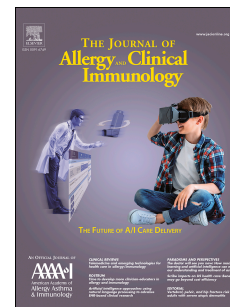


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Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19

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



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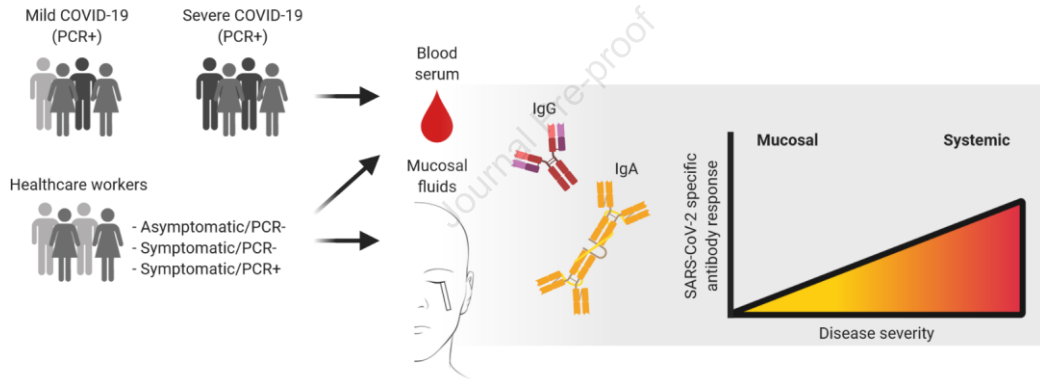
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Systemic and mucosal antibody response specific to SARS-CoV-2 during mild versus severe COVID-19



1 **Systemic and mucosal antibody responses specific to SARS-CoV-2**

2 **during mild versus severe COVID-19**

3 Carlo Cervia, MMed,^{1†} Jakob Nilsson, MD, PhD,^{1†} Yves Zurbuchen, MMed,¹ Alan Valaperti,
 4 PhD,¹ Jens Schreiner, PhD,¹ Aline Wolfensberger, MD,² Miro E. Raeber, MMed,¹ Sarah
 5 Adamo, MMed,¹ Sebastian Weigang, MSc,³ Marc Emmenegger, MSc,⁴ Sara Hasler,¹ Philipp
 6 P. Bosshard, PhD,⁵ Elena De Cecco, PhD,⁴ Esther Bächli, MD,⁶ Alain Rudiger, MD,⁷ Melina
 7 Stüssi-Helbling, MD,⁸ Lars C. Huber, MD,⁸ Annelies S. Zinkernagel, MD, PhD,² Dominik J.
 8 Schaer, MD,⁹ Adriano Aguzzi, MD,⁴ Georg Kochs, PhD,^{3,10} Ulrike Held, PhD,¹¹ Elsbeth
 9 Probst-Müller, MD, PhD,¹ Silvana K. Rampini, MD,⁹ and Onur Boyman, MD^{1,12} *

10

11 ¹Department of Immunology, University Hospital Zurich (USZ), Zurich, Switzerland

12 ²Department of Infectious Diseases and Hospital Epidemiology, USZ, Zurich, Switzerland

13 ³ Institute of Virology, Medical Center, University of Freiburg, Freiburg, Germany

14 ⁴ Institute of Neuropathology, USZ, Zurich, Switzerland

15 ⁵ Department of Dermatology, USZ, Zurich, Switzerland

16 ⁶ Clinic for Internal Medicine, Uster Hospital, Uster, Switzerland

17 ⁷ Department of Medicine, Limmattal Hospital, Schlieren, Switzerland

18 ⁸ Clinic for Internal Medicine, City Hospital Triemli Zurich, Zurich, Switzerland

19 ⁹ Department of Internal Medicine, USZ, Zurich, Switzerland

20 ¹⁰ Faculty of Medicine, University of Freiburg, Freiburg, Germany

21 ¹¹ Department of Biostatistics, at Epidemiology, Biostatistics and Prevention Institute,
 22 University of Zurich, Zurich, Switzerland

23 ¹² Faculty of Medicine, University of Zurich, Zurich, Switzerland

24 [†] Contributed equally

25 * Correspondence: Onur Boyman, MD, Department of Immunology, University Hospital
 26 Zurich, Gloriastrasse 23, 8091 Zurich. E-mail: onur.boyman@uzh.ch; phone: +41 44 255
 27 2069.

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31 **Competing interests**

32 The authors declare no competing financial interests related to this work.

33

34

35 Abstract

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

39 **Objectives.** To evaluate systemic and mucosal antibody responses toward SARS-
40 CoV-2 in mild versus severe coronavirus disease 2019 (COVID-19) cases.

41 **Methods.** Using immunoassays specific for SARS-CoV-2 spike proteins, we
42 determined SARS-CoV-2-specific immunoglobulin A (IgA) and immunoglobulin G
43 (IgG) in sera and mucosal fluids of two cohorts, including SARS-CoV-2 polymerase
44 chain reaction (PCR)⁺ patients (n = 64) as well as PCR⁺ and PCR⁻ healthcare workers
45 (n = 109).

46 **Results.** SARS-CoV-2-specific serum IgA titers in mild COVID-19 cases were often
47 transiently positive, whereas serum IgG titers remained negative or became positive
48 12–14 days after symptom onset. Conversely, patients with severe COVID-19 showed
49 a highly significant increase of SARS-CoV-2-specific serum IgA and IgG titers after
50 symptom onset. Very high titers of SARS-CoV-2-specific serum IgA correlated with
51 severe acute respiratory distress syndrome (ARDS). Interestingly, some healthcare
52 workers with negative SARS-CoV-2-specific serum antibody titers showed SARS-
53 CoV-2-specific IgA in mucosal fluids with virus-neutralizing capacity in some cases.
54 SARS-CoV-2-specific IgA titers in nasal fluids inversely correlated with age.

55 **Conclusions.** Systemic antibody production against SARS-CoV-2 develops mainly in
56 severe COVID-19, with very high IgA titers seen in patients with severe ARDS,
57 whereas mild disease may be associated with transient production of SARS-CoV-2-
58 specific antibodies but stimulate mucosal SARS-CoV-2-specific IgA secretion.

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

Capsule summary

Our study suggests a model where the extent and duration of SARS-CoV-2-related clinical symptoms correlates with the systemic and mucosal virus-specific humoral immune responses, with implications for seroprevalence studies.

Keywords

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

ACE2: angiotensin-converting enzyme 2

ARDS: acute respiratory distress syndrome

COVID-19: coronavirus disease 2019

Ct: cycle threshold

ECD: extracellular domain

ELISA: enzyme-linked immunosorbent assay

HCW: healthcare worker

HCW cohort: healthcare worker cohort

IgA: immunoglobulin A

IgG: immunoglobulin G

IgM: immunoglobulin M

IQR: interquartile range

MERS-CoV: Middle East respiratory syndrome coronavirus

NC: nucleocapsid

OD: optical density

RBD: receptor-binding domain

RT-qPCR: quantitative reverse-transcriptase polymerase chain reaction

S: spike

SARS-CoV: severe acute respiratory syndrome coronavirus

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

WHO: World Health Organization

Introduction

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 (MERS-CoV).¹⁻⁴ The zoonotic introduction of MERS-CoV 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 affected about 35.2 million confirmed cases 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 3–14 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% 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% and more, and critical COVID-19 results in acute respiratory distress syndrome (ARDS) with respiratory failure, multiorgan dysfunction, and shock. The World Health Organization (WHO) proposed a classification of symptomatic COVID-19 into (i) mild illness, (ii) mild pneumonia, (iii) severe pneumonia, (iv) ARDS (based on the Berlin definition of ARDS)¹³, and (v) 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 serological 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 pro-inflammatory cytokines, including interleukin-6. Conversely, other anti-viral 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 and T cells become activated, resulting in the production of SARS-CoV-2-specific antibodies, comprising immunoglobulin M (IgM), immunoglobulin A (IgA), and immunoglobulin G (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 correlate with virus control is a matter of intense discussions.^{16,17,24,25}

Unlike the internal nucleocapsid protein (NC) of SARS-CoV-2, that shares about 90% amino acid sequence homology with the NC 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 correlate with COVID-19 parameters. And, finally, it is unclear whether tissue-associated IgA and IgG secretion, rather than their systemic production, might be evident in SARS-CoV-2-exposed individuals undergoing mild disease.

Methods

Human subjects and patient characteristics

Following written informed consent, patients and healthcare workers (HCWs) were recruited for sampling of blood and mucosal secretions. We studied two cohorts, including patients with quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR)-confirmed SARS-CoV-2 infection ($n = 64$; median age 59.5 years) with mild versus severe COVID-19, and HCWs (termed 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 of all professional groups, both with and without patient contact. Exposure was defined as contact with an RT-qPCR-confirmed COVID-19 patient without adequate safety measures.²⁷ Because of pre-existing comorbidities, six patients were under stable immunosuppressive treatment at time of inclusion (**Table 1**); 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, two subjects with mild COVID-19 were sampled repeatedly during their disease course. Our COVID-19 patients were classified according to the WHO criteria¹⁴ into (a) mild cases, comprising mild illness and mild pneumonia, versus (b) severe cases, 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 the HCW cohort (termed HCW mucosal subgroup; $n = 33$) volunteered to be sampled for blood as well as, simultaneously, tears, nasal fluid, and saliva.

