Received: 28 January 2021 | Revised: 13 May 2021 | Accepted: 22 May 2021

DOI: 10.1111/jch.14308

ORIGINAL PAPER

WILEY

Risk factors for the delayed viral clearance in COVID-19 patients

Xiaoping Chen MD, PhD[1] | Wenjia Hu MT[1] | Miao Yang MD, PhD[2] | Jiaxin Ling PhD[3] | Yongxi Zhang MD, PhD[1] | Liping Deng MD, PhD[1] | Jinlin Li PhD[3,4] | Åke Lundkvist PhD[3] | Johanna F Lindahl PhD[3,5,6] | Yong Xiong MD, PhD[1]

[1]Department of Infectious Diseases, Zhongnan Hospital of Wuhan University, Wuhan, China

[2]Department of Endocrinology, Zhongnan Hospital of Wuhan University, Wuhan, China

[3]Department of Medical Biochemistry and Microbiology, Zoonosis Science Center, University of Uppsala, Uppsala, Sweden

[4]Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden

[5]Department of Biosciences, International Livestock Research Institute, Hanoi, Vietnam

[6]Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence

Yong Xiong, MD & PhD, Department of infectious diseases, Zhongnan Hospital of Wuhan University, No.169, Donghu Road, Wuchang District, Wuhan City, Hubei Province, P.R China.

Johanna F Lindahl, PhD, Department of Medical Biochemistry and Microbiology, Zoonosis Science Center, University of Uppsala, Uppsala, Sweden; Department of Biosciences, International Livestock Research Institute, 100000 Hanoi, Vietnam; Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Emails: yongxiong64@163.com(YX); johanna.lindahl@imbim.uu.se(JFL)

Funding information

This work was supported by the Fundamental Research Funds for the Central Universities (2042020kf1018).

Abstract

Comorbidities are important for the disease outcome of COVID-19, however, which underlying diseases that contribute the most to aggravate the conditions of COVID-19 patients are still unclear. Viral clearance is the most important laboratory test for defining the recovery of COVID-19 infections. To better understand which underlying diseases that are risk factors for delaying the viral clearance, we retrospectively analyzed 161 COVID-19 clinical cases in the Zhongnan Hospital of Wuhan University, Wuhan, China between January 5 and March 13, 2020. The demographic, clinical and laboratory data, as well as patient treatment records were collected. Univariable and multivariable analysis were performed to explore the association between delayed viral clearance and other factors by using logistic regression. Survival analyses by Kaplan-Meier and Cox regression modeling were employed to identify factors negatively influencing the viral clearance negatively. We found that hypertension and intravenous immunoglobulin adversely affected the time of viral RNA shedding. Hypertension was the most important risk factor to delay the SARS-CoV-2 virus clearance, however, the use of Angiotensin-Converting Enzyme Inhibitors(ACEI)/Angiotensin Receptor Blockers(ARB) did not shorten the time for virus clearance in these hypertensive patients' virus clearance. We conclude that patients having hypertension and intravenous immunoglobulin may delay the viral clearance in COVID-19 patients.

Xiaoping Chen, Wenjia Hu and Miao Yang are corporate first authors

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SASR-CoV-2), was first identified in Wuhan, China, and now has developed into the second new pandemic in the 21st century.[1-3] The clinical symptoms of COVID-19 varies and most often displays as fever, cough and mild fatigue, sometimes also dyspnea, myalgia and severe anorexia, and may develop into critical respiratory failure, or even fatal.[4-7] The clinical characterizations of early infections inside of Wuhan are important references for other regions or countries to compare the development of clinical manifestations and epidemiological trends of COVID-19.

