Clinical Infectious Diseases

MAJOR ARTICLE

A Confirmed Case of SARS-CoV-2 Pneumonia With Negative Routine Reverse Transcriptase–Polymerase Chain Reaction and Virus Variation in Guangzhou, China

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(See the Editorial Commentary by Sipahi on pages e434–6.)

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia is a newly recognized disease, and its diagnosis is primarily confirmed by routine reverse transcriptase -polymerase chain reaction (RT-PCR) detection of SARS-CoV-2.

Methods. However, we report a confirmed case of SARS-CoV-2 pneumonia with a negative routine RT-PCR.

Results. This case was finally diagnosed by nanopore sequencing combined with antibody of SARS-CoV-2. Simultaneously, the *ORF* and *NP* gene variations of SARS-CoV-2 were found.

Conclusions. This case highlighted that false-negative results could be present in routine RT-PCR diagnosis, especially with virus variation. Currently, nanopore pathogen sequencing and antibody detection have been found to be effective in clinical diagnosis.

Keywords. SARS-CoV-2; pneumonia; routine RT-PCR; virus variation; nanopore sequencing.

Recently, coronavirus disease 2019 (COVID-19) has become a global epidemic; up to 25 June 2020 there have been 9 296 202 confirmed cases and 479 133 deaths related to COVID-19 worldwide [1, 2]. According to the World Health Organization (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 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, some studies found that routine RT-PCR may present false-negative results [5], but these reports did not clarify the cause of the false-negative results and the significance of their clinical and epidemic impact. Coincidentally, we found a routine RT-PCR to be negative but in a 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 unexpected discovery of this case.

CASE REPORT

The patient is a 57-year-old women who returned to Guangzhou from her hometown, Xiantao city in Hubei Province, with her family on 20 January 2020 (Figure 1). She had no contact with patients with fever or COVID-19 or wild animals. She also has no chronic disease or history of smoking.

On 30 January 2020, she developed a fever as 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 fever outpatient section of our hospital (hospital A) 2 days later (1 February). Considering their clinical symptoms and history of stay in the epidemic area of COVID-19, routine RT-PCR detection of SARS-CoV-2 was carried out with a oropharyngeal swab sample and sent to Guangdong Centers for Disease Control and Prevention (CDC). Unfortunately, the test results from the CDC were positive for 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 woman was subsequently admitted to hospital A as a patient with suspected COVID-19.

On admission (hospital say 1) of this patient in our report, 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% (breathing ambient air). She preserved sanity, and

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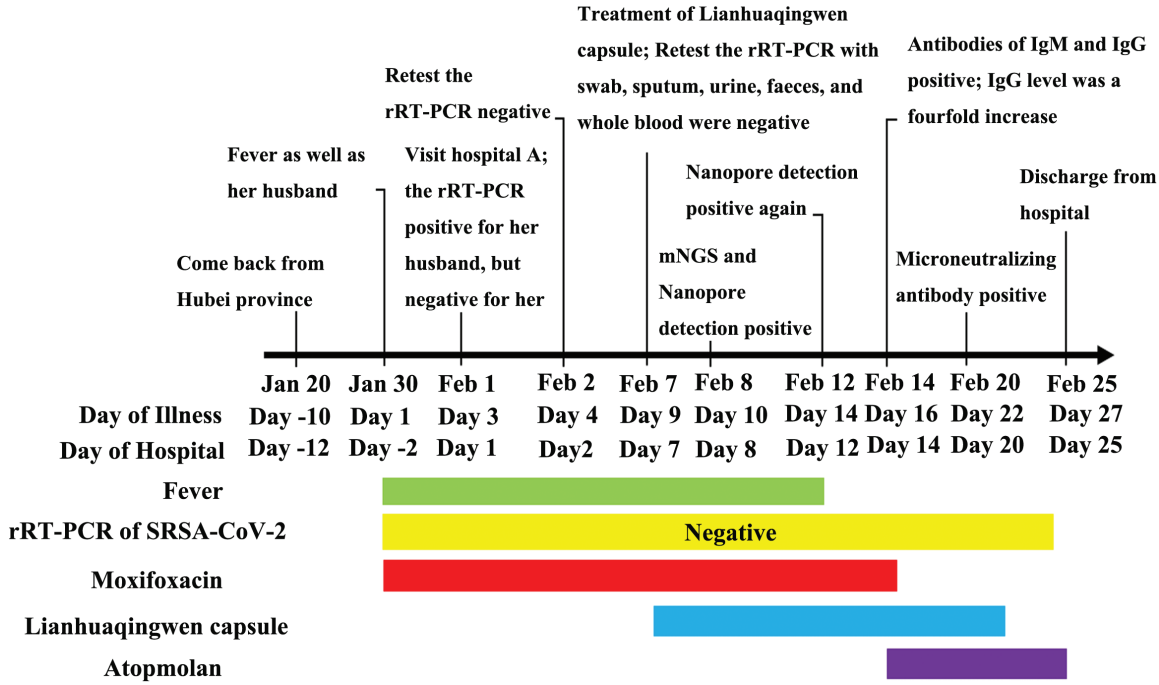

