Investigation of health issues and immunological responses in COVID-19 Survivors in Wuhan

Doctoral thesis

to obtain a doctorate

from the Faculty of Medicine

of the University of Bonn

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2024

Written with authorization of

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Day of oral examination: 13.02.2024

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List of Abbreviation

COVID-19	Coronavirus infectious disease-2019
CT	Computertomographie
IGCSA	Colloidal gold-based immunochromatographic strip assay
IgG / IgM	Immunoglobulin G / M
IQR	Interquartile range
ORF1ab	Open reading frame 1ab
RR	Relative risk
RT qPCR	Real-time quantitative polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome-related coronavirus 2
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease

1. Summary

To the present, several studies have investigated the sequelae and herd immunity of discharged and recovered coronavirus infectious disease-2019 (COVID-19) patients. However, little is known whether the life quality and safety of these COVID-19 survivors may be influenced by prior COVID-19 infection. To clarify this issue, the present study recruited a total of 3,677 discharged COVID-19 patients [median age = 59 years, interquartile range (IQR) = 47–68, range = 10–98; 55.5 % female], who hospitalized in four medical facilities or hospitals in Wuhan, China in the time interval between January 18 and March 29, 2020. Three forms of data including case reports, medical records, and self-reports were included into the present study. All participants were followed to record possible post-COVID-19 sequelae, with a median of 144 days (IQR = 135–157). During this follow-up, approximately a quartal patients (976; 26.5 %) experienced at least one post-COVID-19 sequela. The incidence of post-COVID-19 sequelae of elderly COVID-19 survivors (≥60 years) was moderately higher than that of young COVID-19 survivors (<60 years; relative risk [RR] 1.05, 95 % CI 1.02–1.10, p =.007). Colloidal gold-based immunochromatographic strip assay (ICGSA) was used to measure the titer of anti-SARS-CoV-2 immunoglobulin G (IgG) and immunoglobulin M (IgM) antibody testing. We observed a dramatic reduction of anti-SARS-CoV-2 IgG (88.0 %, 95 % CI = 84.2–90.4) and IgM (93.2 %, 95 % CI = 88.5–96.4) antibodies during this follow-up. We used Real-time quantitative PCR (RT-qPCR) for the detection of the presence of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) detection. In this cohort, we found that 45 (1.2 %) patients were retested positive for SARS-CoV-2 and 37 (1.0 %) patients died during follow-up. Of those who died during this follow-up study, 26 (70.3 %) patients were male and all patients were tested negative for both IgG and IgM, except for one-person retested IgG-positive. The present study documents the presence of post-COVID-19 sequelae that impair functions of multiple organ systems among COVID-19 survivors. This result suggests that the long-term effects of COVID-19 infection have increasing impact by negatively influencing survivors' quality of life, straining health care systems, and resulting in extended periods of lost productivity.

1.1 Introduction

The COVID-19 pandemic has greatly changed the life of people in the past two years. By July 2020, more than 14 million confirmed cases and 0.6 million deaths were reported worldwide (WHO reports, 2021). As COVID-19 is highly infectious and its transmission capacity varies with the seasons, the number of confirmed cases and deaths continues to increase, so will the recovered patients discharge from medical facilities (Dong et al., 2021). Countries have launched public health strategies to control the spread of the virus.

From government aspect, measures were induced to curb the spread of the virus, including necessary social distancing, travel restrictions and wearing masks; from the aspect of medical facilities, COVID-19 testing and strict hospital discharge criteria were implemented, including a mandatory 14-day of post-hospital discharge clinical monitoring at regional shelter hospitals, who play the role of following up COVID-19 patients during and after their stay. In this study, we analyzed a large cohort of COVID-19 survivors released from four medical facilities in Wuhan based on their long-term follow-up data, investigating both the physical and psychological symptoms, including SARS-CoV-2 immune recognition.

Tab. 1: SARS-CoV-2 RT-qPCR Test Result*

ORF1ab	Gene N	Gene E	Result
Positive	Positive	Positive	Positive
Positive	Positive	Negative	Positive
Positive	Negative	Positive	Positive
Positive	Negative	Negative	Positive only when a repeated test shows the same result
Negative	Positive	Negative	Suspicious. Recheck after a certain period
Negative	Negative	Positive	
Negative	Negative	Negative	Negative

* The table is modified from the article" Mei Q, Wang F, Yang Y, Hu G, Guo S, Zhang Q, Bryant A, Zhang L, Kurts C, Wei L, Yuan X, Li J. Health Issues and Immunological Assessment Related to Wuhan's COVID-19 Survivors: A Multicenter Follow-Up Study. Front Med (Lausanne). 2021 May 7;8:617689"

1.2 Materials and Methods

Survivals of COVID-19

Data collection of this study was based on data platforms of Wuhan No.1 Hospital, Wuchang Hospital, Zhongshang Hospital, and Hubei Province Hospital. These government-designated hospitals were mandated to treat all the COVID patients of

varying severity (mild, severe, and critical). From January 18 and March 29, 2020, 3,677 consecutive patients with confirmed COVID-19 infection who were discharged from above hospitals were enrolled. Standard hospital discharge criteria (Yang et al., 2020) included: (1) the axillary temperature was maintained below 37.3 ℃ for more than three days; (2) Computertomographie (CT) scan provide radiological evidence of significant resolution of pneumonia; (3) two sequential negative from nasopharyngeal /oropharyngeal swab samples of SARS-CoV-2 RT qPCR at the interval of at least 24 hours are required.

As a newly recognized disease, little is known about the clinical manifestations, course development, mortality of COVID-19 infection, and the dynamics and range of viral transmission during the beginning stages of disease outbreaks. As a result, all inpatients who met the discharge criteria are directly transferred to a Fangcang-like medical institutions for clinical monitoring for 14 days (Shang et al., 2020). Patients' follow-up started on the day 1 of discharging from primary hospital and ended on July 24, 2020. The data for this investigation are derived from trusted medical records, case reports, and self-reports.

Fangcang-Like Medical Institutions and Their Discharge Criteria

Fangcang is public health concept newly emerged in China during the SARS-CoV-2 outbreak of 2020. Fangcang shelter are large-scale, temporary medical facilities rapidly constructed from converting public venues such as exhibition centers and football stadiums with basic medical devices equipped, close patient monitoring, and rapid patient assessment and referral. The construction and employ of Fangcang-like medical facilities have effectively mobilized medical resources and have greatly reduced the burden of local medical capacity (Shang et al., 2020). In order to restrain the rapidly emerging COVID-19 epidemic while maximizing the value of medical resources, Chinese government designated certain hospitals to treat all the COVID patients of varying severity. Once diagnosed, patients would be admitted in those designated-hospitals and received professional treatment until the disease was cured and discharge criteria were met. Subsequently, the discharged patients were transferred directly from primary hospitals to Fangcang-Like medical institutions for maintenance medical observation and follow-up, typically for 14 days. After this observation period in the Fangcang shelter, patients were discharged home to self-quarantine if they met both the following criteria: (a) no recurrence of any clinical symptom including respiratory-related inflammation and (b) negative SARS-CoV-2 RT-qPCR test on nasopharyngeal/oropharyngeal swab samples after 14 days.

For COVID-19 survivors who failed to reach any one of above criteria, reemerging any

clinical symptom or sign during monitoring in Fangcang shelters, they were immediately readmitted to government-designated hospitals. After discharge home from Fangcang shelters to self-quarantine, the community hospitals play the critical roles for clinical monitoring and diagnostic testing. When clinical symptoms occurred, or other medical circumstances required, or upon personal request, CT imaging and SARS-CoV-2 retesting were performed.

