Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Factors associated with negative conversion of viral RNA in patients hospitalized with COVID-19

Xiaowen Hu,1, Yuhua Xing,1, Jing Jia,1, Wei Ni,1, Jiwei Liang,1, Dan Zhao,1, Xin Song,1, Ruqin Gao,1⁎, Fachun Jiang,1,2,3⁎

1 Municipal Centre of Disease Control and Prevention of Qingdao, Qingdao Institute of Prevention Medicine, Qingdao, Shandong Province, China
2 Department of Paediatrics, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China
3 Qingdao Women and Children's Hospital, Qingdao University, Qingdao, Shandong Province, China

HIGHLIGHTS

• Older age were independently associated with delayed clearance of SARS-CoV-2 RNA.
• Chest tightness were independently associated with delayed clearance of SARS-CoV-2 RNA.
• Intermittent negative status was predicted a probability of 60% if occurring headache.

ABSTRACT

Factors associated with negative conversion of SARS-CoV-2 RNA in hospitalized patients have not yet been systematically determined. We conducted a retrospective cohort study of COVID-19 patients in Qingdao, China. Both univariate and multivariate analysis were performed to identify independent factors for time to viral RNA negative conversion. Data on patients with re-detectable viral RNA after showing negative on RT-PCR test (intermittent negative status) were also analyzed. A total of 59 patients confirmed with COVID-19 were included in this study, with a median duration of 1 (interquartile range, IQR: 0–2) day from symptom onset to hospital admission. Median communicable period (from first day of positive nucleic acid test to first day of consecutive negative results) was 14 (IQR: 10–18) days, and 7 (IQR: 6–10) days for 10 patients with intermittent negative results. Age older than 45 years (hazard ratio, HR: 0.378; 95% confidence interval, CI: 0.205–0.698) and chest tightness (HR: 0.290; 95%CI: 0.091–0.919) were factors independently affecting negative conversion of SARS-CoV-2 RNA. Headache (odds ratio: 7.553; 95%CI: 1.011–28.253) was significantly associated with intermittent negative status, with a predicted probability of 60%. Older age and chest tightness were independently associated with delayed clearance of SARS-CoV-2 RNA in hospitalized patients. These predictors would provide a new perspective.
1. Introduction

On December 12, 2019, 27 cases of pneumonia of unknown causes were reported in Wuhan, Hubei province, China (WMHC, 2020). On February 11, 2020, the world health organization (WHO) officially named this emerging disease as coronavirus disease 2019 (COVID-19), and the etiological agent was subsequently identified and renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses (CSTGV, 2020; WHO, 2020). With the evolving pandemic, COVID-19 has wreaked havoc in China and spread rapidly to around 210 countries and regions all over the world, constituting a global threat with the highest risk impact (Our World in Data, 2020).

Since the identification of the first few cases of COVID-19, numerous studies have mushroomed with a focus on the epidemiological, clinical, laboratory, and radiological characteristics of infected patients, prompting the development of diagnosis and treatment strategies (Ai et al., 2020; Chen et al., 2020; Cortegiani et al., 2020; Huang et al., 2020; Xing et al., 2020; Zhu et al., 2020). However, there is limited data regarding the potential predictors of negative conversion with COVID-19 patients. Possible reasons underlying this research gap may be due to the lack of detailed sequential virology profiles at the early stage of the disease. Another possible reason is that some cases were admitted to hospital after relatively long period of illness onset, making it difficult to determine the start point for the negative conversion of SARS-CoV-2.

Health authorities in Qingdao, a city located in the northeast of China beyond the epidemic center, took a rapid response in the face of COVID-19 outbreak. The local government and municipal center for disease control and prevention in Qingdao has launched series of aggressive measures to screen, quarantine, diagnose, treat and monitor suspected patients and their close contacts (Jia et al., 2020; Xing et al., 2020). COVID-19 cases in Qingdao were identified and admitted to designated hospitals at an early stage, providing a strong foundation for our research. This study aimed to ascertain viral clearance time and factors associated with SARS-CoV-2 RNA negative-conversion in upper respiratory specimens. We described the dynamic changes of viral shedding in laboratory-confirmed COVID-19 patients on presentation till hospital discharge.

