT-qPCR–negative, with a recent history of SARS-CoV-2 exposure (n = 17); (2) symptomatic, RT-qPCR–negative (n = 71); and (3) symptomatic, RT-qPCR–positive (n = 21).

The asymptomatic/PCR-negative group contained very few S1-specific serum IgA-positive subjects and no IgG-positive subjects (Fig 5, *A*). Conversely, there were 4 of 71 participants (6%) with positive IgA and IgG values found in the symptomatic/PCR-negative group, which likely represented individuals who had had a mild SARS-CoV-2 infection (Fig 5, *A*). As expected, the symptomatic/PCR-positive group contained more seropositive individuals, with 8 of 21 subjects (38%) having positive IgA and IgG titers for S1 of SARS-CoV-2 at the time of sampling (Fig 5, *A*).

To investigate S1-specific IgA and IgG titers at mucosal sites, we analyzed tears, nasal fluids, and saliva in a subset of the HCW cohort (ie, the HCW mucosal subgroup) (Fig 4). This subgroup also recorded self-reported clinical symptoms (Tables III and IV). When the symptomatic/PCR-positive members of the HCW mucosal subgroup were assessed, a clear correlation was evident between positivity of S1-specific IgA and IgG in serum (Fig 5, *B*) with the corresponding values in nasal secretions (Fig 5, *C*). Thus, for S1-specific IgG, symptomatic/PCR-positive members with positive serum titers also showed elevated titers of S1-specific IgG in their nasal secretions (Fig 5, *B* and *C*), possibly indicating transfer of S1-specific IgG from serum to the nasal mucosa. Conversely, the relationship of serum versus nasal fluid in symptomatic/PCR-positive members was more variable for S1-specific IgA (Fig 5, *B* and *C*).

To further investigate these findings, we adapted and used our 2 SARS-CoV-2 S protein–specific immunoassays (Figs E5 and E6) to assess the subjects in the HCW mucosal subgroup who tested negative for SARS-CoV-2–specific IgA or IgG in their serum. First, we ruled out an influence of time of sampling or total amount of detectable IgA and IgG in our samples. The mean time of sampling since symptom onset was 26.5 days for both

the symptomatic/PCR-negative and symptomatic/PCR-positive groups of the HCW mucosal subgroup, whereas the asymptomatic/PCR-negative group was tested 11 days or more after exposure. Total IgA and IgG titers were comparable in the serum samples as well as in the tear, nasal fluid, and saliva samples from all 3 groups of participants (see Fig E7 in this article's Online Repository at www.jacionline.org). Notably, whereas total IgG titers were measurable in nasal fluids, they were very low in tear fluid and saliva (see Fig E7). Analyzing S protein–specific IgA and IgG in our mucosal samples, we observed a reliable correlation between our 2 immunoassays for serum IgA and IgG, as well as for tear and nasal fluid IgA, whereas the other measurements were less consistent and were thus not considered for our conclusions (see Fig E6 in this article's Online Repository at www.jacionline.org).

Interestingly, we were able to detect S protein–specific IgA in the mucosal samples from several subjects in the absence of seropositivity. Analyzing individual participants, we found that subjects COV2-M0033, COV2-M0061, and COV2-M0103 showed high S1-specific, ECD-specific, and RBD-specific IgA values in their nasal fluids, whereas the total IgA values were average in nasal fluids of these individuals (Fig 5, *D-F* and see Fig E7). Moreover, the nasal fluid of subject COV2-M0015 contained high S1-specific and RBD-specific IgA values, in the presence of average total IgA values (Fig 5, *D-F* and see Fig E7). When their tear fluid was measured, subjects COV2-M0015 and COV2-M0033 presented with high S1-specific, ECD-specific, or RBD-specific IgA values (Fig 5, *G-I*). Additionally, a few other individuals also had detectable S protein–specific IgG in their nasal fluid despite being IgG seronegative (Fig 5, *D-F*). Notably, some mucosal samples showed comparable neutralizing capacity to serum in an *in vitro* neutralization assay of viable SARS-CoV-2 (see Fig E8 in this article's Online Repository at www.jacionline.org). These findings further supported our detection of humoral immune reponses at mucosal sites in patients with mild COVID-19 in the absence of serum antibodies toward SARS-CoV-2.

In contrast to total IgA titers, when we assessed S protein–specific IgA values in nasal fluid versus age in seronegative HCWs, we found an inverse correlation (R2adj = 0.153; P = .037). The same analysis with S protein–specific IgA titers in serum versus age, however, did not reveal a correlation (P = .58) (Fig 5, *J-L*). Interestingly, the longitudinal subject with a short disease duration (COV2-A0014), transient S protein–specific IgA, and delayed IgG production also had high titers of S protein–specific IgA in her nasal fluid (see Fig E9 in this article's Online Repository at www.jacioniline.org).

Collectively, in 15% to 20% of S protein–seronegative individuals in our cohort, we detected S protein–specific IgA antibodies at several mucosal sites. Furthermore, mucosal S protein–specific IgA titers were inversely correlated with patient age, suggesting increased mucosal antibody responses in younger SARS-CoV-2–exposed individuals.

DISCUSSION

In individuals with severe COVID-19, we found that SARS-CoV-2 S protein–specific serum IgA and IgG titers became positive in samples obtained on average 3 to 5 days after symptom onset, which is in agreement with the findings of earlier publications.[16] These antibody responses showed a strong correlation with disease duration, but they were independent of patient age, sex, and most preexisting comorbidities. Very high serum titers of S protein–specific IgA, but not IgG, were correlated with severe ARDS, thus warranting further studies evaluating the role of IgA in SARS-CoV-2-associated severe ARDS.

Conversely, in patients with mild SARS-CoV-2 infection, S protein–specific serum IgA production was transient, delayed, or even absent and accompanied by an S protein–specific serum IgG response that occurred late or remained negative. Interestingly, however, we found evidence of S protein–specific IgA and IgG at mucosal sites of individuals with mild COVID-19. There, mucosal S protein–specific IgG titers appeared to mirror the systemic (ie, serum) titers of these antibodies. Mucosal S protein–specific IgA titers, however, were even detectable at several mucosal sites of about 15% to 20% of S protein–seronegative individuals in our cohort. Interestingly, mucosal S protein–specific IgA titers were correlated inversely with patient age.