Figure 1. Timeline of the patient's clinical course. Symptoms and treatment according to day of illness and day of hospitalization, 20 January to 25 February 2020. Abbreviations: Ig, immunoglobulin; mNGS, metagenomic next-generation sequencing; rRT-PCR, real-time reverse transcriptase–polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

lung auscultation revealed that her breathing was rough, but without rhonchi or moist crackles. Laboratory examinations were performed, which showed that leukocyte count was 2.60×10^9/L and lymphocyte count was 0.9×10^9/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 showed ground-glass lesions scattered in both lungs, which obviously appeared on the lower right dorsal segment/outer basal segment of the lung (Figure 2). Based on the above-mentioned findings, we classified her as a patient highly suspected of having SARS-CoV-2 pneumonia, which we made as a clinical diagnosis [3]. Thus, she was isolated in a single room and was given a treatment with low-flow oxygen and moxifloxacin (oral, 400 mg once a day [qd]), according to the treatment guidelines of community-acquired pneumonia [6]. However, her fever increased day by day, and a temperature spike occurred on hospital day 3, when the maximum temperature reached 38.5°C. Fortunately, other symptoms did not get worse. The routine RT-PCR detection of SARS-CoV-2 was repeatedly carried out with an 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, Respiratory Panel, Lot 190255); and this was reported back in approximately 1 hour as negative for all pathogens tested, including influenza A and B, parainfluenza, respiratory syncytial virus, rhinovirus, adenovirus, and other coronaviruses, etc.

The patient was diagnosed as being highly suspected of having SARS-CoV-2 pneumonia. Thus, routine RT-PCR detection of SARS-CoV-2 was used as a conventional monitor from 5 to 24 February (hospital day 5 to 24), with samples including oropharyngeal swab, sputum, urine, stool, anal swab, and whole-blood test in hospital A and the Guangdong CDC. However, all of these test results were still negative (Table 2). As her fever continued, 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 (Figure 1), and her temperature gradually returned to normal on hospital day 12. The sputum sample was sent to the laboratory 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 (abundance, 0.05%) (Supplementary Table 1). In order to further validate the existence of SARS-CoV-2, the same sputum sample was tested by nanopore sequencing, and the SARS-CoV-2 genomic sequence was again detected. After that, another sputum sample collected on hospital day 12 continued to show SARS-CoV-2 gene detection by nanopore sequencing (Figure 3). The chest CT scan was performed again on 12 February (hospital day 12), which showed that the inflammatory exudation in the field of the right lower lung was increased and became dense (Figure 2). Moreover, the antibody

