

Comparing Immunoassays for SARS-CoV-2 Antibody Detection in Patients with and without Laboratory-Confirmed SARS-CoV-2 Infection

Karoline Leuzinger,^{a,b} Michael Osthoff,^c Sarah Dräger,^c Hans Pargger,^d Martin Siegemund,^d Stefano Bassetti,^c Roland Bingisser,^e Christian H. Nickel,^e Sarah Tschudin-Sutter,^f Nina Khanna,^f Katharina Rentsch,^g Manuel Battegay,^f Adrian Egli,^{h,i}  Hans H. Hirsch^{a,b,f}

^aClinical Virology, University Hospital Basel, Basel, Switzerland

^bTransplantation & Clinical Virology, Department of Biomedicine, University of Basel, Basel, Switzerland

^cInternal Medicine, University Hospital Basel, Basel, Switzerland

^dIntensive Care Unit, University Hospital Basel, Basel, Switzerland

^eEmergency Medicine, University Hospital Basel, Basel, Switzerland

^fInfectious Diseases & Hospital Epidemiology, University Hospital Basel

^gClinical Chemistry, Laboratory Medicine, University Hospital Basel, Basel, Switzerland

^hApplied Microbiology Research, Department of Biomedicine, University of Basel, Basel, Switzerland

ⁱClinical Bacteriology and Mycology, University Hospital Basel, Basel, Switzerland

ABSTRACT Commercially available SARS-CoV-2-directed antibody assays may assist in diagnosing past exposure to SARS-CoV-2 antigens. We cross-compared the following eight immunoassays detecting antibodies against SARS-CoV-2 nucleocapsid (N) or spike (S) antigens in three cohorts consisting of 859 samples from 622 patients: (#1) EDI novel coronavirus COVID-19 (Epitope), (#2) RecomWell SARS-CoV-2 (Mikrogen), (#3) COVID-19 ELISA (VirCell), (#4) Elecsys anti-SARS-CoV-2 N (Roche), (#5) Liaison SARS-CoV-2 S1/S2 (DiaSorin), (#6) anti-SARS-CoV-2 ELISA (EuroImmun), (#7) Elecsys anti-SARS-CoV-2 S (Roche), and (#8) Liaison SARS-CoV-2 TrimericS (DiaSorin). In cross-sectional cohort 1 (68 sera from 38 patients with documented SARS-CoV-2 infection), agreement between assays #1 to #6 ranged from 75% to 93%, whereby discordance mostly resulted from N-based assays #1 to #4. In cross-sectional cohort 2 (510 sera from 510 patients; 56 documented, 454 unknown SARS-CoV-2 infection), assays #4 to #6 were analyzed further together with assays #7 and #8, revealing 94% concordance (44 [9%] positives and 485 [85%] negatives). Discordance was highest within 2 weeks after SARS-CoV-2/COVID-19 diagnosis and confirmed in the longitudinal cohort 3 (281 sera from 74 COVID-19 patients), using assays #4, #6, #7, and #8. Subanalysis of 20 (27%) initially seronegative cohort 3 patients revealed assay-dependent 50% and 90% seroconversion rates after 8 to 11 days and 14 to 18 days, respectively. Increasing SARS-CoV-2 antibodies were significantly associated with declining levels of viral loads, lactate dehydrogenase, interleukin-6, and C-reactive protein and preceded clearance of SARS-CoV-2 detection in the upper respiratory tract by approximately 1 week. SARS-CoV-2-specific antibody assays show substantial agreement, but interpretation of qualitative and semiquantitative results depends on the time elapsed postdiagnosis and the choice of viral antigen. Mounting of systemic SARS-CoV-2-specific antibodies may predict recovery from viral injury and clearance of mucosal replication.

KEYWORDS COVID-19, spike, nucleocapsid, antibody, seroconversion

Following the outbreak of SARS-CoV-2 (SCoV2) evolving into the current coronavirus pandemic, the global health emergency continues to be highly concerning, as the number of confirmed cases has passed 200 million, with a death toll of more than 4

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Address correspondence to Hans H. Hirsch, hans.hirsch@unibas.ch.

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million (<https://coronavirus.jhu.edu/map.html>). Although approximately three-quarters of people with confirmed SCoV2-infection have been reported as recovered and seem to be at least partly immune and protected from severe clinical outcomes, it is clear that the pandemic can only be countered by a massive effort of global vaccine rollout (1). In this highly dynamic situation of exposure, recovery, and vaccination, the detection of SCoV2-RNA by nucleic acid testing (NAT) remains the gold standard for diagnosing SCoV2 infection and coronavirus disease (COVID-19) (2, 3). However, as hygiene measures and herd immunity are curtailing the prevalence rates, the positive predictive value of NAT also decreases from more than 99% at prevalence rates above 10% to less than 92% at rates of <1%, even for automated dual-target NAT assays (3). Detection of SCoV2-specific antibodies may become important as a supplement to NAT, especially among persons with unknown or negative SCoV2-NAT screening (4; <https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html>). The commercially available methods for detecting virus-specific antibodies in the clinical laboratory include enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA), and electrochemiluminescence immunoassay (ECLIA). Different viral antigens have been explored, but the most frequently used are the structural spike (S) or nucleocapsid (N) protein. The 45.6-kDa N protein is one of the most abundant and conserved viral proteins produced during SCoV2 replication and is required for packaging the viral RNA genome inside the virion (5), thus rendering it a suitable candidate for measuring antibody responses (6). The 141.2-kDa S protein forms the characteristic crown-like spikes on the outside of the virions and represents a trimeric glycosylated membrane structure built by three monomers (7). Each S protein monomer contains an amino-terminal S1 domain with the receptor-binding domain (RBD) binding to the angiotensin-converting enzyme 2 (ACE2) receptor (8, 9). Binding of one monomer to ACE2 facilitates proteolytic cleavage and conformational change of the respective carboxy-terminal S2 domain for subsequent membrane fusion and uncoating (9–11). Developing S protein-specific antibodies has been associated with protection from SCoV2 reinfection (12, 13), whereby the RBD-domain is a main target for virion-neutralizing antibodies (14, 15). To evaluate the performance and utility of commercially available immunoassays in the clinical laboratory, we compared eight assays designed to detect SCoV2-specific antibodies using either the full-length N or S protein or specific domains thereof (S1/S2 subunits, S1 subunit, RBD domain).

MATERIALS AND METHODS

Patient cohorts and clinical samples. Antibody testing was performed in the Division of Clinical Virology of the University Hospital Basel. The samples were obtained from three different patient cohorts attending the University Hospital Basel, a tertiary care center and designated COVID-19 hospital of the City of Basel, Switzerland, between March 2020 and March 2021 (detailed in Table S1 in the supplemental material).