We think that these findings suggest a model according to which the extent and duration of SARS-CoV-2–related clinical symptoms, which are likely correlated with virus replication, dictate the level of virus-specific humoral immunity. This hypothesis is consistent with the findings of previous publications demonstrating that the magnitude of the humoral response toward SARS-CoV-2 is dependent on the duration and magnitude of viral antigen exposure.[33,34] Low antigen exposure will elicit mucosal IgA-mediated responses, which can be accompanied by systemic IgA production; however, systemic virus-specific IgA responses can also be absent, transient, or delayed. This type of "mucosal IgA" antibody response seemed to be particularly prevalent in younger individuals with mild SARS-CoV-2 infection without evidence of pneumonia. These projected longitudinal relationships from cross-sectional evaluations need confirmation in longitudinal studies. Notably, of the 2 subjects in our longitudinal study, patient COV2-A0014 showed milder and shorter-lasting clinical symptoms and more rapid virus clearance, which was associated with transient S protein–specific IgA and delayed IgG production, but high titers of S protein–specific IgA in her nasal fluid.

These data might be a reflection of increased mucosal immunity in the young or decreased mucosal immunity in the old.[35] Along these lines, previous data on coronavirus seroprevalence of HKU1-specific IgG showed an absence of systemic HKU1-specific antibodies in individuals younger than 20 years of age, with increasing seroprevalence with increasing age.[36] Extrapolating this model to also include children and infants, it is conceivable that children and infants have primed mucosal innate and IgA antibody responses on account of their frequent upper respiratory tract infections and therefore respond preferentially in this manner to SARS-CoV-2 infection. This hypothesis might also explain why children rarely present with symptomatic SARS-CoV-2 infection. Looking at the other end of the age sprectrum, previous studies have shown that the kinetics and strength of antiviral immune responses, including T-cell activation and proliferation, become slower with increasing age.[37,38] The elucidation of these questions and the confirmation of our findings will require larger studies. However, because of the transient nature of S protein–specific antibody responses in oligosymptomatic patients, reliance on measurement of SARS-CoV-2–specific serum IgA and IgG titers in asymptomatic patients might underestimate the percentage of individuals who have experienced this coronavirus infection and thus, may be deceiving when estimating the epidemic spread of SARS-CoV-2. Our data suggest that, in

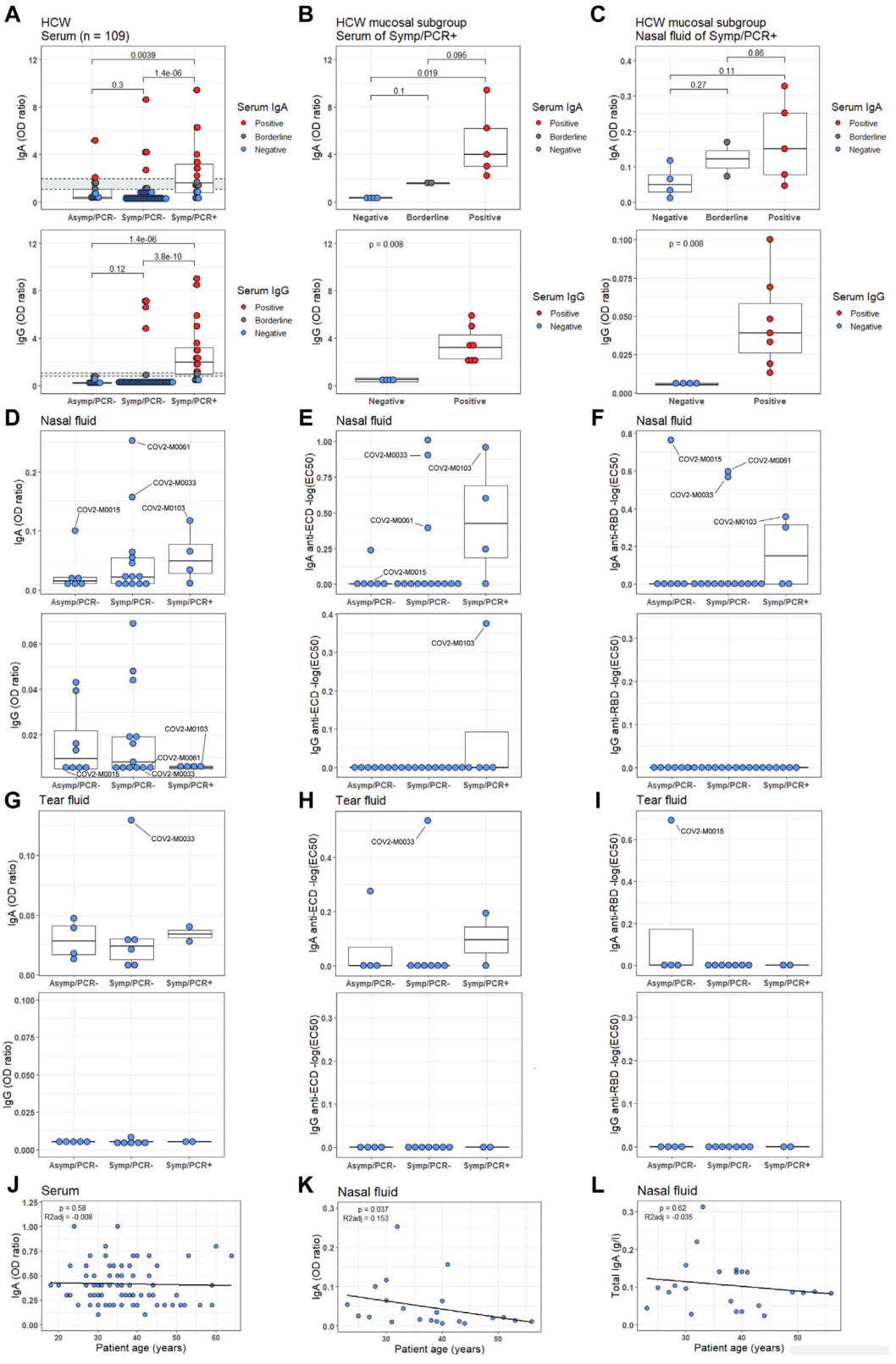


FIG 5. Analysis of SARS-CoV-2 S protein–specific IgA and IgG responses in serum and mucosal fluids. **A,** S protein–specific IgA (*top*) and IgG (*bottom*) serum titers in the HCW cohort (n = 109). Dashed lines indicate borders between positive (*red*), borderline (*gray*), and negative (*blue*) values, with the gray-shaded area showing borderline values. **B** and **C**, S protein–specific IgA (*top*) and IgG (*bottom*) serum (**B**) and nasal fluid (**C**) titers of symptomatic, PCR-positive (Symp/PCR^{+}) individuals (n = 11) in the HCW mucosal subgroup. Comparison of HCWs with negative, borderline, and positive values. **D-F**, S protein–specific IgA (*top*) and IgG (*bottom*) titers