Table 1. Clinical Laboratory Examination Results

Measure	Reference Range	Illness Day 3, Hospital Day 1	Illness Day 5, Hospital Day 3	Illness Day 15, Hospital Day 13	Illness Day 19, Hospital Day 17	Illness Day 26, Hospital Day 24
Blood routine examination						
White-cell count, × 10^9/L	4.0–10.0	4.03	2.60[a]	5.80	4.50	3.80[a]
Neutrophil count, × 10^9/L	1.80–8.00	2.3	1.40	3.90	2.40	2.00
Lymphocyte count, × 10^9/L	0.90–5.20	1.40	0.90[a]	1.30	1.70	1.40
Hemoglobin, g/L	110–150	138	135	115	113	103
Platelet count, × 10^9/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[b]	351	—
Arterial blood gas analysis						
pH	7.35–7.45	—	7.397	7.372	7.391	7.397
PO_2, mmHg	85–108	—	112.50	131.30	91.30	177.10
PCO_2, mmHg	35–48	—	39.10	43.50	42.90	45.40
HCO^{3-}, 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[a]	453	435	610
Liver function analysis						
ALT, U/L	5–40	—	24.60	—	80.20[b]	54.80[b]
TP, g/L	65–85	—	70.30	—	32.10	61.10
TBIL, μmol/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
LDH, U/L	109–255	—	178.80	240.80	189.80	149.90
aTnI, μg/L	0–0.04	—	0.00	0.00	0.00	0.00
MYO, μg/L MYO: Myohemoglobin	<70	—	18.80	13.40	15.50	14.10
Biochemical function analysis						
BUN, mmol/L	2.9–7.2	—	3.20	3.40	3.00	3.70
Cr, μmol/L	44–133	—	76.20	67.20	67.30	63.10
K, mmol/L	3.5–5.3	—	3.87	3.53	3.74	3.42
Na, mmol/L	134–145	—	139.90	140.80	141.30	140.80
Cl, mmol/L	96–111	—	105.10	106.60	108.20	108.90
Ca, mmol/L	2.03–2.7	—	2.09	2.14	2.15	2.18
Antibody of mycoplasma pneumoniae		—	—	1:320[b]	1:160[b]	1:40

"–" indicates that it was not detected at the time.

[a]The value for the patient was below normal.

[b]The value for the patient was above normal.

PT: Prothrombin Time; FIB: Fibrinogen APTT: Activated Partial Thromboplastin Time; S: Second; TP: Total Protein; TBIL: Total Bilirubin.

of *Mycoplasma pneumoniae* was 1:320 (positive range >1:40), which hinted at *M. pneumoniae* infection (Table 1). Hence, the moxifloxacin and Lianhuaqingwen capsule were still prescribed (Figure 1). The immunoglobulin (Ig) M and IgG antibody levels were retested and the results were positive on hospital day 13, and there was an approximate 10-fold (0.067 vs 0.673) increase in IgG. Furthermore, the micro-neutralization antibody (IgM and IgG) of SARS-CoV-2 from Guangdong CDC also was positive (hospital day 20) (Table 2, Supplementary Table 5). Up to this point, taking into consideration the epidemiological history, clinical features, imaging findings, the positive results of SARS-CoV-2 nanopore gene sequencing and the antibodies of SARS-CoV-2, this patient was finally diagnosed with "SARS-CoV-2 pneumonia, mild case," although the routine RT-PCR test remained negative all this time (Table 2). The clinical symptoms of this patient were completely in remission on hospital day 24. The laboratory examinations were essentially normal (Table 1). The chest CT scan showed that the lesions are clearly absorbed compared with that on 12 February (hospital day 12). Therefore, the patient was discharged from the hospital upon recovery on 25 February (hospital day 25; illness day 27).