SARS-CoV-2 belongs to the genus coronavirus (CoV), a group of enveloped, single-stranded, positive-sense RNA viruses. The host cell receptor for SARS-CoV and SARS-CoV-2 is the angiotensin-converting enzyme 2 (ACE2), presented epithelial cells in the respiratory tract.[8-10] Middle East respiratory syndrome virus (MERS), uses dipeptidyl peptidase 4 (DPP4) as cellular receptor. By using ACE2, which is also an enzyme that modulates the renin-angiotensin-aldosterone system (RAAS), SARS-CoV infections can disturb RAAS in the targeted cells by downregulate the expression of ACE2 and increase lung damage and edema.[11]

Clinical studies identified that severe COVID-19 cases often occurred in patients with old age, male, diabetes, hypertension or other comorbidities.[9,12,13] In COVID-19 patients with underlying diseases, a very high case fatality rate (73.3%) has been observed.[14] However, how these comorbidities affect the SARS-CoV-2 clearance is not yet understood. The association between duration of viral clearance and the use of ACE inhibitors and angiotensin receptor blocker (Angiotensin-Converting Enzyme Inhibitors(ACEI)/Angiotensin Receptor Blockers(ARB) for the COVID-19 patients combined with underlying diseases are unknown and such clinical-based studies are still lacking. Potential factors might be that these comorbidities directly accelerate the damage of target tissues or that they favor the virus proliferation during a SARS-CoV-2 infection. Trying to provide more clinical information to answer this question, we analyzed 161 COVID-19 hospitalized patients and evaluated which underlying diseases that had the highest risk to aggravate the conditions of a COVID-19 infection.

2 | METHODS

2.1 | Study design and participants

Between January 5 and March 13, 2020, we collected the records of 161 hospitalized patients in Zhongnan Hospital of Wuhan University, Wuhan, China, whose throat swab specimens had been tested positive for SARS-CoV-2 by qRT-PCR according to a protocol previously described.[4] The patients who repeatedly tested SARS-CoV-2 RNA negative for at least two times with an interval of more than 24h were regarded as viral negative. The admission date was used as the starting time-point for the viral clearance process, and the date of the second negative detection of viral RNA was calculated as the end time-point of viral clearance. According to the duration of viral shedding time, the patients were divided into two groups; those that shedded the virus less than 15 days and those the shedded virus more than 15 days. This study was approved by the ethics board in Zhongnan Hospital of Wuhan University, Wuhan, China (No.2020011). Informed consents were obtained from all patients upon admission to Zhongnan Hospital of Wuhan University, Wuhan, China.

2.2 | Data collection

We recorded patient demographic data including gender, age, and time from onset of disease until seeking hospital care; clinical data of chronic diseases including hypertension, cardiovascular diseases, diabetes, chronic obstructive pulmonary disease (COPD), and chronic liver disease existed as comorbidities; treatment data including usage of oxygen support, antivirals(Arbidol/Lopinavir/Ritonavir), corticosteroid, or immunoglobulin. Two or more researchers were assigned to collect and review relevant patients' data independently.

2.3 | Statistical analysis

Chi-square test or Fisher's exact test were used to evaluate categorical variables. We firstly tested distribution of measurement data, and if the measurement data was normally distributed, the t test was used and the results were expressed as the mean ±standard deviation (SD); or if the measurement data was non-normally distributed, the Mann-Whitney U test was used and the results were expressed as the median (25% – 75% interquartile range, IQR). Univariable and multivariable analysis were performed to explore the association between delayed viral clearance and risk factors by using logistic regression. For the multivariable analysis, factors with a p-value was lower than 0.1 in the univariable analysis were included. We used Kaplan-Meier survival analysis to estimate the cumulative negativity rate of SARS-CoV-2 viral RNA and the stratified logrank statistic to compare SARS-CoV-2 clearance in different groups. p values <.05 were considered statistically significant. All statistical analyses were performed by using SPSS 17.0 software package (Chicago, Illinois, USA).

3 | RESULTS

3.1 | Demographics, comorbidities, treatment, and clinical outcomes of 161 COVID-19 patients with different viral shedding durations

From Table 1, a total of 161 patients, 90 females (90/161, 55.9%) and 71 males (71/161, 44.1%), were included in the study. At the end of observation, 146 patients had cleared the virus infection and

TABLE 1 Demographics, Comorbidities, treatment, and clinical outcomes of 161 COVID-19 patients with different shedding durations