Cohort 1 comprised 38 COVID-19 patients hospitalized between March and May 2020 with SCoV2 detected by NAT in naso-oropharyngeal swabs (NOPS) at the time of hospitalization (day 1). The cohort 1 patients provided 68 plasma samples taken within 45 days after diagnosis and analyzed by six immunoassays, #1 to #6 (see below for technical details).

Cohort 2 consisted of 510 outpatients presenting to our hospital from May 2020 to November 2020. In 56/510 (11%) patients, SCoV2 infection had been documented by NAT (16), while in the remaining 454 patients, a previous SCoV2-NAT was negative (45, 10%) or the SCoV2 exposure was unknown (409, 90%). Cohort 2 patients provided one serum sample ($n = 510$) analyzed by five immunoassays, 4 to 8 (see below for technical details).

Cohort 3 comprised 74 COVID-19 patients hospitalized between May 2020 to March 2021 having a positive SCoV2 NAT at the time of hospitalization (day 1) and at least 2 follow-up NOPS and 2 plasma samples within 45 days postdiagnosis (338 NOPS; 281 plasma samples). Cohort 3 patients provided 281 plasma samples analyzed by four immunoassays (4, 6, 7, and 8; see below for technical details).

Standard baseline demographics and laboratory parameters (lactate dehydrogenase [LDH], C-reactive protein [CRP], interleukin-6 [IL-6], and D-dimer concentrations) were collected by electronic chart review.

Laboratory diagnosis of SCoV2 infection. NOPS were obtained by sampling nasopharyngeal and oropharyngeal sites separately followed by combining the swabs into one universal transport medium tube before shipment to the diagnostic laboratory as described previously (2). SCoV2 NAT was performed from NOPS on the cobas 6800 system targeting the open reading frame 1 (ORF1) and the envelope (E) gene (3).

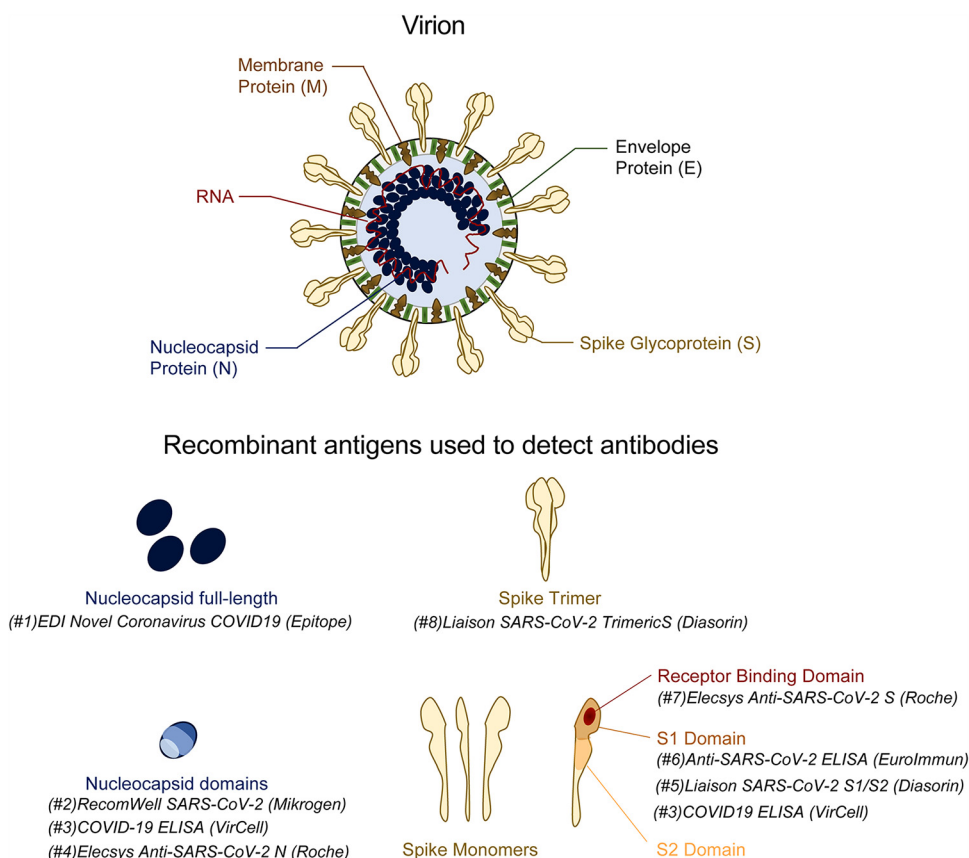


FIG 1 SARS-CoV-2 (SCoV2) virion and recombinant antigens used in antibody assays. (Top) Coronavirus particle. (Bottom) Recombinant structural viral proteins of SCoV2 used as antigens for antibody detection in the indicated immunoassays (see Table S2 in the supplemental material for technical details). N, nucleocapsid; nucleocapsid domain, spike protein (S) trimer, monomer composed of S1, S2, and the receptor-binding domain (RBD) as part of S1.

Total nucleic acids were extracted from the universal transport medium using the MagNA Pure 96 system and the DNA and viral NA small volume kit (Roche Diagnostics, Rotkreuz, Switzerland), and SCoV2 loads were quantified using the laboratory-developed Basel-SCoV2-S-112-bp NAT, targeting specific viral sequences of the spike glycoprotein S gene (3).

Immunoassays. In this retrospective study, patient plasma and serum samples were collected from in-patients on the wards and the outpatient clinics of the University Hospital of Basel. Samples were directly transported to the diagnostic laboratory and stored at 4°C if analyzed within 7 days or stored at -20°C if analyzed later in batch.

The diagnostic performance of the following eight CE-IVD marked immunoassays was assessed (Fig. 1): (#1) EDI novel coronavirus COVID-19 (Epitope, CA, USA) detecting IgG against the recombinant SCoV2 full-length N protein, (#2) RecomWell SARS-CoV-2 (Mikrogen, Neuried, Germany) detecting IgG against recombinant SCoV2 N protein domains, (#3) COVID-19 ELISA (VirCell, Granada, Spain) detecting IgG against recombinant SCoV2 N protein and S1 domains, (#4) Elecsys anti-SARS-CoV-2 N (Roche, Rotkreuz, Switzerland) detecting total immunoglobulins against recombinant SCoV2 N protein domains, (#5) Liaison SARS-CoV-2 S1/S2 (DiaSorin, Dietzenbach, Germany) detecting IgG against recombinant SCoV2 S1/S2 domains, (#6) anti-SARS-CoV-2 ELISA (EuroImmun, Lübeck, Germany) detecting IgG against recombinant SCoV2 S1 domains, (#7) Elecsys anti-SARS-CoV-2 S (Roche) detecting total immunoglobulins against the SCoV2 S protein RBD, and (#8) Liaison SARS-CoV-2 TrimericS (DiaSorin) detecting IgG against the recombinant SCoV2 trimeric S protein.