Venous blood samples were collected in BD Vacutainer CAT serum tubes (Becton Dickinson). Tears were sampled using filter paper produced for Schirmer tear tests (HS Clement Clarke Ophthalmic). Nasal fluids were collected by inserting a dry soft tissue into the nasal cavities for 5 minutes (Vostra). Unstimulated saliva was collected for 5 minutes.

IgA and IgG immunoassays

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

Furthermore, we assessed the samples of the HCW mucosal subgroup using an in-house immunoassay for IgA and IgG against S protein extracellular domain (ECD), RBD, and NC.³⁰ Mucosal samples were pre-diluted 1:2 in sample buffer (PBS Tween-20 0.1%, 1% milk) and serum was pre-diluted 1:20 in sample buffer and transferred to antigen-coated 1536-well assay plates using acoustic dispensing technology (Labcyte ECHO 555) with serial dilutions ranging from 1:5–1:640 (mucosal samples) and 1:50–1:6400 (serum). ODs were measured at 450 nm in a multimode plate reader

(Perkin Elmer EnVision), followed by fitting with a logistic regression and determination of the inflection point of the sigmoidal curve ($-\log(\text{EC}_{50})$). Negative values were depicted as 0.

Quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR)

Nasopharyngeal swabs were subjected to RT-qPCR using the TaqMan SARS-CoV-2 Assay Kit v2 (Thermo Fischer), the 2019-nCoV CDC qPCR Probe Assay (2019-nCoV CDC EUA Kit; Integrated DNA Technologies, Inc.), or the Roche Cobas SARS-CoV-2 Test CE-IVD (Roche) according to 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 DMEM plus 2% fetal calf serum, 20 mM HEPES and 0.05% NaHCO_3 . Two-fold 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 one hour at room temperature and dispersed on 96-well plates containing a semi-confluent VeroE6 monolayer. The plates were incubated for 20 hours at 37°C in a humidified atmosphere with 5% CO_2 , prior to fixation and fluorescent staining with a SARS-CoV NC-specific antibody (biomol #200-401-A50). The assay was evaluated by fluorescence microscopy, and plaque reduction of less than 50% was interpreted as absent, reduction of 50–80% as partial, and reduction of 80–100% as full neutralization, all compared to a control without patient serum or mucosal fluid.

235

236 **Statistics**

237 Descriptive statistics for the cohort of patients (stratified by mild versus severe
238 disease) and the HCW cohort are presented as median and interquartile ranges for
239 continuous variables, as well as numbers and percentages of total for categorical
240 variables. For the comparison of location parameters in two independent groups, the
241 Wilcoxon's rank sum test was used, in a version accounting for ties.³¹ For the
242 comparison of more than two independent groups, the non-parametric Kruskal-Wallis
243 test was used. Multiple linear regression models were used to quantify the association
244 between log-transformed IgA and IgG titers as outcomes as well as a set of pre-
245 defined independent variables. These included disease severity, age, duration of
246 symptoms (days), and patient comorbidities. Generalized additive models were used
247 to evaluate potential non-linear relationships of disease duration with the two
248 outcomes, as described above. Wilcoxon test was used to test for differences between
249 two continuous variables in the tables, and *P* values were adjusted using the Holm
250 method. Fisher's exact test was used for comparing two and Chi-square for comparing
251 three categorical variables. Non-parametric Spearman correlations were used for the
252 comparison between different immunoassays.

253 Statistical analyses were performed with R (version 3.6.1) and using the packages
254 "coin" and "mgcv". Graph-Pad Prism was used for visualization. *P* values in the
255 patient cohort were adjusted for multiple testing, using the method proposed by
256 Benjamini-Hochberg.³² Adjusted *P* values were considered statistically significant if
257 smaller than the significance level of $\alpha = 0.05$. In the HCW mucosal subgroup,
258 evidence was quantified on a continuous scale and these results were considered
259 exploratory.

260

Results

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

Serum samples of 64 RT-qPCR-confirmed mild ($n = 26$) and severe ($n = 38$) COVID-19 cases (**Table 1**) were assessed for IgA and IgG antibodies toward the SARS-CoV-2 S1 protein by using highly-specific immunoassays. The mean period between reported symptom onset and serum collection were 13.5 days (median 9 days) in the mild COVID-19 group and 20.2 days (median 15.5 days) in the severe COVID-19 group of patients, respectively (**Figure E1A**). In comparison to mild COVID-19, patients with severe disease had on average higher serum titers of S1-specific IgA ($P < 0.0001$) and IgG ($P < 0.0001$) (**Figure 1A**). In mild COVID-19 cases, serum titers of S1-specific IgA increased slightly ($P < 0.0001$) as a function of time from serum sampling to symptom onset (**Figure 1B**). Likewise, serum titers of S1-specific IgG increased moderately ($P = 0.002$) in mild cases (**Figure 1B**). These antibody responses revealed no significant pattern associated with patient age ($P = 0.15$ for IgA, and $P = 0.28$ for IgG) (**Figure 1C**), sex (**Figure E1B** and **E1C**), pre-existing 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 2**).

On average, positive S1-specific serum IgA titers became evident in mild COVID-19 cases ten days after symptom onset (**Figure 1B**). S1-specific serum IgA titers peaked in samples drawn at around three weeks from symptom onset, whereas in subjects tested later S1-specific serum IgA tended to be lower. As for S1-specific serum IgG

concentrations, these remained negative or reached positive values in mild COVID-19 patients around 12–14 days after symptom onset (**Figure 1B**).

In stark contrast to mild cases, patients suffering from severe COVID-19 showed a strong correlation of serum titers of S1-specific IgA with disease duration ($P = 0.0008$), which was even more pronounced for serum titers of S1-specific IgG ($P < 0.0001$) (**Figure 1B**). These antibody responses became positive on average in samples obtained on day 3–4 for IgA and day 4–5 for IgG, and they appeared to be independent of patient age ($P = 0.76$ for IgA, and $P = 0.76$ for IgG), sex, and comorbidities (**Figure 1B and 1C, Figure E1B and E1C, and Table 2**).

When grouped according to level of care, we observed in mild cases of COVID-19 that S1-specific serum IgA titers did not show any discernible pattern, whereas S1-specific serum IgG titers were higher in hospitalized compared to outpatient cases (**Figure 2A**). Thus, we next assessed disease severity and, as expected, younger patients tended to have milder disease, whereas older patients had more severe manifestations (**Figure E2**). There was no time-dependent pattern visible for S1-specific serum IgA, whereas S1-specific serum IgG titers showed a stronger increase over time in mild pneumonia cases versus those with mild illness (**Figure 2B**). Strikingly, very high titers (>25 optical density [OD] ratio) of SARS-CoV-2-specific serum IgA, but not serum IgG, correlated with severe ARDS (**Figure 2B and Figure E3**). 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 2**).

In summary, disease severity appears 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 two selected patients with mild COVID-19, as presented below.

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

We followed up two 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 on, followed by fever on day 1, and dysosmia on days 9–16. She (patient COV2-A0014) showed signs of fatigue and sore throat from day 0 on, fever between days 2–5, and cough on day 3 (**Figure 3A**).

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

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 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 7–14, became borderline positive on day 20, and clearly positive at an OD ratio of 4.5 on day 30 (**Figure 3C**). Conversely, S1-specific serum IgA titers in patient COV2-A0014 were borderline on day 4, 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 4–7, 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 (**Figure 3C**).

We compared these results to longitudinal analyses of S1-specific serum IgA and IgG values in two different situations. In asymptomatic controls, S1-specific serum IgA and IgG remained negative throughout the period of assessment, whereas in severe COVID-19 cases both antibody responses increased after day 4–5 and were markedly elevated on day 14–15 (**Figure E4**).

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

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

Having observed that in mild COVID-19 cases, 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; termed HCW cohort). These HCWs either did or did not have clinical symptoms suggestive of COVID-19, and they either tested negative or positive for SARS-CoV-2 in respiratory secretions by RT-qPCR. We grouped them as follows (**Figure 4**): (i) asymptomatic, RT-qPCR negative, with a recent history of SARS-CoV-2 exposure (Asymp/PCR-; n = 17); (ii) symptomatic, RT-qPCR negative (Symp/PCR-; n = 71); and (iii) symptomatic, RT-qPCR positive (Symp/PCR+; n = 21).

The Asymp/PCR- group contained very few S1-specific serum IgA-positive and no IgG-positive subjects (**Figure 5A**). Conversely, there were four out of 71 (6%) participants with positive IgA and IgG values found in the Symp/PCR- group, which likely represented individuals that had had a mild SARS-CoV-2 infection (**Figure 5A**). As expected, the Symp/PCR+ group contained more seropositive individuals,

with 8 out of 21 (38%) subjects having positive IgA and IgG titers for S1 of SARS-CoV-2 at the time of sampling (**Figure 5A**).

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, termed HCW mucosal subgroup (**Figure 4**). This subgroup also recorded self-reported clinical symptoms (**Table 3**). When assessing the Symp/PCR+ members of the HCW mucosal subgroup, a clear correlation was evident between positivity of S1-specific IgA and IgG in serum (**Figure 5B**) with the corresponding values in nasal secretions (**Figure 5C**). Thus, for S1-specific IgG, Symp/PCR+ members with positive serum titers also showed elevated titers of S1-specific IgG in nasal secretions (**Figure 5B and 5C**), possibly indicating transfer of S1-specific IgG from serum to the nasal mucosa. Conversely, the relationship of serum versus nasal fluid in Symp/PCR+ members was more variable for S1-specific IgA (**Figure 5B and 5C**).