TABLE III. Demographic characterstics of the HCW cohort included in the S protein–specific IgA and IgG serology study

Characteristic	Asymptomatic/ PCR-negative (n = 17)	Symptomatic/ PCR-negative (n = 71)	Symptomatic/ PCR-positive (n = 21)	Total (n = 109)	*P* value
Median age (y), median (IQR)	39 (34-44)	36 (30-41)	38 (30-48)	36 (30-43)	.4156
Sex (male/female)	6/11	16/55	3/18	25/84	.3066

TABLE IV. Demographic and clinical characteristics of the HCW mucosal subgroup assessed in the S protein-specific IgA and IgG mucosal fluid study

Characteristic	Asymptomatic/ PCR-negative (n = 9)	Symptomatic/ PCR-negative (n = 13)	Symptomatic/ PCR-positive (n = 11)	Total (n = 33)	*P* value
Median age (y), median (IQR)	38 (36-44)	40 (32-49)	38 (30-42)	39 (31-43)	.8
Sex (male/female)	4/5	6/7	3/8	13/20	.5999
Reported symptoms, no. (%)					
Fatigue	—	6 (46.2)	7 (63.6)	13 (39.4)	.4442
Body temperature >38.0°C	—	4 (30.8)	1 (9.1)	5 (15.2)	.3271
Feeling feverish	—	6 (46.2)	4 (36.4)	10 (30.3)	.6968
Chills	—	1 (7.7)	2 (18.2)	3 (9.1)	.5761
Shivering	—	3 (23.1)	4 (36.4)	7 (21.2)	.6591
Body aches	—	8 (61.5)	8 (72.7)	16 (48.5)	.6792
Back pain	—	5 (38.5)	4 (36.4)	9 (27.3)	>.999
Cough	—	5 (38.5)	6 (54.5)	11 (33.3)	.6824
Dyspnea	—	2 (15.4)	4 (36.4)	6 (18.2)	.3572
Pleuritis	—	3 (23.1)	4 (36.4)	7 (21.2)	.6591
Sore throat	—	11 (84.6)	6 (54.5)	17 (51.5)	.1819
Coryza	—	7 (53.8)	6 (54.5)	13 (39.4)	>.999
Hoarseness	—	5 (38.5)	4 (36.4)	9 (27.3)	>.999
Anosmia/dysosmia	—	2 (15.4)	8 (72.7)	10 (30.3)	.0111
Diarrhea	—	5 (38.5)	2 (18.2)	7 (21.2)	.3864
Nausea	—	3 (23.1)	3 (27.3)	6 (18.2)	>.999
Conjunctivitis	—	2 (15.4)	—	2 (6.1)	.4819

Categoric values were compared by using the Fisher exact test or chi-square if more than 2 groups were being compared. Continuous variables were compared by using the Kruskal-Wallis test.

addition to measurement of serum, measurement of SARS-CoV-2–specific mucosal IgA should be considered.

With increased SARS-CoV-2–related clinical symptoms and hence antigen exposure, we observed a "systemic IgA and IgG" type of antibody response characterized by S protein–specific IgA that may be transient or delayed and the presence of S protein–specific IgG. With even further increasing clinical severity, we found high to very high serum IgA and high IgG responses in patients with severe cases and ARDS. Thus, our findings suggest 4 grades of antibody responses dependent on COVID-19 severity with (1) oligosymptomatic disease and mucosal antibody responses in the absence of systemic antibody production; (2) mild-to-moderate disease and transient or delayed systemic IgA and IgG production; (3) patients with severe COVID-19 with high serum IgA and high IgG responses; and (4) patients with very severe cases of COVID-19, including severe ARDS, with very high serum IgA and high IgG titers.

Whether these S protein–specific antibody responses confer immunity to a secondary infection with SARS-CoV-2 is a matter of intense debate. Previous publications indicated that S protein–specific serum IgG antibodies are correlated with virus neutralization *in vitro*,[16,17,39] although some publications have questioned the efficacy of neutralization by these antibody responses.[25] Our neutralization data showed a correlation between SARS-CoV-2–neutralizing activity and detectable S protein–specific IgA and IgG in both serum and mucosal fluids, suggesting that the observed humoral responses could be protective. On the basis of correlative data from the SARS-CoV outbreak and preclinical SARS-CoV infection models,[40] a contribution of the humoral immune response to immune pathology has been discussed,[41,42] potentially

in nasal fluid, including S1-specific (**D**), SARS-CoV-2 S protein extracellular domain (ECD)-specific (**E**), and S1 protein RBD–specific IgA and IgG (**F**) of S1 protein–seronegative individuals in the HCW mucosal subgroup. Comparison of asymptomatic, PCR-negative (Asymp/PCR$^-$), symptomatic, PCR-negative (Symp/PCR$^-$), and Symp/PCR$^+$ HCWs. HCWs with negative S protein–specific IgA serum values are labeled individually. **G-I**, S protein–specific IgA (*top*) and IgG (*bottom*) titers in tear fluid, including S1-specific (**G**), ECD-specific (**H**), and RBD-specific IgA and IgG (**I**) of S1 protein–seronegative individuals in the HCW mucosal subgroup. (**J-L**) Linear modeling of S1 protein–specific IgA titers in serum (**J**) and nasal fluids (**K**) and total IgA in nasal fluids (**L**), as a function of age in S1 protein–seronegative individuals in the HCW mucosal subgroup.

by augmenting proinflammatory monocytes in the lungs. However, trials with convalescent serum treatments have shown promising results during the current COVID-19 pandemic and also in SARS-CoV.[43] Another caveat relates to the durability of protective humoral immunity. Whether S protein–specific mucosal IgA responses confer immunity to a secondary infection with SARS-CoV-2 remains to be seen. We are currently characterizing the cellular immune responses to SARS-CoV-2 and following up our patient cohort longitudinally to address these important issues.[44,45]

We thank Alessandra Guaita, Jennifer Jörger, Mitchell Levesque, Daniel Pinschewer, Hugo Sax, Urs Steiner, Barbara Turi, Alberto Turi, and the members of the Boyman laboratory for helpful discussions and support. We are also grateful to those HCWs at University Hospital Zurich who helped with sampling and recruitment of the HCW cohort. The graphical abstract was created with BioRender software (BioRender.com).