2. Methods

2.1. Study design and data collection

This retrospective cohort study included patients with confirmed COVID-19 hospitalized in Qingdao, Shandong Province, China. All patients were diagnosed with COVID-19 based on New Coronavirus Pneumonia Diagnosis and Treatment Plan (NHCPRC, 2020a, NHCPRC, 2020b, NHCPRC, 2020c), and were discharged between Jan 29, 2020 and Mar 12, 2020.

Upon identification of a suspected case of COVID-19, the joint expert team comprising members from the Qingdao Municipal Center for Disease Control and Prevention (Qingdao CDC) together with prefecture health authorities would initiate detailed field investigations and collect respiratory specimens for centralized testing at Qingdao CDC.

Data were collected onto standardized forms through interviews of infected persons. Epidemiological, demographic, clinical, laboratory, treatment, and outcome data were extracted from the interview as well as field reports.

This study was approved by the Ethics Commission of Municipal Centre of Disease Control and Prevention of Qingdao and written informed consent was waived considering the emergency of infectious disease.

2.2. Definitions of basic concepts

A case was confirmed by a positive result of real-time reverse-transcription-polymerase-chain-reaction (RT-PCR) analysis for SARS-CoV-2 in nasopharyngeal swabs. A severe case is defined as a patient who fulfills one of the following criteria: respiratory rate ≥ 30 breaths/ min; oxygen saturation ≤ 93% at a rest state; arterial partial pressure of oxygen (PaO2)/oxygen concentration (FiO2) ≤ 300 mmHg. Patients were discharged based on the clinical guideline such as respiratory samples consecutively negative for nucleic acid testing with an interval of at least one day (NHCPRC, 2020a, NHCPRC, 2020b, NHCPRC, 2020c).

Some novel definitions were developed to describe the outcomes and time to RT-PCR negative conversion during hospitalization. Communicable period is defined as the duration from the first day of positive RT-PCR result till the first day showing negative on nucleic acid testing successively, as proposed by a previous study on a similar topic (Gao and Li, 2020). Intermittent negative status is defined as patients with re-detectable viral RNA after showing negative on RT-PCR test, with a duration from the first day of showing negative on nucleic acid test till the beginning of consecutive negative tests.

2.3. Laboratory procedures

SARS-CoV-2 detection in respiratory specimens was conducted by Qingdao CDC using real-time RT-PCR targeted at open reading frame 1ab (ORF1ab) and nucleocapsid protein (N) genes. Tests were carried out in biosafety level 2 facilities, using a commercial kit approved by the China Food and Drug Administration. Nasopharyngeal swabs were obtained from patients on a daily basis within 3 days after illness onset. RNA was extracted from the samples and then underwent real-time RT-PCR with SARS-CoV-2-specific primers and probes. A cycle threshold (Ct) value was used to approximately reflect the viral loads (inversely related to Ct-value) in the respiratory tract, a method as suggested by previous studies (Zhu et al., 2020; Xu et al., 2020). A Ct value <37 was defined as a positive test, and a Ct value of 40 or more was considered as a negative test. An equivocal result, defined as a Ct value between 37 and 40, required confirmation by retesting. If the repeated Ct value was <40 and an obvious peak was observed, or if the repeated Ct value was >37, the result was deemed positive.

2.4. Statistical analysis

In our study, negative conversion of viral RNA during the communicable period, as time-to-event data, was the outcome measure and presented with Kaplan-Meier curves. In order to detect the independent predictors of RNA negative conversion, the univariate and multivariate analysis were performed. The log-rank test was first conducted with a total of 25 factors, such as virological, demographic, epidemiological, clinical, radiological and laboratory variables. Multivariate Cox regression model was then performed with the significant factors selected by univariate analysis to tease out the independent predictors for RNA negative conversion. The association between independent predictors and negative conversion was
A total of 59 patients hospitalized with COVID-19 were included in this study, of whom 43 were cluster cases from eleven outbreaks, including 33 family members and 10 close contacts. Eighteen patients had a travel history to Hubei Province before illness onset, and 11 patients were not from the floating population. The median age of patients was 46 years (IQR: 30–57). As shown in Table 1, 83.1% of the 59 COVID-19 cases in Qingdao were non-severe (mild to moderate cases), and 16.9% were severe cases. Most common symptoms at onset of the disease were fever (46.7%), cough (33.9%), and fatigue (33.3%). The median duration from disease onset to hospital admission was 1 day (IQR: 0–2), with a median of 19 days from illness onset to hospital discharge (IQR: 16–25). As shown in Table 1, 83.1% of the 59 COVID-19 cases in Qingdao were non-severe (mild to moderate cases), and 16.9% were severe cases. Most common symptoms at onset of the disease were fever (46.7%), cough (33.9%), and fatigue (33.3%).

