JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Coronavirus Pandemic

Comparison of clinical characteristics between fecal/perianal swab nucleic acid-positive and -negative patients with COVID-19

Gong-Qi Chen[1]*, Wen-Tao Luo[1]*, Chao-Hui Zhao[1], Chun-Na Li[1], Zhong-Si Hong[1], Jin-Yu Xia[1], Xi Liu[1]

[1] *Department of Infectious Diseases, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, China*

* Authors contributed equally to this work.

Abstract

Introduction: We analyzed the clinical characteristics of COVID-19 fecal/perianal swab nucleic acid-positive patients in our hospital and evaluated the effect of SARS-CoV-2 on the gastrointestinal tract.

Methodology: Ninety-seven patients in the Fifth Affiliated Hospital of Sun Yat-sen University from January 17, 2020 to March 2, 2020 with fecal/perianal swab samples were selected as subjects and the results of real-time fluorescence reverse transcriptase-PCR SARS-CoV-2 nucleic acid detection of fecal/perianal swabs were used to divide subjects into positive and negative groups.

Results: Fecal/perianal swabs of 53.61% (52/97) patients were positive including 31 males (59.62%) and 21 females (40.38%). The negative group had more females than males (P = 0.001). The distribution of case classification based on the most severe condition observed after admission was different between groups: five (5.15%) critical type patients were all from the positive group (P = 0.029). There was no statistical difference in clinical manifestations between the groups. In the positive group, the mean nucleic acid-negative conversion time was 14.13 ± 8.61 days, which was significantly later than the negative group (6.98 ± 5.16 days; P < 0.001). In the positive group, 92% (48/52) had nucleic acid-negative conversion with a mean nucleic acid-negative conversion time of 22.58 ± 10.30 days. Among them, 41 (78.85%) cases were delayed compared with pharynx/nasal swab nucleic acid-negative conversion time.

Conclusions: The positive rate of fecal/perianal swab nucleic acid in male patients was higher than that in female patients. Fecal/perianal swab nucleic acid positive may be an indicator of critical conditions in those with COVID-19.

Key words: COVID-19; SARS-CoV-2; clinical characteristics; fecal swab; ACE2; PCR.

J Infect Dev Ctries 2020; 14(8):847-852. doi:10.3855/jidc.12885

(Received 24 April 2020 – Accepted 20 July 2020)

Introduction

In December 2019, a new respiratory infectious disease was identified [1] and subsequently named Coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). The disease is highly contagious and has spread rapidly between countries and continents. On 11 March 2020, the WHO declared that the outbreak of COVID-19 had become a global pandemic. Early studies of the disease suggested that COVID-19 was mainly transmitted by droplets and contact, but subsequent studies isolated SARS-CoV-2 in stools [2]. Other studies demonstrated that angiotensin-converting enzyme 2 (ACE2) is the receptor of SARS-CoV-2 entry into human cells [3,4] and that ACE2 mRNA is highly expressed in the gastrointestinal system, which provides a prerequisite for SARS-CoV-2 infection. However, whether the fecal-oral pathway is the main route of transmission and whether there is a difference in clinical characteristics between fecal/perianal swab nucleic acid-positive and -negative patients is unclear. This study retrospectively analyzed the clinical characteristics of 97 patients with COVID-19 treated in our hospital and divided them into two groups according to the nucleic acid results of fecal/perianal swabs to evaluate the effect of the novel SARS-CoV-2 in the gastrointestinal tract.

Methodology

The clinical data of 98 patients with COVID-19 treated in the Fifth Affiliated Hospital of Sun Yat-sen University in Zhuhai from January 17, 2020 to March 2, 2020 were collected. One patient was excluded from the study because of a lack of stool specimens. Finally, ninety-seven patients with fecal/perianal swab samples were selected as study subjects and the results of real-time fluorescence RT-PCR SARS-CoV-2 nucleic acid detection of fecal/perianal swabs were used to divide subjects into positive and negative groups. General data, epidemiological history, clinical manifestations, pharynx/nasal swabs, and fecal/perianal swab nucleic

acid detection results of 97 patients with COVID-19 were collected from patients' hospital records retrospectively.