All assays were performed according to the manufacturers' instructions (Table S2). All samples from each of the three patient cohorts, 1, 2, and 3, were analyzed in parallel by the indicated immunoassays. Consensus results were used for cross-comparison of the eight assays in all three patient cohorts.

Serum laboratory markers. Concentrations of LDH and CRP were analyzed on a cobas c702, IL-6 on a cobas e801 (Roche), and D-dimers by Hemosil D-dimer (HS) assay (Werfen, MA, USA).

Statistical analysis. All statistical data analysis was done in R (version 3.6.1; <https://cran.r-project.org>), using Prism (version 8; GraphPad Software, CA, USA) for data visualization. Mann-Whitney U test, Spearman's rank correlation, and nonlinear regression analysis were used as indicated.

Ethics. The study was conducted according to good laboratory practice and in accordance with the Declaration of Helsinki and national and institutional standards and approved by the ethics committee (EKNZ 2020-00769).

RESULTS

To evaluate different commercial SCoV2-specific antibody assays in the clinical laboratory, we cross-compared results from three different patient cohorts, encompassing a total of 859 samples from 622 patients.

Cohort 1 consisted of 38 patients admitted to the hospital with a diagnosis of SCoV2 infection/COVID-19, providing 68 plasma samples (Table S1). Six antibody assays were compared that used antigens derived from the viral N protein (ElecSys-N, EpiTope-N, and MikroGen-N), the viral S protein (Liaison-S and EuroIm-S), or both (VirCell-S/N immunoassay) (Fig. 1). Agreement between the six assays ranged from 75% to 93% (Fig. 2A).

To assess the semiquantitative aspects of SCoV2 antibody assays, we correlated the readouts of the antibody index values in the concordant (black dots) and discordant (red dots) samples (Fig. S1). Significant correlations ($P < 0.001$) were observed for all comparisons, as the Spearman rank correlation coefficient (r_s) ranged from 0.78 to 0.95, being higher among immunoassays using similar viral antigens (EuroIm-S- and Liaison-S-based immunoassays; EpiTope-N- and MikroGen-N-based immunoassays). Despite their overall correlation, however, discordant results were not evenly distributed among the positive/negative or negative/positive categories for specific assay comparisons but tended to cluster for some assays more frequently in one discordance category (Fig. S1). Thus, the EpiTope-N assay yielded more positive results than the other N antigen-containing assays, MikroGen-N, ElecSys-N, and VirCell-S/N (Fig. 2B; Fig. S1). The data suggested assay-inherent differences in sensitivity and specificity, with the EpiTope-N assay being potentially more sensitive or less specific. As expected, the antibody detection rate increased with increasing time after documented SCoV2 infection for all assays (Fig. 2B): discordance was highest, with 56% for samples taken within 7 days after diagnosis and decreased to 12% for samples taken after 14 days (Fig. 2C). Discordant results mostly originated from the EpiTope-N assay, detecting antibodies in 12/68 (18%) plasma samples identified as negative with other N-based assays, i.e., ElecSys-N and MikroGen-N. When excluding EpiTope-N test results, discordance among immunoassays decreased significantly ($P < 0.001$), which was not observed when excluding ElecSys-N results ($P = 1.0$; Fig. 2C). Taken together, the data of cohort 1 indicated differences in assay performance and suggested a significant role of antigen and/or platform within the first 14 days after diagnosis.

To validate these results in a larger cross-sectional data set, 510 serum samples from different outpatients (cohort 2) were tested using three assays (ElecSys-N, Liaison-S, and EuroIm-S) that showed $>85\%$ concordance in cohort 1. In addition, two assays were added that used different viral antigens on the same testing platform, the ElecSys-S using the S protein RBD domain and the Liaison-TriS immunoassays using a trimeric S protein to potentially improve SCoV2-specific antibody detection in the first 7 days after diagnosis. Of note, cohort 2 encompassed 56 patients with NAT-confirmed SCoV2 infection and 454 patients with unknown SCoV2 exposure (Table S1). Overall, a high concordance of 94% was found for all five assays, consisting of 44 (9%) samples with detectable antibodies and 435 (85%) samples with undetectable antibodies (Fig. 3A; Fig. S2). Discordant results were observed in 31 (6%) samples (6 NAT positive, 11 NAT negative, and 14 NAT unknown), consisting of various combinations of the different assays, 13 of which resulted from EuroIm-S-negative and 20 from ElecSys-N-negative results (Fig. S2). Qualitative agreement of the test results among the five assays was high, reaching 98% between the ElecSys-S and ElecSys-N ECLIA, with a Cohen's κ of 0.94, while the ElecSys-S ECLIA and the EuroIm-S ELISA showed 95%, with a Cohen's κ of 0.88 (Fig. 3B).

To assess the quantitative relationship between the different SCoV2 antibody assays, we examined antibody levels in the concordant (black dots) and discordant (red dots) samples. Overall, the correlation was statistically significant for all comparisons ($P < 0.001$). However, the Spearman rank correlation coefficient varied widely from as low as r_s of 0.39 for ElecSys-N versus EuroIm-S to as high as r_s of 0.73 for Liaison-TriS versus ElecSys-S. When comparing the Liaison-S with the Liaison-TriS immunoassay, 10 negative test results were obtained with the Liaison-S compared to the Liaison-TriS immunoassay, all of which were independently confirmed by the other

