Prevalence of IgG antibodies to SARS-CoV-2 in Wuhan –

implications for the ability to produce long-lasting protective antibodies against

SARS-CoV-2

Tables and Figures: 5 tables and 1 figure.

Tao Liu, MD[1]*; Sanyun Wu, MD, PhD[2]*; Huangheng Tao, PhD[3]*; Guang Zeng, MD[1]; Fuling Zhou, MD[2†]; Fangjian Guo, MD, PhD[4,5†]; Xinghuan Wang, MD[1†]

[1]Department of Urology, Zhongnan Hospital of Wuhan University, Wuhan, Hubei

[2]Department of Hematology, Zhongnan Hospital of Wuhan University, Wuhan, Hubei

[3] State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) and Key Laboratory for Oral Biomedicine of Ministry of Education (KLOBM), School and Hospital of Stomatology, Wuhan University, Wuhan, Hubei

[4]Department of Obstetrics & Gynecology, The University of Texas Medical Branch at Galveston, Galveston, TX

[5]Center for Interdisciplinary Research in Women's Health, The University of Texas Medical Branch at Galveston, Galveston, TX

* Contributing equally

† Contributing equally

Correspondence to: Xinghuan Wang, MD
Department of Urology
Zhongnan Hospital of Wuhan University
No. 169 Donghu Road, Wuchang District
Wuhan, Hubei 430071, China
Email: wangxinghuan@whu.edu.cn
and
Fangjian Guo, MD, PhD
Department of Obstetrics & Gynecology
Center for Interdisciplinary Research in Women's Health
The University of Texas Medical Branch at Galveston
301 University Blvd
Galveston, TX 77555-0587
Tel: (409) 772-8599
Email: faguo@utmb.edu
and
Fuling Zhou, MD
Department of Hematology
Zhongnan Hospital of Wuhan University
No. 169 Donghu Road, Wuchang District

Wuhan, Hubei 430071, China
Email: zhoufuling@whu.edu.cn

Primary Funding Sources

Role of the Funder/Sponsor

The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript, and decision to submit the manuscript for publication.

Financial Disclosures

The authors have no conflict of interest to disclose.

Author Contributions: Dr. X. Wang had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs T. Liu, S. Wu and H. Tao contributed equally to the study. Drs F. Zhou, F. Guo, and X. Wang contributed equally as senior authors. Concept and design: F. Zhou and X. Wang. Acquisition, analysis, or interpretation of data: T. Liu, S. Wu and H. Tao, Guang Zeng. Drafting of the manuscript: T Liu, S. Wu, F. Guo. Critical revision of the manuscript for important intellectual content: S. Wu, X. Wang. Statistical analysis: T. Liu, H. Tao.

Data and code availability statement

Data and analyses codes are available from the corresponding authors on request. All request for raw and analyzed data and materials will be reviewed by the corresponding authors to verify whether the request is subject to any intellectual property or confidentiality obligations. Access will be granted after a signed data access agreement is attained.

Abstract

Background

It is to be determined whether people infected with SARS-CoV-2 will develop long-term immunity against SARS-CoV-2 and retain long-lasting protective antibodies after the infection is resolved. This study was to explore to explore the outcomes of IgG antibodies to SARS-CoV-2 in four groups of individuals in Wuhan, China.

Methods

We included the following four groups of individuals who received both COVID-19 IgM/IgG tests and RT-PCR tests for SARS-CoV-2 from February 29, 2020 to April 29, 2020: 1470 hospitalized patients with COVID-19 from Leishenshan Hospital, Zhongnan Hospital of Wuhan University, and Wuhan No. 7 Hospital, 3832 healthcare providers without COVID-19 diagnosis, 19555 general workers, and 1616 other patients to be admitted to the hospital (N=26473). COVID-19 patients who received IgM/IgG tests <21 days after symptom onset were excluded.

Results

IgG prevalence was 89.8% (95% CI 88.2-91.3%) in COVID-19 patients, 4.0% (95% CI 3.4-4.7%) in healthcare providers, 4.6 (95% CI 4.3-4.9 %) in general workers, and 1.0% in other patients (p all <0.001 for comparisons with COVID-19 patients). IgG prevalence increased significantly by age among healthcare workers and general workers. Prevalence of IgM antibodies to SARS-CoV-2 was 31.4% in COVID-19 patients, 1.5% in healthcare providers, 1.3% in general workers, and 0.2% in other patients.

3

Conclusions

Very few healthcare providers had IgG antibodies to SARS-CoV-2, though a significant

proportion of them had been infected with the virus. After SARS-CoV-2 infection, people

are unlikely to produce long-lasting protective antibodies against this virus.

Introduction

Currently, coronavirus disease in 2019 (COVID-19) caused by severe acute respiratory
syndrome coronavirus 2 (SARS-CoV-2)[1-4] has become a global pandemic. The virus
was freely transmitted among residents in the communities[5-8] in Wuhan, China from late
November 2019 till several days after the lockdown of the city on January 23, 2020.
Most of SARS-CoV-2 infections do not require medical attention[9-11] and only about 5%
COVID-19 cases in China need intensive care[12]. As of May 20, 2020, no effective
therapies or vaccines for COVID-19 have been reported. Though, numerous therapeutic
approaches are under investigation around the world, including existing anti-human
immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and
influenza virus medications[13], RNA-dependent RNA polymerase inhibitor Remdesivir[14],
antimalarials (chloroquine/hydroxychloroquine), IL-6 blocker Tocilizumab, serum from
recovered COVID-19 patients, and inactivated virus, subunit and recombinant vaccines.
Among those therapeutic approaches, Remdesivir is most promising so far, although an
underpowered trial failed to demonstrate treatment benefits.[15,16]

