A Confirmed Case of SARS-CoV-2 Pneumonia with Routine RT-PCR Negative and Virus Variation in Guangzhou, China

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Summary

A case was confirmed with SARS-CoV-2 pneumonia by Nanopore pathogen sequencing and antibody detection. However, the routine RT-PCR of SARS-CoV-2 was negative. Furthermore, it was shown to be negative by RT-PCR due to a viral mutation.
Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia is a newly recognized disease, and its diagnosis is primarily confirmed by routine RT-PCR detection of SARS-CoV-2. However, we report a confirmed case of SARS-CoV-2 pneumonia with routine RT-PCR negative. This case has been finally diagnosed by Nanopore sequencing combined with antibody of SARS-CoV-2. Simultaneously, the ORF and NP gene variation of SARS-CoV-2 were found. This case has highlighted that false negative results could be present in the routine RT-PCR diagnosis, especially with virus variation. At the moment, Nanopore pathogen sequencing and antibody detection have been found effective in clinical diagnosis.

Keyword: SARS-CoV-2; Pneumonia; Routine RT-PCR; Virus variation; Nanopore sequencing
Background

Recently, coronavirus disease 2019 (COVID-19) has become a global epidemic, up to June 25, 2020, there have been 9,296,202 confirmed cases and 479,133 deaths of COVID-19 around the world [1, 2]. According to WHO and Chinese interim guidance [3, 4], the confirmation of diagnosis is usually via routine real-time reverse transcriptase polymerase chain reaction (RT-PCR) detection of SARS-CoV-2. However, some studies found that the routine RT-PCR may present false negative results [5], but these reports didn’t clarify the cause of the false negative results and the significance of its clinical and epidemic impact. Coincidentally, we have found a routine RT-PCR negative, but confirmed case of SARS-CoV-2 pneumonia in Guangzhou, China. This report mainly describes the clinical diagnosis process and the new diagnostic methodology, the evolution and mutation analysis of viruses, and the management and some unexpected discovery of this case.

Case Report

The patient is a fifty-seven years old women, who returned to Guangzhou from her hometown, Xiantao city of Hubei Province with her families on January 20, 2020 (Fig. 1). She had no contact with patients with fever or COVID-19, or wild animals. And she has no chronic disease or history of smoking.

On January 30, 2020, she developed fever and so did her husband. She had a maximum temperature of 37.8°C with symptoms of chills, chest and back pain, and no other respiratory or digestive symptoms, without treatment. Due to no easing of the symptoms, she and her husband went to see a doctor in the heat outpatient of our hospital (hospital A) two days later (February 1). Considering their clinical symptoms and history of stay in the epidemic area of COVID-19, the routine RT-PCR detection of SARS-CoV-2 was primarily carried out for them with the oropharyngeal swab sample and sent to Guangdong Centers for Disease Control and Prevention (CDC). Unfortunately, the detection result from CDC was positive of SARS-CoV-2 for her husband, but negative for her. Subsequently, her husband was taken to hospital B, which was the designated treatment hospital of COVID-19, for further treatment, and she was sequentially admitted to hospital A as a suspected patient.
On admission (Hospital Day 1) of this patient in our case, the physical examination revealed a body temperature of 37.3°C, pulse of 87 beats per minute, respiratory rate of 20 breaths per minute, blood pressure of 114/78 mm Hg, and oxygen saturation of 95% (breath ambient air). She preserved sanity, and lung auscultation revealed the sound of breath was rough, but without rhonchi or moist crackles. Laboratory examinations were performed which showed that leukocyte was 2.60X10^9/L, lymphocyte was 0.9X10^9/L. Procalcitonin (PCT), liver and kidney function, enzymatic indicators and D-dimer were normal (Table 1). Chest computed tomographic (CT) scan performed at that time reported the two lungs being scattered with ground glass lesions, which obviously appeared on the lower right dorsal segment/outer basal segment of the lung (Fig. 2). Based on the above mentioned, we classified her as a highly suspected patient of SARS-CoV-2 pneumonia, which made as a clinical diagnosis [3]. Thus she was isolated in a single room, and was given a treatment with low-flow oxygen and moxifoxacin (oral, 400mg qd), according to the treatment guideline of community-acquired pneumonia [6]. However, her fever was rising day by day, and the thermal spike presented on Hospital Day 3, when the maximum temperature reached 38.5°C. Fortunately, other symptoms did not deteriorate. The routine RT-PCR detection of SARS-CoV-2 was repeatedly carried out with oropharyngeal swab sample again on Hospital Day 2, and the result remained negative. Subsequently, a nasopharyngeal swab specimen was obtained and examined for respiratory pathogens using a rapid nucleic acid amplification test (NAAT; QIAstat-Dx, Respiraotry Panel, LOT 190255); and this was reported back in about 1 hour to show negative for all pathogens tested, including influenza A and B, parainfluenza, RSV, rhinovirus, adenovirus and other coronaviruses etc.