SARS-CoV-2 Real-Time Quantitative PCR Test

Nasopharyngeal /oropharyngeal swab, sputum, and other samples were collected for laboratory testing for SARS-CoV-2 infection was based on viral RNA detection by quantitative RT-PCR. Viral RNA was extracted from the patients' samples and was inactivated in 56◦C incubator for 30 min. Primer probes were designed to target on three genes of SARS-CoV-2: open reading frame 1ab (ORF1ab) and genes of nucleocapsid proteins N and E (Table 1). The PCR buffer, reverse transcriptase enzyme, DNA polymerase, and gene primers were mixed together according to protocol and added to a 96-well plate. The extracted virus RNA samples were then added to the wells, the plate sealed, and RT-qPCR amplification was performed as follows: one cycle at 40–45◦C for 10 min, followed by 95◦C for 3 min. Then, DNA denaturation and amplification proceeded for 45 cycles at 95◦C for 15 s and 55–58◦C for 30 s. When the cycle threshold remains below or above 44, the test results for SARS-CoV-2 are reported as positive or negative, respectively (Table 1). The sensitivity of SARS-COV-2 RT-qPCR is 400 copies/ml for the RT-qPCR test can detect SARS-CoV-2 nucleic acid reference materials within 400 copies/ml with a detection rate of 100 %.

SARS-CoV-2 RT-qPCR was performed twice on nasopharyngeal/oropharyngeal swab samples obtained with at least a 24-h interval between samples for clinically cured patients to determine whether they meet the discharge criteria of the hospital, which is consecutive negative results. Subsequently, for discharged patients, this RT-qPCR test was performed at least once in Fangcang shelters during their 14-day monitoring, and any time necessary afterwards when clinical symptoms reoccurred or upon personal requests in community hospitals.

Colloidal Gold-Based Immunochromatographic Strip Assay

The colloidal gold-based immunochromatographic strip assay (IGCSA) was used for IgG and IgM detection. IgM and IgG test cards were numbered sequentially. Citrate-treated anticoagulant blood samples are centrifuged at 500 × g for 5 min and then 10 μl of plasma is added to the sample wells for 10–15 min. A test was considered positive when lines for

the patient sample and the positive control sample appeared simultaneously. Samples were regarded as negative in which a line developed only for the positive control. Samples were deemed invalid when only the patient sample, and not the positive control sample, requiring a repeated test. the sensitivity and specificity of this assay for each immunoglobulin alone and in combination regarding SARS-CoV-2 detection in samples are demonstrated in table 2.

Tab. 2 : Sensitivity and specificity of ICGSA*

Immunoglobulin test	Sensitivity (95% CI)	Specificity (95% CI)
IgM	87.1% (83.9–91.3)	99.7% (98.4–100)
IgG	88.3% (85.6–92.0)	99.4% (98.0–99.8)
Combination of IgG and IgM	91.6% (88.6–93.4)	99.2% (97.6–99.7)

The table is from the article" Mei Q, Wang F, Yang Y, Hu G, Guo S, Zhang Q, Bryant A, Zhang L, Kurts C, Wei L, Yuan X, Li J. Health Issues and Immunological Assessment Related to Wuhan's COVID-19 Survivors: A Multicenter Follow-Up Study. Front Med (Lausanne). 2021 May 7;8:617689"

Statistical Analysis

Continuous variables were showed in median (IQR) and test with the Mann–Whitney U-test; categorical variables were showed in number (percentage) and test by Fisher's exact test. $P < 0.05$ was regarded as statistically significant.

1.3 Results

From Jan. 18 to Mar. 29, 2020, we followed up and analyzed 3,677 consecutive hospitalized COVID-19 survivors met the clinical discharge criteria and were discharged from the aforementioned four hospitals in Wuhan, China. Based on data from four independent medical teams, the median age was 59 years (IQR = 47–68, range = 10–98; 54.1 % female), the median follow-up time was 144 days (IQR = 135–157, range = 117–188; Table 3). Among this cohort, 2,331 (63.4 %) survivors were of mild disease, while 1,239 (33.7 %) and 95 (2.6%) patients suffered from severe and critical condition respectively during their initial hospitalization. During this initial hospitalization, almost all patients received antiviral therapy (3570 of 3677 patients, 97.1 %), most of them were treated with antibiotic (3,026 of 3677patients, 82.3 %). Corticosteroids were use in 2401 patients (65.3 %), interferon nebulization and γ-immunoglobulin were applied in 1,540 (41.9 %) and 1,445 (39.3 %) patients, respectively. Apart from medication strategy, 3086 (83.4 %) survivors received a standard oxygen therapy via nasal catheter, 467 (12.7 %)

received high-flow nasal cannula therapy, 173 (4.7 %) had non-invasive mechanic ventilation, and 30 (0.8 %) required invasive mechanic ventilation. The median time from symptom onset to hospital admission and the length of initial hospitalization was 8.0 days (IQR = 6.0–11.0) and 17.0 days (IQR = 11.0–25.0), respectively.

From the first day of discharge to end of follow-up, 976 (26.5 %) COVID-19 survivors developed at least one sequela (median age = 57, IQR = 47.8–56.4, range = 17–92; 59.0 % female), chest pain/tightness (184, 5.0 %), shortness of breath (136, 3.7 %), and cough/sputum (87, 2.4 %) are the most seen sequelae (Table 3). Impact pulmonary function were observed in 337 (9.2 %) survivors, while 278 (7.6 %) had sequelae related to cardiac function. Sequelae in neurologic and endocrine system are occurred in 289 (7.9 %) and 90 (2.4 %) survivors respectively. Sequelae are listed in Table 4 by 10-year age interval of all the 3,677 survivors. Elderly survivors (age ≥60 years) are slightly more inclined to develop the post-COVID-19 sequelae than young survivors (age <60), with sequelae incidence of 1,795 versus n = 1,882(RR = 1.05, 95 % CI = 1.02–1.10, p = 0.007). Nevertheless, gender difference did not significantly impact the incidence of post-COVID-19 sequelae (Table 4). Apart from physical sequelae, 173 (4.7 %) COVID-19 survivors self-reported various psychological symptoms such as anxiety (103, 2.8 %), depression (70, 1.9 %), and emotional instability (37, 1.0 %), while 132 (3.6 %) survivors failed to report personal feelings. 802(21.8 %) survivors received professional assessment by mental health care specialists and were deemed to have a clinically defined psychological condition. 136 (3.7 %) survivors had better psychological conditions after psychological therapy.

We define the initial phase and late phase of follow-up as days 1-45 and days 100-150, respectively. According to ICGSA results for anti-SARS-CoV-2 viral immunoglobulins, during the initial phase of follow-up, IgM and IgG were both positive in 249 (6.8 %) COVID-19 survivors, 1,274 (34.6 %) survivors were IgG-positive and IgM-negative, 121 (3.3 %) survivors were IgG-negative and IgM-positive, while the rest 2,033 (55.3 %) were negative for both IgG and IgM (Fig. 1). However, when undergoing serological test at the late phase, we saw a dramatically decrease in antibody tilters, with IgG and IgM antibody positivity rates reducing by 88.0 % (95 % CI = 84.2–90.4) and 93.2 % (95 % CI = 88.5–96.4), respectively. To specify, only 25 (0.7 %) survivors were positive for both IgG and IgM, 157 (4.3 %) were IgG-positive and IgM-negative, none were IgG-negative and IgM-positive, and 3,495 (95.1 %), the majority of survivors, were negative for both IgG and IgM (Fig. 1).

It is worth noting that 45 (1.2 %) survivors were retested positive for SARS-CoV-2 (median age = 57 years, IQR = 50–64, range = 25–81; 68.9 % female; Table 3) during follow-up.