The presence of SARS-CoV-2 in COVID-19 patients is usually confirmed using real-
time reverse-transcriptase polymerase chain reaction (RT-PCR) method.[17-19] Due to
sample collection method, discrepancies in personnel skills training and low virus load
in throat swabs after symptom onset[20], RT-PCR methods have high false-negative test
results[19,21,22]. Several days after symptom onset when infected patients start to seek
medical attention, virus load in clinical specimens of upper respiratory tract will become

5

relatively low[20,23]. It often takes two or three repeatedly collected specimens to get a positive test result in COVID-19 patients. SARS-CoV-2 enters respiratory epithelial cells via interactions with angiotensin converting enzyme 2 (ACE2)[24]. The spike (S) protein of SARS-CoV-2 mediates binding of the virus with Spike protein's receptor ACE2 and promotes fusion of viral and host cell membranes and subsequent virus entry into the host cell. In patients infected with SARS-CoV-2, IgM antibodies are detectable around 7 days post infection and IgG antibodies usually take two weeks to develop.[20,24-26] Recently, several COVID-19 IgM/IgG rapid tests have been developed around the world[27]. It is reported that in some recovered COVID-19 patients who had two negative RT-PCR tests on nasal or throat swabs taken at least 24 hours apart, segments of virus RNA were still detected in other types of clinical samples, especially in fecal swabs[28]. Additionally, some patients had recurrent positive RT-PCR tests on nasal or throat swabs after recovered from COVID-19.[28] It still unknown whether COVID-19 patients will develop long-term immunity against SARS-CoV-2 and retain long-lasting protective antibodies after the infection is resolved. Large-scale sero-epidemiological studies are also needed to assess infection attack rates and disease incidence in the population and herd immunity. In this study, we reported the experiences in COVID-19 IgM/IgG testing in Wuhan. We assessed prevalence of IgG antibodies against SARS-CoV-2 in hospitalized patients with COVID-19 from Zhongnan Hospital of Wuhan University and Leishenshan Hospital (set up on an emergency basis to admit COVID-19 patients and managed by Zhongnan Hospital of Wuhan University), and Wuhan No. 7 Hospital, healthcare providers without a confirmed COVID-19 diagnosis working in Zhongnan Hospital of Wuhan University, and people from the general population in Wuhan.

6

Considering that Wuhan was the early epicenter of COVID-19 outbreak, most of these healthcare providers would be inevitably exposed to SARS-CoV-2 during the early days of the outbreak (from late November 2019 to January 20, 2020) when person-to-person transmission was not suspected and little personal protection against this virus was employed among medical personnel, a significant proportion of whom would get infected with the virus.

Results

Characteristics of participants

Mean age was 58.7 years in 1470 COVID-19 patients, 37.1 years in 3832 healthcare providers, 41.6 years in 19555 general workers and 53.3 years in other patients (**Table 1**). Hospitalized COVID-19 patients were composed of more older people, while healthcare providers and general workers were mostly young adults. Among COVID-19 patients, median time from symptom onset to IgM/IgG test was 41 days (interquartile range 33-50 days). The last RT-PCR tests for SARS-CoV-2 were positive in only eight COVID-19 patients and none of three patients was tested negative for IgG antibodies to SARS-CoV-2.

Prevalence of IgG and IgM antibodies to SARS-CoV-2

Prevalence of IgG antibodies to SARS-CoV-2 was 89.8% (95% CI 88.2-91.3%) in

COVID-19 patients (**Table 2**) compared to 4.0% (95% CI 3.4-4.7%) in healthcare providers, 4.6% (95% CI 4.3-4.9%) in general workers, and 1.0% in other patients (p all <0.001 for comparing to COVID-19 patients). Only the comparison of IgG prevalence between healthcare workers and general workers was not significant (p=0.39). IgG prevalence increased significantly by age among healthcare providers, and was 2.8% in those <30 years old, 9.6% in those 60-69 years old and 10.0% in those ≥70 years old (p<0.001 for trend). IgG prevalence also increased significantly by age among general workers. Prevalence of IgM antibodies to SARS-CoV-2 was 31.4% (95% CI 29.0-33.7%) in COVID-19 patients, 1.5% (95% CI 1.1-1.8%) in healthcare providers, 1.3 (95% CI 1.1-1.5%) in general workers, and 0.2% (95% CI 0-0.4%) in other patients (**Table 3**).

IgM and IgG antibodies to SARS-CoV-2 and mortality in COVID-19 patients

Among COVID-19 patients, mean age was similar between those with IgG antibodies to SARS-CoV-2 and those without (**Table 4**). Presence of IgG antibodies to SARS-CoV-2 was not associated with most demographic characteristics, disease severity, presence of comorbidities, treatment received, and clinical characteristics, except for antibiotics treatment, chloroquine/hydroxychloroquine treatment, and needing intubation. IgG prevalence and IgM prevalence among hospitalized patients with COVID-19 by demographic and clinical characteristics are presented in **Table 5**. Mortality rate was 1.3% (95% CI 0.7-1.9%) in those with IgG antibodies to SARS-CoV-2 and was 3.3% (95% CI 0.4-6.2%) in those without (**Figure 1**). Mortality risk was similar between those with IgG antibodies to SARS-CoV-2 and those without (adjusted hazard ratio 0.45 95% CI 0.16-

8

1.24, P=0.12).

