

Dynamics and Correlation Among Viral Positivity, Seroconversion, and Disease Severity in COVID-19

A Retrospective Study

Yu Fu, MD*; Yongsheng Li, MD*; Ensong Guo, MD*; Liang He, MD*; Jia Liu, MD; Bin Yang, MD; Fuxia Li, MD; Zizhuo Wang, MD; Yuan Li, MD; Rourou Xiao, MD; Chen Liu, MD; Yuhang Huang, MD; Xue Wu, MD; Funian Lu, MD; Lixin You, MD; Tianyu Qin, MD; Chaolong Wang, PhD; Kezhen Li, PhD; Peng Wu, PhD; Ding Ma, PhD; Chaoyang Sun, PhD; and Gang Chen, PhD

Background: The understanding of viral positivity and seroconversion during the course of coronavirus disease 2019 (COVID-19) is limited.

Objective: To describe patterns of viral polymerase chain reaction (PCR) positivity and evaluate their correlations with seroconversion and disease severity.

Design: Retrospective cohort study.

Setting: 3 designated specialty care centers for COVID-19 in Wuhan, China.

Participants: 3192 adult patients with COVID-19.

Measurements: Demographic, clinical, and laboratory data.

Results: Among 12 780 reverse transcriptase PCR tests for severe acute respiratory syndrome coronavirus 2 that were done, 24.0% had positive results. In 2142 patients with laboratory-confirmed COVID-19, the viral positivity rate peaked within the first 3 days. The median duration of viral positivity was 24.0 days (95% CI, 18.9 to 29.1 days) in critically ill patients and 18.0 days (CI, 16.8 to 19.1 days) in noncritically ill patients. Being critically ill was an independent risk factor for longer viral positivity (hazard ratio, 0.700 [CI, 0.595 to 0.824]; $P < 0.001$). In patients with laboratory-confirmed COVID-19, the IgM-positive rate was

19.3% in the first week, peaked in the fifth week (81.5%), and then decreased steadily to around 55% within 9 to 10 weeks. The IgG-positive rate was 44.6% in the first week, reached 93.3% in the fourth week, and then remained high. Similar antibody responses were seen in clinically diagnosed cases. Serum inflammatory markers remained higher in critically ill patients. Among noncritically ill patients, a higher proportion of those with persistent viral positivity had low IgM titers (<100 AU/mL) during the entire course compared with those with short viral positivity.

Limitation: Retrospective study and irregular viral and serology testing.

Conclusion: The rate of viral PCR positivity peaked within the initial few days. Seroconversion rates peaked within 4 to 5 weeks. Dynamic laboratory index changes corresponded well to clinical signs, the recovery process, and disease severity. Low IgM titers (<100 AU/mL) are an independent risk factor for persistent viral positivity.

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* Drs. Fu, Li, Guo, and He contributed equally to this work.

Coronavirus disease 2019 (COVID-19), which was first reported in Wuhan, China, in December 2019, has spread throughout the world (1, 2). The pandemic has threatened a substantial portion of the population. By 5 August 2020, COVID-19 had affected more than 18 million persons, spread among 216 countries and regions, and caused nearly 700 000 deaths, according to the situation reports from the World Health Organization (3).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus that causes COVID-19. Knowledge about viral polymerase chain reaction (PCR) positivity patterns, duration, and neutralizing antibody responses is critical for implementing an epidemiologic control strategy, antiviral treatment, and vaccinations. Although studies have described SARS-CoV-2 viral kinetics and positivity (4, 5), those studies were based on small sample sizes and included mostly COVID-19 cases of mild or moderate severity. Furthermore, to our knowledge, no large clinical studies have systematically analyzed the correlations between viral dynamic PCR positivity, seroconversion, and disease severity (6-8). Furthermore, our

understanding remains fragmented about persistent infections and viral PCR positivity kinetics in critically ill patients.

We aimed to gain a comprehensive understanding of viral dynamics, along with its correlations with seroconversion and prognosis, in 3192 patients with COVID-19 admitted to Tongji hospitals.

METHODS

Design Overview, Settings, and Participants

We did a retrospective study of 3192 consecutive patients hospitalized with COVID-19 between 18 January and 31 March 2020 at 3 designated specialty care centers for COVID-19 (Sion-French New City Branch, Optical Valley Branch, and Main District) of Tongji Hospital in Wuhan, China.

Eligible patients were aged 18 years or older and were identified as having COVID-19 according to the diagnostic criteria specified in the COVID-19 Diagnosis and Treatment Plan issued by the National Health Commission of the People's Republic of China (version 7.0) (Appendix Table 1, available at Annals.org) (9).

Specifically, a clinical diagnosis of COVID-19 was made on the basis of relevant epidemiologic history; typical clinical manifestations, especially positive findings on computed tomography scans; and evidence of antibody response, but in the absence of positive results on nucleic acid testing during the entire course. Laboratory-confirmed COVID-19 cases referred to those with positive results on viral testing.

This study was approved by the Ethical Committee of Tongji Hospital of Huazhong University of Science and Technology. Written informed consent was not required because all data were analyzed retrospectively and anonymously.

Definitions

We classified the clinical severity of each COVID-19 case according to the COVID-19 Diagnosis and Treatment Plan (**Appendix Table 1**). Critically ill cases were defined as those that required intubation or involved shock, other organ failure, or admission to the intensive care unit (10). Mildly, moderately, and severely ill patients were categorized as noncritically ill.

The time of disease onset was defined as either the date when signs or symptoms consistent with COVID-19 first appeared or the date of the first positive result on a viral PCR test, whichever was earlier. The time intervals for viral, serologic, and other laboratory indices were calculated from disease onset to the testing date. The viral positivity duration referred to the time from disease onset to the last positive result on a SARS-CoV-2 PCR test before discharge. We retrospectively allocated noncritically ill patients who had RNA-positive test results for more than 30 days after onset to the persistent viral positivity (PVP) group. As a control, noncritically ill patients with virus-negative conversion within 21 days were allocated to the short viral positivity (SVP) group.

Measurements

We obtained demographic, laboratory, and clinical data from electronic medical records. Viral and serologic testing were done according to consensus recommendations from infectious disease specialists at Tongji Hospital. Specifically, viral testing was recommended in the following situations: patients whose condition changed (symptoms and computed tomography scan improved or deteriorated); patients with no clinical changes, with testing repeated weekly until 30 days after onset; and patients with prolonged viral PCR positivity (≥ 30 days after illness onset), with testing repeated more frequently. To monitor the effectiveness of viral clearance, viral tests were recommended every 2 days after convalescent plasma (CP) therapy. The consensus recommended discontinuation of further viral testing when patients satisfied the criteria for discharge, consisting of absence of fever for at least 3 days, substantial improvement in computed tomography imaging and clinical remission of respiratory symptoms, and 2 throat-swab samples negative for SARS-CoV-2 RNA obtained at least 24 hours apart.

For each patient, at least 2 SARS-CoV-2-specific antibody (IgG and IgM) tests were recommended, 2 days after admission and repeated before discharge. Serum

infection markers, including inflammatory cytokines, blood routine indices, C-reactive protein, erythrocyte sedimentation rate, procalcitonin, and other associated indices, were obtained at the discretion of physicians without any systematic approach.