### Table 1

| Factors                          | Count (%) | Negative conversion rate | X² value | P value |
|----------------------------------|-----------|--------------------------|----------|---------|
| Overall                          | 59        |                         |          |         |
| Age <45                          | 28 (47.5) | 7.2 82.2 96.5           | 6.9      | 0.009   |
| Age ≥45                          | 31 (52.5) | 12.9 45.2 87.1          |          |         |
| Sex Male                         | 28 (47.5) | 7.2 67.9 96.5           | 0.8      | 0.40    |
| Sex Female                       | 31 (52.5) | 12.9 58.1 87.1          |          |         |
| Disease severity                 |           |                         |          |         |
| Non-severe                       | 49 (83.1) | 2.5 66.7 93.8           | 1.7      | 0.20    |
| Severe                           | 10 (16.9) | – 44.5 81.9            |          |         |
| Residence                        |           |                         |          |         |
| External                         | 11 (18.6) | – 91.7 100              | 4.2      | 0.06    |
| Local                            | 48 (81.4) | 23.9 55.4 89.4          |          |         |
| Cluster case                     |           |                         |          |         |
| Yes                              | 43 (72.9) | 9.3 51.2 90.7           | 5.3      | 0.02    |
| No                               | 16 (27.1) | 12.5 91.8 93.8          |          |         |
| Wuhan Hubei                      |           |                         |          |         |
| Yes                              | 18 (30.5) | 5.6 83.4 94.5           | 2.1      | 0.15    |
| No                               | 42 (71.2) | 12.1 53.6 90.3          |          |         |
| Fever                            |           |                         |          |         |
| Yes                              | 34 (71.2) | 12.1 63.6 99.1          | 0.2      | 0.66    |
| No                               | 25 (48.8) | 7.7 61.6 92.4           |          |         |
| Non-productive cough             |           |                         |          |         |
| Yes                              | 18 (30.5) | 15.6 55.6 88.9          | 0.2      | 0.62    |
| No                               | 41 (69.5) | 12.2 65.9 92.7          |          |         |
| Sputum production                |           |                         |          |         |
| Yes                              | 7 (11.9)  | – 85.7 85.7             | 0.1      | 0.97    |
| No                               | 52 (88.1) | 11.5 59.6 92.3          |          |         |
| Fatigue                          |           |                         |          |         |
| Yes                              | 7 (11.9)  | 16.7 50.0 66.7          | 3.4      | 0.07    |
| No                               | 52 (88.1) | 9.7 65.4 94.3           |          |         |
| Myalgia                          |           |                         |          |         |
| Yes                              | 4 (6.8)   | 25.0 25.0 75.0          | 1.1      | 0.29    |
| No                               | 55 (93.2) | 9.2 66.7 92.6           |          |         |
| Headache                         |           |                         |          |         |
| Yes                              | 6 (10.2)  | – 60.0 80.0             | 0.6      | 0.46    |
| No                               | 53 (89.8) | 11.4 64.2 92.5          |          |         |
| Chest tightness                  |           |                         |          |         |
| Yes                              | 5 (8.5)   | – 40.0 60.0             | 3.9      | 0.04    |
| No                               | 54 (91.5) | 13.0 64.9 94.5          |          |         |
| Leukocyte count                  |           |                         |          |         |
| <4 × 10⁹/L                       | 7 (11.9)  | 14.3 100 100            | 5.4      | 0.02    |
| ≥4 × 10⁹/L                       | 52 (88.1) | 10.6 53.2 89.4          |          |         |
| Neutrophil percentage            |           |                         |          |         |
| <50%                             | 6 (10.2)  | 16.7 33.3 100           | 0.1      | 0.87    |
| ≥50%                             | 53 (89.8) | 9.4 66.1 90.6           |          |         |
| Neutrophil percentage            |           |                         |          |         |
| ≤70%                             | 16 (27.1) | 13.7 68.2 93.2          | 3.5      | 0.06    |
| >70%                             | 44 (72.9) | – 46.7 86.7            |          |         |
| Lymphocyte percentage            |           |                         |          |         |
| <20%                             | 16 (27.1) | 5.6 52.8 88.2          | 1.7      | 0.19    |
| ≥20%                             | 43 (72.9) | 14.3 66.7 92.9          |          |         |
| Lymphocyte percentage            |           |                         |          |         |
| ≤40%                             | 46 (78.0) | 8.7 60.9 89.2          | 1.9      | 0.17    |
| >40%                             | 13 (22.0) | 15.4 69.3 100           |          |         |
| CT imaging                       |           |                         |          |         |
| Normal                           | 11 (18.6) | 8.4 58.4 91.7          | 0.1      | 0.94    |
| Abnormal                         | 48 (81.4) | 11.8 63.6 91.5          |          |         |
| Involved lung field              |           |                         |          |         |
| Unilateral                       | 17 (35.4) | 27.8 61.2 94.5          | 3.1      | 0.08    |