Diagnosis and clinical classifications were performed with reference to the "COVID-19 Diagnosis and Treatment Protocol (Trial Version 7)" [5] issued by the National Health Commission of China. Briefly, the confirmed cases should conform to the clinical manifestations and/or epidemiological history in the protocol and have a positive real-time RT-PCR SARS-CoV-2 test. The severity of the disease can be divided into 4 types: mild (mild clinical symptoms without CT imaging features of pneumonia), ordinary (fever, respiratory symptoms and CT imaging features of COVID-19 pneumonia), severe (respiratory distress (respiratory rate ≥ 30 breaths/min), oxygen saturation ≤ 93% and arterial oxygen tension (or pressure) (PaO_2)/fractional inspired oxygen (FiO_2) ratio ≤ 300 mmHg) and critical (respiratory failure requiring mechanical ventilation and organ failure).

Real-time RT-PCR SARS-CoV-2 analysis of both pharynx/nasal and fecal/perianal swabs has been performed in Control Disease Center of Zhuhai and/or the Fifth Affiliated Hospital of Sun Yat-sen University. According to the manufacturer's instructions, RNA was extracted from different specimens using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). RT-PCR assays were performed using the SARS-CoV-2 real-time RT-PCR Kit (Shanghai ZJ Bio-Tech Co, Ltd, Shanghai, China), targeting the open reading frame lab (ORF1ab) and nucleoprotein (N) gene regions. If both targets tested positive, the case was considered

Table 1. Comparison of the clinical characteristics of COVID-19 fecal/perianal swab nucleic acid-positive and -negative patients.

		Fecal/perianal swabs nucleic acid test result (%)		
	Total (N = 97)	**Positive group (n = 52)**	**Negative group (n = 45)**	**p value**
Gender				
Male	43 (44.33)	31 (59.62)	12 (26.67)	0.001
Female	54 (55.67)	21 (40.38)	33 (73.33)	
Age	46.54 ± 17.67	45.27 ± 20.47	47.98 ± 13.93	0.447
Hubei Sojourn History				
No	18 (18.56)	7 (13.46)	11 (24.44)	0.165
Yes	79 (81.44)	45 (86.54)	34 (75.56)	
Cluster Epidemic				
No	16 (16.49)	6 (11.54)	10 (22.22)	0.157
Yes	81 (83.51)	46 (88.46)	35 (77.78)	
Coexist Diseases				
cardiovascular and cerebrovascular diseases	22 (22.68)	9 (17.31)	13 (28.89)	0.174
respiratory system diseases	4 (4.12)	2 (3.85)	2 (4.44)	0.716
digestive system diseases	3 (3.09)	1 (1.92)	2 (4.44)	0.899
endocrine system diseases	12 (12.37)	4 (7.69)	8 (17.78)	0.132
malignant tumors	5 (5.15)	1 (1.92)	4 (8.89)	0.277
Smoking History				
Yes	4 (4.12)	1 (1.92)	3 (6.67)	0.509
Case Classification				
Mild	16 (16.49)	12 (23.08)	4 (8.89)	0.029
Ordinary	55 (56.70)	25 (48.08)	30 (66.67)	
Severe	21 (21.65)	10 (19.23)	11 (24.44)	
Critical	5 (5.15)	5 (9.62)	0 (0.00)	
Clinical Manifestation				
Fever	61 (62.89)	36 (69.23)	25 (55.56)	0.164
Cough	49 (50.52)	30 (57.69)	19 (42.22)	0.129
Polypnea	8 (8.25)	4 (7.69)	4 (8.89)	0.876
Pharyngalgia	22 (22.68)	13 (25.00)	9 (20.00)	0.558
Chest distress	7 (7.22)	4 (7.69)	3 (6.67)	0.842
Headache	10 (10.31)	5 (9.62)	5 (11.11)	0.926
Muscle ache	11 (11.34)	5 (9.62)	6 (13.33)	0.565
Fatigue	12 (12.37)	6 (11.54)	6 (13.33)	0.789
Nausea and vomiting	4 (4.12)	1 (1.92)	3 (6.67)	0.509
Diarrhea	8 (8.25)	2 (3.85)	6 (13.33)	0.186
Asymptomatic	6 (6.19)	5 (9.62)	1 (2.22)	0.278