Within 2 hours of the throat or nasopharyngeal swab, total RNA was extracted from samples with the Respiratory Sample RNA Isolation Kit (Shanghai BioGerm Medical Technology Company). A total of 40 μ L of cell lysates in the collection tube was vortexed for 10 seconds and centrifuged at 1000 rpm for 5 minutes. Then, reverse transcriptase PCR (RT-PCR) was done to identify SARS-CoV-2 RNA, according to the manufacturer's protocol. Samples with cycle threshold (Ct) values of 38.0 or lower were considered positive for SARS-CoV-2 RNA. Samples with Ct values above 38.0 were repeated. Repeated samples with Ct values above 38.0 and those with undetectable Ct values were considered to be negative for SARS-CoV-2. Primer sequences are shown in **Appendix Table 2** (available at [Annals.org](#)) (11).

Within 2 hours of the blood draw, serum was extracted. Serum SARS-CoV-2-specific IgG and IgM titers were measured using the SARS-CoV-2 antibody detection kits supplied by YHLO (YHLO Biotech Co.). This chemiluminescence immunoassay used recombinant antigens that contained the nucleoprotein and spike protein of SARS-CoV-2 as targets for IgM and IgG antibodies. A positive result was defined as 10 AU/mL or more and a negative result as less than 10 AU/mL. The kit was previously shown to detect IgM and IgG with sensitivities of 83% and 93%, respectively, and specificities of 99% and 92%, respectively (12).

CP Therapy

Patients with laboratory-confirmed COVID-19 who had fully recovered, been discharged from the hospital, and had no clinical symptoms for more than 2 weeks were recruited as potential CP donors. Those with a spike receptor-binding, domain-specific IgG antibody titer exceeding a 1:160 dilution were selected as eligible donors according to the Convalescent Plasma Therapy for COVID-19 Patients (version 2) (13). Donors and recipients with ABO blood types were compatible, and the transfusion dose was approximately 4 to 13 mL/kg of the recipient's body weight (14).

Statistical Analysis

The median duration of viral positivity was calculated using Kaplan-Meier estimation. The association between disease severity and viral positivity duration was examined using Kaplan-Meier curves with the log-rank test. Multivariable Cox proportional hazards analysis, including disease severity, age, sex, and prognosis-related comorbid conditions, was done to investigate risk factors for longer viral positivity. Multivariable logistic regression analysis, including antibody levels, age, sex, and prognosis-related comorbid conditions, was done to investigate the predictive variables for patients in the PVP group. Results were expressed as hazard ratios or odds ratios, with corresponding 95% CIs.

We expressed categorical variables as numbers and percentages and continuous variables as medians and interquartile ranges (IQRs) (**Appendix Table 3**, available

at Annals.org). We compared the difference for continuous variables between 2 independent groups using the Wilcoxon rank-sum test for data with skewed distribution. Proportions for categorical variables were evaluated by the χ^2 test. The Fisher exact test was used for small sample sizes.

All of the serum laboratory indices with continuous values were applied log2 transformation, except the lymphocyte count. For every serum marker, patients with completely missing observations were removed. Multiple measurements of the same test in 1 patient at the same time point were averaged for the analysis. When testing the differences for serum indices in every week between subpopulations, we dealt with the nonindependent repeating measurements with missing data using a linear mixed model. We used the linear mixed model by including an individual patient identifier as the random intercept to account for within-individual correlation, with adjustment for age, sex, or clinical classification in different conditions. We treated the measurement time points (in weeks) as categorical explanatory variables in the linear mixed model. We derived *P* values of between-group comparison at each time point using the Wald test.

A *P* value less than 0.05 was considered statistically significant. We used GraphPad Prism 8.0; R platform

(www.r-project.org); and SPSS, version 25.0 (IBM), for analyses.

Role of the Funding Source

This study did not receive funding.

RESULTS

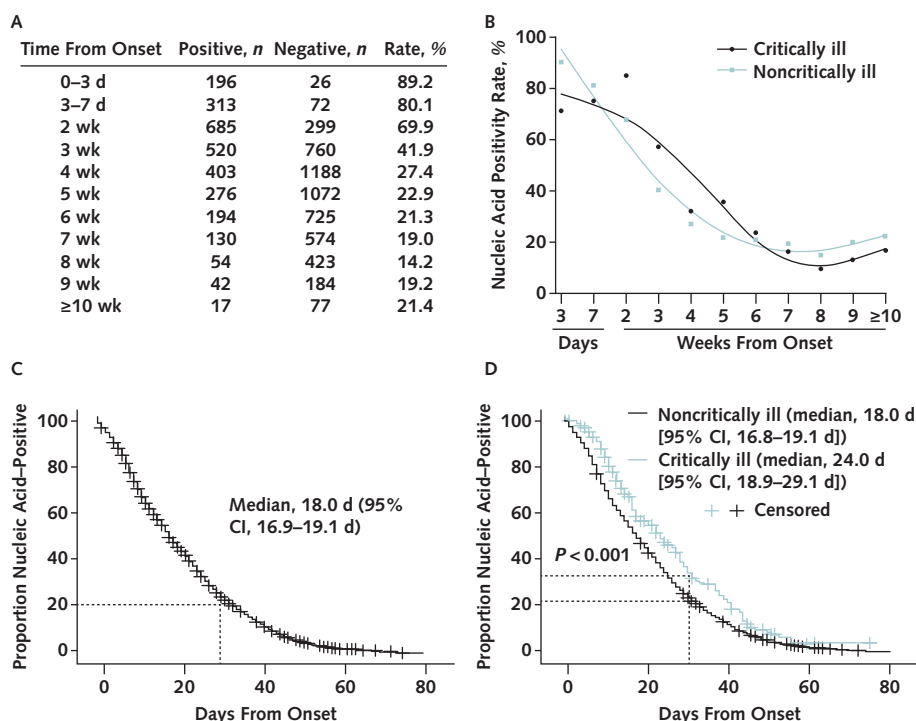
Patient Demographic Characteristics

In this retrospective study, we included 3192 adult patients with clinically diagnosed (*n* = 1050) or laboratory-confirmed (*n* = 2142) COVID-19. Among these patients, 1585 (49.7%) were men and 1607 (50.3%) were women. The median age was 62 years (range, 19 to 96 years; IQR, 51 to 70 years). The most common presenting symptoms were fever (69.5%), cough (51.9%), dyspnea (31.8%), diarrhea (19.2%), and fatigue (15.1%). The most common comorbid conditions were hypertension (29.8%), diabetes (13.8%), and coronary heart disease (7.1%).