Clinical implications: Measurement of SARS-CoV-2–specific serum IgA and IgG titers in asymptomatic patients might underestimate the prevalence of infected individuals.

REFERENCES

1. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5:536-44.
2. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265-9.
3. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579:270-3.
4. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33.
5. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020;20:533-4.
6. V'kovski P, Gultom M, Steiner S, Kelly J, Russeil J, Mangeat B, et al. Disparate temperature-dependent virus – host dynamics for SARS-CoV-2 and SARS-CoV in the human respiratory epithelium [e-pub ahead of print]. bioRxiv http://biorxiv.org/lookup/doi/10.1101/2020.04.27.062315. Accessed November 27, 2020.
7. Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, et al. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility [e-pub ahead of print]. N Engl J Med https://doi.org/10.1056/NEJMoa2008457. Accessed November 27, 2020.
8. Tong Z-D, Tang A, Li K-F, Li P, Wang H-L, Yi J-P, et al. Potential presymptomatic transmission of SARS-CoV-2, Zhejiang Province, China, 2020. Emerg Infect Dis 2020;26:1052-4.
9. Li R, Pei S, Chen B, Song Y, Zhang T, Yang W, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). Science 2020;368:489-93.
10. Wu Z, McGoogan JM. 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 2020;323:1239.
11. Zhang J, Dong X, Cao Y, Yuan Y, Yang Y, Yan Y, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy 2020;75:1730-41.
12. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 2020;8:475-81.
13. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin definition. JAMA 2012;307:2526-33.
14. WHO. Clinical management of severe acute respiratory infection when COVID-19 is suspected [e-pub ahead of print]. Available at: https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. Accessed November 27, 2020.
15. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020; 367:1260-3.
16. To KK-W, Tsang OT-Y, Leung W-S, Tam AR, Wu T-C, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020;20:565-74.
17. Ni L, Ye F, Cheng M-L, Feng Y, Deng Y-Q, Zhao H, et al. Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals. Immunity 2020;52:971-7.e3.
18. Schulz KS, Mossman KL. Viral evasion strategies in type I IFN signaling - a summary of recent developments. Front Immunol 2016;7:498.
19. Hu Y, Li W, Gao T, Cui Y, Jin Y, Li P, et al. The severe acute respiratory syndrome coronavirus nucleocapsid inhibits type I interferon production by interfering with TRIM25-mediated RIG-I ubiquitination. J Virol 2017;91.
20. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, Uhl S, Hoagland D, Møller R, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell 2020;181:1036-45.e9.
21. Amanna IJ, Slifka MK. Mechanisms that determine plasma cell lifespan and the duration of humoral immunity: long-term antibody production. Immunol Rev 2010;236:125-38.
22. Azkur AK, Akdis M, Azkur D, Sokolowska M, Veen W, Brüggen M, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy 2020;75:1564-81.
23. Callow KA, Parry HF, Sergeant M, Tyrrell DAJ. The time course of the immune response to experimental coronavirus infection of man. Epidemiol Infect 1990; 105:435-46.
24. Bryant JE, Azman AS, Ferrari MJ, Arnold BF, Boni MF, Boum Yap, et al. Serology for SARS-CoV-2: apprehensions, opportunities, and the path forward. Sci Immunol 2020;5:eabc6347.
25. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271-80.e8.
26. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 2020;11:1620.
27. Swissnoso. Recommendations for healthcare workers, having had unprotected close contact with COVID-19 cases. Available at: https://www.swissnoso.ch/fileadmin/swissnoso/Dokumente/5_Forschung_und_Entwicklung/6_Aktuelle_Erreignisse/200526_management_of_HCW_with_COVID-19_contact_V4.0.pdf. Accessed October 5, 2020.
28. Kaegi C, Wuest B, Schreiner J, Steiner UC, Vultaggio A, Matucci A, et al. Systematic review of safety and efficacy of rituximab in treating immune-mediated disorders. Front Immunol 2019;10:1990.
29. Meyer B, Torriani G, Yerly S, Mazza L, Calame A, Arm-Vernez I, et al. Validation of a commercially available SARS-CoV-2 serological immunoassay. Clin Microbiol Infect 2020;26:1386-94.
30. Emmenegger M, De Cecco E, Lamparter D, Jacquat RPB, Ebner D, Schneider MM, et al. Early peak and rapid decline of SARS-CoV-2 seroprevalence in a Swiss metropolitan region [e-pub ahead of print]. medRxiv Available at: https://www.medrxiv.org/content/10.1101/2020.05.31.20118554v4. Accessed November 27, 2020.
31. Hollander M, Wolfe DA. Nonparametric statistical methods. 2nd ed. Hoboken, NJ: John Wiley & Sons; 1999.
32. Thissen D, Steinberg L, Kuang D. Quick and easy implementation of the Benjamini-Hochberg procedure for controlling the false positive rate in multiple comparisons. J Educ Behav Stat 2002;27:77-83.
33. Liu Y, Yan L-M, Wan L, Xiang T-X, Le A, Liu J-M, et al. Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis 2020;20:656-7.
34. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054-62.
35. Brodin P, Davis MM. Human immune system variation. Nat Rev Immunol 2017; 17:21-9.
36. Chan CM, Tse H, Wong SSY, Woo PCY, Lau SKP, Chen L, et al. Examination of seroprevalence of coronavirus HKU1 infection with S protein-based ELISA and neutralization assay against viral spike pseudotyped virus. J Clin Virol 2009;45:54-60.
37. van Deursen JM. The role of senescent cells in ageing. Nature 2014;509:439-46.
38. Goronzy JJ, Weyand CM. Successful and maladaptive T cell aging. Immunity 2017;46:364-78.
39. Ng KW, Faulkner N, Cornish GH, Rosa A, Harvey R, Hussain S, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. Science 2020; eabe1107.
40. Liu L, Wei Q, Lin Q, Fang J, Wang H, Kwok H, et al. Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. JCI Insight 2019;4.

41. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol 2020;20:363-74.
42. Yu H, Sun B, Fang Z, Zhao J, Liu X, Li Y, et al. Distinct features of SARS-CoV-2-specific IgA response in COVID-19 patients. Eur Respir J 2020;56:2001526.
43. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA 2020;323:1582.
44. Chevrier S, Zurbuchen Y, Cervia C, Adamo S, Raeber ME, de Souza N, et al. A distinct innate immune signature marks progression from mild to severe COVID-19 [e-pub ahead of print]. bioRxiv Available at: http://biorxiv.org/lookup/doi/10.1101/2020.08.04.236315. Accessed November 27, 2020.
45. Adamo S, Chevrier S, Cervia C, Zurbuchen Y, Raeber ME, Yang L, et al. Lymphopenia-induced T cell proliferation is a hallmark of severe COVID-19 [e-pub ahead of print]. bioRxiv Available at: http://biorxiv.org/lookup/doi/10.1101/2020.08.04.236521. Accessed November 27, 2020.

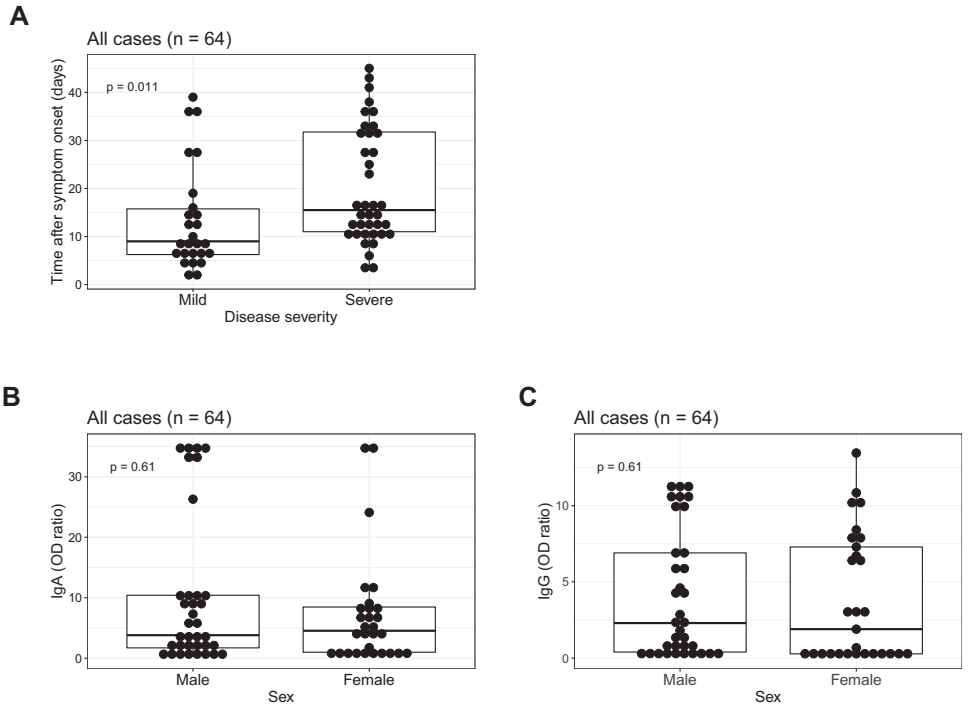


FIG E1. S protein–specific serum IgA and IgG values compared with sampling time point, disease severity, patient age, and sex. **A**, Comparison of days between reported symptom onset and sample collection in patients with mild (n = 26) versus severe COVID-19 (n = 38). **B,** Visualization of age distribution in the generalized additive models of S1 protein–specific IgA and IgG serum titers as a function of days between reported symptom onset and sample collection. Comparison of patients with mild (n = 26) versus severe cases (n = 38). **C** and **D,** Comparison of S1 protein–specific serum IgA (**C**) and IgG (**D**) titers in male (n = 35) versus female (n = 29) patients with COVID-19. The average times between reported symptom onset and sample collection were 17 days (median 13 days) in male patients and 18 days (median 14 days) in female patients.

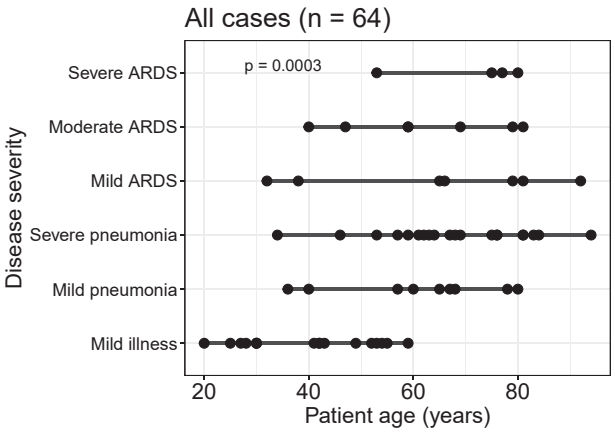


FIG E2. Distribution of disease severity and age of the patients with COVID-19. Comparison in all patients with COVID-19 (n = 64) of patient age distribution with COVID-19 severity at the time of sample collection, ranging from mild COVID-19 to severe ARDS, as defined by the World Health Organization classification criteria.[14] *P* value was computed by using the Kruskal-Wallis test.