The median duration from initial hospital discharge to retest positivity was 32.0 days (IQR = 28.0–40.0, range = 9–58; Table 3). None of these 45 was a health care worker, but none had they taken medicine regularly after their initial hospital discharge. Of these retest-positive survivors, 25 of whom were readmitted to designated-hospitals once the test results reported, and other 20 remained at home for self-quarantine. In terms of serological testing, 2 of the 45 survivors showed positive in both IgG and IgM antibodies, 26 were IgG-positive and IgM-negative, 2 were IgG-negative and IgM-positive, and the remaining 15 were negative for both antibodies. Furthermore, 24 of this retest-positive survivors presented with at least one symptom associated with COVID-19 infection, the most common being dyspnea, cough, and chest tightness, while remaining 21 of them were asymptomatic. As for the conditions during initial hospitalization of this retest-positive subgroup, 19, 24, and 2 of them had mild, sever, and critical condition. By the time of July 24, 2020, all 45 retest-positive survivors were still alive. 20 retested-positive survivors who were readmitted for the second time met the discharge criteria after reinforcing treatment and were once again released to home quarantine. There is no new viral transmission was observed or reported.

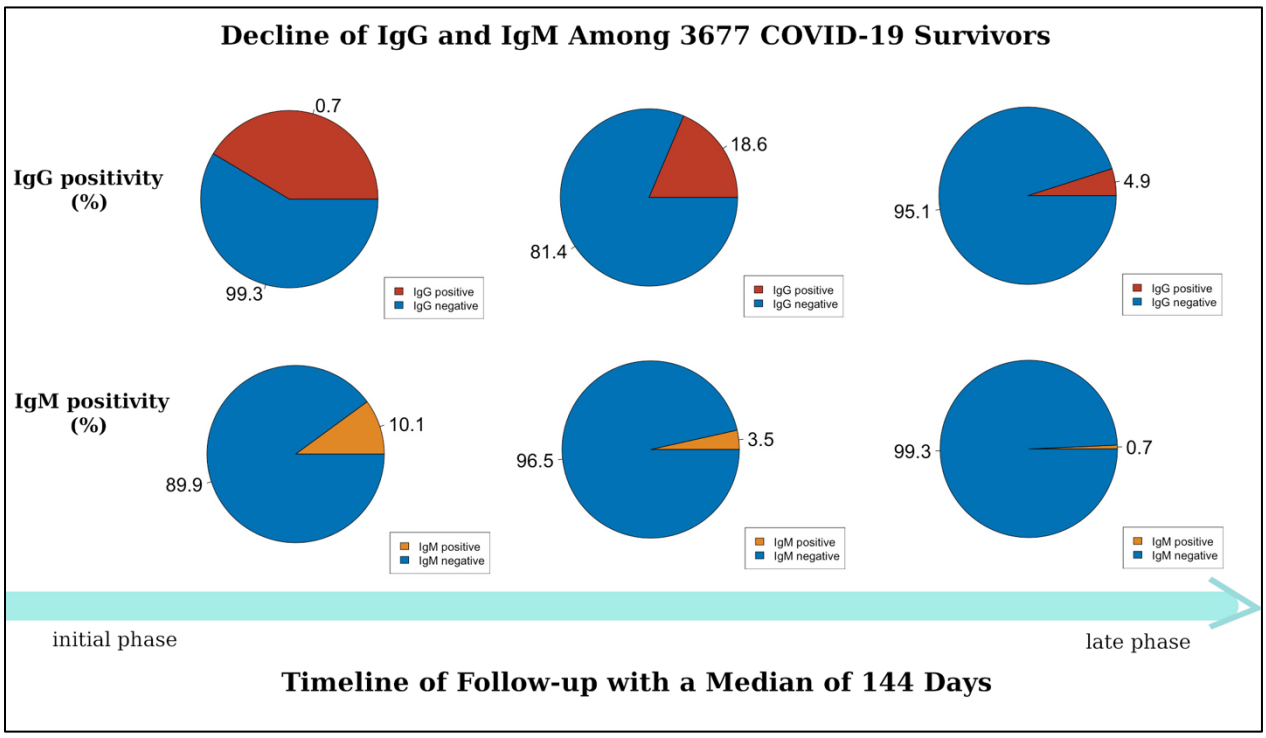

Fig. 1: Dynamic decline of immunoglobulin G and immunoglobulin M among COVID-19 survivors*

The figure is from the article" Mei Q, Wang F, Yang Y, Hu G, Guo S, Zhang Q, Bryant A, Zhang L, Kurts C, Wei L, Yuan X, Li J. Health Issues and Immunological Assessment Related to Wuhan's COVID-19 Survivors: A Multicenter Follow-Up Study. Front Med (Lausanne). 2021 May 7;8:617689"

During the follow-up, 37 (1.0 %) of the 3,677 COVID-19 survivors died (median age = 70.0 years, IQR = 56.0–79.0, range = 31.0–98.0; 29.7 % female; Table 3). None of the death was a health care worker or retested positive. 31 of the deceased were attributed to COVID-19 infection, while the remaining 6 patients died of comorbidities including diabetes, hepatobiliary tube cancer, heart attack, encephalorrhagia, epilepsy, and scurvy. The median duration from hospital discharge to death was 33.0 days (IQR = 18.0–42.0; Table 3). 5 individuals of death cohort were readmitted due to developing deteriorating conditions after initial hospital discharge, and died 4–15 days after readmission. The remaining 32 patients died at home. All of these 37 patients had stable psychological conditions, however, none had they taken any medicine regularly after initial hospital discharge. All deceased individuals were IgG- and IgM-negative but one were IgG-positive/IgM-negative, suggesting there is no immune system recognition of SARS-CoV-2. In this deceased subgroup, 15 (40.5 %), 4(10.8 %), and 18 (48.6 %) individuals were diagnosed as mild, sever, and critical condition during their initial hospitalization. As for treatment during their initial hospitalization, 34 (91.9 %) individuals received antiviral treatments including arbidol, ganciclovir, oseltamivir, ribavirin, and hydroxychloroquine,36 (97.3 %) individuals were given antibiotics, and 36 (97.3 %) were given corticosteroids (Table 3).

Table 3. Clinical characteristics, retest-positivity and sequelae among discharged COVID-19 patients*

Characteristics	All Patients (IQR / %)	Retested-positive group (IQR / %)	Deceased group (IQR / %)
Number of patients	3677	45 (1.2)	37 (1.0)
Median age, years	59.0 (47.0 - 68.0)	57 (50.0 - 64.0)	70.0 (56.0 - 79.0)
Gender	...		
Male	1688 (45.9)	14 (31.1)	26 (70.3)

Female	1989 (54.1)	31 (68.9)	11 (29.7)
Retest-positivity	45 (1.2)	45 (100)	0
Number of new viral transmission	0	0	0
Severity (initial hospitalization)	…		
Mild	2331 (63.4)	19 (42.2)	15 (40.5)
Severe	1239 (33.7)	24 (53.3)	4 (10.8)
Critical	107 (2.9)	2 (4.5)	18 (48.6)
Treatment (initial hospitalization)	…		
antiviral therapy	3570 (97.1)	41 (91.1)	34 (91.9)
antibiotic treatment	3026 (82.3)	38 (84.4)	36 (97.3)
corticosteroids	2401 (65.3)	28 (62.2)	36 (97.3)
interferon nebulization	1540 (41.9)	15 (33.3)	5 (11.1)
γ-immunoglobulin	1445 (39.3)	1 (2.2)	3 (8.1)

Median time from symptom onset to admission, days (IQR)	8.0 (6.0 - 11.0)	8.0 (6.0 - 11.0)	10.0 (7.0 - 13.0)
Median time to hospitalization, days (IQR)	17.0 (11.0 - 25.0)	16.5 (11.0 - 24.0)	16.0 (10.0 - 23.0)
Median follow-up time, days (IQR)	144.0 (135.0 - 157.0)	150.0 (139.5 - 158.5)	33.0 (18.0 - 42.0)
Sequelae	...		
Pulmonary function	337 (9.2)		10 (27.0)
shortness of breath	136	5	3
cough / sputum	87	4	2
pharyngitis / foreign body feeling	42	1	2
dyspnea	30	7	3
pulmonary fibrosis	21		
lung damage	12		
bronchitis	4		