Given she was diagnosed as a highly suspected patient of SARS-CoV-2 pneumonia. Thus, the routine RT-PCR detection of SARS-CoV-2 was used as a conventional monitor from February 5 to 24 (Hospital Day 5 to 24), with samples including oropharyngeal swab, sputum, urine, stool, anal swab and whole blood test in hospital A and Guangdong CDC. However, all of these test results were still negative (Table 2). As her fever went on, the antiviral drug Lianhuaqingwen capsule, which is one kind of natural herbal medicine that has shown to have antiviral effect [7], was used for treatment of the patient on Hospital Day 7 (Fig. 1), and the temperature gradually dropped to normal on Hospital Day 12. The sputum sample was sent to the lab for clinical metagenomic next-generation sequencing (mNGS) testing on Hospital Day 8. Unexpectedly, there was a SARS-CoV-2 genomic segment detected, however, the genome coverage was only 75 bp (aboundacne, 0.05%)
(Supplementary Table 1). In order to further validate the existence of SARS-CoV-2, the same sputum sample was tested by Nanopore. To our excitement, SARS-CoV-2 genomic sequence was again detected. After that, another sputum sample collected on Hospital Day 12 has continued to show SARS-CoV-2 gene detection by Nanopore (Fig. 3). The chest CT was retested on February 12 (Hospital Day 12), which presented that the inflammatory exudation in the field of the right lower lung was increased and became dense (Fig. 2). Moreover, the antibody of *mycoplasma pneumoniae* was 1:320 (positive range> 1:40), which hinted the infection of *mycoplasma pneumoniae* (Table 1). Hence, the Moxifoxacin and Lianhuaqingwen capsule were still prescribed (Fig.1). The IgM and IgG antibody were retested and the results were positive on Hospital Day 13, and there was an approximate tenfold (0.067 vs 0.673) increase of IgG (Fig. 6). Furthermore, the micro-neutralization antibody (IgM and IgG) of SARS-CoV-2 from Guangdong CDC also was positive (Hospital Day 20) (Table 2 and Supplementary Table 5). Up to this point, taking into consideration of the epidemiological history, clinical features, imaging findings, the positive results of SARS-CoV-2 gene sequencing of Nanopore and the antibody of SARS-CoV-2, this patient was finally diagnosed for “SARS-CoV-2 pneumonia, mild case”, although the routine RT-PCR detection kept to be negative all the time (Table 2). The clinical symptoms of this patient were completely in remission on Hospital Day 24. The laboratory examinations were by and large normal (Table 1). The chest CT showed that the inflammation effusion of lung was significantly absorbed compared to that of February 12 (Hospital Day 12). Therefore, the patient was discharged from hospital upon recovery on February 25 (Hospital Day 25; Illness Day 27).
Methods

Specimen Collection

Clinical specimens, including oropharyngeal and anal swab, serum, sputum, urine and stool were obtained in accordance with WHO and Chinese guidelines[3, 4]. Specimens were stored between 2°C to 8°C until ready for shipment to the Guangdong CDC and the lab of hospital A.