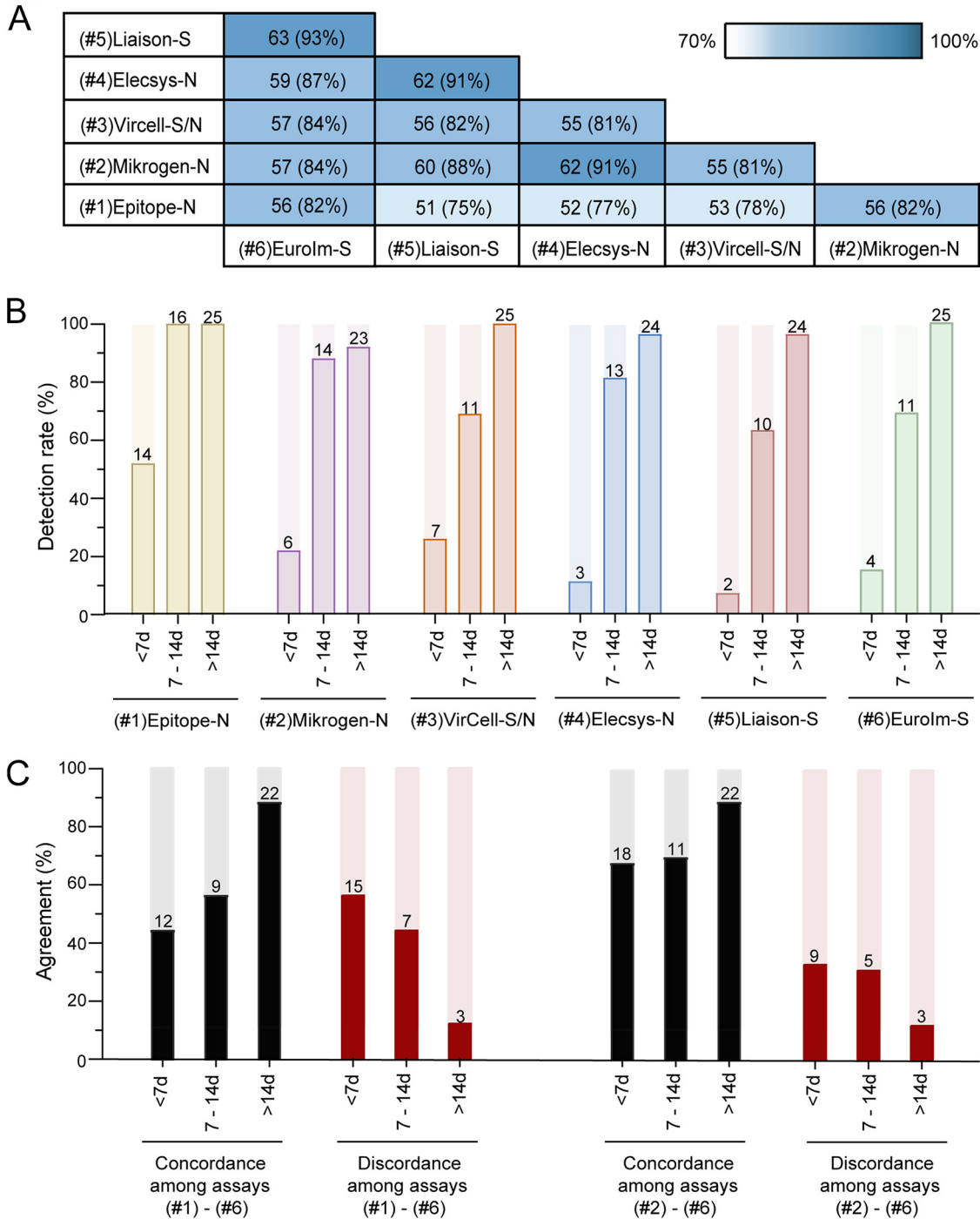


FIG 2 Comparison of 6 antibody assays in 68 plasma samples from 38 COVID-19 patients with confirmed SCoV2 infection (cohort 1). Qualitative comparison of (#1) EDI novel coronavirus COVID-19 (Epitope), (#2) RecombWell SARS-CoV-2 (Mikrogen), (#3) COVID-19 ELISA (VirCell), (#4) Elecsys anti-SARS-CoV-2 N (Roche), (#5) Liaison SARS-CoV-2 S1/S2 (DiaSorin), and (#6) anti-SARS-CoV-2 ELISA (EuroImmun) (see Table S2 in the supplemental material for technical details). (A) Qualitative agreement between immunoassays in percent as visualized by the color code. (B) Antibody detection rate by the indicated assays in samples obtained <7 days ($n = 27$), 7 to 14 days ($n = 16$), and >14 days ($n = 25$) after SCoV2 NAT (representing day 1). (C) Histograms show the concordance/discordance of assays #1 to #6 and #2 to #6, respectively, according to time of blood sampling as described in panel B.

assays, suggesting a higher assay sensitivity of the trimeric compared to the monomeric S antigen (Fig. S3).

To address the performance of different antigens and assays over time after SCoV2 diagnosis, we analyzed 281 plasma samples from 74 COVID-19 patients (cohort 3) admitted

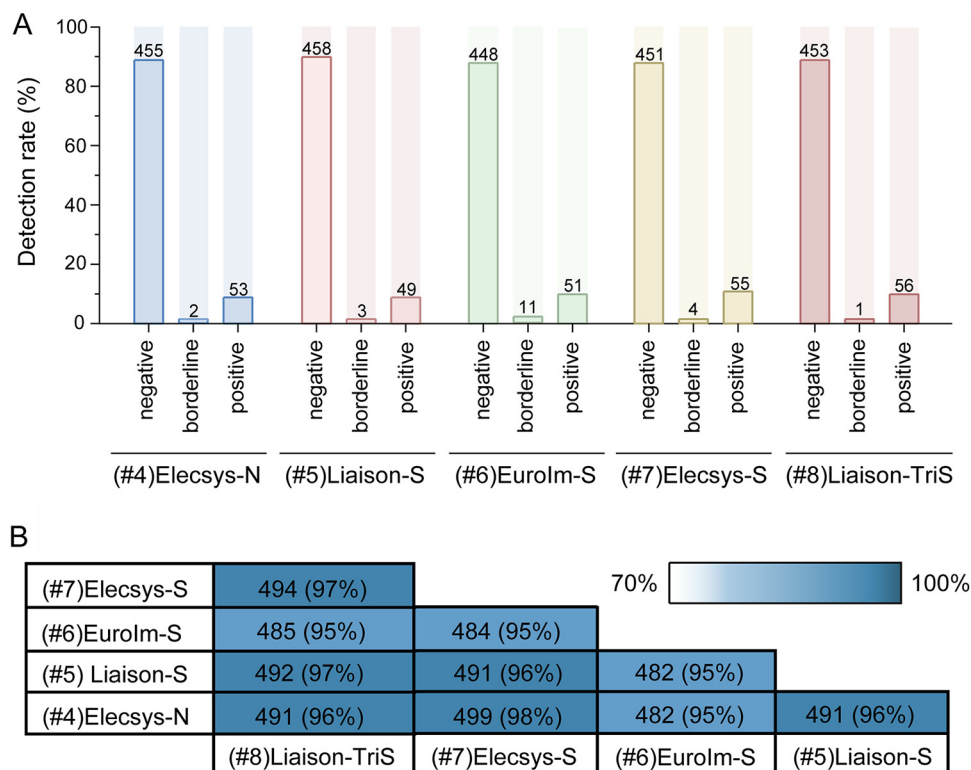


FIG 3 Comparison of five antibody assays in 510 outpatients with detected or unknown SCoV2 infection (cohort 2). Qualitative comparison of (#4) Elecsys anti-SARS-CoV-2 N (Roche), (#5) Liaison SARS-CoV-2 S1/S2 (DiaSorin), (#6) anti-SARS-CoV-2 ELISA (EuroImmun), (#7) Elecsys anti-SARS-CoV-2 S (Roche), and (#8) Liaison SARS-CoV-2 TrimericS (DiaSorin) (see Table S2 in the supplemental material for technical details). (A) SCoV-2 antibody detection by the indicated assays. (B) Qualitative agreement between immunoassays in percent as visualized by the color code (for details, see Fig. S2).

to the hospital with confirmed SCoV2 detection by NAT (Table S1). The Elecsys-S ECLIA (#7) had the highest detection rate of 71% in the first 7 days after SCoV2 diagnosis, followed by 63% for Elecsys-N ECLIA (#4), 56% for Liaison-TriS CLIA (#8), and 53% for EuroIm-S ELISA (#6) (Fig. 4A). Overall concordance was 82%, which increased from 72% in the first week to 98% in the fourth week after diagnosis (Fig. 4B).