Discussion

We analyzed prevalence of IgG antibodies to SARS-CoV-2 in hospitalized COVID-19 patients, healthcare providers without a confirmed COVID-19 diagnosis, general workers, and other patients to be admitted to hospital. The most intriguing finding of this study is that only 4% of healthcare providers without confirmed COVID-19 diagnosis had IgG antibodies to SARS-CoV-2 in their blood. Most of the healthcare providers were exposed to SARS-CoV-2 during the first few months of the outbreak when use of personal protection equipment was sparse as person-to-person transmission was not suspected. COVID-19 IgM/IgG tests in the US and around the world as reported in the news constantly showed that the true infection rate would be 10 to 80 times higher than that had been confirmed by RT-PCR tests for SARS-CoV-2. Seroprevalence of antibodies to SARS-CoV-2 in 1021 people before resuming work from April 3 to 15, 2020 in Wuhan was reported to be ~10%,[29] about 20 times higher than the infection attack rate calculated from the confirmed COVID-19 cases. In New York City, a 21.2% positive rate was reported with young and middle-aged people having the highest positive rate. The proportion of people infected with SARS-CoV-2 who have no symptom or only mild symptoms that do not need medical attention or hospitalization may account for the majority of SARS-CoV-2 infections. Currently, no effective therapeutics are available for treating COVID-19 patients. Detecting patients with SARA-CoV-2 infection who are not in urgent need of hospitalization will provide no

9

benefit to these patients, though it may have important public health applications in tracing their close contacts and preventing these people from spreading the infection to others. In Zhongnan Hospital of Wuhan University, 2.88% (118/4099) healthcare workers were diagnosed with COVID-19 before March 16, 2020. With a moderate estimation, the true infection rate would be ten times that had been confirmed, i.e., >25% of those healthcare providers without diagnosed COVID-19 had been infected. However, only 4% of those infected healthcare workers without confirmed COVID-19 still had IgG antibodies to SARS-CoV-2. They just got infected with SARS-CoV-2 and cleared the virus by their own immune systems. No long-lasting protective antibodies against SARS-CoV-2 were produced in these healthcare providers. Our observed high prevalence of IgG antibodies to SARS-CoV-2 in older groups (60-69 years old and ≥70 years old) among health care workers and general worker in Wuhan also raised the concern that IgG antibodies to SARS-CoV-2 would be lost some time after the infection was cleared, as young or middle-aged people usually took more social responsibilities and had higher chances to get infected during lockdown of the city. We also found that >10% of confirmed COVID-19 cases had no detectable serum levels of IgG antibodies to SARS-CoV-2 after 21 days post symptom onset. They were unlikely to not produce IgG antibodies to SARS-CoV-2 after being infected with the virus[20,24-26,30]. Long et al reported that after 17-19 days post symptom onset, IgG was positive in all patients with COVID-19.[30] Lack of blood samples >17 days post symptom onset may be responsible for negative IgG tests in some patients reported in previous literatures.[25,26] Therefore, >10% patients in our study who had no IgG antibodies after 21 days post symptom onset most likely lost those IgG antibodies after the infection was resolved.

10

After infection with SARS-CoV-1, patients start to produce SARS-specific IgG antibody in the second week, which persists for a long time[31,32]. Even after 210 days after symptom onset, neutralizing viral antibodies (anti-viral IgG) are still detectable in recovered SARS patients[33]. It is believed that the Spike protein and nucleocapsid protein play a central role in the antibody production[31,32,34]. Research has focused on developing vaccines and therapeutics targeting the Spike protein[35,36] and the nucleocapsid protein[37]. However, our findings indicate that people are unlikely to develop long-lasting neutralizing antibodies to SARS-CoV-2 after SARS-CoV-2 infection. Infections with some viruses, such as HIV, do not illicit robust protective immunity[38], while common cold coronavirus only generate partial protective immunity[39]. Cross-reactive antibodies in convalescent SARS patients' sera can neutralize other human betacoronaviruses[40], as those viruses share a significant B-cell epitope overlapping the heptad repeat-2 region of the Spike protein. SARS-CoV-2 also belongs to betacoronaviruses. However, no individuals or populations had shown explicit immunity to SARS-CoV-2 and every human being in the world was susceptible to this virus before it first jumped from wild animals to humans. It might be due to the dramatic differences between SARS-CoV-1 and SARS-CoV-2 in the receptor-binding region of Spike protein and the key amino acid residues involved in the interaction with human ACE2.[24] Neutralizing antibodies to SARS-CoV-1 may not produce reliable immunoprotection against SARS-CoV-2. However, why long-lasting protective antibodies are not produced after SARS-CoV-2 infection is still to be studied.

Are IgG antibodies to SARS-CoV-2 parts of short-term immunity against this virus? It is reported that after infected with SARS-CoV-2, rhesus macaques produced antibodies that exhibited neutralizing activity against SARS-CoV-2 in vitro, and re-challenge with the same viral dose weeks post initial infection was not successful.[41,42] This indicates that short-term neutralizing antibodies may be produced in this animal model. However, infected monkeys have a faster viral clearance mechanism than humans and reinfection protection for humans even in a short time period after the initial infection might not necessarily happen. We found mortality risks were similar between hospitalized COVID-19 patients with IgG antibodies to SARS-CoV-2 and those without, which indicates that absence of IgG antibodies may not affect clinical end outcome and IgG antibodies may even not be part of short-term protective immune response against SARS-CoV-2. Whether therapeutic antibodies targeting the spike protein are effective is also a question. A recent study characterized the viral spike protein receptor-binding domain-specific monoclonal antibodies derived from single B cells of patients with COVID-19.[43] In that study, the three most server cases (one dead) had much higher plasma binding activities to SARS-CoV-2 spike protein, spike protein receptor-binding domain, and nucleocapsid protein than the other five cases with mild symptoms, which casts some doubts on the relationship between antibody response and disease progression and the utilization of neutralizing antibodies as prophylactic and therapeutic SARS-CoV-2 interventions. Antibody-dependent enhancement of viral entry via the binding of those neutralizing antibodies and the spike protein is also a big concern to be closely monitored[44-47].