To further investigate these findings, we adapted and used our two SARS-CoV-2 S protein-specific immunoassays (**Figure E5 and E6**) to assess the subjects in the HCW mucosal subgroup that tested negative for SARS-CoV-2-specific IgA or IgG in serum. Firstly, we ruled out an influence of time of sampling or total amount of detectable IgA and IgG in our samples. Mean time of sampling since symptom onset was 26.5 days for both Symp/PCR- and Symp/PCR+ groups of the HCW mucosal subgroup, whereas the Asymp/PCR- group was tested 11 days or more after exposure. Total IgA and IgG titers were comparable in serum samples as well as tear, nasal fluid, and saliva samples of all three groups of participants (**Figure E7**). Notably, whereas total IgG titers were measurable in nasal fluids, they were very low in tear fluid and saliva (**Figure E7**). Analyzing S protein-specific IgA and IgG in our mucosal samples, we observed a reliable correlation between our two immunoassays for serum IgA and

IgG, as well as tear and nasal fluid IgA, whereas the other measurements were less consistent and were thus not considered for our conclusions (**Figure E6**).

Interestingly, we were able to detect S protein-specific IgA in the mucosal samples of 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 total IgA values were average in nasal fluids of these individuals (**Figure 5D–5F** and **Figure 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 (**Figure 5D–5F** and **Figure E7**). When measuring tear fluid, subjects COV2-M0015 and COV2-M0033 presented with high S1-specific, ECD-specific, or RBD-specific IgA values (**Figure 5G–5I**). Additionally, a few other individuals also had detectable S protein-specific IgG in their nasal fluid despite being IgG seronegative (**Figure 5D–5F**). Notably, some mucosal samples showed comparable neutralizing capacity to serum in an *in vitro* neutralization assay of viable SARS-CoV-2 (**Figure E8**). These findings further supported our detection of humoral immune responses at mucosal sites in mild COVID-19 cases in the absence of serum antibodies toward SARS-CoV-2.

In contrast to total IgA titers, assessing S protein-specific IgA values in nasal fluid versus age in seronegative HCWs, we found an inverse correlation ($R^2_{adj} = 0.153$, $P = 0.037$). The same analysis with S protein-specific IgA titers in serum versus age, however, did not reveal a correlation ($P = 0.58$) (**Figure 5J–5L**). Interestingly, the longitudinal subject (COV2-A0014) with short disease duration, transient S protein-specific IgA and delayed IgG production also had high titers of S protein-specific IgA in her nasal fluid (**Figure E9**).

Collectively, in 15–20% 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 inversely correlated with patient age, suggesting increased mucosal antibody responses in younger SARS-CoV-2-exposed individuals.

Discussion

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

Conversely, in mild cases of SARS-CoV-2 infection, S protein-specific serum IgA production was transient, delayed or even absent, 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, i.e. serum, titers of these antibodies. Mucosal S protein-specific IgA titers, however, were even detectable at several mucosal sites of about 15–20% S protein-seronegative individuals in our cohort. Interestingly, mucosal S protein-specific IgA titers correlated inversely with patient age.

We think these findings suggest a model where the extent and duration of SARS-CoV-2-related clinical symptoms, which likely correlate with virus replication, dictate the level of virus-specific humoral immunity. This hypothesis is consistent with

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 our two longitudinal subjects, patient COV2-A0014 showed milder and shorter 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 comprise also children and infants, it is conceivable that children and infants have primed mucosal innate and IgA antibody responses due to 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 spectrum, previous studies have shown that 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, due to 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, be deceiving when estimating the epidemic spread of SARS-CoV-2. Our data suggest that, in addition to 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 severe cases and ARDS. Thus, our findings suggest four grades of antibody responses dependent on COVID-19 severity with (i) oligosymptomatic disease and mucosal antibody responses in the absence of systemic antibody production, (ii) mild-to-moderate disease and transient or delayed systemic IgA and IgG production, (iii) severe cases with high serum IgA and high IgG responses, and (iv) very severe cases, 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 correlated with virus neutralization in vitro,^{16,17,39} although some publications 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. Based on 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 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}

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Author contributions

CC and JN contributed to study design, patient recruitment, data collection, data analysis, data interpretation, and writing of the manuscript. YZ and AW contributed to patient recruitment, clinical management, data collection, data analysis, and data interpretation. AV, JS, SA, ME, SW, PPB, EDC, AA, GK, and EP-M contributed to experiments, data collection, data analysis, data interpretation. MER, SH, EB, AR, MS-H, LCH, ASZ, and DJS contributed to patient recruitment and clinical management. UH contributed to data analysis and data interpretation, particularly statistical analysis. SKR contributed to study design, patient recruitment, data collection, data analysis, and data interpretation. OB contributed to study conception and design, data analysis, data interpretation, and wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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Ethical approval

The study was approved by the Cantonal Ethics Committee of Zurich (BASEC #2016-01440 and #2020-00363).

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. *bioRxiv* 2020: <http://biorxiv.org/lookup/doi/10.1101/2020.04.27.062315>
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. *N Engl J Med* 2020.

- 552 8. Tong Z-D, Tang A, Li K-F, Li P, Wang H-L, Yi J-P, et al. Potential
553 Presymptomatic Transmission of SARS-CoV-2, Zhejiang Province, China,
554 2020. *Emerg Infect Dis.* 2020;26:1052–4.
- 555 9. Li R, Pei S, Chen B, Song Y, Zhang T, Yang W, et al. Substantial undocumented
556 infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-
557 2). *Science.* 2020;368:489–93.
- 558 10. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the
559 Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a
560 Report of 72 314 Cases From the Chinese Center for Disease Control and
561 Prevention. *JAMA.* 2020;323:1239.
- 562 11. Zhang J, Dong X, Cao Y, Yuan Y, Yang Y, Yan Y, et al. Clinical characteristics
563 of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy.*
564 2020;75:1730–41.
- 565 12. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of
566 critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-
567 centered, retrospective, observational study. *Lancet Respir Med.* 2020;8:475–81.
- 568 13. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT,
569 Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin
570 Definition. *JAMA.* 2012;307:2526–33.
- 571 14. WHO. Clinical management of severe acute respiratory infection when COVID-
572 19 is suspected. 2020: [https://www.who.int/publications-detail/clinical-](https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected)
573 [management-of-severe-acute-respiratory-infection-when-novel-coronavirus-](https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected)
574 [\(ncov\)-infection-is-suspected.](https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected)

- 575 15. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, et al.
576 Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.
577 Science. 2020;367:1260–1263.
- 578 16. To KK-W, Tsang OT-Y, Leung W-S, Tam AR, Wu T-C, Lung DC, et al.
579 Temporal profiles of viral load in posterior oropharyngeal saliva samples and
580 serum antibody responses during infection by SARS-CoV-2: an observational
581 cohort study. Lancet Infect Dis. 2020;20:565–74.
- 582 17. Ni L, Ye F, Cheng M-L, Feng Y, Deng Y-Q, Zhao H, et al. Detection of SARS-
583 CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent
584 Individuals. Immunity. 2020;52:971-977.e3.
- 585 18. Schulz KS, Mossman KL. Viral Evasion Strategies in Type I IFN Signaling – A
586 Summary of Recent Developments. Front Immunol. 2016;7:498.
- 587 19. Hu Y, Li W, Gao T, Cui Y, Jin Y, Li P, et al. The Severe Acute Respiratory
588 Syndrome Coronavirus Nucleocapsid Inhibits Type I Interferon Production by
589 Interfering with TRIM25-Mediated RIG-I Ubiquitination. J Virol 2017;91.
- 590 20. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, Uhl S, Hoagland D, Møller R, et
591 al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-
592 19. Cell. 2020;181:1036-1045.e9.
- 593 21. Amanna IJ, Slifka MK. Mechanisms that determine plasma cell lifespan and the
594 duration of humoral immunity: Long-term antibody production. Immunol Rev.
595 2010;236:125–38.

- 596 22. Azkur AK, Akdis M, Azkur D, Sokolowska M, Veen W, Brüggen M, et al.
597 Immune response to SARS-CoV-2 and mechanisms of immunopathological
598 changes in COVID-19. *Allergy*. 2020;75:1564–81.
- 599 23. Callow KA, Parry HF, Sergeant M, Tyrrell DAJ. The time course of the immune
600 response to experimental coronavirus infection of man. *Epidemiol Infect*.
601 1990;105:435–46.
- 602 24. Bryant JE, Azman AS, Ferrari MJ, Arnold BF, Boni MF, Boum Yap, et al.
603 Serology for SARS-CoV-2: Apprehensions, opportunities, and the path forward.
604 *Sci Immunol*. 2020;5:eabc6347.
- 605 25. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et
606 al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked
607 by a Clinically Proven Protease Inhibitor. *Cell*. 2020;181:271-280.e8.
- 608 26. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike
609 glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity
610 with SARS-CoV. *Nat Commun*. 2020;11:1620.
- 611 27. Swissnoso. Recommendations for healthcare workers, having had unprotected
612 close contact with COVID-19 cases. Available from :
613 [https://www.swissnoso.ch/fileadmin/swissnoso/Dokumente/5_Forschung_und_E](https://www.swissnoso.ch/fileadmin/swissnoso/Dokumente/5_Forschung_und_Entwicklung/6_Aktuelle_Ereignisse/200526_management_of_HCW_with_COVID-19_contact_V4.0.pdf)
614 [ntwicklung/6_Aktuelle_Ereignisse/200526_management_of_HCW_with_COVI](https://www.swissnoso.ch/fileadmin/swissnoso/Dokumente/5_Forschung_und_Entwicklung/6_Aktuelle_Ereignisse/200526_management_of_HCW_with_COVID-19_contact_V4.0.pdf)
615 [D-19_contact_V4.0.pdf](https://www.swissnoso.ch/fileadmin/swissnoso/Dokumente/5_Forschung_und_Entwicklung/6_Aktuelle_Ereignisse/200526_management_of_HCW_with_COVID-19_contact_V4.0.pdf) [Accessed 5 October 2020].
- 616 28. Kaegi C, Wuest B, Schreiner J, Steiner UC, Vultaggio A, Matucci A, et al.
617 Systematic Review of Safety and Efficacy of Rituximab in Treating Immune-
618 Mediated Disorders. *Front Immunol*. 2019;10:1990.