Nucleic Acid Isolation and Routine RT-PCR Detection of SARS-CoV-2

Total RNA was extracted from 200μL specimen with automatic nucleic acid extractor (20190001, GenAct NE-48, Shanghai GeneoDx Biotech Co., LTD, China) according to the manufacturer’s instructions; a 50 μL elution volume was obtained for each sample. A 2μL aliquot of RNA was used for real-time RT-PCR, which targeted the ORF1ab and NP gene using a RT-PCR probe kit (GZ-D2RM, Shanghai GeneoDx Biotech Co., LTD, China). Real-time RT-PCR was performed under the following conditions: 42°C for 5 min and 95°C for 10s, followed by 40 cycles of amplification at 95°C for 10 s and 60°C for 45 s. Criteria for judging results: CT value<37 positive; 37≤CT value≤40 suspicious positive, and ≥40 negative. However, the positive should meet both ORF1ab and NP gene positive simultaneously.

RNA and Gene Detection for SARS-CoV-2

1) Method of Nanopore pathogen sequencing

On the basis of previous research methods [8], our team has developed a Platform of New Generation of Pathogenic Gene Sequencing (the fourth generation) - Nanopore Sequencing and Analysis Platform for this study. Furthermore, the Nanopore sequenced reads were aligned to the complete SARS-CoV-2 genome published on NCBI (NC_045512) [9].

2) Bioinformatic methods

With the raw sequencing data, we performed data filtration with NanoFilt (version 1.7.0) [10], and specie annotation with Kraken [11]. Then, genome alignment (NCBI: MN908947.3) was carried out with Minimap2 (version 2.17-r941) [12], and genome variations were called with bcftools (version 1.8) [13]. With SARS genome (NC_004718.3) and SARS-CoV-2 genomes (one from Guangzhou, China and the other one from
Washingto, USA), SNPs were detected using Mummer (version: 3.23) [14], and the phylogenetic tree was constructed with Mega X (version 10.0.4) [15].

3) RNA detection and Sanger Sequencing

To identify the mutation of NP gene, the amplification of NP gene was performed by PCR with forward primer: 5’- GACCTACACAGGTGCCATCAA-3’ and reverse primer: 5’- CCATCTGCCCTTGTGTGGTCT-3’. The product of PCR was sequenced by Sangon Biotech (Shanghai, China). The gene sequence of Sanger sequencing was shown as supplementary materials (Supplementary Table 2).

IgM/IgG Antibody of SARS-CoV-2 was Detected with ELISA Assay

Anti-Human IgM (μ-chain specific) antibody or N protein of SARS-CoV-2 (IgG) was used as the coating. The plasma of patients was diluted at 1:100 for testing. HRP labeled N protein of SARS-CoV-2 (IgM) or anti-human IgG (H + L) antibody labeled with HRP was used as the secondary antibody. The color was developed by TMB and terminated by H2SO4. Then OD450 was tested. The positive and negative control were set at the same time.

Results

The Sequence Detected by Nanopore was SARS-CoV-2, and the Nucleocapsid (NP) and ORF of SARS-CoV-2 have Variation

The sputum specimens obtained from this patient on Hospital Day 8 and 12 were tested by Nanopore sequencing. A total of 242,889 reads were obtained for the samples (Figure 3A, B), and the aligning ratio was 53.96% when they were mapped to SARS-CoV-2 genome. With specie annotation, virus was the dominant domain with a relative ratio of 60.20% (Figure 3C). Moreover, 99.99% of the reads in virus domain were from SARS-CoV-2 (Figure 3D-F).

After Nanopore reads alignment, 172,62bp of SARS-CoV-2 genome were covered with a depth of 876.3 on average (Figure 4A). However, the alignment depth was not balanced (Figure 4C and Supplementary Fig.1), and 5’ end of NP gene (29,380-29,533) was uncovered. In addition, the sample exhibited 6 single nucleotide polymorphism variations (SNPs, Fig. 4A), which were distributed in ORF1ab, ORF6, and ORF8 (Fig. 4B, detailed in Supplementary Table 3). After the construction of phylogenetic tree, the closer phylogenetic
relationship was discovered between the sample 1 and EPI_ISL_412967, which were both isolated from Guangzhou patients (Fig. 4D). Because the 5’ end of NP gene was uncovered, we used Sanger sequencing to identify the segment. PCR primers was designed to cover 29,380-29,533 region of SARS-CoV-2 genome and the amplified length was 490bp. This sequence only had 6% query cover with SARS-CoV-2, and it could not match any sequence in NCBI database (Figure 4E).