To specifically address seroconversion, we identified a subgroup of 20 COVID-19 patients of cohort 3 with undetectable SCoV2 antibodies in their first plasma sample by assays 4, 6, 7, and 8 (Table S1). The mean SCoV2 loads in NOPS steadily decreased over time, whereby the time to first undetectable SCoV2 load (threshold) was reached in 50% and 90% of patients after 16 and 26 days, respectively (Fig. 4C). Conversely, the time to seroconversion varied among the immunoassays. For the Elecsys-S (#7), seroconversion rates of 50% and 90% were reached on day 8 and day 14, respectively, hence 3 and 4 days earlier than in the comparator assays Liaison-TriS (#8), EuroIm-S (#6), and Elecsys-N (#4) (Fig. 4D). Despite these differences in seroconversion rates, the data indicated an inverse relationship between SCoV2 replication in the upper respiratory tract and the specific antibody responses detected in blood, whereby increasing antibody levels preceded declining SCoV2 loads in NOPS by approximately 5 to 10 days.

Given the inverse relationship between viral load and specific antibody immune response, we explored several laboratory markers of tissue injury, acute phase response, and innate immune activation indicative of severe COVID-19 in the subgroup of 20 cohort 3 patients showing SCoV2 seroconversion (Table S1) (17). With few exceptions, LDH, IL-6, CRP, and D-dimers were highly elevated at the time of diagnosis and admission to hospital (Fig. 5A, left panels). Over the next 10 days, the mean levels

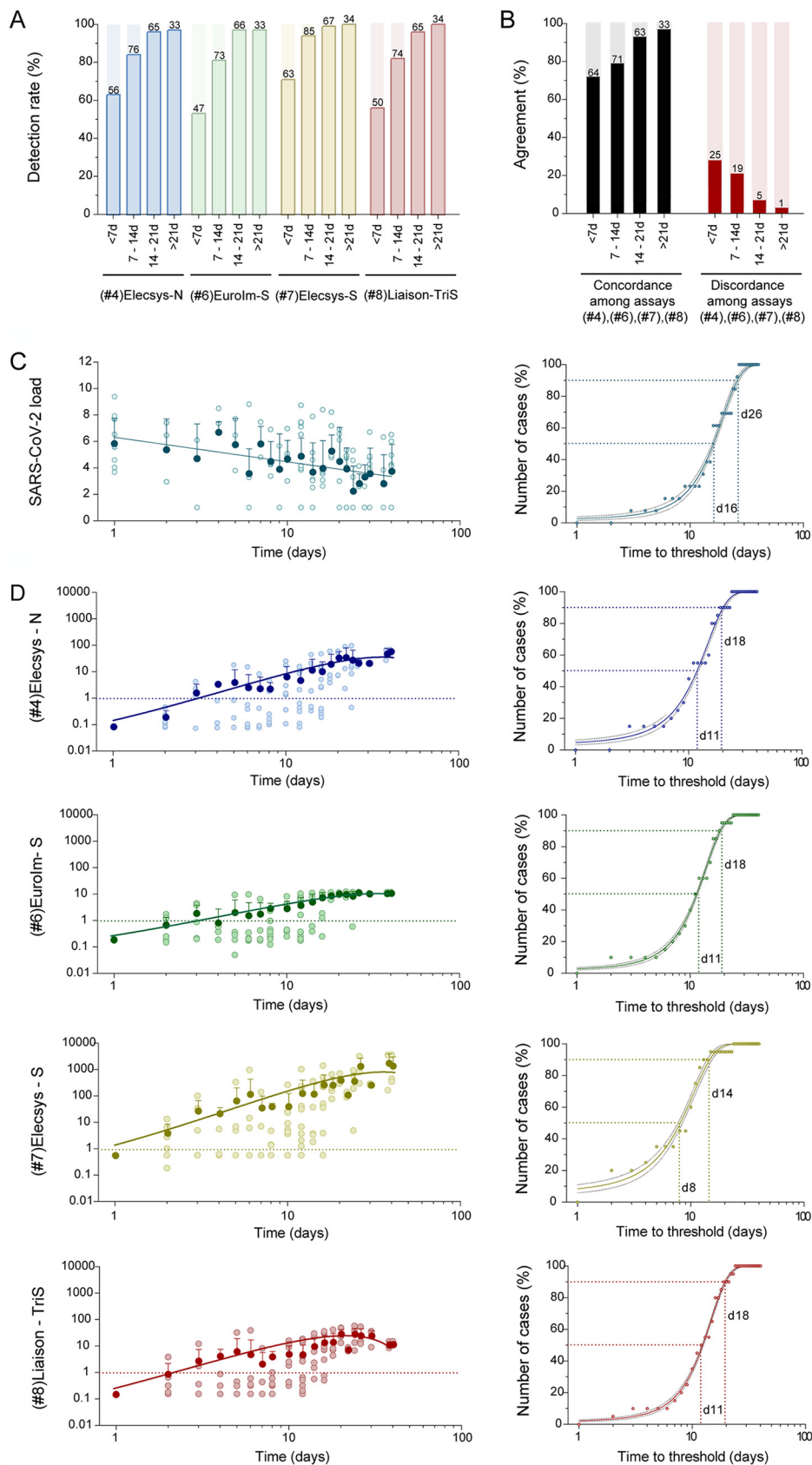


FIG 4 SCov2 loads and antibody titers in SCov2-infected patients admitted to the hospital for COVID-19 (cohort 3). (A) Antibody detection rate by the indicated assays in 281 plasma samples obtained from 74 (Continued on next page)

remained high for LDH, IL-6, and CRP and slightly increased for D-dimers before decreasing at around 20 days after admission (Fig. 5B, left panels). To visualize the respective laboratory marker dynamics, we determined the proportion of cases crossing different arbitrary threshold levels over time (Fig. 5B, right panels). The results revealed that an increasing proportion of patients showed marker levels decreasing below the indicated threshold strata. On day 20, 30% had LDH levels lower than 400 U/liter, 23% had IL-6 levels below 500 ng/liter, 32% had CRP levels below 50 mg/liter, and 40% had D-dimers below 2 ng/liter (Fig. 5B, right panels).