- 619 29. Meyer B, Torriani G, Yerly S, Mazza L, Calame A, Arm-Vernez I, et al.
620 Validation of a commercially available SARS-CoV-2 serological immunoassay.
621 Clin Microbiol Infect. 2020;26:1386–94.
- 622 30. Emmenegger M, De Cecco E, Lamparter D, Jacquat RPB, Ebner D, Schneider
623 MM, et al. Early peak and rapid decline of SARS-CoV-2 seroprevalence in a
624 Swiss metropolitan region. medRxiv 2020:
625 <https://www.medrxiv.org/content/10.1101/2020.05.31.20118554v4>.
- 626 31. Hollander M, Wolfe DA. Nonparametric Statistical Methods. Second Edition ed:
627 John Wiley & Sons; 1999.
- 628 32. Thissen D, Steinberg L, Kuang D. Quick and Easy Implementation of the
629 Benjamini-Hochberg Procedure for Controlling the False Positive Rate in
630 Multiple Comparisons. J Educ Behav Stat. 2002;27:77–83.
- 631 33. Liu Y, Yan L-M, Wan L, Xiang T-X, Le A, Liu J-M, et al. Viral dynamics in
632 mild and severe cases of COVID-19. Lancet Infect Dis. 2020;20:656–7.
- 633 34. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors
634 for mortality of adult inpatients with COVID-19 in Wuhan, China: a
635 retrospective cohort study. The Lancet. 2020;395:1054–62.
- 636 35. Brodin P, Davis MM. Human immune system variation. Nat Rev Immunol.
637 2017;17:21–9.
- 638 36. Chan CM, Tse H, Wong SSY, Woo PCY, Lau SKP, Chen L, et al. Examination
639 of seroprevalence of coronavirus HKU1 infection with S protein-based ELISA

- 640 and neutralization assay against viral spike pseudotyped virus. J Clin Virol.
641 2009;45:54–60.
- 642 37. van Deursen JM. The role of senescent cells in ageing. Nature. 2014;509:439–
643 46.
- 644 38. Goronzy JJ, Weyand CM. Successful and Maladaptive T Cell Aging. Immunity.
645 2017;46:364–78.
- 646 39. Ng KW, Faulkner N, Cornish GH, Rosa A, Harvey R, Hussain S, et al. Pre-
647 existing and *de novo* humoral immunity to SARS-CoV-2 in humans. bioRxiv
648 2020: <http://biorxiv.org/lookup/doi/10.1101/2020.05.14.095414>
- 649 40. Liu L, Wei Q, Lin Q, Fang J, Wang H, Kwok H, et al. Anti-spike IgG causes
650 severe acute lung injury by skewing macrophage responses during acute SARS-
651 CoV infection. JCI Insight 2019; 4.
- 652 41. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19:
653 immunity, inflammation and intervention. Nat Rev Immunol. 2020;20:363–74.
- 654 42. Yu H, Sun B, Fang Z, Zhao J, Liu X, Li Y, et al. Distinct features of SARS-
655 CoV-2-specific IgA response in COVID-19 patients. Eur Respir J.
656 2020;56:2001526.
- 657 43. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 Critically
658 Ill Patients With COVID-19 With Convalescent Plasma. JAMA. 2020;323:1582.
- 659 44. Chevrier S, Zurbuchen Y, Cervia C, Adamo S, Raeber ME, de Souza N, et al. A
660 distinct innate immune signature marks progression from mild to severe

661 COVID-19. bioRxiv 2020:
662 <http://biorxiv.org/lookup/doi/10.1101/2020.08.04.236315>

663 45. Adamo S, Chevrier S, Cervia C, Zurbuchen Y, Raeber ME, Yang L, et al.
664 Lymphopenia-induced T cell proliferation is a hallmark of severe COVID-19.
665 bioRxiv 2020: <http://biorxiv.org/lookup/doi/10.1101/2020.08.04.236521>

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667

Table 1. Demographic and clinical characteristics of the patient cohort.

Patients were divided into mild versus severe COVID-19 cases. Disease severity was defined according to the WHO classification¹⁴. Categorical values between mild and severe COVID-19 cases

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were compared using Fisher's exact test and continuous variables were compared using non-parametric Wilcoxon test.

	Mild cases (n = 26)	Severe cases (n = 38)	Total (n = 64)	P value
Median age (median (IQR ^a) [yrs])	46.0 (31.50 - 48.50)	67.5 (59.0-79.0)	59.5 (42.75-75.25)	<0.0001
Gender (m/f)	11/15	24/14	35/29	0.1282
COVID-19 disease severity sampling				
Mild illness– no. (%)	17 (65.4)	-	17 (26.6)	-
Mild pneumonia – no. (%)	9 (34.6)	-	9 (14.1)	-
Severe pneumonia – no. (%)	-	20 (52.6)	20 (31.3)	-
Mild ARDS – no. (%)	-	7 (18.4)	7 (10.9)	-
Moderate ARDS – no. (%)	-	7 (18.4)	7 (10.9)	-
Severe ARDS – no. (%)	-	4 (10.5)	4 (6.3)	-
Level of care at blood sampling timepoint				
Outpatient – no. (%)	14 (53.8)	-	14 (21.9)	<0.0001
Hospitalized – no. (%)	12 (46.2)	38 (100)	50 (78.1)	<0.0001
Comorbidities				
Hypertension – no. (%)	6 (23.1)	22 (57.9)	28 (43.8)	0.0098
Diabetes – no. (%)	4 (15.4)	12 (31.6)	16 (25)	0.2393
Heart disease – no. (%)	2 (7.7)	17 (44.7)	19 (29.7)	0.0018
Cerebrovascular disease – no. (%)	1 (3.8)	4 (10.5)	5 (7.8)	0.6404
Lung disease – no. (%)	3 (11.5)	6 (15.8)	9 (14.1)	0.7275
Kidney disease – no. (%)	6 (23.1)	10 (26.3)	16 (25)	>0.9999
Malignancy – no. (%)	-	4 (10.5)	4 (6.3)	0.1397
Systemic Immunosuppression – no. (%)	2 (7.7)	4 (10.5)	6 (9.4)	>0.9999
Immunosuppression				
Glucocorticoids – no. (%)	2 (7.7)	4 (10.5)	6 (9.4)	>0.9999
Mycophenolate Mofetil – no. (%)	1 (3.8)	-	1 (1.6)	0.4062
Calcineurin Inhibitors – no. (%)	1 (3.8)	1 (2.6)	2 (3.1)	>0.9999
Azathioprin – no. (%)	1 (3.8)	2 (5.3)	3 (4.7)	>0.9999
Leflunomide – no. (%)	1 (3.8)	-	1 (1.6)	0.4062
Mesalazine – no. (%)	1 (3.8)	-	1 (1.6)	0.4062
^a IQR denotes the interquartile range				
^b COVID-19 severity at sampling according to WHO guidelines ¹⁴				

Table 2. Linear models for prediction of IgA and IgG serum titers.

(A–B) Multiple linear model of S1 protein-specific IgA serum titers (A; logarithmized) and IgG serum titers (B; 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).

SARS-CoV-2-specific IgA serum titers	Coefficient	95% - Confidence Interval	P value
Intercept	-0.90	-1.92 to 0.12	0.083
Severe disease	1.35	0.74 to 1.96	< 0.0001
Days	0.053	0.03 to 0.08	< 0.0001
Age	0.011	-0.01 to 0.03	0.31
Hypertension	0.27	-0.48 to 1.02	0.47
Diabetes	-0.23	-0.89 to 0.42	0.48
Heart Disease	-0.18	-0.87 to 0.52	0.61
Lung disease	-0.18	-0.95 to 0.59	0.63
Malignancy	-1.77	-2.87 to -0.67	0.002
Cerebrovascular disease	0.24	-0.81 to 1.30	0.64
Kidney disease	-0.17	-0.88 to 0.53	0.63
Immunosuppression	-0.44	-1.46 to 0.57	0.39

SARS-CoV-2-specific IgG serum titers	Coefficient	95% - Confidence Interval	P value
Intercept	-1.66	-2.81 to -0.51	0.005
Severe disease	1.42	0.73 to 2.11	0.0001
Days	0.07	0.04 to 0.10	< 0.0001
Age	0.0012	-0.02 to 0.03	0.92
Hypertension	0.41	-0.43 to 1.26	0.33
Diabetes	-0.19	-0.92 to 0.55	0.62
Heart Disease	-0.13	-0.91 to 0.65	0.73
Lung disease	-0.31	-1.18 to 0.55	0.47
Malignancy	-0.89	-2.13 to 0.34	0.15
Cerebrovascular disease	-0.44	-1.63 to 0.75	0.46
Kidney disease	0.21	-0.58 to 1.01	0.59
Immunosuppression	-0.76	-1.90 to 0.39	0.19

670

A

B

Table 3. Demographic and clinical characteristics of healthcare worker cohort.