Data are presented as the mean ± SD or number (%).

laboratory confirmed. The nucleic acid-negative conversion time refers to the duration between the patient's admission time and the first time when his/her SARS-COV-2 RT-PCR analysis shows negative, provided that he/she has received two consecutive negative results for the analysis (with an interval of at least 1-day) since admission.

SPSS 21.0 (IBM Corp., Armonk, NY, USA) statistical software was used for statistical analysis. Counting data were expressed by frequency (percentage) and the χ^2 test was used for inter-group comparisons. If the measurement data were in accord with the normal distribution, it was expressed as the mean ± SD, and the *t*-test was used for inter-group comparisons; if not, it was expressed as the median (interquartile range, IQR) and a nonparametric rank-sum test was used for inter-group comparisons. Kaplan–Meier curves were used to compare differences in nucleic acid-negative conversion time between different groups. $P < 0.05$ was statistically significant.

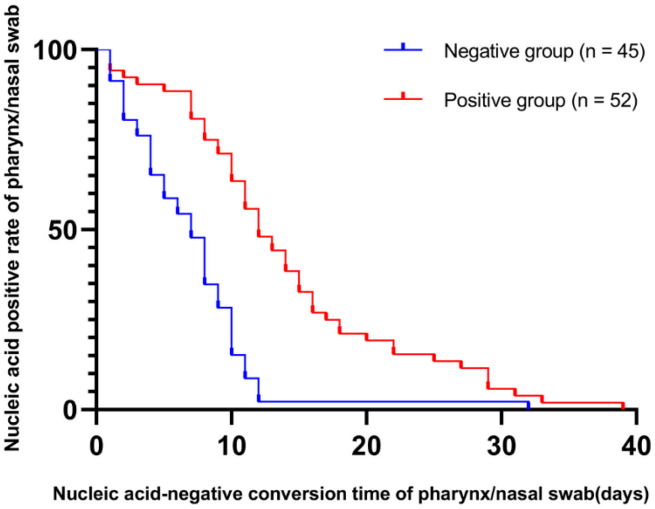
This study was approved by The Research Ethics Committee at the Fifth Affiliated Hospital of Sun Yat-Sen University to allow retrospective access to patients' records and files (No. ZDWY [2020] Lunzi No. K22-1). The study was considered exempt from informed consent as it was an observational cohort study.

Results

General data and epidemiology

Of 97 patients, 52 patients (53.61%) were positive for SARS-CoV-2 nucleic acids by real-time fluorescence RT-PCR of fecal/perianal swabs (Table 1). Forty-five patients (46.39%) were negative for nucleic acids in at least one fecal/perianal swab. In the fecal/perianal swab nucleic acid-positive group, the proportion of males was higher than that of females (59.62% *vs* 40.38%). In the negative group, the proportion of females was higher than that of males (73.33% *vs* 26.67%; P = 0.001). Forty-five (86.54%) cases in the positive group and 34 (77.78%) cases in the negative group had a history of traveling in the Hubei province. A disease cluster contained 46 cases (88.46%) in the positive group and 35 cases (77.78%) in the negative group. There was no significant relationship between the incidence of traveling in Hubei or disease cluster and the detection of nucleic acids in feces/perianal swabs. Among all patients, 22 cases (22.68%) had cardio-cerebrovascular disease, 4 (4.12%) had respiratory system disease, 3 (3.09%) had digestive system disease, 12 (12.37%) had endocrine system disease, and 5 (5.15%) had a history of malignant tumors. Four cases (4.12%) had a history of smoking.