Furthermore, we designed new primers focusing on the non-mutation area of NP and ORF of SARS-CoV-2. The RT-PCR result was positive (Supplementary Table 4), which suggested that the variant virus was SARS-CoV-2.

IgM and IgG Antibody turned from negative to positive, and Micro-neutralization Antibody of SARS-CoV-2 was Positive for This Patient

The initial blood specimen (plasma) obtained from this patient on Hospital Day 8 was negative for the total IgM/IgG antibody of SARS-CoV-2, and the OD value for IgM and IgG were 0.089 and 0.067 (normal range 0.1-0.15). However, the retesting on IgM/IgG antibody turned positive on Hospital Day 13, and the OD value for IgM and IgG were 0.232 and 0.673. From negative to positive, the IgG level in the patient exhibited approximate tenfold increase (OD value: 0.067 vs 0.673). The IgG level continuously increasing, and the highest point appeared on Hospital Day 20 with OD value was 1.01, then decrease gradually (Figure 5).

Discussion

Herein, we report a confirmed case of SARS-CoV-2 pneumonia in Guangzhou, China, which was finally confirmed by Nanopore sequencing and SARS-CoV-2 antibody detection combining with clinical features and chest CT, but negative by routine RT-PCR. Furthermore, we found the newly discovered virus variation in NP and ORF of SARS-CoV-2, which may lead to the routine RT-PCR result negative.

To the situation, we think the best way is to efficiently identify the COVID-19 cases, and provide effective quarantine and clinical treatment to the patients. However, with only clinical features, such as fever (Table 1) and imaging features (Fig. 2), it is difficult to differentiate COVID-19 from other viral infections. Although virus nucleic acid RT-PCR, CT imaging and some hematology parameters are adopted for clinical diagnosis of the infection
[16], yet there proved to be a certain amount of false negative results [5], especially for highly suspected cases as our case (Table 2), which would make the epidemic worse. Therefore, more effective methods should be applied for the clinical diagnosis. The Nanopore sequencing could be an efficient method, which has made nanotechnology achieve a practical breakthrough in single-molecule detection for the first time [17]. This technology has provided rapid detection of South America Zika virus, African Ebola virus and other new viruses [18, 19]. After COVID-19 outbreak, we have established a new Nanopore sequencing method of SARS-CoV-2 (Fig. 3), and it was able to correctly provide positive test results of SARS-CoV-2 where the routine RT-PCR was negative (Table 2 and Supplementary Table 1). Admittedly, Nanopore sequencing is a relatively new method for SARS-CoV-2 detection, so we combined it with antibody test to make a final detection. In 2003 SARS-CoV infection, the antibody detection has shown its value [20], and we have further proved the value of IgM and IgG detection declared in an earlier study [21] in the SARS-CoV-2 infection. The fact that IgM antibody turned from negative to positive, or that there was a fourfold increase of IgG at recovery phase compared with that at the acute phase, have provided meaningful diagnostic value (Fig. 5, and Supplementary Table 5). After all, taking into account the epidemiological history, clinical features, imaging findings (chest CT), the positive results of SARS-CoV-2 Nanopore sequencing and the antibodies (particularly the positive of micro-neutralization antibody), this patient was confirmed for “SARS-CoV-2 pneumonia, mild case”. Additionally, the results from mNGS sequencing have provided a hint that *Prevotella melaninogenica*, *Neisseria meningitidis* and *Campylobacter concisus* (genomic coverage>5%) might be dominant in the upper respiratory tract of the patient (Supplementary Table 1). Since the three bacteria were conditional pathogens [22], we are not certain about the causal relationship between the SARS-CoV-2 infection and the imbalanced respiratory microbiome in this retrospective study.