(A) Healthcare worker cohort (HCW cohort) included in the S protein-specific IgA and IgG serology study.

(B) Healthcare worker subgroup (HCW mucosal subgroup) assessed in the S protein-specific IgA and IgG mucosal fluid study. Categorical values were compared using Fisher's exact test or Chi-square if more than two groups were being compared. Continuous variables were compared using Kruskal-Wallis test.

A					
HCW Cohort Characteristics	Asymptomatic/PCR- (n = 17)	Symptomatic/PCR- (n = 71)	Symptomatic/PCR+ (n = 21)	Total (n = 109)	P value
Median age (median (IQR ^a) [yrs])	39 (34 - 44)	36 (30 - 41)	38 (30 - 48)	36 (30 - 43)	0.4156
Gender (m/f)	6/11	16/55	3/18	25/84	0.3066
^a IQR denotes the interquartile range					

671

B					
HCW Mucosal Subgroup Characteristics	Asymptomatic/PCR- (n = 9)	Symptomatic/PCR- (n = 13)	Symptomatic/PCR+ (n = 11)	Total (n = 33)	P value
Median age (median (IQR ^a) [yrs])	38 (36-44)	40 (32-49)	38 (30-42)	39 (31-43)	0.8
Gender (m/f)	4/5	6/7	3/8	13/20	0.5999
Reported symptoms					
Fatigue – no. (%)	-	6 (46.2)	7 (63.6)	13 (39.4)	0.4442
Body temperature > 38.0°C – no. (%)	-	4 (30.8)	1 (9.1)	5 (15.2)	0.3271
Feeling feverish – no. (%)	-	6 (46.2)	4 (36.4)	10 (30.3)	0.6968
Chills – no. (%)	-	1 (7.7)	2 (18.2)	3 (9.1)	0.5761
Shivering – no. (%)	-	3 (23.1)	4 (36.4)	7 (21.2)	0.6591
Body aches – no. (%)	-	8 (61.5)	8 (72.7)	16 (48.5)	0.6792
Back pain – no. (%)	-	5 (38.5)	4 (36.4)	9 (27.3)	>0.999
Cough – no. (%)	-	5 (38.5)	6 (54.5)	11 (33.3)	0.6824
Dyspnea – no. (%)	-	2 (15.4)	4 (36.4)	6 (18.2)	0.3572
Pleuritis – no. (%)	-	3 (23.1)	4 (36.4)	7 (21.2)	0.6591
Sore throat – no. (%)	-	11 (84.6)	6 (54.5)	17 (51.5)	0.1819
Coryza – no. (%)	-	7 (53.8)	6 (54.5)	13 (39.4)	>0.999
Hoarseness – no. (%)	-	5 (38.5)	4 (36.4)	9 (27.3)	>0.999
Anosmia/Dysosmia – no. (%)	-	2 (15.4)	8 (72.7)	10 (30.3)	0.0111
Diarrhea – no. (%)	-	5 (38.5)	2 (18.2)	7 (21.2)	0.3864
Nausea – no. (%)	-	3 (23.1)	3 (27.3)	6 (18.2)	>0.999
Conjunctivitis – no. (%)	-	2 (15.4)	-	2 (6.1)	0.4819
^a IQR denotes the interquartile range					

672

673 **Figure legends**

674 **Figure 1. Influence of COVID-19 severity, disease duration, and patient age on**
675 **SARS-CoV-2-specific serum IgA and IgG titers. (A)** Comparison of SARS-CoV-2
676 spike (S) protein subunit S1-specific serum IgA and IgG titers (optical density [OD]
677 ratio) in mild (n = 26) versus severe COVID-19 cases (n = 38). Average time between
678 reported symptom onset and sample collection was 13.5 (median 9 days) in mild and
679 20.2 (median 15.5 days) days in severe cases. **(B)** Generalized additive modeling of
680 S1-specific IgA and IgG serum titers as a function of days between reported symptom
681 onset and sample collection in mild (n = 26) versus severe COVID-19 cases (n = 38).
682 Dashed lines indicate borders between positive and borderline/negative serum values
683 of S1-specific IgA (top) and IgG (bottom). **(C)** Linear modeling of S1-specific IgA
684 and IgG serum titers as a function of patient age in mild (n = 26) versus severe cases
685 (n = 38). *P* values and adjusted R^2 (R^2_{adj}) of linear and generalized additive models
686 were computed with logarithmized IgA/IgG titers.

687

Figure 2. S protein-specific serum antibodies compared to level of care and disease severity. (A) Level of patient care at 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 mild cases ($n = 26$). Severe cases were all hospitalized and are thus not depicted. (B) Disease severity at 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 mild ($n = 26$) versus severe cases ($n = 38$).

Figure 3. Longitudinal study of two mild cases of COVID-19. (A) Time of reporting of indicated symptoms in two mild COVID-19 patients, including a 42-year old male (COV2-A0013; left panels) and a 42-year old female (COV2-A0014; right panels). (B) Cycle threshold (Ct) values of SARS-CoV-2 quantitative reverse-transcriptase polymerase chain reaction (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 cut-offs for positive, borderline and negative serum values of S1-specific IgA (top) and IgG (bottom), with the gray shaded area highlighting the borderline values.

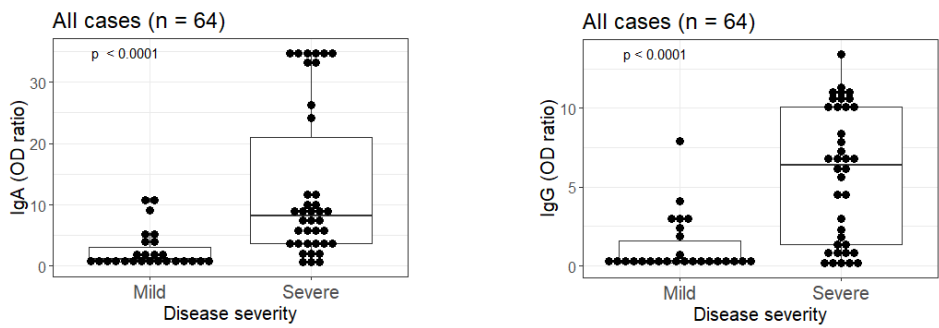
Figure 4. Flow chart showing characterization of healthcare worker cohort. (A)

We grouped our healthcare worker (HCW) cohort ($n = 109$) as follows: (i) asymptomatic, PCR negative (Asymp/PCR- $n = 17$) reporting exposure (see Methods) to a COVID-19 patient 11–24 days prior to sampling; (ii) symptomatic, PCR negative (Symp/PCR-; $n = 71$); and (iii) symptomatic, PCR positive (Symp/PCR+; $n = 21$). All HCWs were analyzed for SARS-CoV-2 S1-specific serum IgA and IgG values. In a subgroup (termed HCW mucosal subgroup), tears, nasal fluid, saliva, and serum were collected simultaneously. Self-reported symptoms were recorded of each participant of the HCW mucosal subgroup. The 33 HCWs of the HCW mucosal subgroup were grouped the same way as the HCW cohort: (i) Asymp/PCR- ($n = 9$); (ii) Symp/PCR- ($n = 13$), sampled on average 26.5 days after symptom onset; and (iii) Symp/PCR+ ($n = 11$), sampled on average 26.5 days after symptom onset.

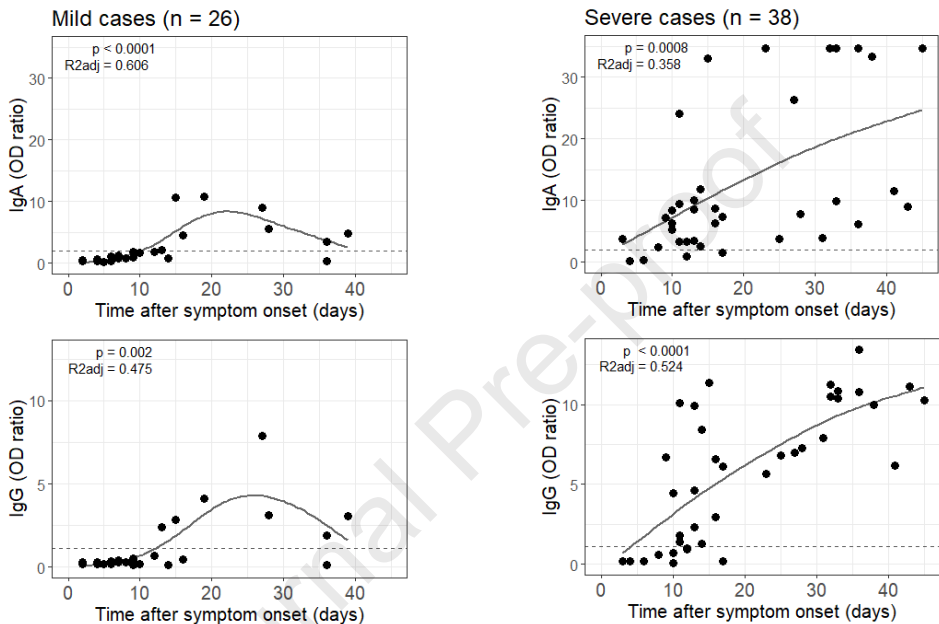
Figure 5. Analysis of SARS-CoV-2 S protein-specific IgA and IgG responses in serum and mucosal fluids.