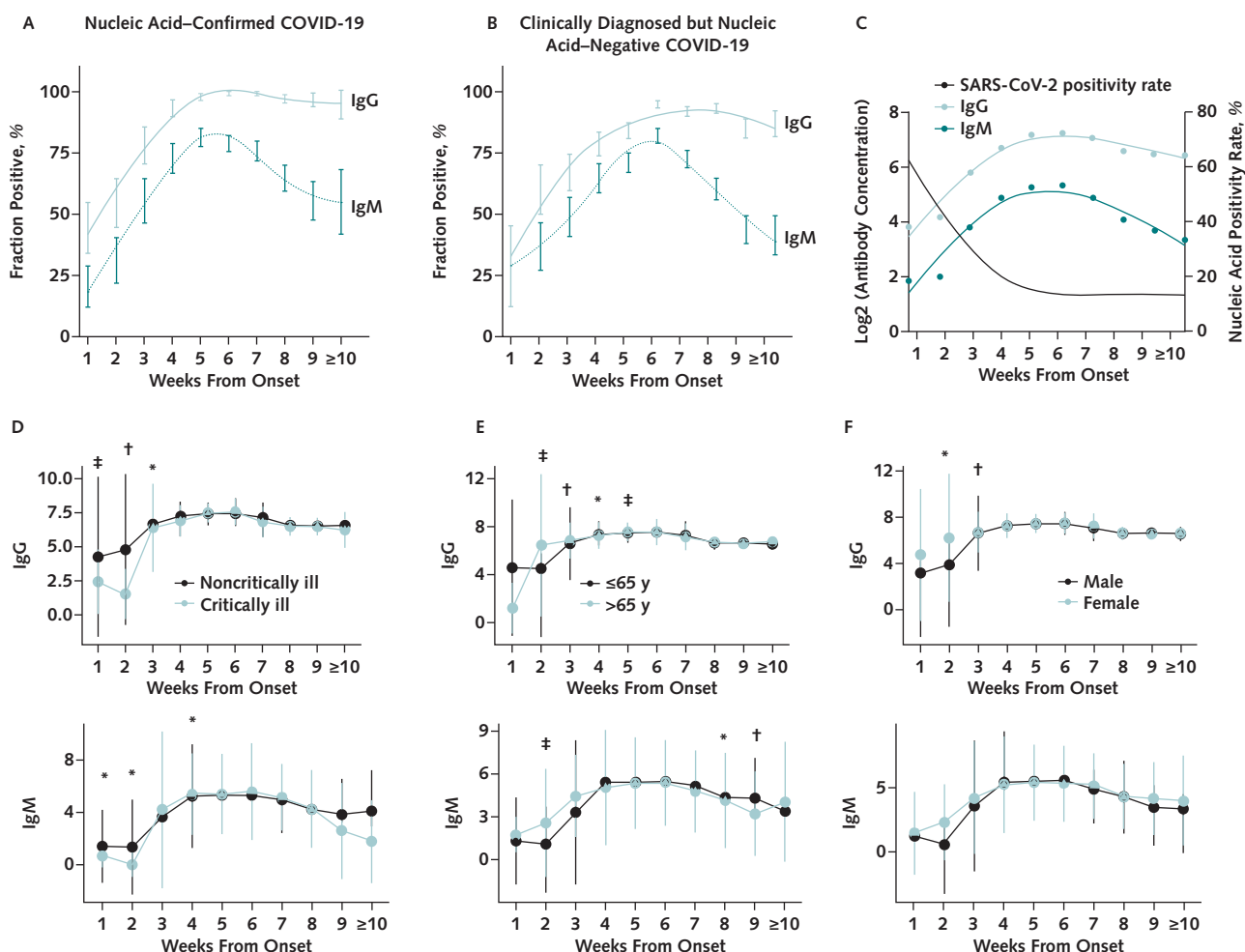
Dynamic SARS-CoV-2 Viral Positivity Rate and Antibody Titer Changes

Results for 12 780 RNA RT-PCR tests for SARS-CoV-2 (11 779 nasopharyngeal and 1001 oropharyngeal swabs) from 3192 participants were available for analysis. The

Figure 1. Dynamics of SARS-CoV-2 virus PCR positivity rate and durations in 2142 patients with laboratory-confirmed COVID-19.



COVID-19 = coronavirus disease 2019; RT-PCR = reverse transcriptase polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. **A.** Variation in RT-PCR positivity rates for SARS-CoV-2 test results from nasopharyngeal swabs during the time after symptom onset in 2142 patients with laboratory-confirmed COVID-19. *n* = total number of specimens in the indicated period. **B.** The time-varying positivity rates of SARS-CoV-2 detection in noncritically ill or critically ill patients with laboratory-confirmed COVID-19. **C.** Kaplan-Meier curve showing the time to the last positive result on SARS-CoV-2 RNA testing in all patients with laboratory-confirmed COVID-19. The dotted line represents day 30 on the x-axis. The median duration of viral positivity and its 95% CI were estimated by the Kaplan-Meier curve. **D.** Kaplan-Meier curve showing the time to the last detected positive result on SARS-CoV-2 RNA testing in noncritically ill or critically ill patients with laboratory-confirmed COVID-19 (log-rank *P* < 0.001). The median durations of viral positivity and their 95% CIs were estimated by the Kaplan-Meier curve. Censored = still positive at the last observation.

Figure 2. Dynamics of the seroconversion rate and SARS-CoV-2 titer changes.

COVID-19 = coronavirus disease 2019; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. **A** and **B**. Dynamic seroconversion rates in 2142 patients with laboratory-confirmed and 1050 patients with clinically diagnosed COVID-19. **C**. Time-varying changes in antibody titers (log₂-transformed values) compared with rate of positive viral RNA test results. **D** to **F**. Serum SARS-CoV-2-specific IgG and IgM changes in all 3192 patients with COVID-19 divided by the clinical classification, age, and sex. Data represent the median and interquartile range. *P* values in each figure are based on the linear mixed model and the Wald test, adjusting for the other 2 characteristics.

* *P* < 0.05.

† *P* < 0.01.

‡ *P* < 0.001.

median testing frequency per patient was 4 times (IQR, 3 to 5 times). We obtained 8364 testing intervals, which is the time between 2 consecutive viral RNA tests in a person. The average testing interval was 4.7 days. Among the 12 780 specimens, SARS-CoV-2 RNA was detected in 3064 swabs (24.0%). We found no discernible differences in detection rates between the nasopharyngeal and oropharyngeal swabs among patients that had both types available. Therefore, we focused on nasopharyngeal swabs from 2142 patients with laboratory-confirmed COVID-19 for subsequent analysis. Of note, the rate of positive viral testing reached its peak (nearly 90%) in the first 3 days, then decreased to 69.9% in the second week, after which it declined sharply to 27.4% in the fourth week. We found that the positivity rates

remained around 20% in samples tested even after the fifth week. The most prolonged duration of viral PCR positivity was more than 10 weeks (Figure 1, A and B).