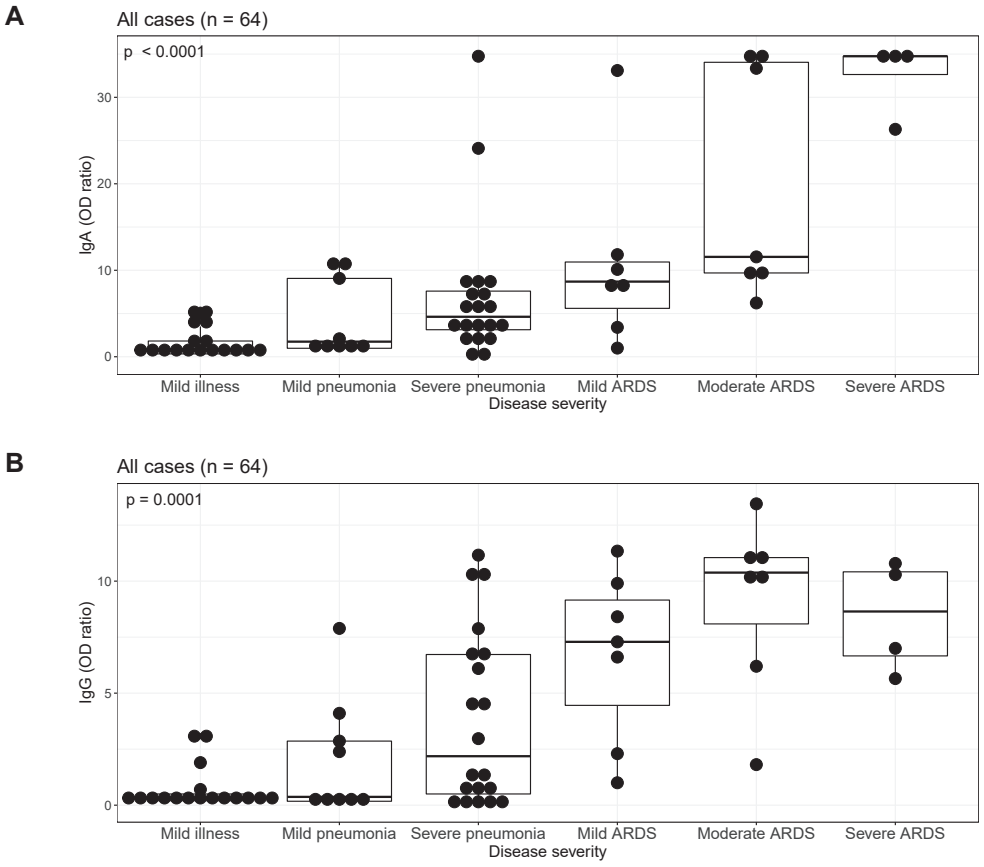


FIG E3. S protein–specific serum IgA and IgG values compared with severity of symptoms of patients with COVID-19. **A** and **B,** Comparison of S1 protein–specific serum IgA (**A**) and IgG (**B**) titers with disease severity in our cohort of patients with COVID-19 (n = 64), ranging from mild COVID-19 to severe ARDS, as defined by the World Health Organization classification criteria.[14] Data are shown as boxplots. Each dot represents an independent and unrelated donor. The significance of between-group differencies was explored by using the Kruskal-Wallis test.

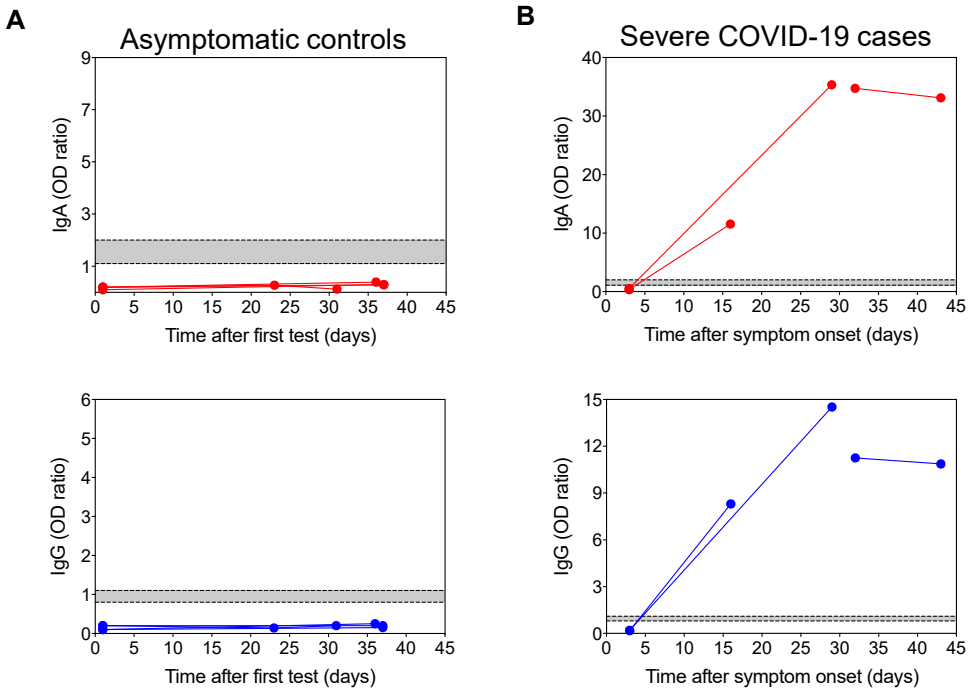


FIG E4. Longitudinal measurement of S protein–specific serum IgA and IgG values in asymptomatic controls and severe cases of COVID-19. **A** and **B**, S1 protein–specific serum IgA (*top*) and IgG (*bottom*) titers in asymptomatic donors (n = 4) (**A**) and patients with severe cases of COVID-19 (n = 3) (**B**). The connected dots represent sequential measurements of the same individual.

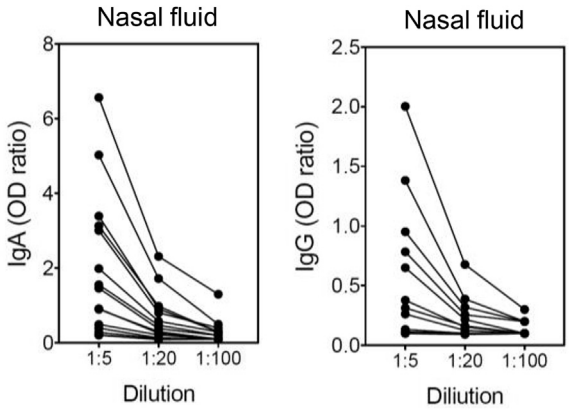


FIG E5. Titration of nasal fluids to detect S protein–specific IgA and IgG. Measurement of S1 protein–specific IgA (*top*) and IgG (*bottom*) by using different dilutions of nasal fluids in a subset of the HCW mucosal subgroup (n = 15).