COPD	3		
haemoptysis	2		
Cardiac Function	278 (7.6)		8 (21.6)
Chest-pain / -tightness	184	4	4
palpitation	63	3	
cardiac disease	14		2
tachycardia	13	1	1
angina pectoris	3		
heart attack	1		1
Neurologic Function	289 (7.9)		5 (13.5)
Insomnia	78	4	4
Joint pain / back pain / lumbago	71	3	
fatigue	55		
headache / dizziness / poor memory	49		

change of taste and smell	10		
myalgia	8		
impaired vision	5		
leg numbness / finger stiffness	5		
neuralgia	2		
paralysis	2		
tinnitus	2		
confusion	1		1
coma	1		
celebral infarction	1		
Endorine System	90 (2.4)		
hair loss	67		
bitter / dryness in mouth	12		
high blood sugar	6		
diabetes	5		
Gastrointestinal function	42 (1.1)		1 (2.7)

gastrointestinal complaints / poor appetite	31	3	1
diarrhoea	8		
constipation	2		
emesia	1		
Dermatological system	33 (0.9)		
hidrosis	24		
erythra	7		
allergy	2		
Hepatic System	16 (0.4)		1 (2.7)
hepatic insufficiency	8		1
oedema	7		
antiadocus	1		
Kidney Function	12 (0.3)		1 (2.7)
hypertension	6		
kidney insufficiency	6		1

Various	66 (1.8)		5 (13.5)
reduction of physical strength	64		5
dryness / excessive secretion in eye	2		

* The table is from the article" Mei Q, Wang F, Yang Y, Hu G, Guo S, Zhang Q, Bryant A, Zhang L, Kurts C, Wei L, Yuan X, Li J. Health Issues and Immunological Assessment Related to Wuhan's COVID-19 Survivors: A Multicenter Follow-Up Study. Front Med (Lausanne). 2021 May 7;8:617689"

Table 4. Sequelae Disposition by 10-year Age intervals of discharged COVID-19 patients*

Age intervals (years)	Sequelae Nr./Nr.		Relative risk ratio (95% CI, *p*-value)
	Male	Female	Male vs. female
10–19	2/10	0/4	1.25 (0.92–1.70, $p = 1$)
20–29	7/56	14/91	0.98 (0.85–1.10, $p = 0.81$)
30–39	46/183	61/206	0.94 (0.83–1.06, $p = 0.36$)
40–49	77/256	78/250	0.98 (0.88–1.10, $p = 0.84$)
50–59	88/353	163/473	0.87 (0.80–0.95, $p = 0.0036$)
60–69	117/452	185/592	0.92 (0.86–1.00, $p = 0.063$)
70–79	48/265	56/256	0.95 (0.88–1.04, $p = 0.323$)
80–89	12/95	19/112	0.95 (0.85–1.06, $p = 0.438$)
>90	3/18	0/5	1.2 (0.98–1.48, $p = 1$)

*The table is from the article" Mei Q, Wang F, Yang Y, Hu G, Guo S, Zhang Q, Bryant A, Zhang L, Kurts C, Wei L, Yuan X, Li J. Health Issues and Immunological Assessment Related to Wuhan's COVID-19 Survivors: A Multicenter Follow-Up Study. Front Med (Lausanne). 2021 May 7;8:617689"

1.4 Discussion

To our knowledge, this study describes the longest clinical follow-up time in the largest cohort of discharged COVID-19 patients. The respiratory system is not the only system that acute COVID-19 infection impairs; many other organ systems, for instance, the circulatory system (Nalleballe et al., 2020), the cardiovascular system (Böhm et al., 2020), the renal system (Su et al., 2020), the gastrointestinal system (Parasa et al., 2020), the endocrine system (Apicella et al., 2020), the nervous system (Ellul et al., 2020), and the skin system (Marzano et al., 2020), are also damaged in varying degrees. It is revealed in our study that some patients who were thought to have recovered from primary acute COVID infection also developed a wide variety of multi-organ dysfunction after discharge. Particularly, 976 (26.5 %) of COVID-19 survivors suffered from cardiovascular, neurological and endocrine system malfunction over a medium follow-up period of 144 days. The risk of sequelae occurring is independent of age and sex. Apart from this, 173 (4.7 %) of discharged patients emerged relevant psychological conditions after COVID-19 infection and medical treatment. These COVID-19-associated sequelae have greatly impacted the long-term quality of life of patients and will make healthcare resources even more strained.

In accordance with the results of recent studies (Ng et al., 2021; He et al., 2021; Wang et al., 2020), our study also found that anti-SARS-CoV-2 antibodies IgG and IgM decreased significantly with prolonged follow-up periods, which strongly suggests that herd immunity is difficult to establish, and antibody titers are unlikely to maintain in an effective level to fully function for a long time. From the other aspect, it is necessary to extend public health practices like social distancing procedures, face covering, and hygiene-based measures to minimize viral transmission.

The overall mortality rate among Wuhan’s COVID-19 survivors is 1 %, where the majority of death cases occurred in individuals who tested negative for both IgG and IgM. These results show that immune responses to SARS-CoV-19 infection may lower patients’ risk of developing life-threatening post-discharge sequelae. Among this deceased cohort, the median age is 70 years, suggesting that advanced age is not only a risk factor for death from primary COVID-19 infection, but it also influences the mortality from post-COVID-19 sequelae. 15 of 37 deceased persons were diagnosed as mild course of COVID-19 during the initial hospitalization, indicating that the primary state of COVID-19 infection is not the sole factor associated with mortality in the post-COVID-19 period. On top of that, 26 male

patients contributed 70.3 % death cases during follow-up, implying that female gender could play an important role in survival during the post-COVID-19 period.

Lastly, this study describes a 1.2 % rate of COVID-19 retest positivity but with no new viral transmission. It is difficult to confirm whether retest-positive survivors are able to infect others, but reinfection via retest-positive people can likely be avoided as long as the appropriate social health measures are practiced.

However, there are some limitations in this study: 1) our study is a national multi-center study, the suitability of the conclusions for global application has yet to be confirmed by additional international studies; 2) this study was not able to determine the effects of treatment on the follow-up outcome; 3) a recent study revealed the clinical characteristics of family members for COVID-19 infection (Su et al., 2020). Our study has not yet been able to determine the post-COVID-19 sequelae-related genetic relationship, warranting further investigation.

1.5 Conclusion

This study shows that COVID-19 survivors suffer persistent and often severe post-infectious sequelae leading to reduced quality of life, lost productivity, and requiring prolonged and repeated visits to medical institutions. Closer follow-up with prompt sequelae medical intervention for COVID-19 survivors may improve their long-term prospect.

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2. Publication

Health Issues and Immunological Assessment Related to Wuhan's COVID-19 Survivors: A Multicenter Follow-Up Study