In this study, we have discovered that most regions on SARS-CoV-2 genome could be covered [9], which provided us with a sufficient method for the diagnosis of patients. In combination with Sanger sequencing (Fig. 4E), the general genomic features and variations could be detected for the virus isolated from the patient. This virus exhibited 4 non-synonymous mutations on gene ORF1ab and ORF8 (Fig. 4 and Supplementary Table 3). Since ORF1ab involved in the transcription and replication of viral RNA, the mutations could be a signal that the evolution of the virus is still underway. That said, it would cause increasing difficulties for the traditional detection methods of SARS-CoV-2 which mainly
adopt ORF1ab as the specified target region for the virus examination [23]. In addition, the non-synonymous mutation on ORF8 (location: 28,144) has been recognized as an important virus typing mutation [23]. According to previous reports, we knew that the virus of this patient could be the older SARS-CoV-2 strain (S typing). The phylogenetic analysis on this virus and previously published SARS-CoV-2 illustrated that its genome exhibited closer relationship with the virus isolated from the same region (Guangzhou, China), and was separated from the virus isolated from Wuhan region (Fig. 4D). Therefore, the evolution process and the origin of SARS-CoV-2 still need to be explored. Furthermore, Sanger sequencing has also proved that the NP gene in this virus has variation (Fig. 5 and Supplementary Table 4). Since the examination of SARS-CoV-2 requires that the NP and ORF1ab genes of SARS-CoV-2 in the same specimen are both positive for RT-PCR [24], the gene variations in these genes might lead to the RT-PCR negative results for the patient.

Admittedly, there are some deficiencies in this paper, the biggest drawback is the lack of the virus culturing for this patient due to a low viral load and laboratory condition and qualification, and the unavailability of the full viral sequence. However, most part of virus genome was detected by Nanopore sequencing, and more samples from other patients in Guangzhou will be tested and reported in subsequent studies.

We report the clinical features, clinical diagnosis and virus mutation of a confirmed patient of SARS-CoV-2 pneumonia with RT-PCR negative in Guangzhou, China. The study of this case highlights that the Nanopore sequencing could be used in the clinical pathogenic diagnosis of SARS-CoV-2 pneumonia, especially when the virus mutation leads to routine RT-PCR negative. This report also demonstrates that we should be focusing on the evolution and variation of virus with epidemic development, which hold the possibility to lead to false negative test results and hence to increase the difficulty of epidemic prevention and control in practice. Finally, it suggests that multiple detection methods, including the antibody detection, should be used to make clinical diagnosis, especially when the routine RT-PCR results are found negative for highly suspected patients.
Notes

Disclaimer. This study has received ethical approval from the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (Ethical number: 2020-36), and the patient’s informed consent authorization has been obtained (Supplementary 2).

Funding. This work was funded by the Scientific and Technological Emergency Special of Guangdong Province for Prevention and Controlling of COVID-19 (Grant number: 2020B111340004); the Emergency Project of COVID-19 Clinical Research on Prevention and Treatment with Traditional Chinese Medicine (TCM) in the Guangdong Bureau of TCM (Grant number: 2020ZYYJ05).

Potential conflicts of interest. The authors report no conflicts of interest.
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Figure legends

Figure 1. **Timeline of the patient’s clinical course.** Symptom and treatment according to day of illness and day of hospitalization, January 20 to February 25, 2020.

Figure 2. **Imaging of chest computed tomographic (CT) scan.** Panel A shows pulmonary and mediastinal window of chest CT on disease onset (Hospital Day 1; Illness Day 3). The two lungs were scattered with ground glass lesions, particularly in the dorsal/outer basal segments of the right lower lung (pulmonary window), suggesting a high possibility of viral pneumonia, and no abnormalities were noted in mediastinal window. The recheck was on Hospital Day 12, it showed ground-glass opacity and consolidation, and the lesion range was enlarged (Panel B). And the exudation of inflammation was well absorbed on Hospital Day 24 after treatment (Panel C).