12

Our findings have important implications for herd immunity, antibody-based therapeutics, public health strategies, and vaccine development. First, as infected people do not develop long-lasting protecting antibodies against SARS-CoV-2, the idea of immune certificate for recovered COVID-19 patients is invalid. This finding also raises concerns for reinfection, chronic infection, and validity of the herd immunity theory for SARS-CoV-2. Second, COVID-19 IgG antibodies as tested by the kits may simply serve as a sign of the infection status and might not be protective neutralizing antibodies. The utilization of convalescent serum from recovered COVID-19 patients in clinical settings[48] would be questionable. Clinical trials on the efficacy of convalescent serum from recovered COVID-19 patients in treating COVID-19 cases were conducted in Wuhan. Their results will provide further evidence to help elucidate the role of COVID-19 IgG antibodies. Third, as serum COVID-19 IgM/IgG level can become undetectable after recovery in patients with SARS-CoV-2 infections, COVID-19 IgM/IgG test will not be a reliable tool for the surveillance of past infections of SARS-CoV-2 in areas where the epidemic is over. Finally, in our study, most of the healthcare providers without a confirmed COVID-19 diagnosis have been exposed to the wild-type SARS-CoV-2 virus in a highly contagious environment. However, none of them developed long-lasting protective antibodies against SARS-CoV-2. These findings raise the concern if the inactivated virus, subunit, and recombinant vaccines currently under development will be able to induce effective immune protections against SARS-CoV-2.

The main strength of this study is that we analyzed data on COVID-19 IgM/IgG tests on a large cohort of individuals from two hospitals in Wuhan, the epicenter of COVID-19

13

outbreak in China. Antibody tests on healthcare providers provided valuable information on outcomes of SARS-CoV-2 infections. Limitations of this study include that we do not have long-term follow-up data on recovered COVID-19 patients. Whether or not those patients will lose COVID-19 IgG antibodies in the next few months is still to be studied. Additionally, COVID-19 IgM/IgG test does not have perfect sensitivity and specificity for detecting IgG antibodies to SARS-CoV-2 in the serum. Nevertheless, we observed that very few healthcare providers without a confirmed COVID-19 diagnosis had a positive test result. This observed phenomenon strongly suggests that long-term protective antibodies are unlikely produced after SARS-CoV-2 infection.

In conclusion, very few healthcare providers without confirmed COVID-19 diagnosis in Wuhan have IgG antibodies to SARS-CoV-2, though a substantial portion of them had been infected with the virus. More than 10% of COVID-19 patients did not have those antibodies after 21 days post symptom onset. After SARS-CoV-2 infection, people are unlikely to produce long-lasting protective antibodies against this virus.

Online Methods

Study Design and Participants

The study was approved by the institutional ethics board at Zhongnan Hospital of

Wuhan University. Requirement for written informed consent was waived by the

institutional ethics board for emerging infectious diseases. We included the following

four groups of individuals who received both COVID-19 IgM/IgG tests and RT-PCR

tests for SARS-CoV-2 from February 29, 2020 to April 29, 2020: hospitalized patients

with COVID-19 from Leishenshan Hospital, Zhongnan Hospital of Wuhan University,

and Wuhan No. 7 Hospital who received these tests before being discharged from

hospital, healthcare providers (doctors, nurses, and nursing workers) without a

confirmed COVID-19 diagnosis working in Zhongnan Hospital of Wuhan University who

received these tests before resuming normal clinical services for patients without

COVID-19, general workers in Wuhan before returning to work, and other patients who

received these screening tests before being admitted to Zhongnan Hospital of Wuhan

University. There were 1603 patients with COVID-19 who received both COVID-19

IgM/IgG tests and RT-PCR tests for SARS-CoV-2 from February 29 to April 5, 2020 (the

last test result was used for analyses). Eighty-four patients were transferred from

Zhongnan Hospital of Wuhan University to Leishenshan Hospital and were only counted

once each. We excluded 133 patients with COVID-19 whose IgM/IgG tests were less

than 21 days after symptom onset to allow enough time for IgG antibodies against

SARS-CoV-2 to develop. There were 4099 healthcare providers working in Zhongnan

15

Hospital of Wuhan University, of whom 118 were diagnosed of COVID-19 before March

16, 2020 and 3835 healthcare providers without diagnosed COVID-19 received both

tests before resuming normal clinical services. Three healthcare providers who were

tested positive for SARS-CoV-2 by RT-PCR tests in their throat swabs were also

excluded from the analyses. Before returning to work, 19570 general workers in Wuhan

without a diagnosis of COVID-19 who received both tests at Zhongnan Hospital of

Wuhan University, of whom 15 were tested positive for SARS-CoV-2 by RT-PCR tests

in their throat swabs and were excluded from the analyses. Before admitted to

Zhongnan Hospital of Wuhan University for other conditions, 1628 patients without

COVID-19 diagnosis received both tests for screening for SARS-CoV-2, of whom 12

were tested positive for SARS-CoV-2 by RT-PCR tests in their throat swabs and were

removed from the analyses. In total, we included 1470 patients with COVID-19, 3832

healthcare providers, 19555 workers, and 1616 other patients in the final analyses

(N=26473). Follow up time for death among hospitalized COVID-19 patients was

calculated as from symptom onset until either discharge from hospital or April 15, 2020,

which came first.

Diagnosis of COVID-19 was based on epidemiological history, clinical manifestations

and presence of SARS-CoV-2 in clinical samples confirmed by using real-time RT-PCR

method.[11] There were changes in diagnosis of COVID-19 in China, and the case

definition was gradually broadened to allow for detection of milder cases.[49] The

confirmed cases were estimated to be 4 times less than that if the later broader case

definition had been adopted earlier. Severity of status of patients with COVID-19 at

16

admission was defined as moderate, severe, or critical. Patients with mild diseases

were not admitted to the above three hospitals and were generally admitted to

Fangcang Hospitals (makeshift hospitals).