Viral shedding duration after admission to hospital				
	Total (161)	**<15 Days (n = 115)**	**≥15 Days (n = 46)**	***P* value**
Sex				**.018**
female	90 (55.9%)	71 (61.7%)	19 (41.3%)	
male	71 (44.1%)	44 (38.3%)	27 (58.7%)	
Age (years)	54.0 (36.0, 66.0)	51.0 (33.0, 62.0)	62.0 (47.0, 71.3)	**.001**
Comorbidities				
Hypertension	37 (23.0%)	22 (19.1%)	15 (32.6%)	**<.0001**
Cardiovascular disease	7 (4.3%)	3 (2.6%)	4 (8.7%)	.087
Diabetes	15(9.3%)	9 (7.8%)	6 (13.0%)	.304
COPD	6 (3.7%)	4 (3.5%)	2 (4.3%)	.792
CKD	6 (3.7%)	4 (3.5%)	2 (4.3%)	.792
Chronic liver diseases	13 (8.1%)	8 (7.0%)	5 (10.9%)	.410
Days from illness onset to hospital, median (IQR), d	7 (4, 10)	7 (4, 10)	6 (3.0, 10.0)	.642
Hospital stays, median (IQR), d	15.0 (9.0, 21.0)	12 (8.0, 17.0)	22.0 (18.0, 29.0)	**<.0001**
Severe type	49 (30.4%)	28 (24.3%)	21 (45.7%)	**.008**
Treatment				
Oxygen support	93 (57.8%)	60 (52.2%)	33 (71.7%)	**.023**
Arbidol	92 (57.1%)	66 (57.4%)	26(56.5%)	.920
Lopinavir/Ritonavir	36 (22.4%)	22 (19.1%)	14(30.4%)	.120
Corticosteroid	80 (49.7%)	51 (22.6%)	29 (63.0%)	**.032**
Intravenous immunoglobulin	33 (20.5%)	16 (13.9%)	17 (37.0%)	**.001**
Clinical outcome				
Discharged	146 (90.7%)	106 (92.2%)	40 (87.0%)	.304
Death	15 (9.3%)	9 (7.8%)	6 (13.0%)	

Abbreviations: CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease.

15 had died (15/161, 9.3%). The age of the patients spanned from 20 to 96 years with a median of 54.0 years (25% – 75% IQR, 36.0–66.0 years). Patients that had different underlying diseases included 37 patients with hypertension (23.0%), seven with cardiovascular disease (4.3%), 15 with diabetes (9.3%), six with chronic obstructive pulmonary disease (COPD) (3.7%), 13 with chronic liver disease (8.1%), and six with chronic kidney disease (CKD) (3.7%). During their hospitalization, oxygen support was used to 93 patients (93/161, 57.8%), the antiviral treatment had been applied to 92 (Arbidol, 57.1%) and 36 (Lopinavir/Ritonavir, 22.4%) patients, respectively. Corticosteroid and intravenous immunoglobulin had been applied to 80 (49.7%) and 33 (20.5%) patients, respectively. The duration of viral shedding was divided into two groups: 46 patients (46/161, 28.6%) had more than 15 days of viral shedding, while the rest 115 patients (115/161, 71.4%) had shedded virus for less than 15 days. We compared these two groups and we found that male, old age, hypertension, cardiovascular disease, severe type, oxygen support, corticosteroid and intravenous immunoglobulin showed statistically differences, that is, these factors can negatively affect the viral shedding time in COVID-19 patients.

3.2 | Univariable and multivariable analyses of associated factors that can delay viral clearance of COVID-19 patients

Univariable analyses were performed to analyze which factors are associated with delaying viral clearance in COVID-19 patients. We found that male, the elderly patients (≥60 years), and the patients with hypertension, severe type, oxygen support, use of corticosteroid, and intravenous immunoglobulin can prolong the duration of viral shedding significantly (≥15 days) ($p < .05$, Table 2). Furthermore, based on the multivariable analyses, hypertension (OR = 4.228; 95%CI, 1.739-10.280; $p = .001$) and intravenous immunoglobulin (OR = 2.818; 95%CI, 1.111-7.148; $p = .029$) were independent factors which were highly associated higher odds to have viral shedding time more than 15 days.