Figure 3. **Length and quality distributions of the nanopore data and Taxonomic annotation results of sample.** A. The distribution of read length. In this plot, x coordinate represents the read length, and the y coordinate represents the base number for the reads with different length. B. The distributions of read quality and read length. In this plot, each read was represented by a dot, and the x and y coordinates represent their length and average quality respectively. The upper and right-side histograms exhibited the distributions of reads length and quality respectively. C. The relative abundances of domains in the sample. D, E and F plots exhibited the compositions and relative abundances of Viruses, Eukaryota and Bacteria at the specie level respectively.
Figure 4. Genome comparison and phylogenetic relationships between sample and SARS-CoV-2. A. Genome alignment between the consensus sequence from the sample and SARS-CoV-2 genome. In this plot, the red and blue dots represent the forward and backward alignment respectively, and the x and y coordinates represent the genome of SARS-CoV-2 and the sample respectively. B. The distributions of SNPs in sample as compared with SARS-CoV-2. Gene names are list on the x coordinate, and the corresponding SNP numbers are list on the y coordinate. The blue and red bars represent the synonymous and non-synonymous mutations respectively. C. Alignment of sequencing reads from sample on SARS-CoV-2 genome. The x and y coordinates represent the locations of SARS-CoV-2 genome and the aligned depth respectively. D. Phylogenetic relationships among the samples. In this phylogenetic tree, MN908947 was taken as the representative genome for SARS-CoV-2, and the SNPs in other genomes were detected using Mummer software. EPI_ISL_412967 and EPI_ISL_412970 were the published SARS-CoV-2 genomes from Guangzhou (China) and Washington (USA) patients, while NC_004718 was the representative genome for SARS. E. The 5’ end of Nucleocapsid gene of SARS-CoV-2 had about 490bp mutation, proved by Sanger Sequencing.

Figure 5. The IgM and IgG antibody of SARS-CoV-2 detected in this patient. The plasma obtained from patient on Hospital Day 8, 14 and 24. The level of IgM and IgG antibody were tested with ELISA method, and the results showed with OD value.
Table 1. Clinical Laboratory Examination Results.

| Measure                          | Reference Range | Illness Day 3 | Illness Day 5 | Illness Day 15 | Illness Day 19 | Illness Day 26 |
|---------------------------------|-----------------|---------------|---------------|---------------|---------------|---------------|
|                                 |                 | Hospital Day 1 | Hospital Day 3 | Hospital Day 13 | Hospital Day 17 | Hospital Day 24 |
| Blood routine examination       |                 |               |               |               |               |               |
| White-cell count (X10^9/L)      | 4.0-10.0        | 4.03          | 2.60#         | 5.80          | 4.50          | 3.80#         |
| Neutrophil count (X10^9/L)      | 1.80-8.00       | 2.3           | 1.40          | 3.90          | 2.40          | 2.00          |
| Lymphocyte count (X10^9/L)      | 0.90-5.20       | 1.40          | 0.90#         | 1.30          | 1.70          | 1.40          |
| Hemoglobin (g/L)                | 110-150         | 138           | 135           | 115           | 113           | 103           |
| Platelet count (X10^9/L)        | 100-400         | 141           | 127           | 177           | 202           | 160           |
| Coagulation function            |                 |               |               |               |               |               |
| PT (S)                          | 11-14.50        |               | 12.90         | 14.10         | 13.40         |               |
| FIB (g/L)                       | 2-4             |               | 3.400         | 3.97          | 3.39          |               |
| APTT (S)                        | 28-42.80        |               | 39.90         | 37.60         | 35.00         |               |
| D-dimer (ng/ml FEU)             | 68-494          |               | 206           | 3204#         | 351           |               |
| Arterial blood gas analysis     |                 |               |               |               |               |               |
| PH                              | 7.35-7.45       |               | 7.397         | 7.372         | 7.391         | 7.397         |
| PO₂ (mmHg)                      | 85-108          |               | 112.50        | 131.30        | 91.30         | 177.10        |
| PCO₂ (mmHg)                     | 35-48           |               | 39.10         | 43.50         | 42.90         | 45.40         |
| HCO₃⁻ (mmol/L)                  | 21.40-27.30     |               | 23.60         | 24.70         | 25.50         | 27.30         |
| LAC (mmol/L)                    | 0.70-2.10       |               | 1.47          | 1.56          | 1.89          |               |
| Oxygenation index (mmHg)        | 400-500         |               | 388#          | 453           | 435           | 610           |
| Liver function analysis         |                 |               |               |               |               |               |
| ALT (U/L)                       | 5-40            |               | 24.60         |               | 80.20*        | 54.80*        |
| TP (g/L)                        | 65-85           |               | 70.30         |               | 32.10         | 61.10         |
| TBIL (umol/L)                   | 1.7-22.20       |               | 6.70          |               | 10.40         | 12.80         |
| Enzymatic indicators            |                 |               |               |               |               |               |
| CK (U/L)                        | 10-190          |               | 59.80         | 49.50         | 49.10         | 62.70         |
| CKMB (U/L)                      | 3-25            |               | 11.00         | 13.00         | 6.00          | 9.00          |
| Biochemical function analysis | BUN (mmol/L) | Cr (umol/L) | Na (mmol/L) | Ca (mmol/L) |
|------------------------------|-------------|-------------|-------------|-------------|
| LDH (U/L)                    | 109-255     | 178.80      | 149.90      |             |
| aTnI (ug/L)                  | 0-0.04      | 0.00        | 0.00        | 0.00        |
| MYO (MYO)                    | <70         | 18.80       | 14.10       |             |
| BUN (mmol/L)                 | 2.9-7.2     | 3.20        | 139.90      | 2.03-2.7    |
| Cr (umol/L)                  | 44-133      | 76.20       | 140.80      |             |
| K (mmol/L)                   | 3.5-5.3     | 3.87        | 141.30      |             |
| Na (mmol/L)                  | 134-145     | 139.90      | 140.80      |             |
| Cl (mmol/L)                  | 96-111      | 105.10      | 108.90      |             |
| Antibody of mycoplasma pneumoniae |           |             | 1:320*      |             |