RT-PCR test for SARS-CoV-2 virus RNA

Clinical specimens collection and RT-PCR test for SARS-CoV-2 were previously

described[18]. Clinical specimens in COVID-19 patients included nasal swabs, throat

swabs, sputum, anal swabs, and bronchoalveolar lavage (BAL), and clinical specimens

in healthcare providers without confirmed COVID-19 diagnosis were only throat swabs.

In brief, clinical specimens were collected from these people by trained nurses or

physicians wearing proper personal protection equipment. RT-PCR tests for SARS-

CoV-2 were performed using a nucleic acid detection kit following the manufacturer's

protocol. The test simultaneously amplifies and detects two target genes, including open

reading frame 1ab (ORF1ab) and nucleocapsid protein (N). Primers used for those two

target genes are as follows: ORF1ab: forward primer CCCTGTGGGTTTTACACTTAA,

reverse primer ACGATTGTGCATCAGCTGA; and the probe 5′-VIC-

CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3′; N: forward primer

GGGGAACTTCTCCTGCTAGAAT, reverse primer CAGACATTTTGCTCTCAAGCTG,

and the probe 5′-FAM- TTGCTGCTGCTTGACAGATT-TAMRA-3′. Conditions for the

amplifications were incubation at 50 °C for 15 minutes and 95 °C for 5 minutes, followed

by 40 cycles of denaturation at 94 °C for 15 seconds and extension at 55 °C for 45

seconds. The diagnostic criteria for positive and negative RT-PCR results were based

17

on the recommendation by the National Institute for Viral Disease Control and

Prevention (China): positive result <37 cycle threshold value (Ct-value) and negative

result ≥40. A Ct-value of 37-39 required retesting.

COVID-19 IgM/IgG test for antibodies against SARS-CoV-2

Serum samples from these people were collected. Methods for testing serum IgM and

IgG antibodies to SARS-CoV-2 were previously described.[50] COVID-19 IgM/IgG test kits

contained recombinant SARS-CoV-2 antigens (spike protein and nucleocapsid protein)

labelled with magnetic beads (tested on a fully automated chemiluminescence

immunoassay analyzer) or colloidal gold (test card), anti-human IgM monoclonal

antibody, and anti-human IgG monoclonal antibody. These test kits were reported to

have high sensitivity and specificity[27,50]. According to the manufacturers, the sensitivity

and specificity are ~90% and >99% for IgM, and ~98% and ~98% for IgG, respectively.

Two physicians extracted the following data using data collection form from electronic

medical records: demographic information such as age and sex, RT-PCR test date and

results, COVID-19 IgM/IgG test date and results, date of symptom onset for COVID-19

patients, treatments received, and clinical outcomes. Another physician in the research

team reviewed the collected data.

Statistical Analysis

18

Continuous variables were reported using mean and 95% confidence interval (CI) if
normally distributed or median and interquartile if nonnormally distributed. Categorical
variables were described as frequency rates and percentages. The χ2 test was used for
the comparison of categorical variables and Fisher's exact test was used when
frequency was too low. Multigroup comparisons were performed using ANOVA test,
following by Tukey test for adjusting for multiple comparisons. Prevalence of positive
IgG test results and 95% CI was also reported. For the assessment of RT-PCR test
results of SARS-CoV-2 and IgM/IgG test results, the last test result for each person was
used in the analyses. Kaplan-Meier curves were plotted to show survival differences in
hospitalized COVID-19 patients by the status of IgG antibodies to SARS-CoV-2.
Adjusted hazard ratio and 95% CI were calculated by fitting a Cox proportional hazard
model, controlling for age, sex, and severity of COVID-19 at admission. Statistical
analyses were conducted using SAS software version 9.4 (SAS Institute; Carey, NC). A
2-sided p value of <0.05 was considered statistically significant.

References

1. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med.* 2020.
2. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* 2020.
3. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet.* 2020.
4. Viruses CSGotICoTo. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol.* 2020.
5. Chan JF, Yuan S, Kok KH, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet.* 2020.
6. Rothe C, Schunk M, Sothmann P, et al. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. *N Engl J Med.* 2020.
7. Li Q, Guan X, Wu P, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *N Engl J Med.* 2020.
8. Phan LT, Nguyen TV, Luong QC, et al. Importation and Human-to-Human Transmission of a Novel Coronavirus in Vietnam. *N Engl J Med.* 2020.
9. Li LQ, Huang T, Wang YQ, et al. 2019 novel coronavirus patients' clinical characteristics, discharge rate and fatality rate of meta-analysis. *J Med Virol.* 2020.
10. Jiang F, Deng L, Zhang L, Cai Y, Cheung CW, Xia Z. Review of the Clinical Characteristics of Coronavirus Disease 2019 (COVID-19). *J Gen Intern Med.* 2020.
11. Deng SQ, Peng HJ. Characteristics of and Public Health Responses to the Coronavirus Disease 2019 Outbreak in China. *J Clin Med.* 2020;9(2).
12. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med.* 2020.
13. Li G, De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). *Nat Rev Drug Discov.* 2020;19(3):149-150.
14. Holshue ML, DeBolt C, Lindquist S, et al. First Case of 2019 Novel Coronavirus in the United States. *N Engl J Med.* 2020;382(10):929-936.
15. Grein J, Ohmagari N, Shin D, et al. Compassionate Use of Remdesivir for Patients with Severe Covid-19. *https://doiorg/101056/NEJMoa2007016.* 2020.
16. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. 2020.
17. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020.
18. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA.* 2020.
19. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA.* 2020.

20. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature.* 2020:1-10.

21. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis.* 2020.

22. Peiris JS, Chu CM, Cheng VC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet.* 2003;361(9371):1767-1772.

23. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *N Engl J Med.* 2020.

24. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature.* 2020;579(7798):270-273.

25. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clin Infect Dis.* 2020.

26. Xiang F, Wang X, He X, et al. Antibody Detection and Dynamic Characteristics in Patients with COVID-19. *Clin Infect Dis.* 2020.