3.3 | Kaplan-Meier survival analysis

Hypertension was manifested in 37 out of 161 patients. In these 37 patients with hypertension, 28 patients had received anti-hypertensive medications, and they were further be divided into two groups: 13 patients who received ACEI/ARB and 15 patients who did not receive ACEI/ARB. Kaplan-Meier survival analysis was used to evaluate the time points for viral clearance in the groups with hypertension or not, and in the groups which received ACEI/ARB or not (Figure 1). From Figure 1A, the cumulative probability of viral clearance was higher in the non-hypertension group than that in the hypertension group ($p < .0001$, log-rank). Patients with hypertension showed a higher probability for a prolonged shedding of SARS-CoV-2 viral RNA as compared to the patients without hypertension. The duration of virus clearance was significantly delayed in the hypertension group. However, the use of anti-hypertension medications (ACEI/ARB) or not had no effects on the cumulative probability of viral negative conversion, with statistically no difference (Figure 1B). Intravenous immunoglobulin had been applied to 33 patients, from Figure 1C, the cumulative probability of viral negative conversion was higher in non-intravenous immunoglobulin group than in the 33 intravenous immunoglobulin patients ($p = .0015$, log-rank). Patients with intravenous immunoglobulin showed a higher probability to have a longer time of clearing SARS-CoV-2 viral RNA than those patients without intravenous immunoglobulin. The duration of virus clearance was significantly delayed in the intravenous immunoglobulin group.

3.4 | Duration of virus shedding durations of COVID-19 patients

The median duration of viral RNA shedding for these 161 patients was 11.0 (7–15.5, IQR) days. Patients with hypertension needed 17.0 days (11.0–22.5, IQR), and patients without hypertension needed only

TABLE 2 Univariable and multivariable analysis of factors associated with delayed viral clearance of COVID-19 patients

	Total (n = 161)	Viral shedding duration≥15 days (n = 46)	Univariable analysis			Multivariable analysis		
			OR	95% CI	*p* value	OR	95% CI	*p* value
Sex	Female	21.1%	Ref.			Ref.		
	Male	38.0%	2.293	1.142–4.604	**.018**	1.027	0.752–3.768	.205
Age (years)	<60	19.2%	Ref.			Ref.		
	≥60	43.5%	3.248	1.599–6.598	**.001**	1.802	0.791–-4.109	.161
Hypertension	No	19.4%	Ref.			Ref.		
	Yes	59.5%	6.111	2.765–13.509	**<.0001**	4.300	1.781–10.378	**.001**
Severe type	No	22.3%	Ref.			Ref.		
	Yes	42.9%	2.610	1.271–5.360	**.008**	1.027	0.373–2.842	.960
Oxygen support	No	19.1%	Ref.			Ref.		
	Yes	35.5%	2.327	1.112–4.871	**.023**	1.333	0.498–3.567	.567
Corticosteroid	No	20.1%	Ref.			Ref.		
	Yes	36.3%	2.141	1.060–4.322	**.032**	0.886	0.337–2.328	.806
Intravenous immunoglobulin	No	22.7%	Ref.			Ref.		
	Yes	51.5%	3.627	1.633–8.059	**.001**	2.806	1.106–7.116	**.030**

Abbreviations: CI, confidence interval; OR, odds ratio.