Quantified by hazard ratio (HR). As negative conversion of viral RNA is a beneficial event, HR value >1 would promote virological clearance, whereas HR value <1 means the independent predictor would delay negative conversion.

Additionally, we investigated factors associated with intermittent negative status. Mann–Whitney U test, χ² test, or Fisher’s exact test was first conducted to compare differences between groups with and without intermittent negative status. Next, factors with statistically significance (P < 0.05) were further analyzed using the logistic regression model, and odds ratio (OR) and predicted probability were calculated.

Continuous and categorical variables were presented as median (interquartile range, IQR) and n (%), respectively. A P value <0.05 (two-tailed) was considered statistically significant. Analyses were performed using SPSS software (version 20.0) and R software (version 3.6.2).

### 3. Results

#### 3.1. Demographical and epidemiological characteristics of study population

A total of 59 patients hospitalized with COVID-19 were included in this study, of whom 43 were cluster cases from eleven outbreaks, including 33 family members and 10 close contacts. Eighteen patients had a travel history to Hubei Province before illness onset, and 11 patients had been to Wuhan in January 2020. Female accounted for 52.5% (31) of cases and 47.5% (28) were male. The majority of patients (48) were local residents in Qingdao, and 11 patients were not from the floating population. The median age of patients was 46 years (IQR: 30–57). More than a quarter of patients (17) had underlying diseases, such as cardiovascular and cerebrovascular diseases (9), endocrine disease (3), gastrointestinal disease (3), respiratory disease (1) and neurological disorder (1). Detailed demographical and epidemiological information was represented in Table 1.

#### 3.2. Clinical presentations, laboratory and radiological findings

The median duration from disease onset to hospital admission was 1 day (IQR: 0–2), with a median of 19 days from illness onset to hospital discharge (IQR: 16–25). As shown in Table 1, 83.1% of the 59 COVID-19 cases in Qingdao were non-severe (mild to moderate cases), and 16.9% were severe cases. Most common symptoms at onset of the
disease were fever and dry cough. Other symptoms included fatigue, headache, chest tightness, myalgia, and sputum production. All patients received standard care including supportive and anti-viral treatment according to the latest clinical guidelines (NHCPRC, 2020a, NHCPRC, 2020b, NHCPRC, 2020c). Antibiotics were only prescribed to patients who were at risk of or presented with bacterial infection. Patients with symptoms of respiratory distress received oxygen therapy. Corticosteroids were used shortly to inhibit inflammatory cascade in patients with progressing disease. Critically ill patients were transferred to intensive care unit.

On admission, 7 patients had leucopenia (white blood cell count <4 × 10⁹/L), 16 patients had increased proportion of neutrophils (>70%), and 13 patients showed elevated lymphocyte percentage (>40%). Abnormalities in chest computed tomograms (CT) were detected in 46 patients (80.0%). The most common pattern of CT changes was high-density shadow over bilateral lungs (54.2%).