27. Li Z, Yi Y, Luo X, et al. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis. *J Med Virol.* 2020.

28. Lan L, Xu D, Ye G, et al. Positive RT-PCR Test Results in Patients Recovered From COVID-19. *JAMA.* 2020.

29. Wu X, Fu B, Chen L, Feng Y. Serological tests facilitate identification of asymptomatic SARS-CoV-2 infection in Wuhan, China. *J Med Virol.* 2020.

30. Long Q-X, Liu B-Z, Deng H-J, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nature Medicine.* 2020:1-4.

31. Zhu M. SARS Immunity and Vaccination. *Cell Mol Immunol.* 2004;1(3):193-198.

32. Ho MS, Chen WJ, Chen HY, et al. Neutralizing antibody response and SARS severity. *Emerg Infect Dis.* 2005;11(11):1730-1737.

33. Shi Y, Wan Z, Li L, et al. Antibody responses against SARS-coronavirus and its nucleocaspid in SARS patients. *J Clin Virol.* 2004;31(1):66-68.

34. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV--a target for vaccine and therapeutic development. *Nat Rev Microbiol.* 2009;7(3):226-236.

35. ter Meulen J, van den Brink EN, Poon LL, et al. Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. *PLoS Med.* 2006;3(7):e237.

36. Liu J, Shao H, Tao Y, et al. Production of an anti-severe acute respiratory syndrome (SARS) coronavirus human monoclonal antibody Fab fragment by using a combinatorial immunoglobulin gene library derived from patients who recovered from SARS. *Clin Vaccine Immunol.* 2006;13(5):594-597.

37. Zhao A, Qin W, Han Y, et al. Isolation and identification of an scFv antibody against nucleocapsid protein of SARS-CoV. *Microbes Infect.* 2007;9(8):1026-1033.

38. M A, TM A, XG Y, et al. HIV-1 Superinfection Despite Broad CD8+ T-cell Responses Containing Replication of the Primary Virus. *Nature.* 2002;420(6914).

39. KA C, HF P, M S, DA T. The Time Course of the Immune Response to Experimental Coronavirus Infection of Man. *Epidemiology and infection.* 1990;105(2).

40. Chan KH, Chan JF, Tse H, et al. Cross-reactive antibodies in convalescent SARS patients' sera against the emerging novel human coronavirus EMC (2012) by both immunofluorescent and neutralizing antibody tests. *J Infect.* 2013;67(2):130-140.
41. Bao L, Deng W, Gao H, et al. Reinfection could not occur in SARS-CoV-2 infected rhesus macaques. 2020.
42. Chandrashekar A, Liu J, Martinot AJ, et al. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. 2020.
43. Ju B, Zhang Q, Ge X, et al. Potent human neutralizing antibodies elicited by SARS-CoV-2 infection. 2020.
44. Walls AC, Xiong X, Park YJ, et al. Unexpected Receptor Functional Mimicry Elucidates Activation of Coronavirus Fusion. *Cell.* 2019;176(5):1026-1039.e1015.
45. Wan Y, Shang J, Sun S, et al. Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry. *J Virol.* 2020;94(5).
46. Cao X. COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol.* 2020.
47. Iwasaki A, Yang Y. The potential danger of suboptimal antibody responses in COVID-19. *Nature Reviews Immunology.* 2020:1-3.
48. Casadevall A, Pirofski L-a. The convalescent sera option for containing COVID-19. 2020.
49. TK T, P W, Y L, EHY L, GM L, BJ C. Effect of Changing Case Definitions for COVID-19 on the Epidemic Curve and Transmission Parameters in Mainland China: A Modelling Study. *The Lancet Public health.* 2020;5(5).
50. Zeng H, Department of Laboratory Medicine ZHoWU, Wuhan, China, Xu C, et al. Antibodies in Infants Born to Mothers With COVID-19 Pneumonia. *JAMA.* 2020.

Figure Legends

Figure 1. Survival among hospitalized patients with COVID-19 who had IgG antibodies

to SARS-CoV-2 and those who did not.

Positive: patients who had IgG antibodies to SARS-CoV-2.

Negative: patients who did not have IgG antibodies to SARS-CoV-2.

Adjust hazard ratio: adjusted for age, sex, and severity of COVID-19 at admission.

Table 1. Characteristics of hospitalized COVID-19 patients, healthcare providers without confirmed COVID-19, general workers, and other patients in Wuhan (n=26473).

	Mean (95% Confidence Interval) or n (%)				P value
	COVID-19 Patients	Healthcare Providers	General Workers	Other Patients	
	(n=1470)	(n=3832)	(n=19555)	(n=1616)	
Age (years)	58.7 (58.0-59.4)	37.1 (36.7-37.4)	41.6 (41.4-41.8)	53.3 (52.4-54.2)	<0.001
Age group (years)					
<30	30 (2.0)	1236 (32.3)	3960 (20.3)	154 (9.5)	<0.001
30-39	130 (8.8)	1265 (33.0)	6068 (31.0)	166 (10.3)	
40-49	203 (13.8)	607 (15.8)	3688 (18.9)	239 (14.8)	
50-59	356 (24.2)	600 (15.7)	4031 (20.6)	459 (28.4)	
60-69	430 (29.3)	114 (3.0)	1315 (6.7)	357 (22.1)	
≥70	321 (21.8)	10 (0.3)	493 (2.5)	241 (14.9)	
Sex					
Female	745 (50.7)	2596 (67.7)	9735 (49.8)	708 (43.8)	<0.001
Male	725 (49.3)	1236 (32.3)	9820 (50.2)	908 (56.2)	

COVID-19 patients: hospitalized patients with COVID-19 pneumonia from Leishenshan Hospital, Zhongnan Hospital of Wuhan University, and Wuhan No. 7 Hospital who received both COVID-19 IgM/IgG tests and RT-PCR tests before being discharged from hospital.