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2.1 Abstract

Introduction: Currently, a large number of hospitalized coronavirus infectious disease-2019 (COVID-19) patients have met the clinical discharge criteria and have been discharged. Little is known about the sequelae and herd immunity, two important factors influencing the life quality and safety of COVID-19 survivors. **Methods:** Discharged COVID-19 patients from four medical facilities in Wuhan, China, were followed in order to record and investigate possible post-COVID-19 sequelae and herd immunity. After hospital discharge, patients reported to Fangcang shelter hospitals for an initial 14-day period of mandatory clinical monitoring. After release from these shelter hospitals, patients returned home for self-quarantine. Real-time quantitative PCR (RT-qPCR) was used for severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) detection. Colloidal gold-based immunochromatographic strip assay (ICGSA) was used for anti-SARS-CoV-2 immunoglobulin G (IgG) and immunoglobulin M (IgM) antibody testing. The data for this study are derived from case reports, medical records, and self-reports. **Results:** A total of 3,677 COVID-19 survivors [median age = 59 years, interquartile range (IQR) = 47–68, range = 10–98; 55.5% female] who were released from four hospitals in Wuhan, China, between January 18 and March 29, 2020 were followed for a median of 144 days (IQR = 135–157). During follow-up, 976 (26.5%) patients had at least one post-COVID-19 sequela. The incidence of post-COVID-19 sequelae among elderly COVID-19 survivors (age ≥60 years) was slightly increased compared to that of young COVID-19 survivors (age <60 years; relative risk = 1.05, 95% CI = 1.02–1.10, p = 0.007). During follow-up, a dramatic reduction of anti-SARS-CoV-2 IgG (88.0%, 95% CI = 84.2–90.4) and IgM (93.2%, 95%CI = 88.5–96.4) antibodies was observed. Among these COVID-19 survivors, 1.2%(n = 45) retested positive for SARS-CoV-2 and 1.0% (n = 37) died during follow-up. Of those who died during follow-up, 70.3% were male and all were negative for both IgG and IgM, except for one person who was IgG-positive. **Conclusions:** Our study documents significant post-COVID-19 sequelae that impair functions of multiple organ

systems in COVID-19 survivors, suggesting that the long-term effects of this disease will negatively impact survivors' quality of life, continue to strain health care systems, and result in extended periods of lost productivity. Furthermore, female gender and anti-SARS-CoV-2 immunity may play an essential role in the survival after COVID-19 infection.

frontiers in Medicine

ORIGINAL RESEARCH
published: 07 May 2021
doi: 10.3389/fmed.2021.617689

Health Issues and Immunological Assessment Related to Wuhan's COVID-19 Survivors: A Multicenter Follow-Up Study

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: 15 October 2020
Accepted: 23 March 2021
Published: 07 May 2021

Citation:
Mei Q, Wang F, Yang Y, Hu G, Guo S, Zhang Q, Bryant A, Zhang L, Kurts C, Wei L, Yuan X and Li J (2021) Health Issues and Immunological Assessment Related to Wuhan's COVID-19 Survivors: A Multicenter Follow-Up Study. Front. Med. 8:617689. doi: 10.3389/fmed.2021.617689

Background: Currently, a large number of hospitalized coronavirus infectious disease-2019 (COVID-19) patients have met the clinical discharge criteria and have been discharged. Little is known about the sequelae and herd immunity, two important factors influencing the life quality and safety of COVID-19 survivors.

Methods: Discharged COVID-19 patients from four medical facilities in Wuhan, China, were followed in order to record and investigate possible post-COVID-19 sequelae and herd immunity. After hospital discharge, patients reported to Fangcang shelter hospitals for an initial 14-day period of mandatory clinical monitoring. After release from these shelter hospitals, patients returned home for self-quarantine. Real-time quantitative PCR (RT-qPCR) was used for severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) detection. Colloidal gold-based immunochromatographic strip assay (ICGSA) was used for anti-SARS-CoV-2 immunoglobulin G (IgG) and immunoglobulin M (IgM) antibody testing. The data for this study are derived from case reports, medical records, and self-reports.

Results: A total of 3,677 COVID-19 survivors [median age = 59 years, interquartile range (IQR) = 47–68, range = 10–98; 55.5% female] who were released from four hospitals in Wuhan, China, between January 18 and March 29, 2020 were followed for a median of 144 days (IQR = 135–157). During follow-up, 976 (26.5%) patients had at least one post-COVID-19 sequela. The incidence of post-COVID-19 sequelae among elderly COVID-19 survivors (age ≥60 years) was slightly increased compared to that of young COVID-19 survivors (age <60 years; relative risk = 1.05, 95% CI = 1.02–1.10, $p = 0.007$). During follow-up, a dramatic reduction of anti-SARS-CoV-2 IgG (88.0%, 95% CI = 84.2–90.4) and IgM (93.2%, 95% CI = 88.5–96.4) antibodies was observed. Among these COVID-19 survivors, 1.2% ($n = 45$) retested positive for SARS-CoV-2 and 1.0% ($n = 37$) died during follow-up.

Of those who died during follow-up, 70.3% were male and all were negative for both IgG and IgM, except for one person who was IgG-positive.

Conclusions: Our study documents significant post-COVID-19 sequelae that impair functions of multiple organ systems in COVID-19 survivors, suggesting that the long-term effects of this disease will negatively impact survivors' quality of life, continue to strain health care systems, and result in extended periods of lost productivity. Furthermore, female gender and anti-SARS-CoV-2 immunity may play an essential role in the survival after COVID-19 infection.

Keywords: post-COVID-19, SARS-CoV-2, mortality, hospital discharge, post-COVID-19 sequela, physical and psychological symptoms, antibody test, IgG and IgM

INTRODUCTION

The coronavirus infectious disease-2019 (COVID-19) pandemic continues to affect people worldwide. As of the end of July 2020, there are more than 14 million confirmed cases, with more than 0.6 million deaths (1). While the numbers of cases and deaths are expected to rise, a larger number of COVID-19 patients have recovered and have been discharged from medical facilities worldwide (1). However, little is known about post-COVID-19 sequelae among the discharged patients and related potential risk factors. Wuhan, China, was the first city to experience the emergence of COVID-19 (2). The central government launched timely public health strategies for virus control, including mandatory curfews and face coverings. At the medical facility level, COVID-19 testing was implemented and strict hospital discharge criteria were developed, including a mandatory 14-day period of post-hospital discharge clinical monitoring at regional shelter hospitals. M [a]any medical facilities continued to follow COVID-19 patients after primary hospital discharge, including the time periods during and after their stay at secondary shelter hospitals. We utilized these long-term follow-up data to investigate both the physical and psychological symptoms, including severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) immune recognition, among a large cohort of COVID-19 survivors released from four medical facilities in Wuhan.

METHODS

COVID-19 Survivors Studied

This study investigated post-infection sequelae among all patients with confirmed COVID-19 infection who were discharged from four hospitals in Wuhan, China, between January 18 and March 29, 2020. These government-designated COVID-19 hospitals included Wuhan No.1 Hospital, Wuchang Hospital, Zhongshang Hospital, and Hubei Province Hospital. The hospitals were mandated to treat all infected patients regardless of disease severity (i.e., mild, severe, and critical). Standard hospital discharge criteria (3) included: (1) absence of fever for more than 3 days; (2) radiological evidence of significant resolution of pneumonia via CT scan; and (3) two sequential negative SARS-CoV-2 real-time quantitative PCR (RT-qPCR) tests on nasopharyngeal/oropharyngeal swab samples with at least a 24-h interval between sampling.

Early in the disease outbreak, little was known regarding the clinical characteristics, disease course, and mortality of COVID-19 infection, or the dynamics and range of viral transmission. Thus, all hospitalized patients who met the discharge criteria were immediately transferred to a Fangcang-like medical facility for a mandated 14-day period of clinical monitoring (4). The date of primary hospital discharge is the start of the follow-up. The last day of follow-up for the COVID-19 survivors included in this study was July 24, 2020. The data for this study are derived from case reports, medical records, and self-reports. This study was approved by the institutional ethics board of Wuhan No.1 Hospital, China (no. [2020] 6). Informed consent was obtained from each participant.

Fangcang-Like Medical Facility and Its Discharge Criteria

Fangcang is a public health concept that was instituted for the first time in China during the SARS-CoV-2 outbreak of 2020. It was highly efficient at the mobilization of medical resources and dramatically reduced the burden on local medical capacity (4). Fangcang shelter hospitals are large-scale, temporary hospitals that are rapidly constructed by converting public venues (e.g., exhibition centers and football stadiums) into medical facilities equipped for basic medical care, frequent patient monitoring, and rapid patient assessment and referral. To maximize medical resources and to contain the rapidly emerging COVID-19 epidemic, government-designated hospitals in China discharged all COVID-19 patients directly to a Fangcang shelter hospital for a defined period of clinical observation, typically 14 days. This clinical observation serves as the initial phase of follow-up. After this observation period in the Fangcang hospital, patients were discharged home to self-quarantine if they met the following criteria: (a) no recurrence of any clinical symptom including respiratory-related inflammation and (b) negative SARS-CoV-2 RT-qPCR test on nasopharyngeal/oropharyngeal swab samples after 14 days.