3.3. Negative conversion of SARS-CoV-2 RNA

Viral loads in nasopharyngeal swabs were estimated with Ct values by RT-PCR. Median Ct values for RT-PCR on ORF 1ab gene was 26 (IQR: 24–31), and median Ct value for N gene was 28 (IQR: 26–31). All patients were discharged base on two consecutive negative tests at least 24 h apart. Median duration of communicable period was 14 (IQR: 10–18) days, ranging from 4 to 25 days. Moreover, 10 patients were in intermittent negative status after first showing negative for RT-PCR, with a median duration in between of 7 days (IQR: 6–10, Fig. 1). The rate of RNA negative conversion within 7 days, 14 days and 21 days...
among all patients were 10.2% (95% confidence interval, 95% CI: 2.1–17.5%), 62.7% (48.1–73.2%), and 91.2% (80.4–96.4%), respectively (Fig. 2).

3.4. Predictors of negative conversion

Table 1 summarized the results of univariate analysis. We evaluated the effect of each factor on negative conversion of COVID-19 patients by Log rank test. Age, cluster cases, chest tightness and leukocyte count were significantly related to RNA negative conversion. Multivariate Cox regression was then performed with the significant factors selected by univariate analysis. Fig. 3 showed that age older than 45 years (HR: 0.378; 95% CI: 0.205–0.698) and chest tightness (HR: 0.290; 95% CI: 0.091–0.919) were independently associated with negative conversion of viral RNA, suggesting that older age and presentation of chest tightness would delay virological clearance in COVID-19 patients (Fig. 4).

Sensitivity analysis was conducted to guarantee robust results. A general linear model (GLM) was performed to quantify the association between four significant factors detected by univariate analysis and time to RNA negative conversion. The effect values were largely consistent, indicating age older than 45 years (RR: 0.553; 95% CI: 0.451–0.869) and chest tightness (RR: 0.444; 95% CI: 0.289–0.978) were inversely correlated with time to negative conversion (Table 2).

| Factors         | Level          | HR (95% CI)       | P value |
|-----------------|----------------|-------------------|---------|
| Age             | <45            | Reference         |         |
|                 | ≥45            | 0.378 (0.205–0.698) | 0.002** |
| Cluster case    | No             | Reference         |         |
|                 | Yes            | 0.765 (0.355–1.650) | 0.495   |
| Leukocyte counts| ≤4.0×10⁹/L    | Reference         |         |
|                 | >4.0×10⁹/L     | 2.536 (0.917–7.313) | 0.073   |
| Chest tightness | No             | Reference         |         |
|                 | Yes            | 0.290 (0.065–0.919) | 0.035*  |

* p<0.05; ** p<0.01; HR, hazard ratio; 95% CI, 95% confidence interval

Fig. 3. Independent predictors of negative conversion by multivariate Cox regression.

Fig. 4. Negative conversion curves in COVID-19 patients according to predictors (A: age; B: chest tightness).
Besides, older patients had more underlying diseases as compared with younger patients infected with SARS-CoV-2 (Liu et al., 2020). Although disease severity and comorbidities were not significant predictors from univariate and multivariate analysis, meaning the two factors had no direct effect on time to RT-PCR conversion, they may indirectly influence viral nucleic acid clearance via effect of age. Further spearman correlation analysis showed that disease severity ($r = 0.283, P = 0.03$) and comorbidities ($r = 0.450, P < 0.001$) were positively correlated with age. These age-related defects may make it more difficult for the host cell immunity to eradicate the invasive pathogen, resulting in prolonged viral shedding in the elderly. However, when we compared the 5 patients presented with chest tightness with the other patients, no obvious differences could be found in terms of epidemiological, laboratory and radiological characteristics. A previous study on SARS-CoV (2002–2003) demonstrated that urine positive for RT-PCR analysis on presentation was associated with delayed RNA negative conversion, while treatment with ribavirin and corticosteroid was related to a sooner virological clearance (Chu et al., 2005). In our study, ribavirin was empirically prescribed to all patients confirmed with COVID-19, whereas corticosteroids were rarely given. We could not detect any relationship between treatment strategies and time to RT-PCR conversion in our patients. The clinical significance of these predictors is still unknown and further studies would be warranted. The potential risk factors for a delayed viral nucleic acid clearance would shed light on early recognition of patients with poor prognosis.