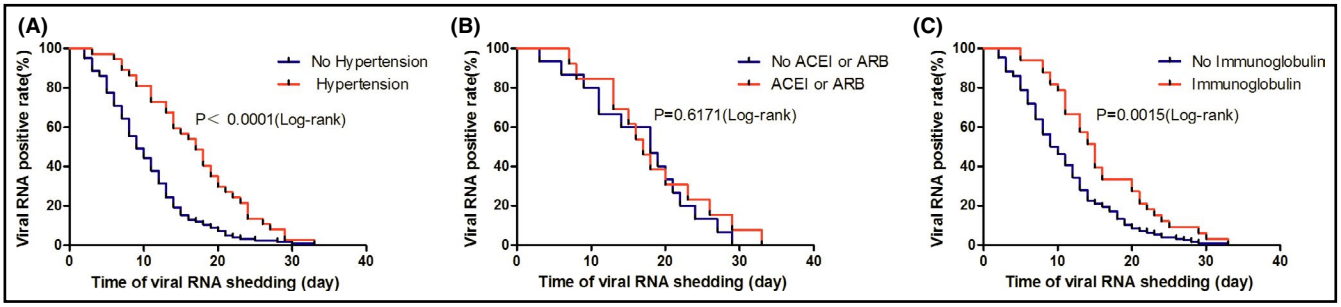

FIGURE 1 Kaplan-Meier estimates of SARS-CoV-2 viral clearance rate of COVID-19 patients with or without hypertension (p=<.0001, log-rank, A) and use of (ACEI/ARB) or not ($p = .6171$, log-rank, B) and intravenous immunoglobulin or not ($p = .0015$, log-rank, C)

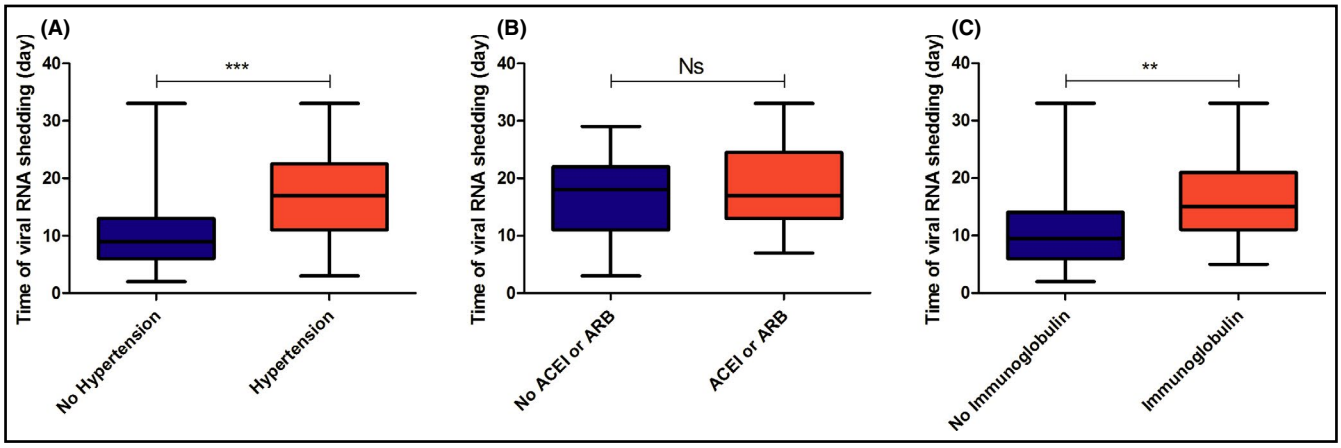

FIGURE 2 Viral RNA shedding durations of COVID-19 patients with or without hypertension ($p < .0001$, A) and use of (ACEI/ARB) or not ($p = .8177$, B), and intravenous immunoglobulin or not ($p = .0002$, C)

9.0 days (6.0–13.0, IQR) for viral clearance. As a result, patients with hypertension showed that having a longer time of viral RNA shedding compared to those without hypertension ($p < .0001$, Figure 2A). We further compared viral RNA shedding durations in the patients that received ACEI/ARB and in patients without treatment. The median duration of viral RNA shedding of 13 patients that received ACEI/ARB was 17.0 days (13.0 - 24.5, IQR), while it was 18.0 days (11.0 −22.0, IQR) for the other 16 patients without ACEI/ARB treatment. There was no significant difference between these two groups ($p = .8177$, Figure 2B). For 33 patients with intravenous immunoglobulin, the median duration of viral RNA shedding was 15.0 (11.0–21.0, IQR) days, while it was 9.5 (6.0–14.0, IQR) days in patients without intravenous immunoglobulin, patients with intravenous immunoglobulin showed that having a longer time of viral RNA shedding compared to those without intravenous immunoglobulin ($p = .0002$, Figure 2C).

4 | DISCUSSION

Hypertension has been considered to cause disease aggravation in coronavirus infections, including COVID-19, SARS, and MERS,[9,14,15] but less attention has been made on risk factors for viral RNA shedding. Our analyses pinpointed hypertension as a strong factor for prolonged viral RNA shedding in COVID-19 patients.