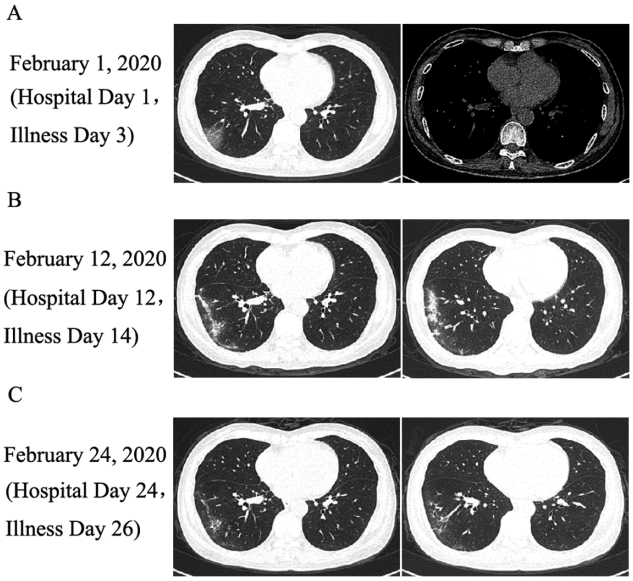

Figure 2. Imaging of chest CT scan. *A*, Pulmonary and mediastinal window of chest CT scan at disease onset (hospital day 1; illness day 3). The two lungs showed scattered ground-glass lesions, particularly in the dorsal/outer basal segments of the right lower lung (pulmonary window), suggesting a high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

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 and 8°C until ready for shipment to the Guangdong CDC and the laboratory at hospital A.

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

Total RNA was extracted from 200-μL specimens 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 2-μL aliquot of RNA was used for real-time RT-PCR, which targeted the ORF1ab and NP gene using an 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 minutes and 95°C for 10 seconds, followed by 40 cycles of amplification at 95°C for 10 seconds and at 60°C for 45 seconds. Criteria for judging results were as follows: cycle threshold (CT) value <37, positive; 37 ≤ CT value ≤40, suspicious positive; and ≥40, negative. However, the positive value should meet both ORF1ab and NP gene positive values simultaneously.

RNA and Gene Detection for SARS-CoV-2

Method of Nanopore Pathogen Sequencing

On the basis of previous research methods [8], our team has developed a 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 National Center for Biotechnology Information (NCBI) (NC_045512) [9].

Bioinformatic Methods

With the raw sequencing data, we performed data filtration with NanoFilt (version 1.7.0) [10], and species annotation with Kraken [11]. Then, genome alignment (NCBI: MN908947.3) was carried out with Minimap2 (version 2.17-r941) [12], and genome variations were detected with bcftools (version 1.8) [13]. With the SARS genome (NC_004718.3) and SARS-CoV-2 genomes (one from Guangzhou, China, and the other from

Table 2. Results of Nucleic Acid and Antibody Detection of SARS-CoV-2

	Samples									
	Routine RT-PCR					mNGS	Nanopore Sequencing	Microneutralization Antibody	IgM or IgG Antibody	New RT-PCR
Date	Oropharyngeal Swab	Sputum	Urine	Stool/Anal Swab	Whole Blood	Sputum	Sputum	Serum	Serum	Sputum
1 February[a] (day 3)	N	–	–	–	–					
2 February (day 4)	N	–	–	–	–					
5 February (day 7)	N	–	–	–	–				+	
8 February (day 10)	–	–	N	N	N	+	+			+
9 February (day 11)	–	N	–	N	–				+	
12 February (day 14)	N	N	–	–	–		+		+	
17 February (day 19)	–	N	–	–	–				+	
20 February[b] (day 22)	N	N	N	N	N			+	+	
24 February (day 26)	–	N	–	N	N					

Abbreviations: Ig, immunoglobulin; mNGS, metagenomic next-generation sequencing; N, negative; RT-PCR, reverse transcriptase–polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; +, positive; –, not detected.

[a]Results from the laboratory of hospital A and Guangdong Centers for Disease Control and Prevention.

[b]Results from Guangdong Centers for Disease Control and Prevention.