COVID-19 survivors who developed any clinical symptom or sign during monitoring at the Fangcang shelter hospitals

TABLE 1 | Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) real-time quantitative PCR (RT-qPCR) test results.

ORF1ab	Gene N	Gene E	Result
+	+	+	Positive
+	+	−	Positive
+	−	+	Positive
+	−	−	Positive only when a repeated test shows the same result
−	+	−	Suspicious. Recheck after a certain period
−	−	+	
−	−	−	Negative

were immediately readmitted to hospitals. After discharge from Fangcang shelter hospitals, the community hospitals were responsible for clinical monitoring and diagnostic testings. When clinical symptoms occurred, or other medical circumstances required, or upon personal request, CT imaging and SARS-CoV-2 retesting were performed.

SARS-CoV-2 Real-Time Quantitative PCR Test

RT-qPCR for SARS-CoV-2 was performed on nasopharyngeal/oropharyngeal swabs for viral detection. Viral RNA was extracted from the patients' nasopharyngeal/oropharyngeal swabs, sputum, and other samples, which were placed in a 56°C incubator for 30 min to inactivate the virus. Primer probes targeted three genes of SARS-CoV-2: open reading frame 1ab (*ORF1ab*) and genes of nucleocapsid proteins N and E (**Table 1**). The PCR buffer, reverse transcriptase enzyme, DNA polymerase, and gene primers were mixed together and added to a 96-well plate. The extracted RNA samples were then added to the wells, the plate sealed, and RT-qPCR amplification was performed as follows: one cycle at 40–45°C for 10 min, followed by 95°C for 3 min. Then, DNA denaturation and amplification proceeded for 45 cycles at 95°C for 15 s and 55–58°C for 30 s. The test results of SARS-CoV-2 were reported as positive or negative when the cycle threshold values remained below 44 or exceeded 43, respectively. The results in **Table 1** were used to determine whether a sample was COVID-19-positive. This RT-qPCR test can detect SARS-CoV-2 nucleic acid standard substance within 400 copies/ml with a detection rate of 100%. Therefore, the sensitivity of the SARS-CoV-2 RT-qPCR is 400 copies/ml.

For each patient, this SARS-CoV-2 RT-qPCR was performed twice on nasopharyngeal/oropharyngeal swab samples obtained with at least a 24-h interval between samples. Both tests must be negative in order to meet the discharge criteria of the hospital, as noted above. Subsequently, this test was performed at least once during Fangcang-medical monitoring; afterwards, this test was performed when clinical symptoms reoccurred, or upon personal requests, or for other reasons such as entering medical facilities and community centers.

TABLE 2 | Sensitivity and specificity of the colloidal gold-based immunochromatographic strip assay (ICGSA).

Immunoglobulin test	Sensitivity (95% CI)	Specificity (95% CI)
IgM	87.1% (83.9–91.3)	99.7% (98.4–100)
IgG	88.3% (85.6–92.0)	99.4% (98.0–99.8)
Combination of IgG and IgM	91.6% (88.6–93.4)	99.2% (97.6–99.7)

Colloidal Gold-Based Immunochromatographic Strip Assay

The colloidal gold-based immunochromatographic strip assay (IGCSA) was used for immunoglobulin G (IgG) and immunoglobulin M (IgM) detection. In brief, the IgM and IgG test cards were numbered sequentially. Anticoagulated (citrated) blood samples were centrifuged for 5 min at 500 × *g* and 10 μl of plasma was added to the sample well for 10–15 min. A test was considered positive when lines for the patient sample and the positive control sample appeared simultaneously. Samples in which a line developed only for the positive control sample were regarded as negative. Tests in which only the patient sample, and not the positive control sample, was positive were deemed invalid, requiring another test. **Table 2** indicates the sensitivity and specificity of this assay for each immunoglobulin alone and in combination regarding SARS-CoV-2 detection in samples.

Statistical Analysis

Continuous variables were expressed as median (interquartile range, IQR) and compared with the Mann–Whitney *U*-test; categorical variables were expressed as number (percentage) and compared by Fisher's exact test. $P < 0.05$ was regarded as statistically significant.

RESULTS

Between Jan 18 and Mar. 29, 2020, 3,677 hospitalized COVID-19 patients met the clinical discharge criteria and were discharged from the aforementioned four hospitals in Wuhan, China. All these COVID-19 survivors were included in our analyses. The median age was 59 years (IQR = 47–68, range = 10–98; 54.1% female). The survivors were followed for a median of 144 days (IQR = 135–157, range = 117–188; **Table 3**) by four independent medical teams. Among this cohort, 2,331 (63.4%) survivors had mild, 1,239 (33.7%) severe, and 95 (2.6%) had critical condition during their initial hospitalization. During this initial hospitalization, 3,570 (97.1%) survivors received antiviral therapy, 3,026 (82.3%) antibiotic treatment, 2,401 (65.3%) corticosteroids, 1,540 (41.9%) interferon nebulization treatment, and 1,445 (39.3%) γ-immunoglobulin treatment. Three thousand and sixty-six (83.4%) survivors were given a standard oxygen therapy via nasal catheter, 467 (12.7%) received high-flow nasal cannula therapy, 173 (4.7%) had non-invasive mechanic ventilation, and 30 (0.8%) required invasive mechanic ventilation. The median time from symptom onset to hospital admission was 8.0 days (IQR = 6.0–11.0). The median length of initial hospitalization was 17.0 days (IQR = 11.0–25.0).

TABLE 3 | Clinical characteristics, retest positivity, and sequelae among discharged coronavirus infectious disease-2019 (COVID-19) patients.

Characteristics	All patients (IQR/%)	Retested positive group (IQR/%)	Deceased group (IQR/%)
No. of patients	3,677	45 (1.2)	37 (1.0)
Median age (years)	59.0 (47.0–68.0)	57 (50.0–64.0)	70.0 (56.0–79.0)
Gender			
Male	1,688 (45.9)	14 (31.1)	26 (70.3)
Female	1,989 (54.1)	31 (68.9)	11 (29.7)
Retest positivity	45 (1.2)	45 (100)	0
No. of new viral transmission	0	0	0
Severity (initial hospitalization)			
Mild	2,331 (63.4)	19 (42.2)	15 (40.5)
Severe	1,239 (33.7)	24 (53.3)	4 (10.8)
Critical	107 (2.9)	2 (4.5)	18 (48.6)
Treatment (initial hospitalization)			
Antiviral therapy	3,570 (97.1)	41 (91.1)	34 (91.9)
Antibiotic treatment	3,026 (82.3)	38 (84.4)	36 (97.3)
Corticosteroids	2,401 (65.3)	28 (62.2)	36 (97.3)
Interferon nebulization	1,540 (41.9)	15 (33.3)	5 (11.1)
γ-Immunoglobulin	1,445 (39.3)	1 (2.2)	3 (8.1)
Median time from symptom onset to admission (days, IQR)	8.0 (6.0–11.0)	8.0 (6.0–11.0)	10.0 (7.0–13.0)
Median time to hospitalization (days, IQR)	17.0 (11.0–25.0)	16.5 (11.0–24.0)	16.0 (10.0–23.0)
Median follow-up time (days, IQR)	144.0 (135.0–157.0)	150.0 (139.5–158.5)	33.0 (18.0–42.0)
Sequelae			
Pulmonary function	337 (9.2)		10 (27.0)
Shortness of breath	136	5	3
Cough/sputum	87	4	2
Pharyngitis/foreign body feeling	42	1	2
Dyspnea	30	7	3
Pulmonary fibrosis	21		
Lung damage	12		
Bronchitis	4		
COPD	3		
Hemoptysis	2		
Cardiac function	278 (7.6)		8 (21.6)
Chest pain/tightness	184	4	4
Palpitation	63	3	
Cardiac disease	14		2
Tachycardia	13	1	1
Angina pectoris	3		
Heart attack	1		1
Neurologic function	289 (7.9)		5 (13.5)
Insomnia	78	4	4
Joint pain/back pain/lumbago	71	3	
Fatigue	55		
headache/dizziness/poor memory	49		
Change of taste and smell	10		
Myalgia	8		
Impaired vision	5		
Leg numbness/finger stiffness	5		
Neuralgia	2		
Paralysis	2		