In the current study, 10 patients had re-detectable positive results for SARS-CoV-2 RNA test during hospitalization. We found that headache was correlated with intermittent negative results in these patients. Previous studies have reported a proportion of recovered patients who met criteria for hospital discharge showed re-detectable positive results for RT-PCR testing (An et al., 2020; Lan et al., 2020; Ling et al., 2020). In general, patients with intermittent negative RT-PCR were younger and had milder symptoms ($r = 0.220, P = 0.041$; $r = 0.242, P = 0.033$, respectively). Cautionary measures should be considered when dealing with patients with recurrence of positive RT-PCR results as SARS-CoV could be transmitted from convalescent patients to others via close contact (Chan et al., 2003). It might be advisable to extend the duration of hospitalization and isolation of patients with intermittent negative RT-PCR results. These findings provide important implications for decision making on quarantine protocols and facilitating optimal antiviral interventions.

Additionally, we analyzed Ct values of RT-PCR test (inversely related to viral load) on admission as accumulating evidence supported the notion that viral loads of SARS-CoV-2 are among the highest in COVID-19 patients early after onset (Pan et al., 2020; To et al., 2020; Zou et al., 2020). A high viral load on presentation was an independent factor contributing to worse prognosis in patients with SARS (Chu et al., 2004). Although we failed to find significant relationship between Ct values and severity of illness, older age was associated with higher viral loads of the RT-PCR test on admission (as reflected by lower Ct values, $r = -0.345\ P = 0.007$). Our findings verified the results from a recent study showing that older age, rather than disease severity, was positively associated with viral RNA copy numbers (To et al., 2020). Studies for SARS-CoV also indicated that increased age was independently associated with higher viral load (Chen et al., 2006).

There are several limitations of this study. First, our sample size is relatively small in comparison with other studies. There were 59 COVID-19 patients in Qingdao during the study period. However, we believe our population was a good preventative of patients outside of the epidemic center. Second, we did not detect the existence of viral RNA in patients’ excretions such as urine and fecal specimens. Previous studies indicated the possibility of viral transmission via fomite and viral loads in the stools might be greater than that in the respiratory specimens (Wölfel et al., 2020; Xu et al., 2020). Third, we did not record the dynamic profiles of viral loads (as reflected by Ct values). How the
chronological changes in viral loads correlate with disease prognosis merits further investigation.

5. Conclusion

This study identified factors of older age and chest tightness were associated with delayed clearance of viral RNA in patients hospitalized with COVID-19. We hope these predictors could provide clues for early identification of patients with prolonged viral shedding, paving the way for development of optimal isolation protocols and treatment strategies.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Xiaowen Hu: Conceptualization, Writing - original draft. Yuhuan Xing: Formal analysis, Writing - original draft. Jing Jia: Investigation, Writing - original draft. Wei Ni: Formal analysis, Writing - original draft. Jiewei Liang: Investigation. Dan Zhao: Investigation. Xin Song: Investigation. Ruqin Gao: Investigation, Writing - original draft. Fachun Jiaow: Conceptualization, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are deeply thankful to all health-care workers involved in the diagnosis and treatment of patients in Qingdao.

References

Ai, T., Yang, Z., Hou, H., Zhan, C., Chen, C., Lv, W., Tao, Q., Sun, Z., Xia, L., 2020. Coronavirus disease 2019 in China: the Epidemiological and Clinical Characteristics of 1014 Patients with 2019-nCoV. JAMA Cardiol. 5, 536–544.

Cortegiani, A., Ingoglia, G., Ippolito, M., Gaiaattano, A., Elaw, S., 2020. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J. Crit. Care. [Epub ahead of print].

Gao, W., Li, L., 2020. Advances on asymptomatic or symptomatic carrier transmission of COVID-19 (in Chinese). Chin. J. Epidemiol. 41, 485–488.

Goronyz, J.J., Lee, W.W., Weyand, C.M., 2007. Aging and T-cell diversity. Exp. Gerontol. 42, 402–408.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., et al., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497–506.

Jia, J., Hu, X.W., Yang, F., Song, X., Dong, L., Zhang, J., Jiang, F., Gao, R., 2020. Epidemiological characteristics on the clustering nature of COVID-19 in Qingdao City, 2020: a descriptive analysis. Disaster. Med. Public 31, 1–5.

Lan, L., Xu, D., Ye, G., Xia, C., Wang, S., Li, Y., Xu, H., 2020. Positive RT-PCR Test Results in Patients Recovered From COVID-19. JAMA https://doi.org/10.1001/jama.2020.2783 (Epub ahead of print).