To explore the relationship of SCoV2 loads, serum biomarkers, and SCoV2-specific antibody levels over time, we analyzed the respective levels in the densely sampled initially seronegative subgroup of 20 COVID-19 patients over four times strata of follow-up (Fig. 6A). The SCoV2 loads showed a stepwise decline from the time of admission to >21 days, whereas N and S protein antibody titers increased (Fig. 6A). Mean IL-6 and D-dimer levels were increasing until day 21 before decreasing thereafter, whereas CRP levels were high before decreasing after day 21 (Fig. 6A). The results suggested that the SCoV2-specific antibody response preceded the decline in acute phase and inflammatory markers. To obtain a more detailed resolution of the quantitative relationship, we performed nonlinear regression analysis of viral loads, antibody, IL-6, and CRP levels and marked the time strata in different shades of color (Fig. 6B). As expected, the anti-S and anti-N levels showed the highest quantitative association ($P < 0.001$; $R^2 = 0.87$) with corresponding distribution in the time strata. Anti-N levels and anti-S levels were negatively associated with CRP levels ($P < 0.001$), with R^2 values of 0.77 and 0.67, respectively. By comparison, the association with IL-6 levels or SCoV2 loads in the upper respiratory tract samples was less tight (Fig. 6B).

DISCUSSION

To evaluate SCoV2-specific antibody responses for routine use in the diagnostic laboratory, we compared eight commercially available immunoassays in cross-sectional and longitudinal patient cohorts. Our study presents three major observations.

First, the overall agreement between these assays ranged from 75% to more than 95% in patient cohort 1 and cohort 2. While the observed range may cast some doubts on their clinical utility, it should be kept in mind that, unlike serological tests for systemic virus infections such as HIV or cytomegalovirus, antibody testing for community-acquired respiratory viruses (CARVs), replicating mostly transiently in the upper respiratory tract, is little developed and hardly used in clinical practice (18). One exception is influenza serology when studying vaccine responses or epidemiological questions, but it rarely informs clinical counseling despite its projected utility in immunocompromised hosts (19). Therefore, and also in view of the difference in antigens, detection formats, instrumentation, and automation, we consider the agreement of these SCoV2-specific antibody results substantial and very likely to pave the way for other CARV serology assays as potential markers of risk and immunity.

Second, the choice of SCoV2 antigen target played a decisive role regarding concordance and magnitude of the respective antibody responses. This resulted not only from the

FIG 4 Legend (Continued)

COVID-19 patients at <7 days ($n = 89$), 7 to 14 days ($n = 90$), 14 to 21 days ($n = 68$), and >21 days ($n = 34$) after SCoV2 detection by NAT (representing day 1). (B) Rate of concordant and discordant results of the (#4) Elecsys anti-SARS-CoV-2 N (Roche), (#5) Liaison SARS-CoV-2 S1/S2 (DiaSorin), (#6) anti-SARS-CoV-2 ELISA (EuroImmun), (#7) Elecsys anti-SARS-CoV-2 S (Roche), and (#8) Liaison SARS-CoV-2 TrimeriCS (DiaSorin) assays according to time of blood sampling as described in panel A (see Table S2 in the supplemental material for technical details). (C) SCoV2 RNA loads in upper respiratory tract samples of 20 COVID-19 patients with SCoV2 seroconversion over time after diagnosis. (Left) Mean, dark blue dots with standard deviation; SCoV2 loads below the limit of detection are arbitrarily set to 1. (Right) Proportion of cases with undetectable SCoV2 as a function of time (95% confidence interval; time to undetectable in 50% and 90% of cases). (D) SCoV2 antibody levels of the indicated assays in 108 plasma samples of 20 COVID-19 patients over time (Left) Dark-colored dots indicate mean, and bars indicate standard deviation. (Right) Respective proportion of patients with seroconversion over time of follow-up. Immunoassay values are normalized to a cutoff threshold of 1 (dotted horizontal lines).