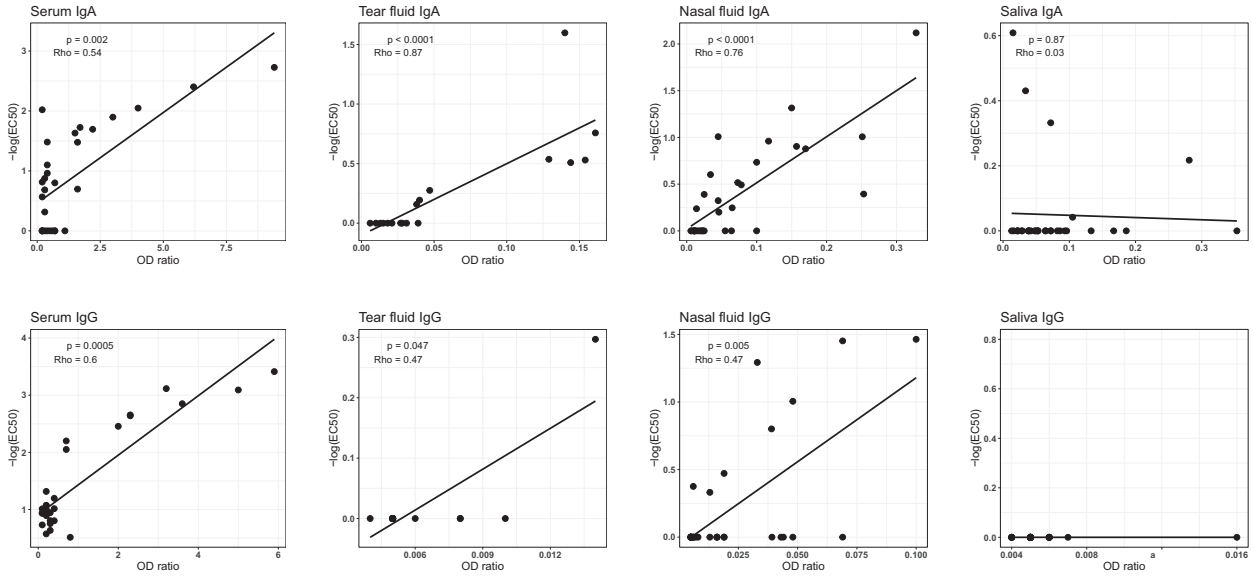


FIG E6. Comparison of immunoassays to measure S protein–specific IgA and IgG in samples from serum, tears, nasal fluid, and saliva. Comparison of OD ratios of IgA (*top*) and IgG (*bottom*) obtained with a commercial ELISA specific for the S1 protein of SARS-CoV-2 (x-axes) and the inflection point of the sigmoidal curve (–log(EC50) (y-axes), the latter determined by measuring IgA (*top*) and IgG (*bottom*) against SARS-CoV-2 S ECD and SARS-CoV-2 S1 protein RBD in serial dilutions using an in-house immunoassay (see the Methods section). S protein–specific IgA (*top*) and IgG (*bottom*) were measured in serum, tear fluid, nasal fluid, and saliva of members of the HCW mucosal subgroup. Data are shown as scatter plots. Each dot represents an independent and unrelated donor. The Spearman correlation coefficient (ρ) is shown with the corresponding *P* value.

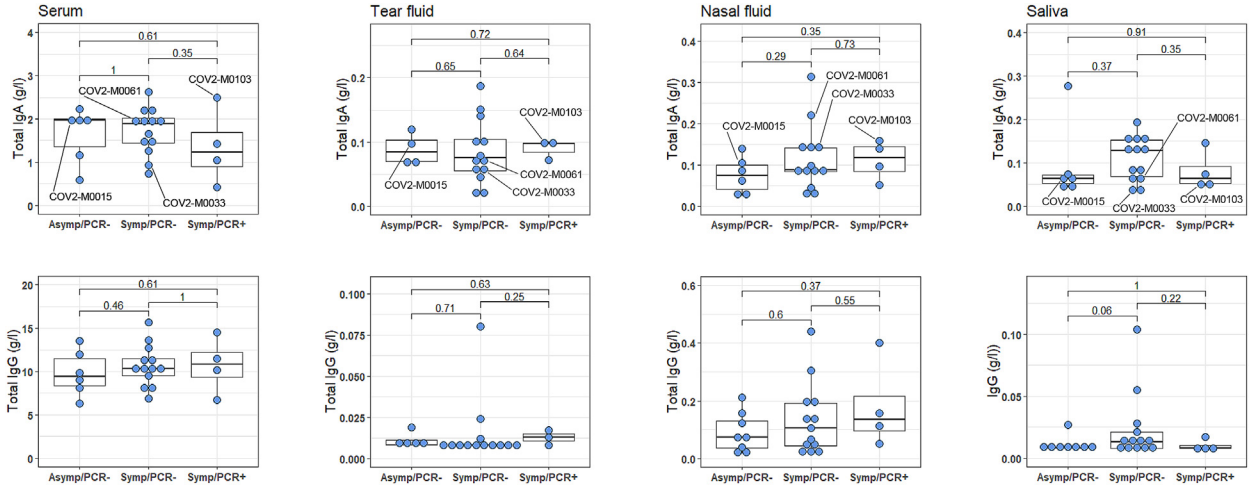


FIG E7. Analysis of total IgA and IgG serum titers in the HCW mucosal subgroup. Total IgA (*top*) and IgG (*bottom*) titers in serum, tear fluid, nasal fluid, and saliva were assessed in individuals in the HCW mucosal subgroup who tested negative for S1 protein–specific serum IgA (*top*) and IgG (*bottom*). The results of a comparison of aymptomatic, PCR-negative (Asymp/PCR$^-$), symptomatic PCR-negative (Symp/PCR$^-$), and symptomatic, PCR-positive (Symp/PCR$^+$) HCWs are shown. Four PCR-negative HCWs with negative S protein–specific IgA values in their serum but increased S protein–specific IgA titers in their nasal fluids are labeled with their corresponding study code. The significance of between-group differences was explored by using the Wilcoxon test.