(Continued)

TABLE 3 | Continued

Characteristics	All patients (IQR/%)	Retested positive group (IQR/%)	Deceased group (IQR/%)
Tinnitus	2		
Confusion	1		1
Coma	1		
cerebral infarction	1		
Endocrine system	90 (2.4)		
Hair loss	67		
Bitter/dryness in mouth	12		
High blood sugar	6		
Diabetes	5		
Gastrointestinal function	42 (1.1)		1 (2.7)
Gastrointestinal complaints/poor appetite	31	3	1
Diarrhea	8		
Constipation	2		
Emesia	1		
Dermatological system	33 (0.9)		
Hidrosis	24		
Erythra	7		
Allergy	2		
Hepatic system	16 (0.4)		1 (2.7)
Hepatic insufficiency	8		1
Edema	7		
Antiadoncus	1		
Kidney function	12 (0.3)		1 (2.7)
Hypertension	6		
Kidney insufficiency	6		1
Various	66 (1.8)		5 (13.5)
Reduction of physical strength	64		5
Dryness/excessive secretion in eye	2		

TABLE 4 | Sequelae disposition by 10-year age intervals of discharged coronavirus infectious disease-2019 (COVID-19) patients.

Age intervals (years)	Sequelae Nr./Nr.		Relative risk ratio (95% CI, *p*-value)
	Male	Female	Male vs. female
10–19	2/10	0/4	1.25 (0.92–1.70, $p = 1$)
20–29	7/56	14/91	0.98 (0.85–1.10, $p = 0.81$)
30–39	46/183	61/206	0.94 (0.83–1.06, $p = 0.36$)
40–49	77/256	78/250	0.98 (0.88–1.10, $p = 0.84$)
50–59	88/353	163/473	0.87 (0.80–0.95, $p = 0.0036$)
60–69	117/452	185/592	0.92 (0.86–1.00, $p = 0.063$)
70–79	48/265	56/256	0.95 (0.88–1.04, $p = 0.323$)
80–89	12/95	19/112	0.95 (0.85–1.06, $p = 0.438$)
>90	3/18	0/5	1.2 (0.98–1.48, $p = 1$)

During follow-up, 976 (26.5%) COVID-19 survivors had at least one sequelae (median age = 57, IQR = 47.8–56.4, range = 17–92; 59.0% female), the most common being chest pain/tightness (184, 5.0%), shortness of breath (136, 3.7%), and cough/sputum (87, 2.4%) (**Table 3**). Three hundred thirty-seven (9.2%) survivors had sequelae affecting pulmonary function, 278 (7.6%) had sequelae related to cardiac function, and 289 (7.9%) and 90 (2.4%) had sequelae impairing the neurologic system and endocrine function, respectively. Sequelae disposition by 10-year age interval of all the 3,677 survivors is included in **Table 4**. The incidence of post-COVID-19 sequelae of elderly COVID-19 survivors (age ≥60 years, $n = 1{,}795$) was slightly increased compared to that of young survivors (age <60, $n = 1{,}882$) [relative risk ratio (RR) = 1.05, 95% CI = 1.02–1.10, $p = 0.007$]. However, gender did not significantly influence the incidence of post-COVID-19 sequelae (**Table 4**). Additionally, 173 (4.7%) survivors self-reported diverse psychological symptoms such as anxiety (103, 2.8%), depression (70, 1.9%), and emotional instability (37, 1.0%). One hundred and thirty-two (3.6%) survivors refused to report personal feelings. Eight hundred two (21.8%) survivors were assessed by mental health care specialists and were deemed to have a clinically defined psychological condition. The psychological conditions of 136 (3.7%) survivors improved after psychological therapy.

At the initial phase of the follow-up (days 1–45 post-hospital discharge), the results of the ICGSA for anti-SARS-CoV-2 viral immunoglobulins showed that 249 (6.8%) COVID-19 survivors

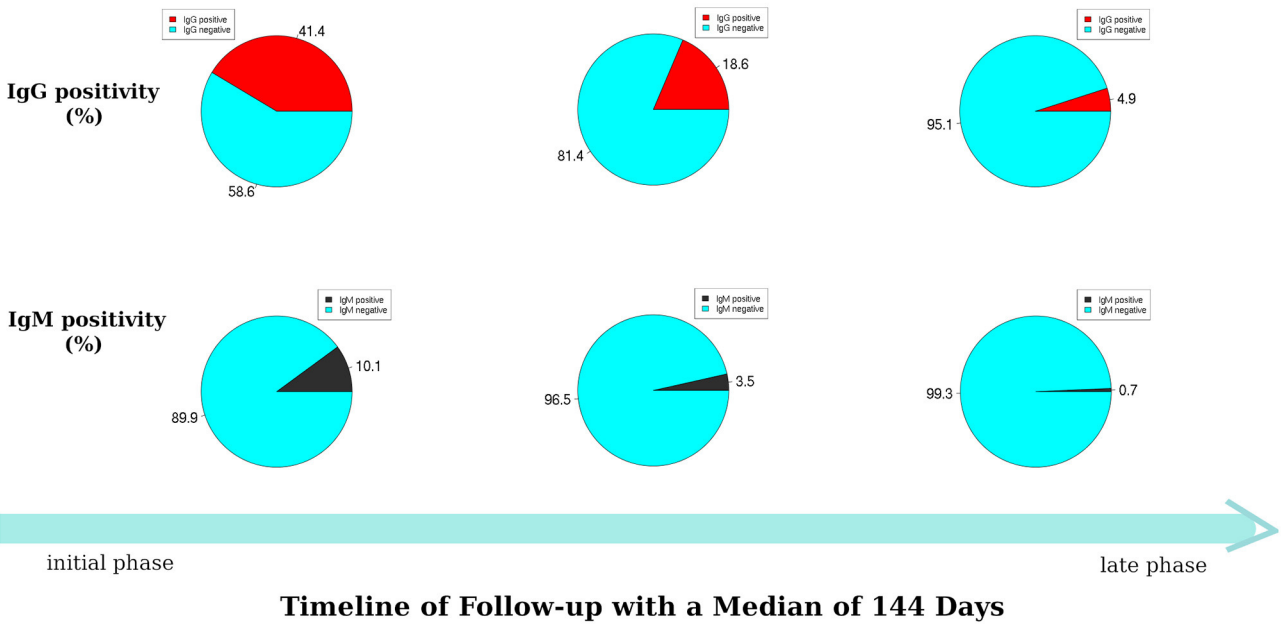

FIGURE 1 | Dynamic decline of immunoglobulin G (IgG) and immunoglobulin M (IgM) among 3,677 coronavirus infectious disease-2019 (COVID-19) survivors during follow-up.

were positive for both IgM and IgG, 1,274 (34.6%) were IgG-positive and IgM-negative, 121 (3.3%) were IgG-negative and IgM-positive, and 2,033 (55.3%) were negative for both IgG and IgM (**Figure 1**). At the late phase of the follow-up (days 100–150 post-hospital discharge), the IgG and IgM antibody positivity rates were reduced by 88.0% (95% CI = 84.2–90.4) and 93.2% (95% CI = 88.5–96.4), respectively. Specifically, only 25 (0.7%) survivors were positive for both IgG and IgM, 157 (4.3%) were IgG-positive and IgM-negative, none were IgG-negative and IgM-positive, and 3,495 (95.1%) were negative for both IgG and IgM (**Figure 1**).