Figure 1. Nucleic acid-negative conversion time of pharynx/nasal swabs in fecal/perianal swab nucleic acid-positive and -negative groups.



The nucleic acid-negative conversion time of the fecal/perianal swab nucleic acid-positive group was significantly longer than that of the negative group. The mean nucleic acid-negative conversion time of the positive group was 14.13 ± 8.61 days and that of negative group was 6.98 ± 5.16 days ($P < 0.001$).

Case classification and clinical manifestation

According to the most frequent condition observed after admission, 12 cases among patients with positive SARS-CoV-2 fecal/perianal swabs were mild type (23.08%), 25 were ordinary type (48.08%), 10 cases were severe type (19.23%), and 5 were critical type (9.62%). In the negative group, there were 4 cases of mild type (8.89%), 30 cases of ordinary type (66.67%), 11 cases of severe type (24.44%), and no critical cases (P = 0.029). In the positive group, there were 36 cases (69.23%) with fever, 30 cases (57.69%) with cough, 4 cases (7.69%) with polypnea, 13 cases (25.00%) with pharyngalgia, 4 cases (7.69%) with chest distress, 5 cases (9.62%) with headache, 5 cases (9.62%) with muscle aches, 6 cases (11.54%) with fatigue, 1 case (1.92%) with nausea and vomiting, 2 cases (3.85%) with diarrhea. Five patients (9.62%) had no obvious symptoms. In the negative group, there were 25 cases (55.56%) with fever, 19 cases (42.22%) with cough, 4 cases (8.89%) with polypnea, 9 cases (20.00%) with pharyngalgia, 3 cases (6.67%) with chest distress, 5 cases (11.11%) with headache, 6 cases (13.33%) with muscle ache, 6 cases (13.33%) with fatigue, 3 cases (6.67%) with nausea and vomiting, 6 cases (13.33%) with diarrhea, and 1 case (2.22%) without symptoms.

The statistical difference between aforementioned symptoms and real-time RT-PCR detection of SARS-CoV-2 in fecal/perianal swabs has not been observed.

Viral nucleic acid-negative conversion

Of 97 patients, only 1 case (1.03%) died, and the remaining 96 cases were cured and discharged from hospital. The mean nucleic acid-negative conversion time of SARS-CoV-2 nucleic acid in pharynx/nasal swabs was 10.81 ± 8.03 days. In the positive group, the mean nucleic acid-negative conversion time was 14.13 ± 8.61 days, which was significantly longer than that in the negative group (6.98 ± 5.16 days; $P < 0.001$) (Figure 1). We also observed that 13 patients (13.40%) became positive again after SARS-CoV-2 nucleic acid-negative conversion of pharynx/nasal swabs when measured twice consecutively (at least 1-day interval): the nucleic acid-positive group accounted for 92.31% (12/13) of cases and the negative group accounted for 7.69% (1/13) ($P = 0.003$). The median time from admission to nucleic acid positive again was 16 days (IQR, 14.0–19.5). Of 52 patients with positive fecal/perianal swab nucleic acids, 92.31% (48/52) had nucleic acid-negative conversion with a mean nucleic acid-negative conversion time of 22.58 ± 10.30 days. Of these, 78.85% (41/52) of cases had a longer fecal/perianal swab nucleic acid-negative conversion time compared with their pharynx/nasal swab nucleic acid-negative conversion time（22.58 *vs* 10.81 days）. Three patients (5.77%) became positive again after SARS-CoV-2 nucleic acid-negative conversion of fecal/perianal swab when measured twice consecutively (at least 1-day interval) and the median time from admission to positive again was 17 days (IQR, 13–28).