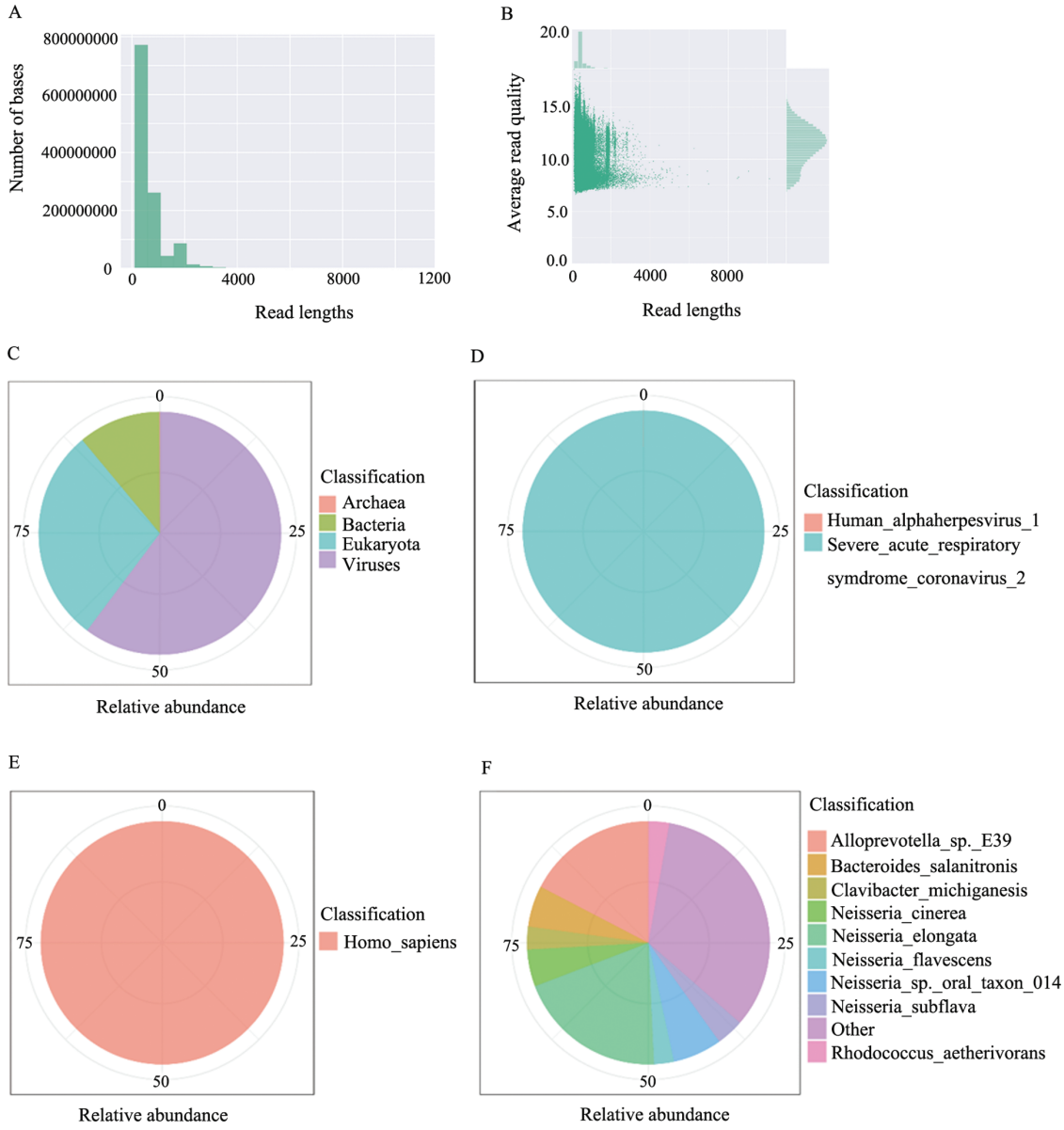

Figure 3. Length and quality distributions of the nanopore data and taxonomic annotation results of samples. *A*, The distribution of read length. In this plot, the "x" coordinate represents the read length and the "y" coordinate represents the base number for the reads with different length. *B*, The 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 show the distributions of read length and quality, respectively. *C*, The relative abundances of domains in the sample. *D–F*, Plots show the composition and relative abundances of viruses, Eukaryota, and bacteria at the species level, respectively.