Our study showed that hypertension was a significant risk factor for a delay of SARS-CoV-2 virus clearance. Abnormal regulation of RASS and ACE2 might play a role during the viral infection.[9] ACE2 is a membrane-related aminopeptidase, which is often expressed in vascular endothelium, kidney and cardiovascular tissues.[16] During the SARS outbreak in 2003, it was discovered that the efficiency of ACE2 usage was a key factor in its infection. The receptor binding domains of SARS-CoV and SARS-CoV-2 are 72% similar, and even SARS-CoV-2 has a higher affinity for ACE2.[17] It was thought that undifferentiated cells that express less ACE2 are rarely infected by coronaviruses, while well-differentiated cells that express more ACE2 are more likely to be infected.[18] Therefore, it is reasonable to infer that the increase in ACE2 expression will promote the increase in SARS-CoV-2 infection rate or susceptibility, during viral entry during the early infection phase. Previous studies have shown that high expression of ACE2 in patients with hypertension might facilitate SARS-CoV-2 to enter the targeted cells in the respiratory system.[9,19] However, after the virus enter into the cells, the degradation of ACE2 was reported due to different mechanisms [11,20] and ACE2 shows protective effects such as vasodilation, vasoprotection, the inhibition of lung damage and edema.[21,22] The expression regulation of ACE2 probably play distinct roles in SARS-CoV-2 early infection and late replication phase.

Above-mentioned viral entry and lung injury mediated by SARS-CoV-2 make the clinical use of ACEI/ARB still debated and whether ACEI/ARB will affect viral RNA shedding remains controversial. It has been speculated that patients with hypertension that had been treated with ACE2 inhibitors, angiotensin-converting enzyme inhibitors (ACEI), and angiotensin receptor blocker (ARB), which could have affected the presence of these receptors, increased the receptor usage of SARS-CoV-2.[8] Although the use of ACEI and ARB may stimulate the increase of ACE2 mRNA expression, an animal study has suggested that these increased gene expressions were not completely related to ACE2 activity.[23] Until now, the usage of ACEI/ARB has no proven association with increased risks of COVID-19 disease or severity of the disease neither,[13,24] similar to the results in our study. In addition, we also found out that the usage of ACEI/ARB had no effects on the time for virus shedding. However, in a Chinese retrospective study of 51 hospitalized patients and a control group of 25 individuals, 17 patients with COVID-19 and hypertension were treated with ACEI/ARB and shown a lower peak of viral load.[25] However, our study suggested that the use of ACEI/ARB did not make a statistically difference regarding to the time for viral clearance in 49 hypertensive patients. We also need to take consideration of small sample sizes in our study.

On the other hand, the males and elderly usually had a high risk of hypertension,[26] making it difficult to discern. According to the

univariable analysis, we also found that male and elderly (≥60 years) were significantly related to longer viral shedding. Sex, age, and immunity are notable biological factors during combating infectious pathogens.[27] The immune system is different between females and males.[28] Females may have a generally lower susceptibility to viral infections. The possible reason may be that estrogen and progesterone can help to increase both the innate and adaptive immune responses. In addition, many immune genes are X-linked, which could also explain for the lower infection rate in women.[29] Higher immune reactivities post-viral infection in women can accelerate the process of viral clearance. On the other hand, it may lead to increased immune-pathogenicity and autoimmunity. Gender differences can also affect risk factors, with men, for example, being more likely to develop hypertension.[30] Males have a higher ratio of hypertension in mice models due to decreased adipose ACE2 activity.[31] However, although the ACE2 gene is located in the X chromosome, there is no evidence of different expression of ACE2 between the sexes.[32] Some research demonstrated that chronic kidney disease(CKD) was the strong independent risk factors for the poor prognosis of COVID-19.[33,34] In our study, we try to find whether there were correlation between CKD and SARS-CoV-2 virus clearance and find that CKD was not associated with SARS-CoV-2 virus clearance.