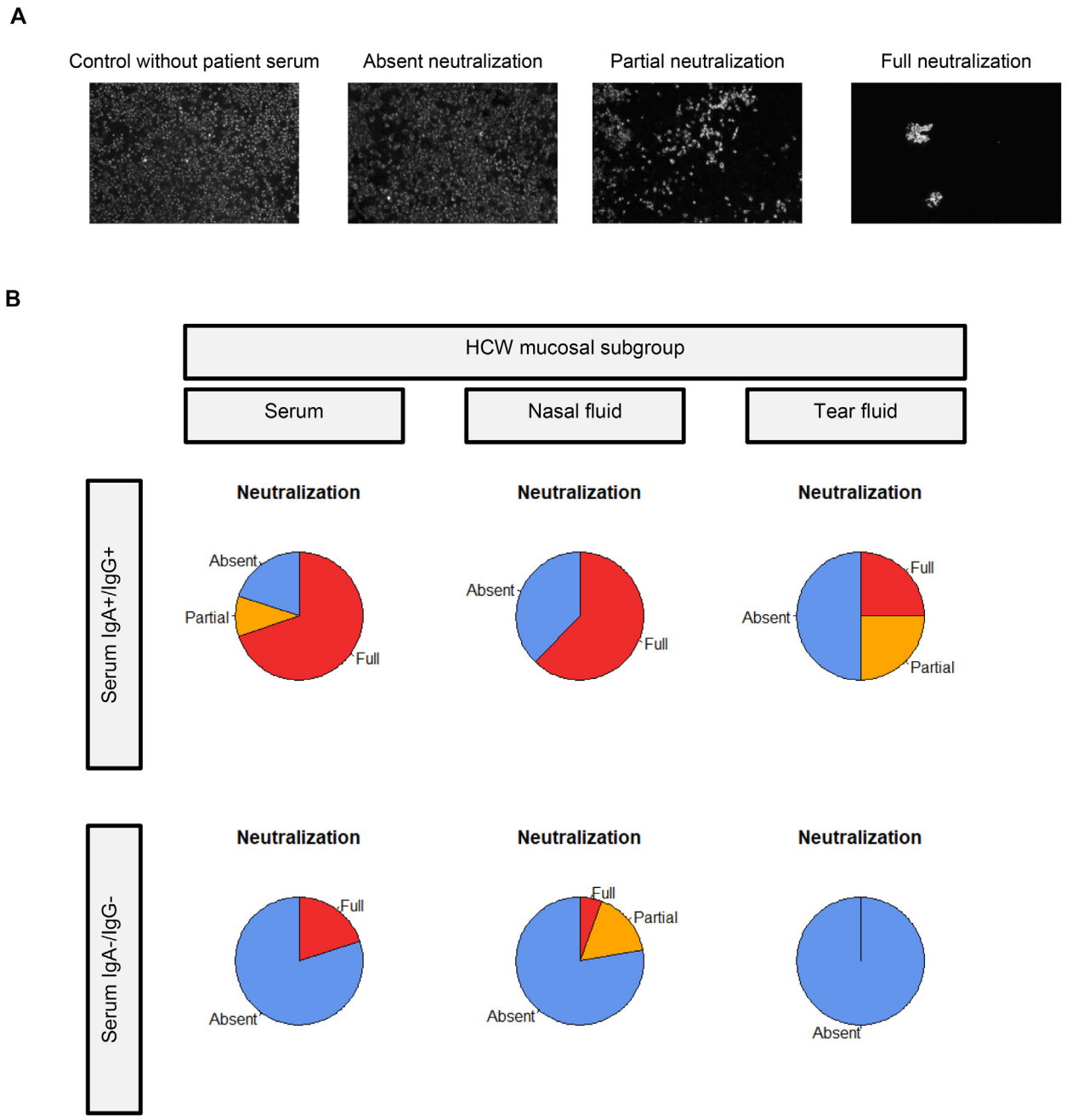


FIG E8. SARS-CoV-2 neutralization in nasal and tear fluids of individuals testing negative for SARS-CoV-2–specific antibodies in serum. **A,** Representative photographs of SARS-CoV-2–infected VeroE6 cells, showing either absent, partial, or full neutralization of SARS-CoV-2 in the presence or absence of patient serum. **B,** Shown are the proportions of full (*red*), partial (*orange*), and absent (*blue*) neutralizing ability of serum (n = 20), nasal fluid (n = 26), and tear fluid samples (n = 7) obtained from the HCW subgroup with either positive to borderline (*top row*) or negative (*bottom row*) S1 protein–specific IgA and IgG titers in their serum.

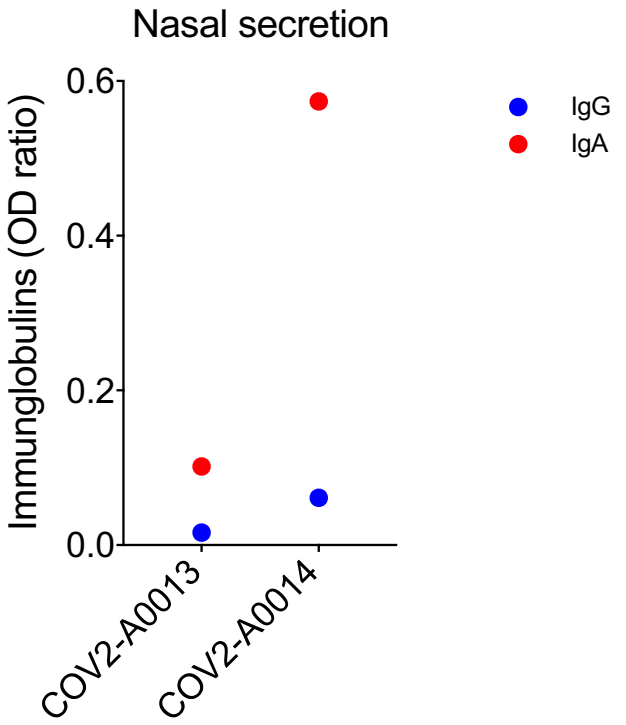


FIG E9. Mucosal S protein–specific IgA and IgG in 2 patients with mild COVID-19. Shown are S1 protein–specific IgA and IgG titers in the nasal fluids of patients COV2-A0013 and COV2-A0014 (see Fig 3) on day 25.