Washington, DC), single nucleotide polymorphisms (SNPs) were detected using Mummer (version 3.23) [14], and the phylogenetic tree was constructed with Mega X (version 10.0.4) [15].

RNA Detection and Sanger Sequencing

To identify the mutation of the NP gene, the amplification of the NP gene was performed by PCR with the forward primer 5′-GACCTACACAGGTGCCATCAA-3′ and reverse primer 5′-CCATCTGCCTTGTGTGGTCT-3′. The product of PCR was sequenced by Sangon Biotech (Shanghai, China). The gene sequence of Sanger sequencing is shown in Supplementary Table 2.

IgM/IgG Antibodies of SARS-CoV-2 Detected With Enzyme-linked Immunosorbent 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. Horseradish peroxidase (HRP)–labeled N protein of SARS-CoV-2 (IgM) or anti-human IgG heavy chain + light chain (H + L) antibody labeled with HRP was used as the secondary antibody. The color was

developed by tetramethylbenzidine (TMB) and terminated by H_2SO_4. Then, OD450 was tested. The positive and negative controls were set at the same time.

RESULTS

SARS-CoV-2 Sequence Detected by Nanopore and Nucleocapsid (NP) and ORF of SARS-CoV-2 Showed Variation

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

After alignment of nanopore reads, 17 262 bp of the SARS-CoV-2 genome was covered, with a depth of 876.3 on average (Figure 4A). However, the alignment depth was not balanced (Figure 4C, Supplementary Figure 1), and the 5′ end of the NP gene (29 380–29 533) was uncovered. In addition, the sample exhibited 6 SNP variations (Figure 4A), which were distributed in ORF1ab, ORF6, and ORF8 (Figure 4B, which is 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 (Figure 4D). Because the 5′ end of the NP gene was uncovered, we used Sanger sequencing to identify the segment. PCR primers were designed to cover the 29 380–29 533 region of the SARS-CoV-2 genome and the amplified length was 490 bp. This sequence only had 6% coverage cover with SARS-CoV-2, and it could not match any sequence in the NCBI database (Figure 4E).

Furthermore, we designed new primers focusing on the nonmutation 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.