(A) S protein-specific IgA (top) and IgG (bottom) serum titers in HCW cohort (n = 109). Dashed lines indicate borders between positive (red), borderline (gray) and negative (blue) values, with gray shaded area showing borderline values. (B–C) S protein-specific IgA (top) and IgG (bottom) serum (B) and nasal fluid (C) titers of Symp/PCR+ individuals (n = 11) in HCW mucosal subgroup. Comparison of HCWs with negative, borderline, and positive values. (D–F) S protein-specific IgA (top) and IgG (bottom) titers in nasal fluid, including S1-specific (D), SARS-CoV-2 S protein extracellular domain (ECD)-specific (E), and S1 protein receptor-binding domain (RBD)-specific IgA and IgG (F) of S1 protein-seronegative individuals in HCW mucosal subgroup. Comparison of Asymp/PCR-, 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 HCW mucosal subgroup. (J–L) Linear modeling of S1 protein-specific IgA titers in serum (J), nasal fluids (K), and total IgA in nasal fluids (L), as a function of age in S1 protein-seronegative individuals of HCW mucosal subgroup.

A



B



C

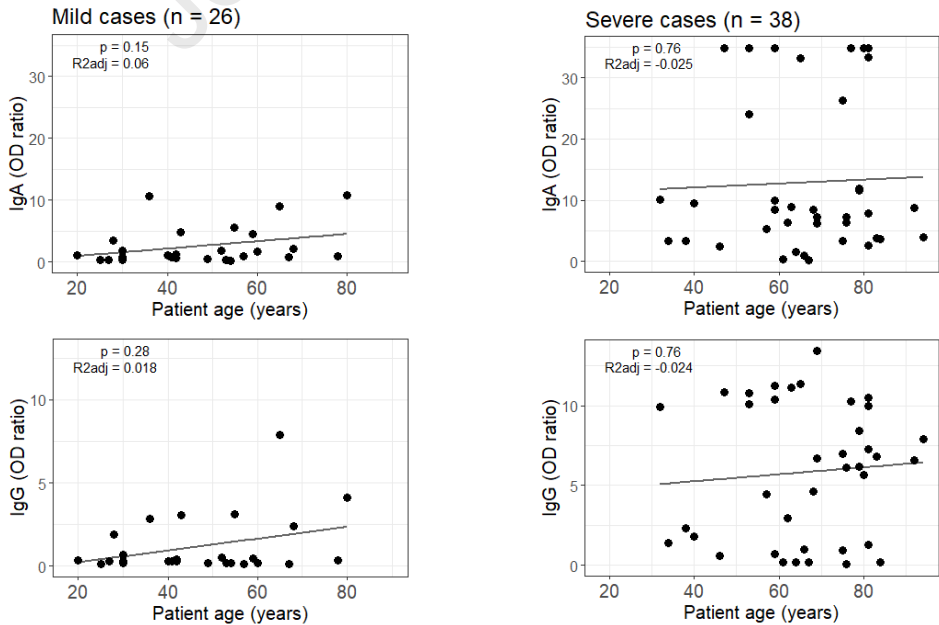
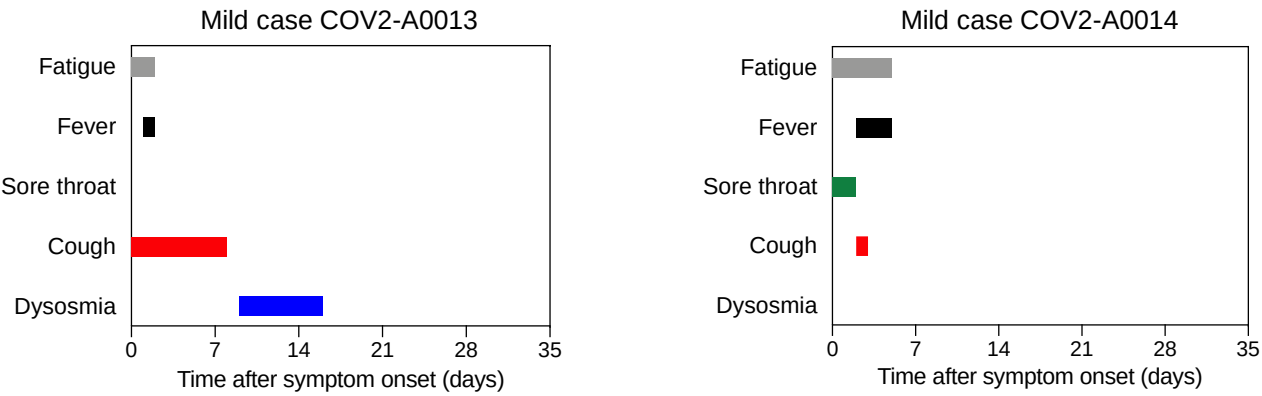