Ling, Y., Xu, S.B., Lin, Y.X., Tian, D., Zhu, Z.Q., Dai, F.H., Wu, F., Song, Z.G., Huang, W., Chen, J., et al., 2020. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. Chin. Med. J. https://doi.org/10.1097/CM9.0000000000000774 (Epub ahead of print).

Liu, K., Chen, Y., Lin, R., Han, K., 2020. Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients. J. Infect. https://doi.org/10.1016/j.jinf.2020.03.003 (Epub ahead of print).

National Health Commission of the People’s Republic of China (NHICPRC), 2020a. Diagnosis and treatment plan of Corona virus disease 2019 (tentative fifth edition). [Assessed 5 Feb, 2020]. Available from http://www.nhc.gov.cn/yzygj/s7653p/202002/3059698c4b4c204b79b0d88962644480.html.

National Health Commission of the People’s Republic of China (NHICPRC), 2020b. Diagnosis and Treatment Plan of Corona Virus Disease 2019 (tentative sixth edition), [Feb 19, 2020]. Available from: http://www.nhc.gov.cn/yzygj/s7653p/202002/83148a32f69d25f5157a8a6e5c2.html.

National Health Commission of the People’s Republic of China (NHICPRC), 2020c. Diagnosis and Treatment Plan of Corona Virus Disease 2019 (tentative seventh edition). Available from http://www.nhc.gov.cn/yzygj/s7653p/202003/4692954fd7ef4cebf80d976919ebe1899.html, Accessed date: 4 March 2020.

Our World in Data, 2020. Coronavirus Disease (COVID-19) – Statistics and Research. Oxford Martin School. The University of Oxford, Global Change Data Lab Available from https://ourworldindata.org/coronavirus/. Accessed date: 30 March 2020.

Pan, Y., Zhang, D., Yang, P., Poon, L.M., Wang, Q., 2020. Viral load of SARS-CoV-2 in clinical samples. Lancet. Infect. Dis. 20, 411–412.

To, K.K., Tsang, O.T., Leung, W.S., Tam, A.R., Wu, T.C., Lung, D.C., Yap, C.C., Cai, J.P., Chan, J.M., Chik, T.S., et al., 2020. Temporal profiles of viral load in post-infectious oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet. Infect. Dis. https://doi.org/10.1016/S1473-3099(20)30196-1 (Epub ahead of print).

Weber, R.G., 2000. Immunity to influenza in the elderly. Vaccine 18, 1686–1689.

Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Sange, Z., Müller, M.A., Niemeyer, D., Jones, T.C., Vollmar, P., Rothe, C., et al., 2020. Virological assessment of hospitalized patients with COVID-2019. Nature https://doi.org/10.1038/s41586-020-2196-x (Epub ahead of print).

World Health Organization (WHO), 2020. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected: interim guidance. Available from: https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. Accessed date: 20 January 2020.

Wuhan Municipal Health Commission (WMHC), 2020. Report of clustering pneumonia of unknown etiology in Wuhan City. Available from http://wjw.wuhan.gov.cn/front/web/showDetail?infId=253517&dkey=8a5aef2c.shtml.

Xing, Y.H., Ni, W., Wu, Q., Li, W.J., Li, G.J., Wang, W.D., Tong, J.N., Song, X.F., Weng, G.W.K., Xing, Q.S., 2020. Prolonged Viral Shedding in Feces of Pediatric Patients with Coronavirus Disease 2019. J. Microbiol. Immunol. Infect. 2020. https://doi.org/10.1016/j.jmii.2020.03.021 (Epub ahead of print).

Xu, Y., Li, X., Zhu, B., Liang, H., Fang, C., Gong, Y., Cuo, Q., Sun, X., Zhao, D., Shen, J., et al., 2020. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nat. Med. 26, 502–505.

Zhou, F., Yu, T., Duan, R., Fan, G., Liu, Y., Li, Z., Xiang, J., Wang, Y., Song, B., Gu, X., et al., 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 395, 1054–1062.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., et al., 2020. A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. 382, 727–733.

Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., Yu, J., Kang, M., Song, Y., Xia, J., et al., 2020. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N. Engl. J. Med. 382, 1177–1179.