Healthcare providers: doctors, nurses, and nursing workers without a confirmed COVID-19 diagnosis working in Zhongnan Hospital of Wuhan University who received both COVID-19 IgM/IgG tests and RT-PCR tests before resuming normal clinical services for patients without COVID-19.

General workers: general workers in Wuhan who received both COVID-19 IgM/IgG tests and RT-PCR tests before returning to work.

Other patients who received both COVID-19 IgM/IgG tests and RT-PCR tests to screen for SARS-CoV-2 infections before being admitted to Zhongnan Hospital of Wuhan University.

24

Table 2. Prevalence of IgG antibodies to SARS-CoV-2 among hospitalized COVID-19 patients, healthcare providers without confirmed COVID-19, general workers, and other patients in Wuhan (n=26473).

	Prevalence % (95% Confidence Interval)			
	COVID-19 Patients (n=1470)	Healthcare Providers (n=3832)	General Workers (n=19555)	Other patients (n=1616)
All	89.8 (88.2-91.3)	4.0 (3.4-4.7)	4.6 (4.3-4.9)	1.0 (0.5-1.5)
Age group (years)				
<30	90.0 (78.6-100.0)	2.8 (1.9-3.8)	3.5 (2.9-4.1)	0
30-39	89.2 (83.8-94.6)	3.8 (2.7-4.8)	4.7 (4.2-5.3)	0.6 (0.0-1.8)
40-49	92.1 (88.4-95.9)	4.4 (2.8-6.1)	4.6 (3.9-5.3)	1.3 (0.0-2.7)
50-59	92.1 (89.3-94.9)	5.5 (3.7-7.3)	4.8 (4.1-5.4)	0.7 (0.0-1.4)
60-69	89.1 (86.1-92.0)	9.6 (4.1-15.2)	6.8 (5.4-8.1)	1.1 (0.0-2.2)
≥70	86.9 (83.2-90.6)	10.0 (0.0-32.6)	5.9 (3.8-8.0)	2.1 (0.3-3.9)
Sex				
Female	90.3 (88.2-92.5)	3.7 (3.0-4.4)	5.0 (4.5-5.4)	1.0 (0.3-1.7)
Male	89.2 (87.0-91.5)	4.8 (3.6-6.0)	4.3 (3.9-4.7)	1.0 (0.3-1.6)

25

Table 3. Prevalence of IgM antibodies to SARS-CoV-2 among hospitalized COVID-19 patients, healthcare providers without confirmed COVID-19, general workers, and other patients in Wuhan (n=26473).

	Prevalence % (95% Confidence Interval)			
	COVID-19 Patients (n=1470)	Healthcare Providers (n=3832)	General Workers (n=19555)	Other patients (n=1616)
All	31.4 (29.0-33.7)	1.5 (1.1-1.8)	1.3 (1.1-1.5)	0.2 (0.0-0.4)
Age group (years)				
<30	23.3 (7.3-39.4)	1.1 (0.5-1.6)	0.7 (0.4-1.0)	0
30-39	38.5 (30.0-46.9)	1.2 (0.6-1.8)	1.0 (0.7-1.2)	0
40-49	28.6 (22.3-34.8)	2.1 (1.0-3.3)	1.7 (1.3-2.1)	0.4 (0.0-1.2)
50-59	25.6 (21.0-30.1)	2.0 (0.9-3.1)	1.7 (1.3-2.1)	0
60-69	31.2 (26.8-35.6)	2.6 (0.0-5.6)	1.7 (1.0-2.5)	0.6 (0.0-1.3)
≥70	37.7 (32.4-43.0)	0	2.4 (1.1-3.8)	0
Sex				
Female	27.5 (24.3-30.7)	1.5 (1.0-2.0)	1.7 (1.4-1.9)	0.1 (0.0-0.4)
Male	35.3 (31.8-38.8)	1.4 (0.7-2.0)	0.9 (0.7-1.1)	0.2 (0.0-0.5)

Table 4. Demographic and clinical characteristics of hospitalized patients with COVID-19 pneumonia from Leishenshan Hospital, Zhongnan Hospital of Wuhan University, and Wuhan No. 7 Hospital (n=1470).

	Mean (95% Confidence Interval) or n (%)		
	Positive	Negative	P Value
	(n=1320)	(n=150)	
Age (years)	58.5 (57.8-59.2)	60.8 (58.3-63.2)	0.061
Age group (years)			
<30	27 (2.0)	3 (2.0)	
30-39	116 (8.8)	14 (9.3)	
40-49	187 (14.2)	16 (10.7)	0.26
50-59	328 (24.8)	28 (18.7)	
60-69	383 (29.0)	47 (31.3)	
≥70	279 (21.1)	42 (28.0)	
Sex			
Female	673 (51.0)	72 (48.0)	0.49
Male	647 (49.0)	78 (52.0)	
Severity of COVID-19 at admission			
Moderate	1004 (76.1)	108 (72.0)	
Severe	263 (19.9)	34 (22.7)	0.51
Critical	53 (4.0)	8 (5.3)	
Comorbidities			
Any	739 (56.0)	87 (58.0)	0.64
Hypertension	390 (29.5)	50 (33.3)	0.34
Cardiovascular disease (any)	418 (31.7)	55 (36.7)	0.21
Cerebrovascular disease	40 (3.0)	7 (4.7)	0.32
Diabetes	165 (12.5)	23 (15.3)	0.32
Chronic bronchitis/asthma	23 (1.7)	2 (1.3)	0.71
Treatment received			
Anti-virus	594 (45.0)	65 (43.3)	0.7
Antibiotics	566 (42.9)	91 (60.7)	0.001
Corticosteroids	148 (11.2)	18 (12.0)	0.77
Chloroquine/hydroxychloroquine	123 (9.3)	6 (4.0)	0.029
Vitamin C	216 (16.4)	22 (14.7)	0.59
Immunoglobulin	12 (0.9)	2 (1.3)	0.64
Traditional Chinese Medicine	1142 (86.5)	121 (80.7)	0.051
Clinical characteristics			
Progression to critical status[*]	71 (5.6)	13 (9.2)	0.09
ICU admission	76 (5.8)	14 (9.3)	0.084
Need intubation	28 (2.1)	11 (7.3)	0.001
Need Ventilator	71 (5.4)	11 (7.3)	0.32
Death	17 (1.3)	5 (3.3)	0.065

ICU: Intensive Care Unit.