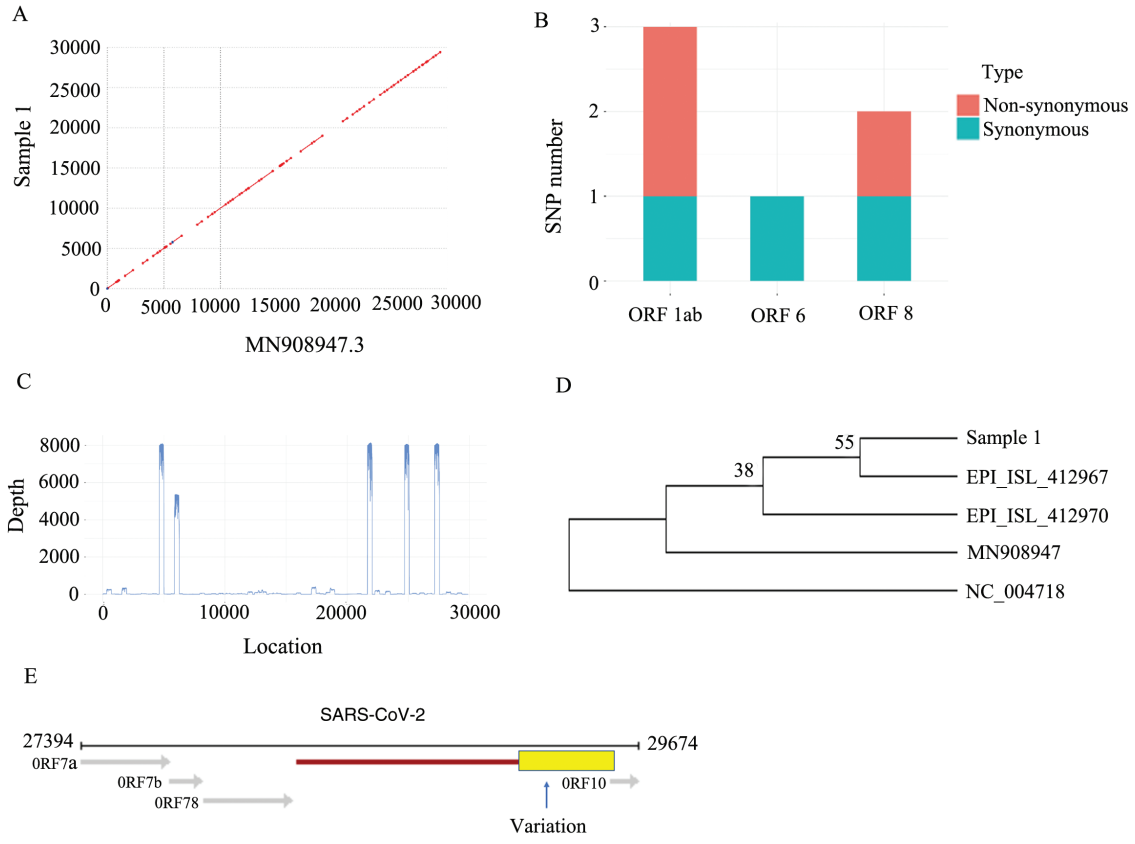

Figure 4. Genome comparison and phylogenetic relationships between samples and SARS-CoV-2. *A*, Genome alignment between the consensus sequence from the sample and SARS-CoV-2 genome. In this plot, the red and blue dots represent the forward and backward alignments, respectively, and the x and y coordinates represent the genome of SARS-CoV-2 and the sample, respectively. *B*, The distributions of SNPs in the sample as compared with SARS-CoV-2. Gene names are listed on the x coordinate, and the corresponding SNP numbers are listed on the y coordinate. The blue and red bars represent the synonymous and nonsynonymous mutations, respectively. *C*, Alignment of sequencing reads from the sample on the SARS-CoV-2 genome. The x and y coordinates represent the locations of the 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, DC, patients, while NC_004718 was the representative genome for SARS. *E*, The 5′ end of the nucleocapsid gene of SARS-CoV-2 had an approximately 490 bp mutation, which was proven by Sanger sequencing. Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SNP, single nucleotide polymorphism.

IgM and IgG Antibodies Turned From Negative to Positive and the Microneutralization Antibody of SARS-CoV-2 Was Positive in This Patient

The initial blood specimen (plasma) obtained from this patient on hospital day 8 was negative for total IgM/IgG antibodies of SARS-CoV-2, and the Optical density (OD) values for IgM and IgG were 0.089 and 0.067 (normal range, 0.1–0.15). However, retesting of IgM/IgG antibodies showed positive results on hospital day 13, and the OD values for IgM and IgG were 0.232 and 0.673. From negative to positive, the IgG level in the patient exhibited an approximate 10-fold increase (OD value, 0.067 vs 0.673). The IgG level continuously increased, with the highest value on hospital day 20 with an OD value of 1.01, then decreased 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 combined with clinical features and chest CT but was negative by routine RT-PCR. Furthermore, we found the newly discovered virus variation in NP and ORF of SARS-CoV-2, which may have led to the negative routine RT-PCR results.