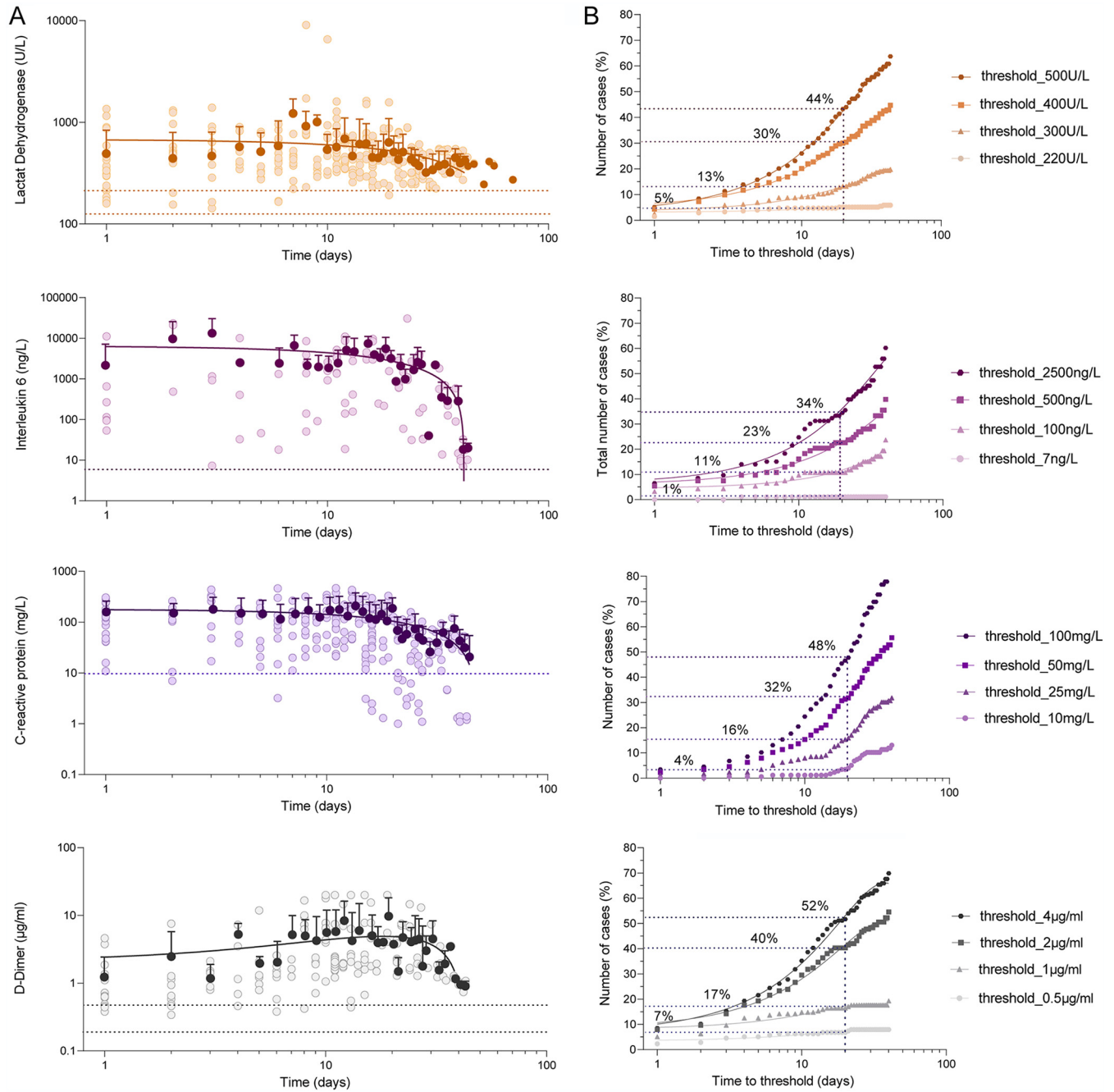


FIG 5 LDH, IL-6, CRP, and D-dimer levels in 20 hospitalized COVID-19 patients with SCoV2 seroconversion (cohort 3). (A) Lactate dehydrogenase, interleukin-6, C-reactive protein, and D-dimer measurements over time (mean, dark-colored dots with error bars of standard deviation). Serum biomarker cutoff thresholds as indicated (dotted horizontal lines). (B) Respective proportion of patients under the cutoff thresholds over time of follow-up (patients under threshold at day 20 indicated with a dotted line).

principal difference of using the nucleocapsid versus the spike proteins as viral antigens but also from the choice of full length, subdomains, or combinations (Fig. 1; Table S2 in the supplemental material). The former is illustrated by the considerable variation in diagnostic performance among the four anti-N-detecting assays. Instead of being randomly distributed around both cutoffs, discordant anti-N antibody responses clustered as reactive in the Epitope-N assay, but were nonreactive in the Mikrogen-N and Elecsys-N assays. While this suggests systematic, possibly test-inherent, issues, it is notable that particularly full-length N protein-based immunoassays have been reported to suffer from lower specificity due to cross-reactive antibodies mounted after previous infections with circulating

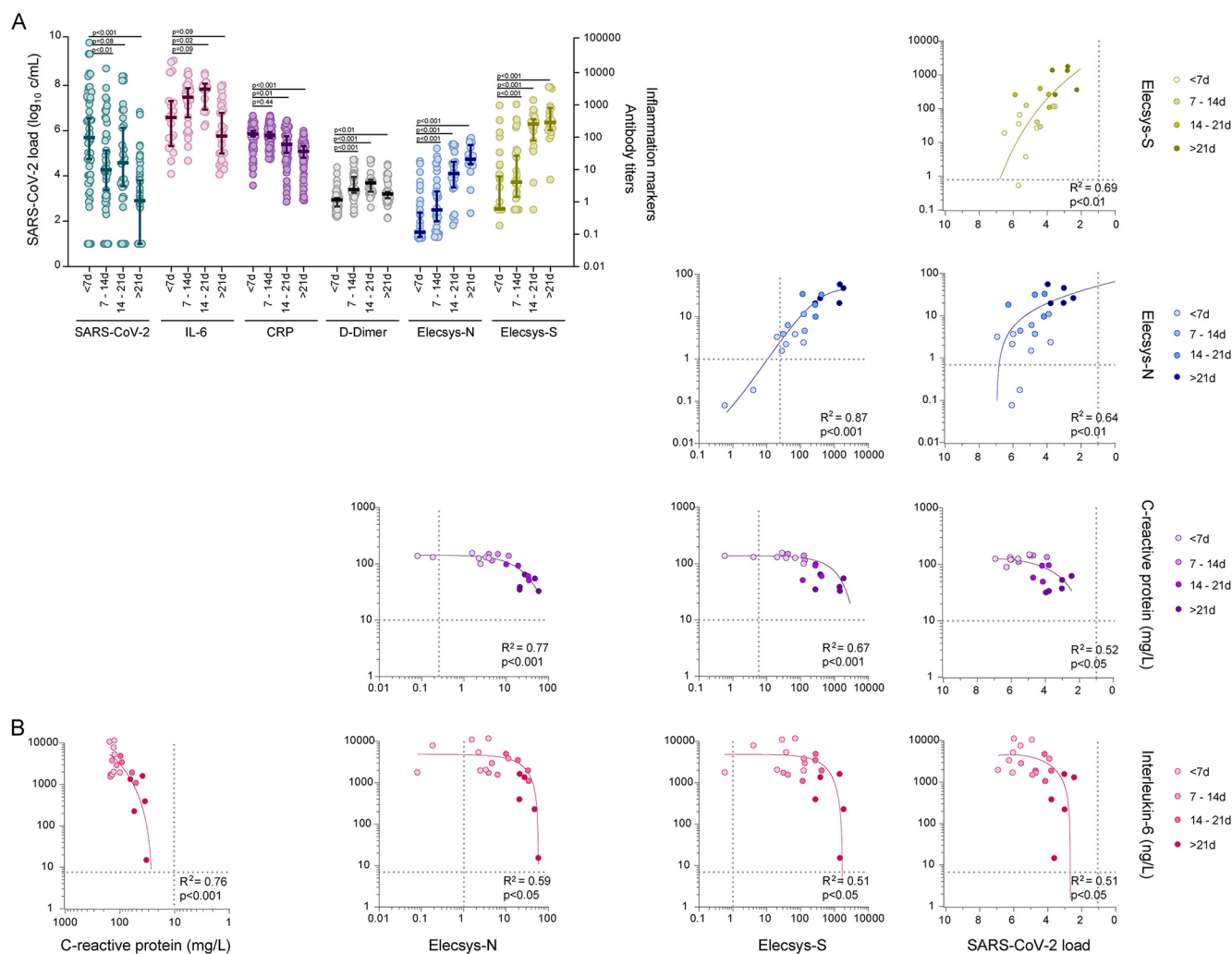


FIG 6 SCoV2 loads, SCoV2 antibody titers, and nonspecific laboratory markers of tissue injury and acute phase response. (A) SCoV2 loads, IL-6, CRP, D-dimer, and SCoV2 antibody titers to the viral nucleocapsid and the spike protein were followed in 20 initially seronegative COVID-19 patients of cohort 3. The respective samples were obtained in 4 weekly strata of <7, 7 to 14, 14 to 21, and >21 days after diagnosis of SCoV2 detection by NAT (median, 25th and 75th percentiles; *P* values by Mann-Whitney U test). (B) Nonlinear regression of SCoV2 loads, inflammatory biomarkers, and SCoV2 antibody titers (correlation coefficient *R*² and *P* values indicated for each comparison).

human coronaviruses, which is less pronounced for the surface-exposed S protein (20, 21). Importantly, no functional control assay or gold standard exists for antibodies to the nucleocapsid antigen located inside the virion, hence it being inaccessible for surface-directed neutralizing antibodies (Fig. 1). The latter is supported by the overall lower variability in detection rates among the anti-S assays observed here, despite using trimeric or monomeric S1/S2 subunits, S1 subunit, or RBD antigens. However, Liaison-TriS CLIA appeared to be slightly more sensitive than the monomeric Liaison-S CLIA, using a common testing platform, or than the EuroIm-S ELISA. This suggests the possibility that the S trimer may not only capture antibodies that bind to further epitopes outside the S1 subunit or RBD, but since conformational change subsequent to receptor binding is needed for membrane fusion, antibodies binding to the prefusion trimers and preventing conformational change subsequent to RBD-ACE2 binding by interlocking the prefusion trimer may also be relevant for neutralization besides direct RBD blocking. However, details of epitope accessibility and relative contribution remain to be defined for these assays.