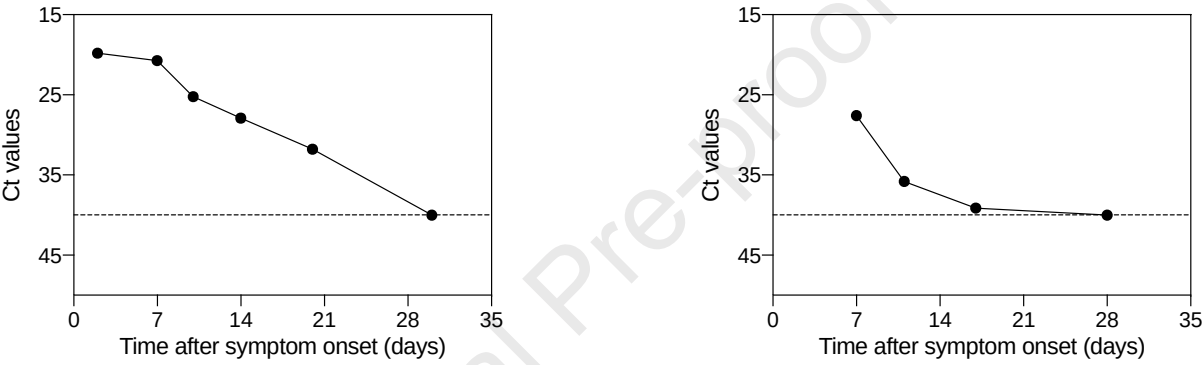
Figure 1

Figure 2

A



B



C

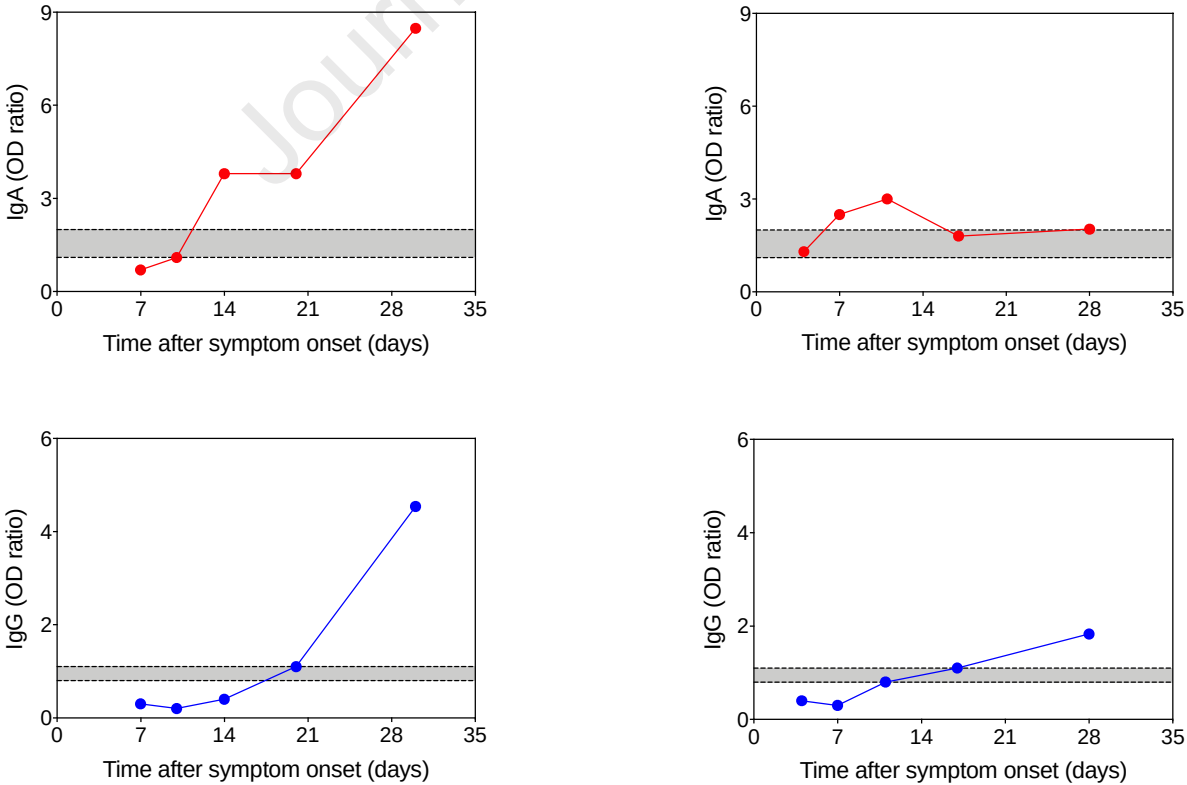


Figure 3

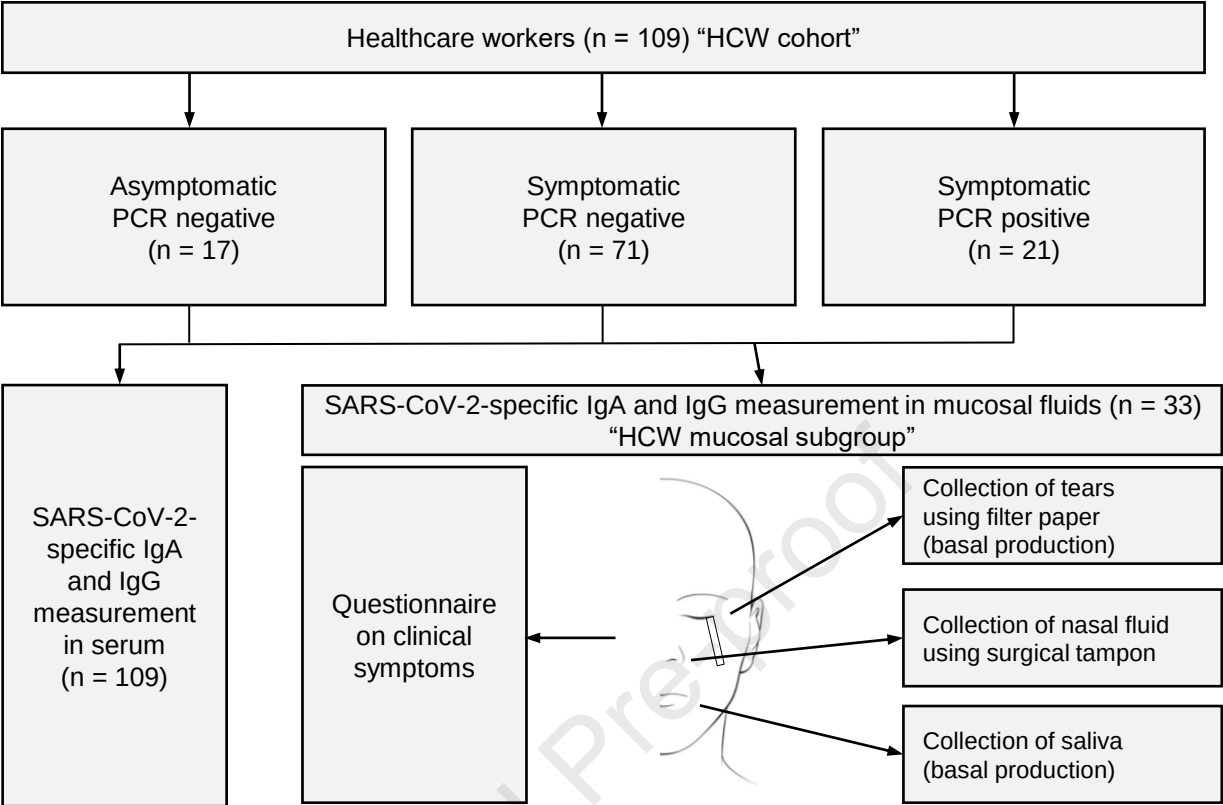


Figure 4

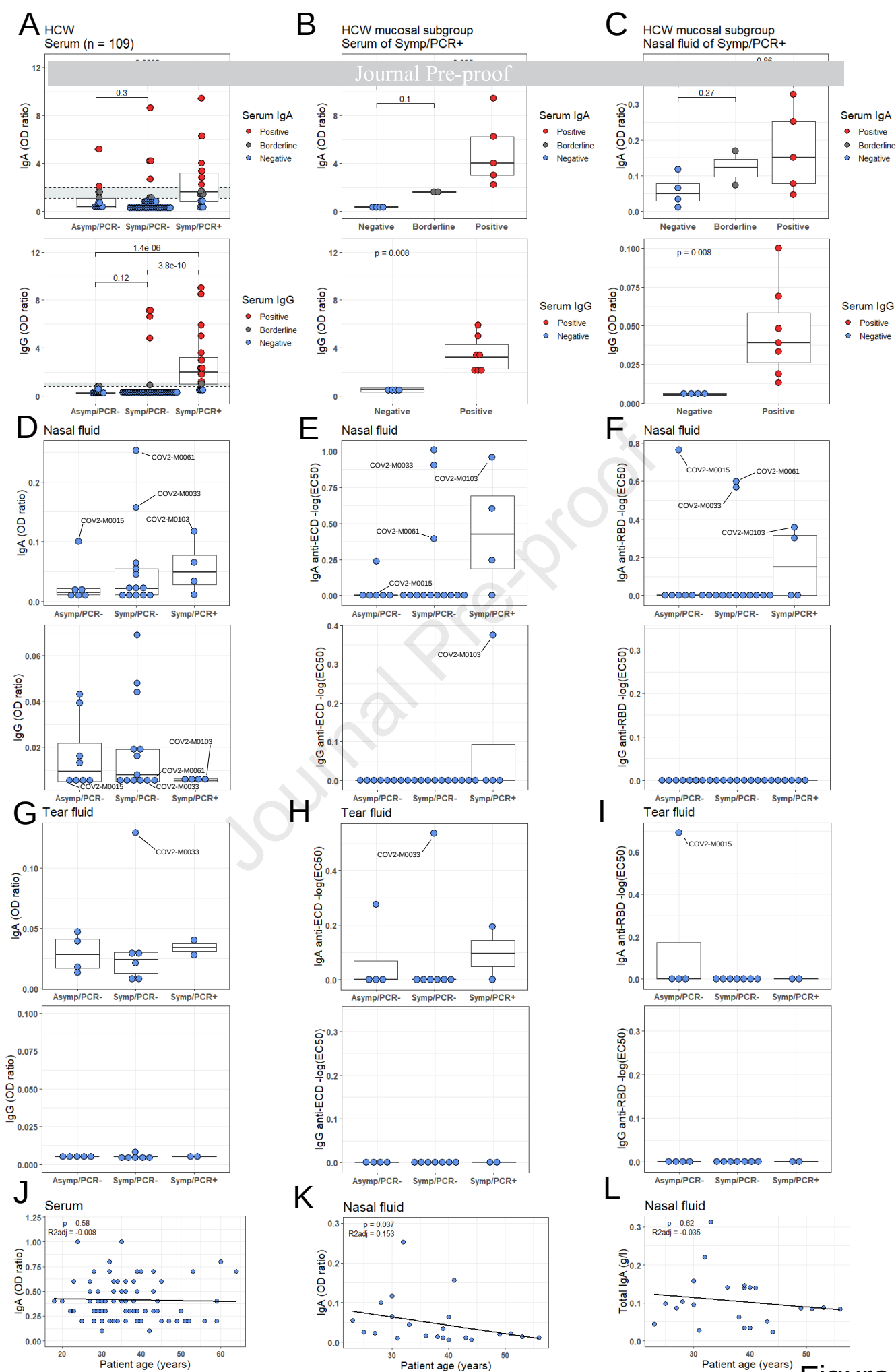
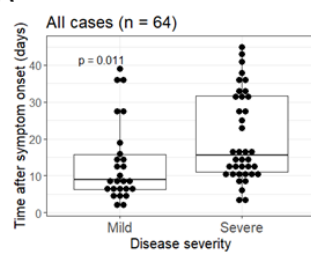
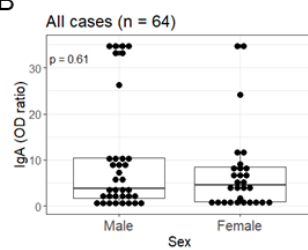


Figure 5

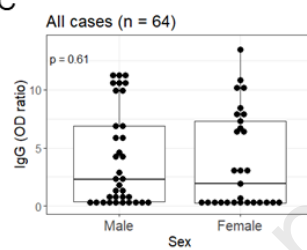
A

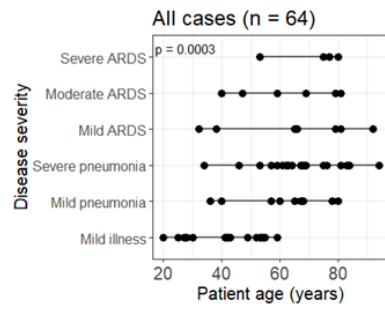


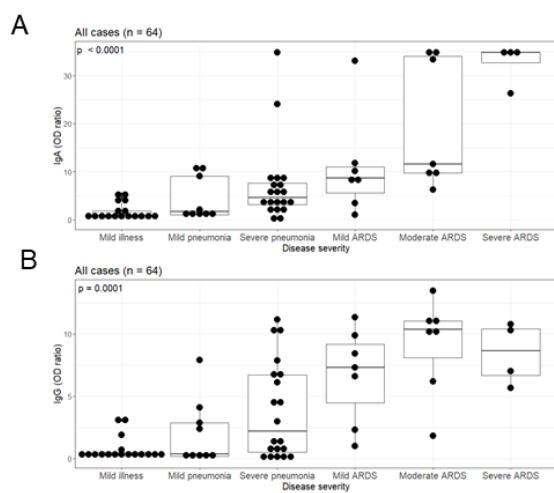
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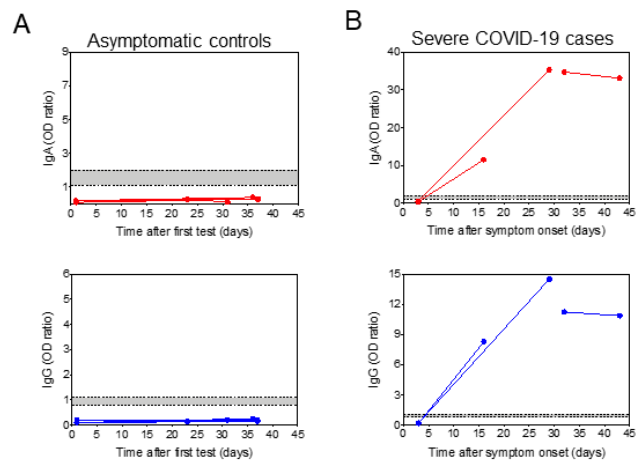