During follow-up, 45 (1.2%) survivors retested positive for SARS-CoV-2 (median age = 57 years, IQR = 50–64, range = 25–81; 68.9% female; **Table 3**). None of these 45 was a health care worker, and none had taken medicine regularly after their initial hospital discharge. Of these, 25 survivors were immediately readmitted to hospitals and 20 remained at home under self-quarantine. Two of the 45 survivors had both IgG and IgM antibodies, 26 were IgG-positive and IgM-negative, two were IgG-negative and IgM-positive, and the remaining 15 were negative for both antibodies. The median duration between initial hospital discharge and retest positivity was 32.0 days (IQR = 28.0–40.0, range = 9–58; **Table 3**). Furthermore, 21 survivors in this retest-positive subgroup were asymptomatic, while 24 had at least one symptom associated with COVID-19, the most common being dyspnea, cough, and chest tightness. During their initial hospitalization, 19 of the 45 survivors had mild disease, 24 had severe condition, and two had critical condition. As of July 24, 2020, all 45 retest-positive survivors were alive. Twenty readmitted and retested positive survivors met the discharge criteria and were once again released to home quarantine. During follow-up, no new viral transmission was observed or reported.

During follow-up of the 3,677 COVID-19 survivors, 37 (1.0%) individuals died (median age = 70.0 years, IQR = 56.0–79.0, range = 31.0–98.0; 29.7% female; **Table 3**). None of the deceased was a health care worker. Thirty-one of the deaths were attributed to COVID-19, while six deaths were caused by comorbidities including diabetes, hepatobiliary tube cancer, heart attack, encephalorrhagia, epilepsy, and scurvy. The median duration from hospital discharge to death was 33.0 days (IQR = 18.0–42.0; **Table 3**). None of these deceased retested positive. Five of these 37 individuals had a worsened condition after hospital discharge and were therefore readmitted to the hospital; they died 4–15 days after readmission. The remaining 32 died at home. None of the deceased had taken any medicine regularly after initial hospital discharge and their psychological conditions had been stable. Except for one IgG-positive/IgM-negative individual, all other deceased individuals were IgG- and IgM-negative, indicating no immune system recognition of SARS-CoV-2. Within this deceased subgroup, 15 (40.5%) individuals had mild, four (10.8%) severe, and 18 (48.6%) had critical condition during their initial hospitalization. Also, during this initial hospitalization, 34 (91.9%) individuals were given antiviral treatments including arbidol, ganciclovir, oseltamivir, ribavirin, and hydroxychloroquine (**Table 3**), 36

(97.3%) individuals received antibiotics, and 36 (97.3%) were given corticosteroids.

DISCUSSION

To our knowledge, this study describes the longest duration of clinical follow-up in the largest cohort of discharged COVID-19 patients. Clearly, acute COVID-19 infection damages pulmonary function, but it has also been associated with the dysfunction of many other organ systems including the circulatory (5), cardiovascular (6), renal (7), gastrointestinal (8), endocrine (9), nervous (10), and skin (11) systems. In contrast, our study shows that diverse multi-organ functional impairments also occur well after hospital discharge in patients deemed recovered from primary acute infection. Specifically, during a mean follow-up time of 144 days, 976 (26.5%) of the COVID-19 survivors developed functional abnormalities of the cardiovascular, neurological, and endocrine systems. The risk of the development of such physical abnormalities was independent of age and gender. Furthermore, we show that 173 (4.7%) discharged patients had an associated psychological condition post-COVID-19 infection. These post-COVID-19 sequelae greatly impact the patients' long-term quality of life and will continue to strain the health care system.

Our study also reports a dramatic reduction of the anti-SARS-CoV-2 antibodies IgG and IgM during this long-term follow-up, which is consistent with the results of several recent studies (12–14). This result strongly suggests that adequate herd immunity may not develop or be maintained for a sufficient period to quell the pandemic. Such a finding supports the need to extend public health practices including social distancing procedures, face covering, and hygiene-based measures in order to limit viral transmission until an effective vaccine is developed and made widely available.

Our study describes a 1% overall mortality rate among Wuhan's COVID-19 survivors. Importantly, the majority of these deaths occurred in individuals who tested negative for both IgG and IgM. These results suggest that immune responses to SARS-CoV-19 infection may lower patients' risk of life-threatening post-discharge sequelae. The median age of this deceased group is 70 years, indicating that advanced age is not only a risk factor for death from primary COVID-19 infection but it also influences the mortality from post-COVID-19 sequelae. Fifteen of the 37 deceased persons had a mild course of COVID-19 during the initial hospitalization, indicating that the disease severity of primary COVID-19 infection is not the sole factor contributing to mortality in the post-COVID-19 period. Furthermore, the majority of deaths during follow-up were those of male survivors (26, 70.3%), implying that female gender could play an important role in survival during the post-COVID-19 period.

Lastly, we report a 1.2% rate of COVID-19 retest positivity among all COVID-19 survivors, with no new viral transmission. We cannot confirm whether or not retest-positive people are able to infect others; however, as long as the appropriate social health measures are practiced, reinfection via retest-positive people can likely be avoided.

Our study has some limitations. Being a national multicenter study, the findings must be further verified by international studies. Secondly, this study was not designed or able to determine the effects of treatment on the follow-up outcome. Thirdly, a recent study revealed the clinical characteristics of family members for COVID-19 infection (15). However, our study was not able to determine the post-COVID-19 sequelae-related genetic relationship. This warrants further investigation.

In summary, our results indicate that persistent and often severe morbidity is prevalent among COVID-19 survivors. Individuals with such post-viral sequelae may have reduced quality of life, including lost productivity, and may continue to strain health care systems. Closer follow-up of COVID-19 survivors with prompt medical intervention for developing sequelae may improve the long-term outlook for these individuals.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional ethics board of Wuhan No.1 Hospital, China (No. [2020] 6). Informed consent was obtained from each participant. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QM, FW, YY, GH, JL, CK, QZ, and SG contributed to the acquisition, analysis, or interpretation of data. QM, YY, JL, and AB drafted the manuscript. QM and JL did the statistical analysis. AB, FW, GH, LW, XY, and LZ contributed to the critical revision of the manuscript for important intellectual content. FW obtained funding. YY and GH gave administrative, technical, or material support. LW, XY, and JL helped with conception design and supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by the SGC's Rapid Response Funding for Bilateral Collaborative Emergence COVID-19 Project between China and Germany (Grant No. C-0065), the Public Health and Family Planning Research Project of Hubei Province (Grant No. WJ2019M128), Natural Science Foundation of Hubei Province (Grant No. 2019CFB449), and General Program of National Natural Science Foundation of China (Grant No. 81372664). The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

3. Acknowledgement

First of all, I would like to express all my gratitude to the supervisor

Prof. Dr. med. Christian Kurts

for giving me the opportunity to do my MD study under his guidance, supporting me whenever I needed his help and introducing me into the world of science. Throughout my doctoral work, Prof. Kurts taught me how to perform scientific work, present research data in an accurate way, and encouraged me to develop the ability of creativity and new skills. I would also like to thank Prof. Kurts for great support in writing thesis and publication and for all the helpful and productive discussions and advices. I would also like to thank Dr. Jian Li for providing me the opportunity to study in Bonn universuty. In addition, I would like to thank Institute of Molecular Medicine and Experimental Immunology, Bonn University for supporting my study.

Moreover, I would like to thank Prof. Amy Bryant who patiently answered my questions, made productive discussions and encouraged me when I was lost. I would also like to thank Dr. Junping Yin who helped me to search for useful information and prepare my doctor thesis. I extend sincere thanks to them for German translation in my thesis.

My thanks should also go to Dr. Alexander Böhner for the kind technical support during my work and I also like to thank them for the nice and friendly atmosphere, making it really comfortable to work with them. Finally, I would like to express my gratitude to my parents and my wife for supporting me.