In our study, intravenous immunoglobulin was an independent factor associated with delayed viral clearance. As an immunomodulatory agent, intravenous immunoglobulin was applied to treat many autoimmune diseases, such as ITP and SLE.[35,36] The mechanism has not been illuminated completely. However, immunosuppression was observed by immunoglobulin in vitro.[37] These mechanisms may cause the delayed virus clearance of SARS-CoV-2 in COVID-19 patients.

Until now, there is no effective medicine available to treat COVID-19. Accordingly, the usage of antivirals and cortisone had no effect on the viral clearance, according to our results. The usage of cortisone had unexpectedly a negative effect on the process of viral clearance, although cortisone was commonly used in the SARS patients.[38] However, it is possible that cortisone was used only for particularly severe cases, which may have confounded the results. The outcome of using corticosteroids in COVID-19 patients is still unclear.[5,39] Given that corticosteroids as immune-modulators that can decline circulating specific B- and T-cell subsets [40] and based on our present results, the usage of corticosteroid should be considered carefully.

In conclusion, COVID-19 patients with hypertension and intravenous immunoglobulin have statistically significant increased risk for later viral clearance of the virus. The general profile of hazards in COVID-19 hospitalized patients over time will provide us more important information concerning decision-making and personalized treatment during the clinical practice.

ACKNOWLEDGEMENTS

Informed consents were obtained from all patients upon admission to Zhongnan Hospital of Wuhan University, Wuhan, China. This study was approved by the ethics board in Zhongnan Hospital of Wuhan University, Wuhan, China (No.2020011).

CONFLICT OF INTEREST

There are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Yong Xiong and Johanna F Lindahl designed the study. Xiaoping Chen, Wenjia Hu, and Miao Yang collected and analyzed the data. Jinlin Li, Jiaxin Ling, Miao Yang, Åke Lundkvist, and Johanna F Lindahl drafted and revised the paper. Yongxi Zhang, Liping Deng, and Yong Xiong administration Project. All the authors read and approved the final manuscript.

ORCID

Yong Xiong https://orcid.org/0000-0003-0678-4064

REFERENCES

1. Guarner J. Three emerging coronaviruses in two decades. *Am J Clin Pathol*. 2020;153(4):420-421.
2. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol*. 2016;14(8):523-534.
3. Hilgenfeld R, Peiris M. From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses. *Antiviral Res*. 2013;100(1):286-295.
4. 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;323(11):1061-1069.
5. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506.
6. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese center for disease control and prevention. *JAMA*. 2020;323(13):1239-1242.
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;382(13):1199-1207.
8. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. *J Virol*. 2020;94(7):e00127-20.
9. Fang LK, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med*. 2020;8(4):e21.
10. Lu G, Wang Q, Gao GF. Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. *Trends Microbiol*. 2015;23(8):468-478.
11. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med*. 2005;11(8):875-879.
12. Garg S, Kim L, Whitaker M, et al. Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019 - COVID-NET, 14 states, march 1–30, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(15):458-464.
13. Hippisley-Cox J, Young D, Coupland C, et al. Risk of severe COVID-19 disease with ACE inhibitors and angiotensin receptor blockers: cohort study including 8.3 million people. *Heart*. 2020;106(19):1503-1511.
14. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-1720.
15. Alqahtani FY, Aleanizy FS, El Hadi A, et al. Prevalence of comorbidities in cases of Middle East respiratory syndrome coronavirus: a retrospective study. *Epidemiol Infect*. 2018;147:1-5.