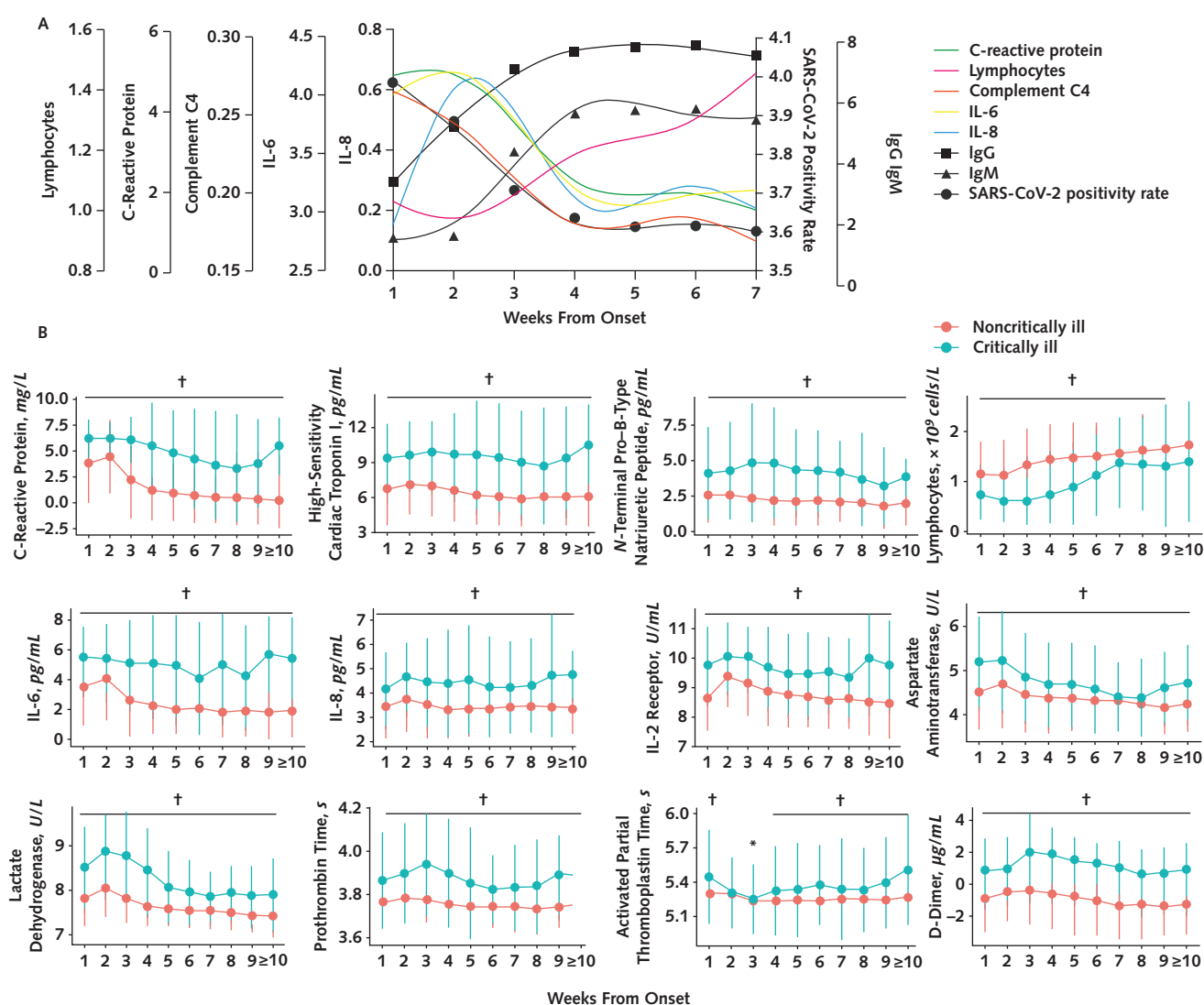
Further, we analyzed the SARS-CoV-2 positivity duration in individual patients. The median duration was 18.0 days (95% CI, 16.9 to 19.1 days) (Figure 1, C). The median duration in critically ill patients was significantly longer than in noncritically ill patients by the log-rank test (24.0 days [CI, 18.9 to 29.1 days] vs. 18.0 days [CI, 16.8 to 19.1 days]; *P* < 0.001) (Figure 1, D). Moreover, being critically ill with COVID-19 was an independent risk factor for longer viral positivity, with adjustment for age, sex, and prognosis-related comorbid conditions (hazard ratio, 0.700 [CI, 0.595 to 0.824]; *P* < 0.001) (Appendix Figure 1, available at [Annals.org](https://www.annals.org)).

The average time interval between 2 consecutive serologic tests was 6.3 days (SD, 3.8 days) in all 3192 patients. In 2142 patients with laboratory-confirmed COVID-19, the IgM-positive rate was 19.3% in the first week, increased gradually to 55.6% in the third week, and peaked in the fifth week (81.5%). Then, it decreased steadily to around 55% after the ninth week. The IgG-positive rate was 44.6% in the first week and 54.2% in the second. It then increased to 93.3% in the fourth week and remained high in the following weeks. The seroconversion for IgG approached 100% at 4 to 5 weeks after onset (Figure 2, A). In 1050 patients with clinically

diagnosed COVID-19, the IgG-positive rate was 28.6% in the first week and 60.9% in the second. Then, it peaked at 96.7% in the sixth week (Figure 2, B). The kinetics and peaks of antibody positivity rates in the clinically diagnosed group were similar to those in the laboratory-confirmed group.

Consistent with seroconversion curves, the temporal dynamics of serum antibody levels showed that both antibody levels gradually increased during the disease progression (Figure 2, C). Levels of IgM were relatively low in the first week but increased to a peak in the fourth week and then decreased steadily. For IgG, levels

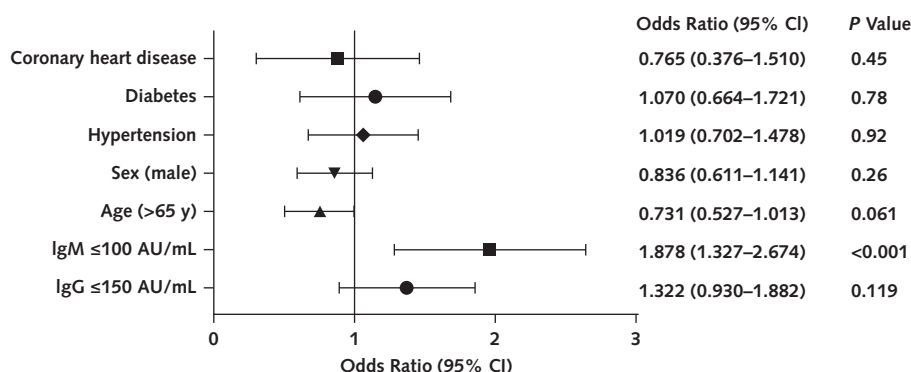
Figure 3. Dynamics of laboratory measurements for patients with COVID-19.



COVID-19 = coronavirus disease 2019; IL = interleukin. **A.** Dynamic changes in serum laboratory indices (log2-transformed values, except for lymphocyte count) over time, together with the time-varying positivity rate of viral testing results and changes in antibody titers in all 3192 patients. **B.** Dynamic changes in each serum laboratory marker (log2-transformed values, except for lymphocyte count) over time in noncritically and critical cases. *P* values demonstrate the differences between the 2 groups in each week and are based on the linear mixed model and the Wald test, adjusting for age and sex. The *P* values in weeks under black horizontal lines were the same and are labeled above.

* *P* < 0.01.

† *P* < 0.001.

Figure 4. The predictive variables to evaluate the PVP and SVP patients.

The forest plot shows odds ratios and 95% CIs based on multivariate logistic regression analysis models for PVP and SVP groups. All variables were binary. IgG ≤150 AU/mL and IgM ≤100 AU/mL indicate that SARS-CoV-2-specific antibodies of patients with COVID-19 had never been more than these values during the entire course. COVID-19 = coronavirus disease 2019; PVP = persistent viral positivity; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SVP = short viral positivity.

increased sharply and remained high for more than 10 weeks after onset (Figure 2, C). Next, we aligned the temporal dynamics of serum antibody levels to viral PCR positivity rate. It seems that as antibodies increased over time, the viral positivity rate decreased. The ranges of antibody titers largely overlapped over the disease course between subpopulations according to COVID-19 severity, age, and sex, although there were statistically significant but modest differences in antibody titers between the subpopulations at a few time points (Figure 2, D to F).