Discussion

SARS-CoV-2 is a newly discovered β coronavirus, which has the general characteristics of coronavirus, but with genomic characteristics different from those of other coronaviruses such as SARS-CoV [4,6]. In this study, gender was significantly related to the detection of SARS-CoV-2 nucleic acid in fecal/perianal swabs. In the positive group, males accounted for a higher proportion than females (59.62% *vs* 40.38%). In earlier studies, it was reported that male may be a predictor of disease aggravation [7]. However, the specific mechanism underlying why males have a higher positive rate of nucleic acids in fecal/perianal swabs is not clear and requires further study. In addition, we found that the severity of the disease was also associated with fecal/perianal swab nucleic acids. All five (5.15%) critical type patients were positive for fecal/perianal swab nucleic acids. Critical patients were in a serious condition with a high viral load and virulence, and were more likely to have virus infection of the gastrointestinal tissues. Therefore, we speculate that positive nucleic acid in fecal/perianal swabs may be a predictor of critical condition in COVID-19 patients.

In this study, there was no statistical difference in clinical manifestations between the two groups. Fever and cough were prominent symptoms of the disease, consistent with previous studies [8,9]. Of note, the incidences of nausea, vomiting, and diarrhea were significantly lower than that in fecal/perianal swab nucleic acid-positive rate, and there was no significant correlation between the occurrence of these clinical symptoms and fecal/perianal swab nucleic acid results. Therefore, we could not expect positive result of real-time RT-PCR from a patient's fecal/perianal swabs based on the presence of digestive tract symptoms. In addition, it is important to consider that some patients have an asymptomatic onset. In this study, six patients (6.19%) had no obvious clinical symptoms. Studies have confirmed that asymptomatic COVID-19 patients are contagious [10] but the infectivity of asymptomatic patients is not clear. We suggest that nucleic acid testing should be carried out for all close contacts of patients diagnosed as COVID-19, not just those under medical isolation, to detect asymptomatic infection at an early stage.

Viral nucleic acid detection is an important standard for the diagnosis and discharge of COVID-19 patients. In this study, the mean nucleic acid-negative conversion time of nucleic acid-positive fecal/perianal swabs was significantly delayed compared with the negative group, with a difference of up to 7 days. In addition, 78.85% (41/52) of patients with nucleic acid-positive fecal/perianal swabs had a delayed nucleic acid-negative conversion time when compared with those with nucleic acid-positive pharynx/nasal swabs. In addition, the proportion of pharynx/nasal swabs returning to positive after negative conversion in the fecal/perianal swab nucleic acid-positive group was higher than that in the fecal/perianal swab-negative group. The existence of SARS-CoV-2 in the gastrointestinal tract has been confirmed [2] and an autopsy report of a COVID-19 patient also found that the small intestine showed segmental dilatation and stenosis (similar to beaded) [11], we speculate that the digestive tract may be a virus repository during disease. Patients with a negative conversion of nucleic acids from pharynx/nasal swabs but not from fecal/perianal

swabs should be carefully removed from isolation because these patients still have a high risk of transmission through the fecal-oral route [12]. In addition, because nucleic acid detection is affected by sample quality, collection time, infection cycle, kit performance, and other factors [13], we suggest that nucleic acids should be reviewed multiple times to reduce the possibility of missed diagnosis and misdiagnosis.

Conclusion

The findings of our study have shown that the positive rate of fecal/perianal swab nucleic acids in male patients was higher than that in female patients and that a positive result of nucleic acid in fecal/perianal swabs may be one of the indicators of critical condition. There was no significant relationship between digestive tract symptoms and fecal/perianal swab nucleic acids but the digestive tract may be a SARS-CoV-2 repository. Therefore, the further researches should pay attention to the effects of virus on the digestive tract. During diagnosis and treatment, clinicians should be aware that the nucleic acid-negative conversion time of some patients with positive fecal/perianal swabs is delayed compared with those with pharynx/nasal swabs, and is more likely to return to positive after negative conversion of pharynx/nasal swabs. For these patients, nucleic acid detection should be reviewed multiple times and patients should be released from isolation with caution.