16. Donoghue M, Hsieh F, Baronas E, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ Res*. 2000;87(5):E1-9.
17. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. 2020;181(2):271-280 e8.
18. Jia HP, Look DC, Shi L, et al. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. *J Virol*. 2005;79(23):14614-14621.
19. Li XC, Zhang J, Zhuo JL. The vasoprotective axes of the renin-angiotensin system: physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. *Pharmacol Res*. 2017;125(Pt A):21-38.
20. Glowacka I, Bertram S, Herzog P, et al. Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. *J Virol*. 2010;84(2):1198-1205.
21. Wosten-van Asperen RM, Lutter R, Specht PA, et al. Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1–7) or an angiotensin II receptor antagonist. *J Pathol*. 2011;225(4):618-627.
22. Hendrickson CM, Matthay MA. Viral pathogens and acute lung injury: investigations inspired by the SARS epidemic and the 2009 H1N1 influenza pandemic. *Semin Respir Crit Care Med*. 2013;34(4):475-486.
23. Ferrario CM, VonCannon J, Jiao Y, et al. Cardiac angiotensin-(1–12) expression and systemic hypertension in rats expressing the human angiotensinogen gene. *Am J Physiol Heart Circ Physiol*. 2016;310(8):H995-1002.
24. Li J, Wang X, Chen J, Zhang H, Deng A. Association of Renin-Angiotensin System Inhibitors With Severity or Risk of Death in Patients With Hypertension Hospitalized for Coronavirus Disease 2019 (COVID-19) Infection in Wuhan, China. *JAMA Cardiol*. 2020;5(7):825-830.
25. Meng J, Xiao G, Zhang J, et al. Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension. *Emerg Microbes Infect*. 2020;9(1):757-760.
26. Rådholm K. *Cardiovascular Risk Factors in Elderly with Special Emphasis on Atrial Fibrillation, Hypertension and Diabetes*. Linköping University; 2015.
27. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. 2016;16(10):626-638.
28. Sex TV. Sex hormones determine immune response *Front Immunol*. 2018;9:1931.
29. Ghosh S, Klein RS. Sex drives dimorphic immune responses to viral infections. *J Immunol*. 2017;198(5):1782-1790.
30. Vasan RS, Beiser A, Seshadri S, et al. Residual lifetime risk for developing hypertension in middle-aged women and men: the Framingham Heart Study. *JAMA*. 2002;287(8):1003-1010.
31. Ji H, Menini S, Zheng W, Pesce C, Wu X, Sandberg K. Role of angiotensin-converting enzyme 2 and angiotensin(1–7) in 17beta-oestradiol regulation of renal pathology in renal wrap hypertension in rats. *Exp Physiol*. 2008;93(5):648-657.
32. AlSiraj, YWC, Thatcher, ES, Cassia, AL. Sex Differences and the Role of the Renin-Angiotensin System in Atherosclerosis and Abdominal Aortic Aneurysms, In *Sex Differences in Cardiovascular Physiology and Pathophysiology* vol. 11. Academic press; 2019. https://www.elsevier.com/books/T/A/9780128131978
33. ERA-EDTA Council; ERACODA Working Group. Chronic kidney disease is a key risk factor for severe COVID-19: a call to action by the ERA-EDTA. *Nephrol Dial Transplant*. 2021;36(1):87-94.
34. Ishii M, Terai H, Kabata H, et al. Clinical characteristics of 345 patients with coronavirus disease 2019 in Japan: a multicenter retrospective study. *J Infect*. 2020;81(5):e3-e5.
35. Debre M, Bonnet MC, Fridman WH, et al. Infusion of Fc gamma fragments for treatment of children with acute immune thrombocytopenic purpura. *Lancet*. 1993;342(8877):945-949.
36. Perez EE, Orange JS, Bonilla F, et al. Update on the use of immunoglobulin in human disease: a review of evidence. *J Allergy Clin Immunol*. 2017;139(3S):S1-S46.
37. Kondo N, Kasahara K, Kameyama T, et al. Intravenous immunoglobulins suppress immunoglobulin productions by suppressing Ca(2+)-dependent signal transduction through Fc gamma receptors in B lymphocytes. *Scand J Immunol*. 1994;40(1):37-42.
38. Stockman LJ, Bellamy R, Garner P. SARS: systematic review of treatment effects. *PLoS Medicine*. 2006;3(9):e343.
39. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet. *Respir Med*. 2020;8(4):420-422.
40. Olnes MJ, Kotliarov Y, Biancotto A, et al. Effects of systemically administered hydrocortisone on the human immunome. *Sci Rep*. 2016;6:23002.

How to cite this article: Chen X, Hu W, Yang M, et al. Risk factors for the delayed viral clearance in COVID-19 patients. *J Clin Hypertens*. 2021;23:1483–1489. https://doi.org/10.1111/jch.14308