[*]61 patients with a critical status at admission were excluded.

Table 5. Prevalence of IgM and IgG antibodies to SARS-CoV-2 among hospitalized patients with COVID-19 from Leishenshan Hospital, Zhongnan Hospital of Wuhan University, and Wuhan No. 7 Hospital (n=1470).

		Prevalence % (95% Confidence Interval)	
	n	IgG	IgM
All	1470	89.8 (88.2-91.3)	31.4 (29.0-33.7)
Age group (years)			
<30	30	90.0 (78.6-100.0)	23.3 (7.3-39.4)
30-39	130	89.2 (83.8-94.6)	38.5 (30.0-46.9)
40-49	203	92.1 (88.4-95.9)	28.6 (22.3-34.8)
50-59	356	92.1 (89.3-94.9)	25.6 (21.0-30.1)
60-69	430	89.1 (86.1-92.0)	31.2 (26.8-35.6)
≥70	321	86.9 (83.2-90.6)	37.7 (32.4-43.0)
Sex			
Female	745	90.3 (88.2-92.5)	27.5 (24.3-30.7)
Male	725	89.2 (87.0-91.5)	35.3 (31.8-38.8)
Severity of COVID-19 at admission			
Moderate	1112	90.3 (88.5-92.0)	28.7 (26.0-31.3)
Severe	297	88.6 (84.9-92.2)	37.4 (31.8-42.9)
Critical	61	86.9 (78.2-95.6)	50.8 (37.9-63.7)
Comorbidities			
Any			
No	644	90.2 (87.9-92.5)	32.3 (28.7-35.9)
Yes	826	89.5 (87.4-91.6)	30.6 (27.5-33.8)
Hypertension			
No	1030	90.3 (88.5-92.1)	31.9 (29.1-34.8)
Yes	440	88.6 (85.7-91.6)	30.0 (25.7-34.3)
Cardiovascular disease (any)			
No	997	90.5 (88.6-92.3)	31.7 (28.8-34.6)
Yes	473	88.4 (85.5-91.3)	30.7 (26.5-34.8)
Cerebrovascular disease			
No	1423	90.0 (88.4-91.5)	31.3 (28.9-33.7)
Yes	47	85.1 (74.5-95.7)	34.0 (20.0-48.1)
Diabetes			
No	1282	90.1 (88.5-91.7)	31.1 (28.6-33.7)
Yes	188	87.8 (83.0-92.5)	33.0 (26.2-39.8)
Chronic bronchitis/asthma			
No	1445	89.8 (88.2-91.3)	31.2 (28.8-33.6)
Yes	25	92.0 (80.6-100.0)	40.0 (19.4-60.6)
Treatment received			
Anti-virus			
No	811	89.5 (87.4-91.6)	31.6 (28.4-34.8)
Yes	659	90.1 (87.9-92.4)	31.1 (27.6-34.7)
Antibiotics			
No	813	92.7 (91.0-94.5)	30.1 (27.0-33.3)
Yes	657	86.1 (83.5-88.8)	32.9 (29.3-36.5)
Corticosteroids			
No	1304	89.9 (88.2-91.5)	29.1 (26.7-31.6)
Yes	166	89.2 (84.4-93.9)	48.8 (41.1-56.5)
Chloroquine/hydroxychloroquine			
No	1341	89.3 (87.6-90.9)	30.8 (28.3-33.3)
Yes	129	95.3 (91.7-99.0)	37.2 (28.8-45.7)

Vitamin C			
No	1232	89.6 (87.9-91.3)	30.4 (27.8-32.9)
Yes	238	90.8 (87.0-94.5)	36.6 (30.4-42.7)
Immunoglobulin			
No	1456	89.8 (88.3-91.4)	30.8 (28.5-33.2)
Yes	14	85.7 (64.7-100.0)	85.7 (64.7-100.0)
Traditional Chinese Medicine			
No	207	86.0 (81.2-90.8)	35.3 (28.7-41.8)
Yes	1263	90.4 (88.8-92.0)	30.7 (28.2-33.3)
Clinical characteristics			
Progression to critical status[a]			
No	1325	90.3 (88.7-91.9)	30.0 (27.5-32.4)
Yes	84	84.5 (76.6-92.4)	38.1 (27.5-48.7)
ICU admission			
No	1380	90.1 (88.6-91.7)	30.5 (28.1-32.9)
Yes	90	84.4 (76.8-92.1)	44.4 (34.0-54.9)
Need intubation			
No	1431	90.3 (88.8-91.8)	30.8 (28.4-33.2)
Yes	39	71.8 (57.0-86.6)	51.3 (34.9-67.7)
Need Ventilator			
No	1388	90.0 (88.4-91.6)	30.6 (28.2-33.0)
Yes	82	86.6 (79.1-94.1)	43.9 (32.9-54.9)
Death			
No	1448	90.0 (88.4-91.5)	31.0 (28.6-33.4)
Yes	22	77.3 (58.3-96.3)	54.5 (31.9-77.1)

ICU: Intensive Care Unit.

[a] 61 patients with a critical status at admission were excluded.

29

Figure 1.