Acknowledgements

We thank all the patients involved in this research.

Funding

This work was supported in part by National key Research and Development (R&D plan "Public security risk prevention and control and emergency technical equipment": COVID-19 diagnosis and control key technology research (2020YFC082400), Guangdong Research and Development (R&D) Program in Key Areas: Guangdong Scientific and Technological Research Special fund for Prevention and Treatment of COVID-19, Special fund for emergency research on SARS-CoV-2 from the Guangzhou Regenerative Medicine and Health Guangdong Laboratory, Cultivation Project of "Three Major Scientific Research Projects" of Sun Yat-sen University in 2020: the Emergency Project of SARS-CoV-2 Prevention and Control Science and Technology of Sun Yat-sen University(89000-31143405), and Zhuhai "SARS-CoV-2 infection Prevention and Control" Emergency Science and Technology Research Project: Screening of specific molecular markers of SARS-CoV-2 infection based on double transcriptome analysis techniques.

Authors' Contributions

GC, CZ, CL, and ZH were responsible for the collection of clinical data; Statistical analyses were completed by WL; GC and WL jointly completed the first draft of this manuscript; JX and XL designed the whole study, provided guidance, and reviewed and submitted the article. All authors have read and agreed with the published version of the manuscript.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W (2020) A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 382: 727–733.
2. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H (2020) Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology 158: 1831-1833.
3. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579: 270–273.
4. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395: 565–574.
5. National Health Commission of China (2020) COVID-19 diagnosis and treatment protocol (trial version 7). Available: http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml. Accessed: 4 April, 2020 [Article in Chinese].
6. Chan JFW, Kok KH, Zhu Z, Chu H, To KKW, Yuan S, Yuen Y (2020) Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect 9: 221–236 .
7. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395: 507–513.
8. Wu J, Liu J, Zhao X, Liu C, Wang W, Wang D, Xu W, Zhang C, Yu J, Jiang B, Cao H, Li L (2020) Clinical characteristics of imported cases of COVID-19 in Jiangsu Province: A multicenter descriptive study. Clin Infect Dis 71: 706-712.
9. Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, Li SB, Wang HY, Zhang S, Gao HN, Sheng JF, Cai HL, Qiu YQ, Li LJ (2020) Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ 368: m606.
10. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, Wang M (2020) Presumed asymptomatic carrier transmission of COVID-19. JAMA 323: 1406-1407.

11. Liu Q, Wang R, Qu G, Wang Y, Liu, Zhu Y, Fei G, Ren L, Zhou Y, Liu L (2020) Gross examination report of a COVID-19 death autopsy. Fa Yi Xue Za Zhi 36: 21–23. [Article in Chinese].
12. He Y, Wang Z, Li F, Shi Y (2020) Public health might be endangered by possible prolonged discharge of SARS-CoV-2 in stool. J Infect 80: e18–e19.
13. Nolan T, Hands RE, Bustin SA (2006) Quantification of mRNA using real-time RT-PCR. Nat Protoc 1: 1559–1582.

Corresponding author
Jin-Yu Xia, Professor
Department of Infectious Diseases, The Fifth Affiliated Hospital, Sun Yat-sen University, No. 52 Meihua East Road, Xiangzhou District, Zhuhai, China.
Tel: +86-07562528527
E-mail: xiajinyu@mail.sysu.edu.cn

Xi Liu, PhD
Department of Infectious Diseases, The Fifth Affiliated Hospital, Sun Yat-sen University, No. 52 Meihua East Road, Xiangzhou District, Zhuhai, China.
Tel: +86-07562528527
E-mail: liuxi26@mail.sysu.edu.cn

Conflict of interests: No conflict of interests is declared.