Dynamic Serum Marker Changes and Their Correlation With Disease Severity

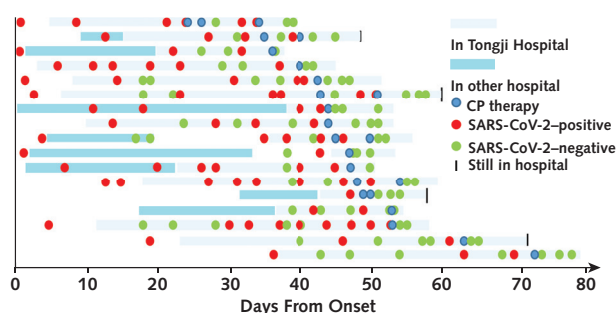
We aligned the dynamic changes of serum markers to viral positivity rates and seroconversion rates (Figure

3, A). Serum C-reactive protein, complement C4, and interleukin-6 (IL-6) reached their peak levels within the first week after disease onset, when the viral positivity rate was high. Interleukin-8 increased sharply and reached a peak in the second week. These indices remained at elevated levels throughout the second week (Figure 3, A); then, they all began to decline in the third week and returned to normal by the fourth week. The kinetics were relatively consistent with the dynamics of the viral positivity rates. Lymphopenia recovered to the normal range by 4 weeks. All of these dynamic laboratory index changes corresponded well with the clinical progression and recovery process of COVID-19.

Next, we tried to show the correlation between temporal dynamics of these indices and disease severity (Figure 3, B). The levels of most serum markers were elevated in the first 2 to 3 weeks and normalized by 4 weeks in noncritically ill patients. In contrast, all of the serum markers except lymphocyte count were already much higher in critically ill patients at disease onset and remained at high levels for a longer period, some until the end of observation. Moreover, male patients and patients aged 65 years or older seemed to have worse laboratory index values than female patients and younger patients, respectively (Appendix Figure 2, available at [Annals.org](#)).

Demographic and Clinical Characteristics and Predictors for PVP in Noncritically Ill Patients With COVID-19

About 20% of patients with noncritical COVID-19 had either PVP or positive results on a viral test repeated after discharge at more than 30 days after onset (Figure 1, A). Most of these patients' clinical symptoms had markedly improved despite PVP. To show potential predictors of a persistent presence of viral RNA in noncritically ill patients, we categorized PVP and SVP groups. These 2 groups showed no differences in demographic characteristics, clinical characteristics, or symptoms (Appendix Table 3). Moreover, laboratory findings did

Figure 5. The disease timeline for 17 patients that received CP therapy.

The time course of each patient shows the times and results for each RT-PCR test and the times for each CP administration. The RT-PCR test results are shown as either positive (red spots) or negative (green spots); CP administrations are shown as blue spots. The time was calculated from onset. The stripe colors represent the time spent in either Tongji Hospital (light blue) or other hospitals (dark blue), such as Fangcang Hospital. The black vertical lines indicate that the patients remained in the hospital at the end of the data collection period. CP = convalescent plasma; RT-PCR = reverse transcriptase polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

not differ between these patients at any time point (Appendix Figure 3, available at [Annals.org](#)). Patients with high IgM levels (>100 AU/mL) at least once during the disease course were observed more frequently in the SVP group (34%) than in the PVP group (22%) ($P < 0.001$). The percentage of patients with strong IgG response (IgG level >150 AU/mL) was lower in the PVP group (70%) than in the SVP group (75%) ($P = 0.072$) (Appendix Table 3). Further, multivariable analysis confirmed that a low IgM antibody level was an independent risk factor for PVP, with adjustment for age, sex, and prognosis-related comorbid conditions by logistic regression analysis (odds ratio, 1.878 [CI, 1.327 to 2.674]; $P < 0.001$) (Figure 4).

Seventeen noncritically ill patients received transfusions of ABO-compatible CP therapy because of persistent, intractable viral replication (Figure 5). Two patients had positive SARS-CoV-2 test results when the test was repeated after discharge. Convalescent plasma was administered between 24 and 73 days from onset considering the persistent and intractable viral replication. Viral RNA became undetectable within 1 to 14 days (median, 1 day [IQR, 1 to 6 days]) after the first dose of CP in all 17 cases. Of note, in 9 of 17 patients, the virus became undetectable just 1 day after CP administration.

DISCUSSION

We did a comprehensive analysis of SARS-CoV-2 nucleic acid test results, dynamic serologic responses, and the correlations between clinical manifestations and serum markers in 3192 inpatients with COVID-19. The large cohort allowed us to identify characteristics and viral PCR positivity patterns associated with patients with COVID-19.

The viral positivity rate in nasopharyngeal specimens peaked within the initial few days of the first symptoms. This rate profile was consistent with previous studies, which showed that SARS-CoV-2 viral shedding began 2 to 3 days before symptom onset (15), peaked during the first week of the illness, then gradually declined during the following weeks (15, 16). This is in sharp contrast to the SARS-CoV-1 profile, where the viral positivity rate peaks 10 to 14 days after symptom onset (17). These findings have several practical implications. First, they indicate that the maximal viral load likely exists in the very early phase of the disease, even before symptom onset. This feature may have contributed to the high transmissibility of SARS-CoV-2. Of note, we found that many patients had already passed the peak phase of viral PCR positivity when first tested. Therefore, it is crucial for a COVID-19 containment strategy to combine immediate and strict isolation of patients and contact tracing (18). For example, the temporary Fangcang shelter hospitals in Wuhan were built rapidly and at massive scales to isolate patients and provide care (19).

Consistent with other studies (16, 20), antibody levels increased rapidly during the first 2 weeks in our study cohort, with median seroconversion times of 12 to 15 days for IgM and 3 to 6 days for IgG. Here, we showed that IgG concentrations peaked around 4 weeks after

symptom onset. Consistent with a previous study on the dynamics of seroconversion (8), we found that the IgM positivity rate was lower than that of IgG during the disease course. Indeed, the detection kits in our study had a lower sensitivity for IgM than for IgG (12). Nucleic acid detection is the gold-standard diagnostic approach for COVID-19, but it has a high false-negative rate (6, 21, 22). Information to date on the antibody responses to SARS-CoV-2 infection is conflicting (23). Although serologic tests should not replace direct viral testing as the primary tool for diagnosing an active SARS-CoV-2 infection, they could be offered as a method to support diagnosis of acute COVID-19 illness for persons who present late, when the sensitivity of RT-PCR testing may be decreasing.

More important, this large, patient-based study provides us an opportunity to show and characterize a specific population of patients with persistently positive or recurrent-positive viral tests after discharge. Although positive viral RNA in specimens may not necessarily indicate viral transmissibility, continued detection of SARS-CoV-2 for long periods or recurrent-positive detection after discharge has raised concerns that these patients are still infectious. Demographic and clinical characteristics did not differ significantly between patients who recovered within 3 weeks and those with PVP. Low IgM antibody titer levels (≤ 100 AU/mL) may serve as a risk factor for PVP. Additional data about virus infectivity based on virus isolation and culture, better predictors for these populations, and more effective antiviral treatment are urgently needed. Furthermore, CP treatment neutralized the virus within 1 to 14 days among patients with persistent SARS-CoV-2 RNA positivity in our study, which is consistent with previous case studies (24, 25). These findings suggest that CP therapy may be a useful option in the management of persistent SARS-CoV-2 infection.

Critically ill patients with COVID-19 in our cohort had positive viral testing that persisted during their disease course and until death among those who did not survive the illness. Liu and colleagues (26) also reported that critical COVID-19 cases tended to be associated with a high viral load and a long viral-positive period. We also found that extremely high levels of inflammatory cytokines, like C-reactive protein, IL-6, and IL-8, were present throughout the disease course in critically ill patients. This persistent viral-positive pattern is relevant to disease management and development of treatment strategies. For critically ill patients with PVP, it would be worth further exploring intensive supportive therapy combined with antiviral therapy (for example, remdesivir [27, 28] or CP [24, 25]) and anti-inflammatory treatment (for example, IL-6 and IL-6 receptor antagonists [29]). Moreover, further investigation is needed to determine whether PVP is associated with a defect in virus-eliminating immune response, like defective type 1 interferons and T-cell or B-cell responses, which would allow SARS-CoV-2 to evade the immune attack.