In this 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 (Figure 2), it is difficult to differentiate COVID-19 from other viral infections. Although viral nucleic acid RT-PCR, CT imaging, and some hematology parameters are used for clinical diagnosis of the infection [16], there has proved to be a certain amount of false-negative results [5], especially for highly suspected cases, such as our case (Table 2), which would make the epidemic worse. Therefore, more effective methods should be applied for the clinical diagnosis. Nanopore sequencing could be an efficient method, which has to make practical breakthroughs in nanotechnology 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 the COVID-19 outbreak, we established a new nanopore sequencing method of SARS-CoV-2 (Figure 3), which was able to correctly provide positive test results of SARS-CoV-2 in cases in which routine RT-PCR was negative (Table 2, Supplementary Table 1). Admittedly, nanopore sequencing is a relatively new method for SARS-CoV-2 detection, so we combined it with an antibody test to make a final detection. In the 2003 SARS-CoV infection the antibody detection method showed its value [20], and we have further proved the value of IgM and IgG detection as reported in an earlier study [21] in SARS-CoV-2 infection. The fact that IgM antibody turned from negative to positive, or that there was a 4-fold increase in IgG in the recovery phase compared with that in the acute phase, provides meaningful diagnostic value (Figure 5, Supplementary Table 5). 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 microneutralization antibody), this patient was confirmed to have "SARS-CoV-2 pneumonia, mild case." Additionally, the results from mNGS sequencing suggest 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 3 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 discovered that most regions on the 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 (Figure 4E), the general genomic features and variations could be detected for the virus isolated from our patient. This virus exhibited 4 nonsynonymous mutations on the genes ORF1ab and ORF8 (Figure 4, Supplementary Table 3). Since ORF1ab is 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, this would cause increasing difficulty 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 nonsynonymous mutation on ORF8 (location 28 144) has been recognized as an important virus mutation [23]. According to previous reports, we knew that the virus in this patient could be the older SARS-CoV-2 strain (S typing). The phylogenetic analysis of this virus and previously published SARS-CoV-2 reports illustrated that its genome exhibited a closer relationship with the virus isolated from the same region (Guangzhou, China), and was separate from the virus isolated from the Wuhan region (Figure 4D). Therefore, the evolution process and the origin of

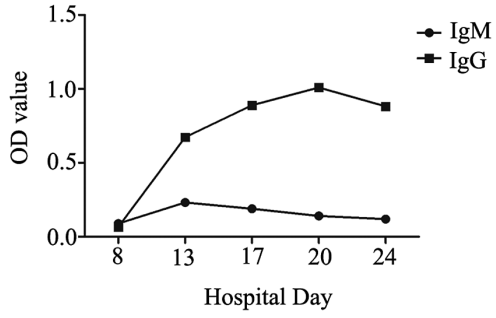

Figure 5. IgM and IgG antibodies of SARS-CoV-2 detected in this patient. The plasma obtained from the patient on hospital days 8, 14, and 24. The levels of IgM and IgG antibody were tested with the ELISA method, and the results shown with the OD value. Abbreviations: ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; OD, optical density.

SARS-CoV-2 still need to be explored. Furthermore, Sanger sequencing has also proved that the NP gene in this virus shows variation (Figure 5, 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 on RT-PCR [24], the gene variations in these genes might have led to the RT-PCR–negative results for this patient.

Admittedly, there are some deficiencies in this study, with the biggest drawback being the lack of 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 of the 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 patient with confirmed SARS-CoV-2 pneumonia with a negative RT-PCR in Guangzhou, China. The study of this case highlights that nanopore sequencing could be used in the clinical pathogenic diagnosis of SARS-CoV-2 pneumonia, especially when the virus mutation leads to negative routine RT-PCR results. This report also demonstrates that we should focus on the evolution of and variation in the virus as the epidemic develops, and the possibility of false-negative test results and hence to increased difficulty of epidemic prevention and control in practice. Finally, it suggests that multiple detection methods, including antibody detection, should be used to make a clinical diagnosis, especially when routine RT-PCR results are found to be negative for patients highly suspected to have SARS-CoV-2.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

The article design and writing group: Zhengtu Li, Yinhu Li, Nanshan Zhong, Feng Ye; The patient management and treatment group: Zhengtu Li, Shaoqiang Li, Feng Yang, Qian Jiang, Liyan Chen,Nanshan Zhong, Feng Ye; The clinical sample collection and detection group: Lingdan Chen, Le Yu, Airu Zhu, Jincun Zhao, Wenju Lu.

Acknowledgments. This study has received ethical approval from the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (ethics number: 2020–36), and the patient's informed consent authorization has been obtained.

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