# The value for the patient was below normal.

*The value for the patient was above normal.

- It wasn’t detected at the time.
Table 2. The Results of Nucleic Acid and Antibody Detection of SRSA-CoV-2.

| Date       | Samples | Routine RT-PCR | mNGS | Nanopore sequencing | Micro-neutralization antibody | IgM or IgG Antibody | New RT-PCR |
|------------|---------|----------------|------|---------------------|------------------------------|---------------------|------------|
|            |         | Oropharyngeal swab | Sputum | Urine | Stool/Anal swab | Whole blood | Sputum | Sputum | Serum | Serum | Sputum |
| Feb 1\(^a\) | N       | -              | -    | -      | -               | -                  | N       | N       | N      | N      | N      |
| (Day 3)    |         |                |      |        |                 |                    |         |         |        |        |        |
| Feb 2\(^b\) | N       | -              | -    | -      | -               | -                  | N       | N       | N      | N      | N      |
| (Day 4)    |         |                |      |        |                 |                    |         |         |        |        |        |
| Feb 5\(^c\) | N       | -              | -    | -      | -               | -                  | N       | N       | N      | N      | N      |
| (Day 7)    |         |                |      |        |                 |                    |         |         |        |        |        |
| Feb 8\(^d\) | -       | -              | N    | N      | N               | N                  | +       | +       | +      | +      | +      |
| (Day 10)   |         |                |      |        |                 |                    |         |         |        |        |        |
| Feb 9\(^e\) | -       | N              | -    | N      | -               | -                  | +       | +       | +      | +      | +      |
| (Day 11)   |         |                |      |        |                 |                    |         |         |        |        |        |
| Feb 12\(^f\) | N      | N              | -    | -      | -               | -                  | +       | +       | +      | +      | +      |
| (Day 14)   |         |                |      |        |                 |                    |         |         |        |        |        |
| Date     | Test Result |
|----------|-------------|
| Feb 17   | N           |
| (Day 19) |             |
| Feb 20   | N           |
| (Day 22) | N           |
| Feb 24   | N           |
| (Day 26) |             |

**Notes:**

a) N means negative; + means positive; - means not detected;

b) # means the results from the lab of hospital A and Guangdong Centers for Disease Control and Prevention (CDC);

c) & means the results from Guangdong Centers for Disease Control and Prevention (CDC).
Figure 2

A
February 1, 2020
(Hospital Day 1, Illness Day 3)

B
February 12, 2020
(Hospital Day 12, Illness Day 14)

C
February 24, 2020
(Hospital Day 24, Illness Day 26)
Figure 5

OD value vs. Hospital Day

- IgM
- IgG