This study has some limitations. First, it was a retrospective study with inevitable missing values. Second, the viral and serologic testing were not consistently done

at regular intervals because of the sudden outbreak of the epidemic and the limited medical resources at that time.

In summary, the findings of this study have enriched our understanding of the various patterns of SARS-CoV-2 positivity and the disease course of COVID-19. Appropriate prevention and therapeutic approaches should be established on the basis of viral kinetics, clinical manifestations, and laboratory testing. Further work is urgently needed to improve our understanding of the potential effects of the virus, host innate and adaptive immune responses, and their interactions during the acute and convalescent phases of COVID-19.

From National Clinical Research Center for Gynecology and Obstetrics and Cancer Biology Research Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China (Y.F., E.G., L.H., J.L., B.Y., F.L., Z.W., Y.L., R.X., C.L., Y.H., X.W., F.L., L.Y., T.Q., K.L., P.W., D.M., C.S., G.C.); Key Laboratory of Tropical Translational Medicine of Ministry of Education, College of Biomedical Informatics and Engineering, Hainan Medical University, Haikou, and College of Bioinformatics Science and Technology, Harbin Medical University, Harbin, China (Y.L.); and Ministry of Education Key Laboratory of Environment and Health, State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China (C.W.).

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Reproducible Research Statement: *Study protocol:* Available from Dr. Sun (e-mail, suncydoctor@gmail.com). *Statistical code:* Available from Dr. Fu (e-mail, fyth@qq.com). *Data set:* Available from Dr. Ma (e-mail, dingma424@126.com).

Corresponding Authors: Gang Chen, PhD (e-mail, tjchengang@hust.edu.cn); Chaoyang Sun, PhD (e-mail, suncydoctor@gmail.com); Ding Ma, PhD (e-mail, dingma424@126.com); and Peng Wu, PhD (e-mail, Pengwu8626@126.com), National Clinical Research Center for Gynecology and Obstetrics and Cancer Biology Research Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095, Jiefang Avenue, Qiaokou District, Wuhan, Hubei, China 430030.

Current author addresses and author contributions are available at Annals.org.

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Current Author Addresses: Drs. Fu, Guo, He, Liu, Yang, Li, Wang, Li, Xiao, Liu, Huang, Wu, Lu, You, Qin, Li, Wu, Ma, Sun, and Chen: Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095, Jiefang Avenue, Qiaokou District, Wuhan, Hubei, China 430030.

Dr. Yongsheng Li: Key Laboratory of Tropical Translational Medicine of Ministry of Education, College of Biomedical Informatics and Engineering, Hainan Medical University, Haikou, China 571119.

Dr. Chaolong Wang: School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China 430030.

Author Contributions: Conception and design: Y. Fu, Yongsheng Li, E. Guo, L. He, P. Wu, D. Ma, C. Sun, G. Chen.

Analysis and interpretation of the data: Y. Fu, Yongsheng Li, E. Guo, L. He, J. Liu, B. Yang, F. Li, Z. Wang, Yuan Li, R. Xiao, C. Liu, Y. Huang, X. Wu, F. Lu, L. You, T. Qin, C. Wang, K. Li, P. Wu, D. Ma, C. Sun, G. Chen.

Drafting of the article: Y. Fu, E. Guo, B. Yang, F. Li, Yuan Li, R. Xiao, C. Liu, Y. Huang, X. Wu, F. Lu, L. You, T. Qin, K. Li, P. Wu, C. Sun.

Critical revision of the article for important intellectual content: Y. Fu, Yongsheng Li, E. Guo, L. He, C. Wang, P. Wu, D. Ma, C. Sun, G. Chen.

Final approval of the article: Y. Fu, Yongsheng Li, E. Guo, L. He, J. Liu, B. Yang, F. Li, Z. Wang, Yuan Li, R. Xiao, C. Liu, Y. Huang, X. Wu, F. Lu, L. You, T. Qin, C. Wang, K. Li, P. Wu, D. Ma, C. Sun, G. Chen.

Provision of study materials or patients: L. He, Z. Wang, P. Wu, C. Sun.

Statistical expertise: Y. Fu, E. Guo, C. Wang, C. Sun.

Administrative, technical, or logistic support: Yongsheng Li, L. He, P. Wu, C. Sun.

Collection and assembly of data: Y. Fu, J. Liu, B. Yang, F. Li, Z. Wang, Yuan Li, R. Xiao, C. Liu, Y. Huang, X. Wu, F. Lu, L. You, T. Qin, K. Li, P. Wu, D. Ma, C. Sun.

Appendix Table 1. Diagnostic Criteria and Definitions for Patients With COVID-19

| Case | Diagnostic Criteria | Patients, <i>n</i> |
|----------------------------|--|--------------------|
| Confirmed cases | Both epidemiologic history and clinical manifestations as follows: Epidemiologic history: 1. Travel in areas with reported cases within 14 d before onset 2. Direct contact history with certain patients with COVID-19 within 14 d before onset 3. Clustering occurrence of cases Clinical manifestations: 1. Fever or respiratory symptoms 2. Typical imaging features of viral pneumonia 3. Normal or decreased total leukocyte counts and decreased lymphocyte counts One of the following evidence types: 1. A positive result of SARS-CoV-2 testing by real-time PCR 2. A virus gene sequence highly homologous to SARS-CoV-2 3. A positive result of SARS-CoV-2-specific IgM and IgG testing, seroconversion of IgG, or an increase in IgG of >4-fold between the acute and convalescent phases | 3192 |
| Clinically diagnosed cases | Clinically diagnosed patients were based on epidemiologic history; typical clinical manifestations, especially positive findings on CT scans; and evidence of antibody response. | 1050 |
| Laboratory-confirmed cases | Patients with ≥ 1 positive result of SARS-CoV-2 testing by real-time PCR. | 2142 |
| Critically ill patients | Patients with COVID-19 and any of the following conditions: intubation required, shock, other organ failure, or admission to the intensive care unit. | 502 |
| Noncritically ill patients | Mild cases had clinical symptoms only. Moderate cases had respiratory symptoms and pneumonic images shown in the CT scan of the chest. Severe cases had any of the following criteria: respiratory distress, respiratory rate ≥ 30 breaths/min, finger oxygen saturation in resting state $\leq 93\%$, or $P_{aO_2}/F_{iO_2} \leq 300$ mm Hg. Mildly, moderately, and severely ill patients were categorized as noncritically ill. | 2690 |

COVID-19 = coronavirus disease 2019; CT = computed tomography; PCR = polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Appendix Table 2. Primer Sequences for the Real-Time Reverse Transcriptase Polymerase Chain Reaction Assay for SARS-CoV-2

| Primer Name | Sequence |
|--|---|
| Target 1 (ORF1ab) comprised forward primer | CCCTGTGGGTTTT ACACTTAA |
| Reverse primer | ACGATTGTGCATCAGCTGA |
| The probe | 5'-VIC-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3' |
| Target 2 (N) comprised forward primer | GGGGAACCTCTCCTGCT AGAA T |
| Reverse primer | CAGACATTTTGCTCTCAAGCTG |
| The probe 5'-FAM- | TTGCTGCTGCTTGACAGATT-T AMRA-3' |

SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Appendix Table 3. Baseline Characteristics of Patients With COVID-19 and PVP or SVP*

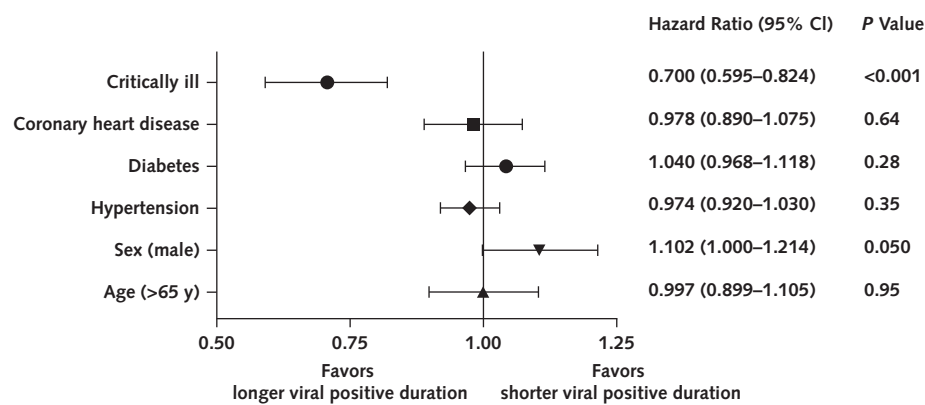
| Characteristic | Patients, n (%) | | | P Value |
|--|------------------|------------------|------------------|---------|
| | Total (n = 1251) | PVP (n = 330) | SVP (n = 921) | |
| Median age (IQR), y | 62.0 (48.0–70.0) | 62.0 (48.3–69.0) | 62.0 (47.0–70.0) | 0.53 |
| <65 y | 703 (56) | 191 (58) | 512 (56) | 0.52 |
| ≥65 y | 548 (44) | 139 (42) | 409 (44) | 0.52 |
| Sex | | | | |
| Female | 686 (55) | 185 (56) | 501 (54) | 0.60 |
| Male | 565 (45) | 145 (44) | 420 (46) | 0.60 |
| Comorbid conditions | | | | |
| Hypertension | 347 (28) | 92 (28) | 255 (28) | 0.95 |
| Coronary heart disease | 71 (6) | 16 (5) | 55 (6) | 0.45 |
| Diabetes | 172 (14) | 44 (13) | 128 (14) | 0.80 |
| Chronic obstructive pulmonary disease | 8 (<1) | 1 (<1) | 7 (<1) | 0.62 |
| Cancer | 47 (4) | 14 (4) | 33 (4) | 0.59 |
| Cerebrovascular disease | 30 (2) | 6 (2) | 24 (3) | 0.42 |
| Tuberculosis | 16 (1) | 3 (1) | 13 (1) | 0.68 |
| Signs and symptoms at disease onset | | | | |
| Fever | 917 (73) | 228 (69) | 689 (75) | 0.044 |
| Cough | 680 (54) | 193 (58) | 487 (53) | 0.079 |
| Fatigue | 216 (17) | 58 (18) | 158 (17) | 0.86 |
| Chill | 98 (8) | 28 (8) | 70 (8) | 0.61 |
| Dyspnea | 384 (31) | 115 (35) | 269 (29) | 0.057 |
| Chest tightness | 172 (14) | 44 (13) | 128 (14) | 0.80 |
| Headache | 60 (5) | 18 (5) | 42 (5) | 0.51 |
| Chest pain | 36 (3) | 10 (3) | 26 (3) | 0.85 |
| Abdominal pain | 13 (1) | 3 (1) | 10 (1) | 0.96 |
| Nausea | 71 (6) | 22 (7) | 49 (5) | 0.36 |
| Vomiting | 43 (3) | 11 (3) | 32 (3) | 0.90 |
| Anorexia | 96 (8) | 29 (9) | 67 (7) | 0.38 |
| Myalgia | 103 (8) | 28 (8) | 75 (8) | 0.85 |
| Vital signs on admission | | | | |
| Arterial pressure | | | | 0.101 |
| <90 mm Hg | 6 (<1) | 2 (<1) | 9 (1) | |
| 90–140 mm Hg | 952 (79) | 240 (76) | 725 (81) | |
| ≥140 mm Hg | 242 (20) | 73 (23) | 159 (18) | |
| Median pulse (IQR), beats/min | 80 (17) | 88 (80–100) | 88 (79–102) | 0.63 |
| Respiratory rate | | | | 0.54 |
| <24 breaths/min | 1188 (95) | 312 (95) | 876 (95) | |
| 24–30 breaths/min | 48 (4) | 10 (4) | 38 (4) | |
| ≥30 breaths/min | 8 (<1) | 3 (1) | 5 (<1) | |
| Percutaneous oxygen saturation, <93% | 30 (2) | 6 (2) | 25 (3) | 0.37 |
| Antibodies† | | | | |
| IgG >150 AU/mL | 492 (73) | 217 (70) | 275 (75) | 0.072 |
| IgM >100 AU/mL | 191 (28) | 67 (22) | 124 (34) | <0.001 |

COVID-19 = coronavirus disease 2019; IQR = interquartile range; PVP = persistent viral positivity; SVP = short viral positivity.

* The means for continuous variables in 2 independent groups were tested by the Wilcoxon rank-sum test for data with skewed distribution. Proportions for categorical variables were tested by the χ^2 test. The Fisher exact test was used when the sample sizes were small.