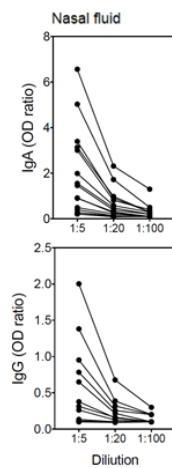
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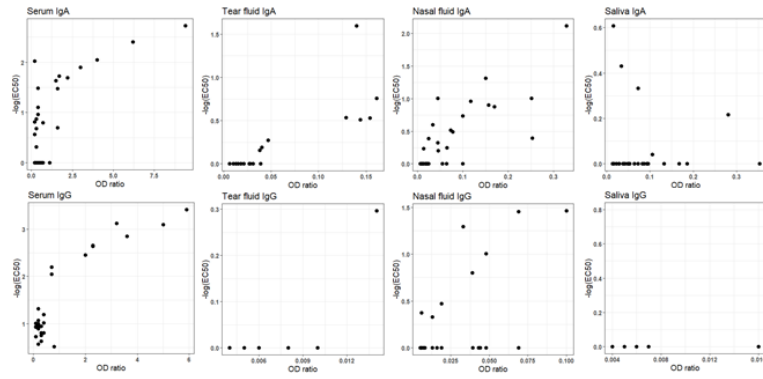


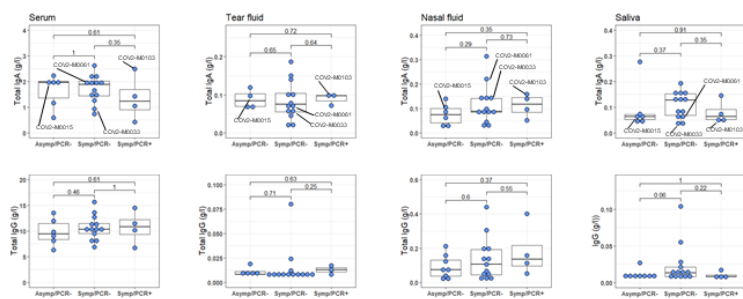


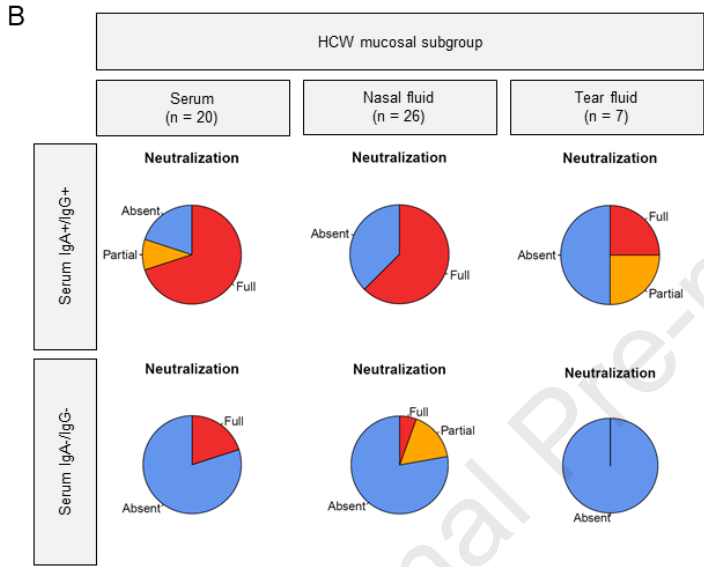
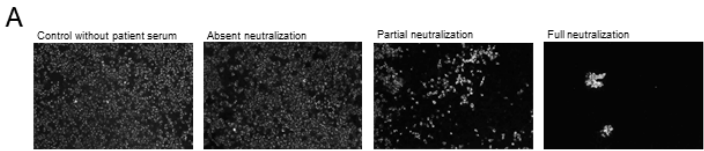


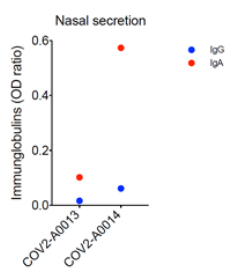












Online Repository Material Figure Legends to:**Systemic and mucosal antibody response specific to SARS-CoV-2 during mild versus severe COVID-19**

Carlo Cervia, MMed,^{1†} Jakob Nilsson, MD, PhD,^{1†} Yves Zurbuchen, MMed,¹ Alan Valaperti, PhD,¹ Jens Schreiner, PhD,¹ Aline Wolfensberger, MD,² Miro E. Raeber, MMed,¹ Sarah Adamo, MMed,¹ Sebastian Weigang, MSc,³ Marc Emmenegger, MSc,⁴ Sara Hasler,¹ Philipp P. Bosshard, PhD,⁵ Elena De Cecco, PhD,⁴ Esther Bächli, MD,⁶ Alain Rudiger, MD,⁷ Melina Stüssi-Helbling, MD,⁸ Lars C. Huber, MD,⁸ Annelies S. Zinkernagel, MD, PhD,² Dominik J. Schaer, MD,⁹ Adriano Aguzzi, MD,⁴ Georg Kochs, PhD,^{3, 10} Ulrike Held, PhD,¹¹ Elsbeth Probst-Müller, MD, PhD,¹ Silvana K. Rampini, MD,⁹ and Onur Boyman, MD^{1,12 *}

Figure E1. S protein-specific serum IgA and IgG values compared to sampling timepoint, disease severity, patient age, and sex.

(A) Comparison of days between reported symptom onset and sample collection in mild ($n = 26$) versus severe COVID-19 cases ($n = 38$). (B) Visualization of age distribution in the generalized additive models of S1 protein-specific IgA and IgG serum titers as function of days between reported symptom onset and sample collection. Comparison of mild ($n = 26$) versus severe cases ($n = 38$). (C–D) Comparison of S1 protein-specific serum IgA (C) and IgG (D) titers in male ($n = 35$) versus female ($n = 29$) COVID-19 patients. Average days between reported symptom onset and sample collection were 17 (median 13) in male and 18 (median 14) days in female patients.

Figure E2. Distribution of disease severity and age of COVID-19 patients.

Comparison in all COVID-19 cases ($n = 64$) of patient age distribution to COVID-19 severity at time of sample collection, ranging from mild COVID-19 to severe ARDS, as defined by the WHO classification criteria¹⁴. P-value was computed using Kruskal-Wallis test.

Figure E3. S protein-specific serum IgA and IgG values compared to severity of symptoms of COVID-19 patients.

(A–B) Comparison of S1 protein-specific serum IgA (A) and IgG (B) titers with disease severity in our COVID-19 patient cohort (n = 64), ranging from mild COVID-19 to severe ARDS, as defined by the WHO classification criteria¹⁴. Data are shown as boxplots. Each dot represents an independent and unrelated donor. The significance of between-group differences was explored by using Kruskal-Wallis test.

Figure E4. Longitudinal measurement of S protein-specific serum IgA and IgG values in asymptomatic controls and severe cases of COVID-19.

(A–B) S1 protein-specific serum IgA (top) and IgG (bottom) titers in asymptomatic donors (A; n = 4) and severe cases of COVID-19 (B; n = 3). The connected dots represent sequential measurements of the same individual.

Figure E5. Titration of nasal fluids to detect S protein-specific IgA and IgG.

Measurement of S1 protein-specific IgA (top) and IgG (bottom) using different dilutions of nasal fluids in a subset of the HCW mucosal subgroup (n = 15).

Figure E6. Comparison of immunoassays to measure S protein-specific IgA and IgG in samples of serum, tears, nasal fluid, and saliva.

Comparison of optical density (OD) ratios of IgA (top) and IgG (bottom) obtained with a commercial enzyme-linked immunosorbent assay specific for the S1 protein of SARS-CoV-2 (x-axes) and the inflection point of the sigmoidal curve ($-\log(\text{EC}_{50})$; y-axes), the latter determined by measuring IgA (top) and IgG (bottom) against SARS-CoV-2 S protein extracellular domain (ECD) and SARS-CoV-2 S1 protein receptor-binding domain (RBD) in serial dilutions using an in-house immunoassay (see Methods). S protein-specific IgA (top) and IgG (bottom) were measured in serum, tear fluid, nasal fluid, and saliva of the healthcare worker mucosal subgroup (HCW mucosal subgroup). Data are shown as scatterplots. Each dot represents an independent and unrelated donor. Spearman's correlation coefficient (Rho) is shown with the corresponding p value.

Figure E7. Analysis of total IgA and IgG serum titers in HCW mucosal subgroup.

Total IgA (top) and IgG (bottom) titers in serum, tear fluid, nasal fluid, and saliva were assessed in individuals of the HCW mucosal subgroup that tested negative for S1 protein-specific serum IgA (top) and IgG (bottom). Comparison of Asymp/PCR-, Symp/PCR- and Symp/PCR+ HCWs is shown. Four PCR-negative healthcare workers with negative S protein-specific IgA values in serum, but increased S protein-specific IgA titers in nasal fluids, are labeled with their corresponding study code. The significance of between-group differences was explored by using Wilcoxon test.

Figure 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 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 serum.

Figure E9. Mucosal S protein-specific IgA and IgG in two mild COVID-19 cases.

Shown are S1 protein-specific IgA and IgG titers in nasal fluids of patients COV2-A0013 and COV2-A0014 (see Figure 3) on day 25.