Third, the discordance among the different antibody assays was most pronounced in the first week after diagnosis and then almost disappeared, independent of the respective assay or platform used. Specifically, the discordance ranged from 30% to almost 60% in the first 7 days after diagnosis in the cohorts studied here and declined

to less than 10% over the next 21 days. In parallel, the antibody levels increased by 1 to 3 orders of magnitude, suggesting that the assays' analytical sensitivity in the first 1 to 2 weeks after diagnosis may be a limiting factor. Additional insight was obtained from our detailed analysis of a subgroup of 20 patients in cohort 3 without detectable SCoV2-specific antibodies in their first blood sample by assays 4, 6, 7, and 8. The data showed that the Elecsys-S assay detected seroconversion at least 3 days earlier than the other assays whereby the 50% and 90% seroconversion rates occurred on day 8 and day 14, respectively. In contrast, the seroconversion rates of 50% and 90% of patients occurred on day 11 and day 18, respectively, using the Liaison-TriS, EuroIm-S, or Elecsys-N assays. Notably, seroconversion preceded the clearance of SCoV2 loads in the upper respiratory tract by approximately 1 week, which occurred in 50% and 90% of the patients on day 16 and day 26, respectively. Although prolonged replication of CARVs is a typical feature of immunocompromised patients (18), extended shedding from mucosal surfaces in the nasopharyngeal and gastrointestinal tract after seroconversion is well-known even in immunocompetent hosts, as reported for entero- and polioviruses (22, 23). Together, these observations suggest that systemic antibody responses in blood may predict the dynamics of virus replication in the upper respiratory tract and aid in the interpretation of virus-specific laboratory tests and in clinical decisions regarding the use and interpretation of antiviral interventions, whereby a clinical benefit may no longer become apparent at later stage of the disease, with the possible exception of immunocompromised patients (17).

In view of the inverse relationship between virus-specific antibody responses and viral loads, we took a closer look at other laboratory markers of tissue injury, innate immune activation, and acute-phase response (17). Our results suggested that increasing anti-S and anti-N antibody responses were inversely correlated with IL-6 and CRP levels, thereby providing a temporary association of virus-specific immunity with recovery from injury and acute-phase response in this longitudinal analysis of densely sampled patients. As expected, anti-S and anti-N levels were highly correlated and increased over time. Previous studies suggested that these serum laboratory markers predict the risk of COVID-19 severity (24–26), with proposed IL-6 and CRP cutoff thresholds of 35 ng/liter and 33 mg/liter to identify patients eventually needing invasive ventilation (25). In our longitudinal cohort 3, seven COVID-19 patients showed IL-6 and CRP levels above these thresholds at hospital admission and subsequently required mechanical ventilation. During follow-up, these markers decreased, whereby week 2 appeared to be decisive, a time when SCoV2-specific antibodies also increased significantly. However, high and prolonged IL-6 and CRP levels persisted in some patients, to which secondary complications may have contributed, such as bacterial and fungal superinfections (27).

Several limitations of this report should be noted. The retrospective nature of this study may have introduced unrecognized biases which may preclude generalizing the results without confirmatory prospective studies. Obviously, the availability of blood samples limits our observations in the two patient cohorts, 1 and 3, to symptomatic patients who presented to the emergency room and were found to have indications for a blood draw. Patients in cohort 2 with a negative SCoV2 NAT or unknown SCoV2 exposure may have had this viral infection in the past without laboratory confirmation. Indeed, approximately 6% of the 454 patients with undefined SCoV2 history had detectable antibodies, which corresponds well to the reported local epidemiology at that time (28). Conversely, our data may not apply to patients with a- or oligosymptomatic SCoV2 infections who did not have clinical indications for a laboratory workup. Data from various studies have indicated that antibody levels in these patients may be lower or not detectable, which may be partly assay dependent (13, 29–32). Further, we could not evaluate the contribution of the detection platform, instrumentation, or automation of ECLIA, CLIA, or ELISA in addition to the differences in the choice and preparation of recombinant antigen, all of which were used in the analyzed assays. We focused on total antibody and IgG antibody responses in blood and did not evaluate the impact of different immunoglobulin classes or IgG subclasses with respect to

microneutralization (33) or functionality (34). Exploratory data from our center and the available literature suggest that detection of IgA is too variable and cannot be used reliably in approximately 1% of patients with selective or partial IgA deficiency (35). IgM appearance has been reported to mostly coemerge in blood together with IgG antibodies during the first 2 weeks postdiagnosis, thereby decreasing its value in addition to being less specific after infections with circulating human coronaviruses (29, 36–39). Also, the inverse relationship between serum biomarkers, viral loads, and SCoV2-specific antibody responses observed in a subgroup of 20 patients of cohort 3 is intriguing and should be examined in larger prospective patient cohorts.

Finally, we did not compare the available assays for antibody responses elicited after vaccination nor the levels associated with protection from SCoV2-replication, escape from viral variants, and severity of COVID-19 or defined antibody responses in immunocompromised patients. However, none of the patients analyzed in our cohorts were vaccinated. The currently licensed vaccines in Switzerland are BioNTech/Pfizer and Moderna and generate antibody responses against the SCoV2 S protein but not against the N protein. After vaccination became available in Switzerland, a brief survey of our hospital database among 110 vaccinees revealed average anti-S levels of 967 U/ml as determined with the Elecsys-S assay. In contrast, hospitalized COVID-19 patients showed mean anti-S titers of 449 U/ml at >21 days postdiagnosis (Fig. 4D), corresponding to approximately 2-fold lower SCoV-2 antibody titers than in vaccinated patients. However, the S-positive and N-negative discordance after vaccination will readily change over time upon vaccination of previously infected persons, reinfection of vaccinated persons, or after licensing of inactivated whole-virus vaccines in the future (40).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.8 MB.

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We have no competing interests to declare.

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