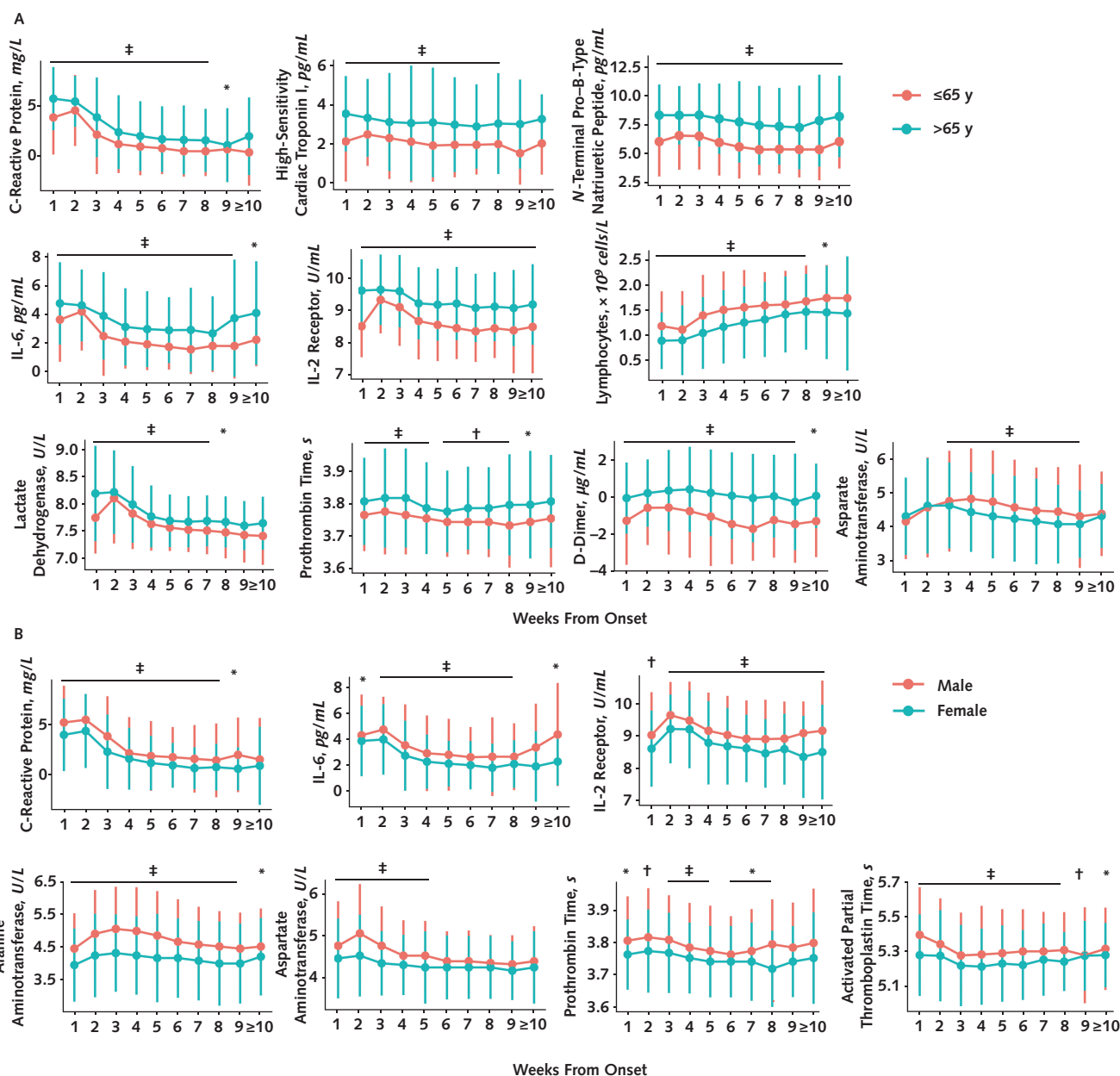
† The seroconversion data were collected in 365 patients in the SVP group and 309 patients in the PVP group.

Appendix Figure 1. The predictor variables for viral positivity duration in 2142 laboratory-confirmed cases.



The forest plot shows hazard ratios and 95% CIs from multivariate Cox proportional hazards models for risk factors for viral positivity duration in 2142 laboratory-confirmed cases. Variables included demographic information, common comorbid conditions, and clinical disease severity. All variables were binary.

Appendix Figure 2. The dynamic laboratory indices for patients with COVID-19, categorized by age and sex.



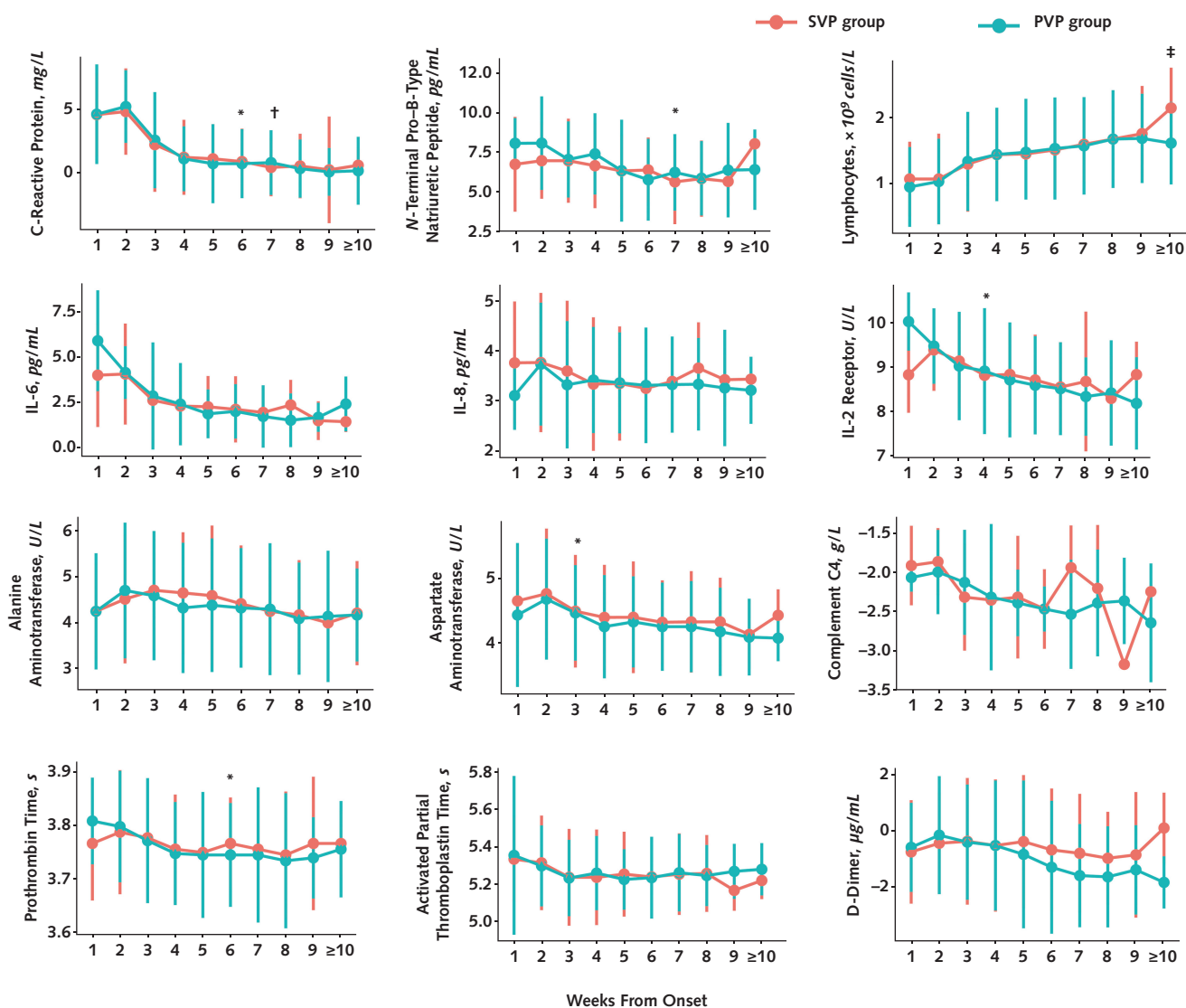
The time-varying changes in laboratory indices (log2-transformed values, except for lymphocyte count) are shown for patients with COVID-19 in different groups divided by age and sex. All *P* values are based on the linear mixed model and the Wald test, adjusting for clinical classification, as well as sex or age. The *P* values in weeks under black horizontal lines were the same and are labeled above. COVID-19 = coronavirus disease 2019; IL = interleukin.

* *P* < 0.05.

† *P* < 0.01.

‡ *P* < 0.001.

Appendix Figure 3. The dynamic laboratory indices for patients with COVID-19 who have PVP or SVP.



The time-varying changes in laboratory indices for patients with COVID-19 are shown, dichotomized by viral positivity duration. Data represent the median values (log2-transformed values, except for lymphocyte count) and interquartile ranges. All *P* values are based on the linear mixed model and the Wald test, adjusting for age and sex. COVID-19 = coronavirus disease 2019; IL = interleukin; PVP = persistent viral positivity; SVP = short viral positivity.

* *P* < 0.05.

† *P* < 0.01.

‡ *P* < 0.001.