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Case report

Repeat laboratory testing of SARS-CoV-2 is necessary to diagnose COVID-19

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The pandemic of corona virus disease 2019 (COVID-19) caused by SARS-CoV-2 is ravaging the world. Diagnosis and isolation of persons who are infected with SARS-CoV-2 is very important medical emergency to contain the epidemic of COVID-19. To date, the diagnosis of COVID-19 is mainly depending on positive quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) results for SARS-CoV-2. In the present study, we reported that two cases with uncommon symptoms from a family cluster were ultimately diagnosed as COVID-19 after more than twice of collecting samples and qRT-PCR tests were done. It is easy to miss diagnosis of COVID-19 especially for patients with uncommon symptoms. More attention should be paid to observe the clinical characteristics of it and invent more accurate and convenient methods to detect SARS-CoV-2 as soon as possible.

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Introduction

Since December 2019, corona virus disease 2019 (COVID-19), caused by SARS-CoV-2, has been spread to a lot of countries in the world [1–3]. On Jan 30, 2020, the World Health Organization (WHO) announced that the epidemic of COVID-19 is a public health emergency of international concern. In order to control the spread of SARS-CoV-2 infection as soon as possible, diagnosing and isolating patients with COVID-19 is the most important medical emergency. Nowadays, the diagnosis of COVID-19 is mainly depending on positive quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) results for SARS-CoV-2 [4], which is recommended by National Health Commission of the People’s Republic of China (Diagnosis and treatment of pneumonitis caused by new coronavirus (trial version 6) http://www.nhc.gov.cn/xcs/zhcwwj/202003/4856d5b0458141fa9f376853224d41d7.shtml). Here, we reported that two cases with uncommon symptoms from a family cluster were ultimately diagnosed as COVID-19 after more than twice of collecting samples and qRT-PCR tests were performed (Fig. 1). We obtained the written consent from the patients to publish the piece.

Case report

Patient 1, a 36-year-old man, drove a car with his parents (Patient 2, Patient 3) from Wuhan, Hubei Province to Shenzhen, Guangdong Province, China on Jan 22, 2020. On Feb 1, 2020, he had dizziness, mild diarrhea, and no fever and cough. He was admitted to Fever Clinic, Longgang Central Hospital of Shenzhen. Computed-tomography (CT) scans of the chest showed bilateral multiple lobular and subsegmental areas of ground-glass opacity and consolidation (Fig. 2). Then he was hospitalized. Blood routine tests showed that white blood cell were normal and lymphocyte counts did not decrease (Table 1). Twice of nasopharyngeal swabs were collected (on Feb 1, 3, respectively) and both were negative for SARS-CoV-2 tested by qRT-PCR. As a result, he was diagnosed as community-acquired pneumonia (CAP), acute enteritis and treated with antibiotics. Several days later, he felt well and was discharged from hospital on Feb 4. Then he was isolated at home. On Feb 5, he had dry cough, and no fever and shortness of breath. On Feb 8, he was referred to Fever Clinic of Longgang Central Hospital of Shenzhen again. Compared with Chest CT images on Feb 2, Chest CT images on Feb 8 became severer (Fig. 2). Then he was hospitalized again. Blood routine tests showed that white blood cell counts were normal and lymphocyte counts did not decrease (Table 1). Twice of nasopharyngeal swabs were collected (on Feb 8, 10, respectively) and only one (on Feb 10) was positive for SARS-CoV-2 tested by qRT-PCR. Then he was diagnosed with COVID-19, and transferred to the Infectious Diseases Unit of the Third People’s Hospital of Shenzhen for isolation and treatment.
Fig. 1. Relationship of the family members and chronology of symptoms onset, positive chest CT and positive qRT-PCR of them.

Fig. 2. CT scans of the chest of Patient 1. Chest CT images showed bilateral multiple lobular and subsegmental areas of ground-glass opacity and consolidation two days after symptoms onset. Compared with chest CT images on Feb 2, chest CT images on Feb 8 were severer.
Table 1

|                  | WBC count $\times 10^9$/L (3.5–9.5) | NEUT $\times 10^9$/L (1.8–6.3) | NEUT % (40–75%) | LYM $(1.1–3.2) \times 10^9$/L (1.1–3.2) | LYM % (20–50) | PLT count $\times 10^9$/L (125–350) | RBC count $\times 10^9$/L (4.3–5.8) | Hb g/L (130–175) | CRP mg/L (0–10) |
|------------------|------------------------------------|------------------------------|-----------------|------------------------------------------|--------------|------------------------------------|-------------------------------|-----------------|---------------|
| Patient 1        |                                    |                              |                 |                                          |              |                                    |                               |                 |               |
| Feb 2            | 4.72                               | 2.20                         | 46.7            | 2.06                                     | 43.6         | 137                                | 5.16                         | 151             | 19.44         |
| Feb 9            | 4.85                               | 2.12                         | 43.8            | 2.17                                     | 44.7         | 299                                | 5.28                         | 154             | 4.16          |
| Patient 2        |                                    |                              |                 |                                          |              |                                    |                               |                 |               |
| Feb 7            | 4.75                               | 3.39                         | 71.4            | 0.94                                     | 19.8         | 295                                | 4.22                         | 132             | 6.42          |
| Patient 3        |                                    |                              |                 |                                          |              |                                    |                               |                 |               |
| Feb 10           | 5.17                               | 3.02                         | 58.4            | 1.49                                     | 28.8         | 241                                | 4.06                         | 122             | 6.15          |
| Feb 20           | 7.95                               | 5.18                         | 65.2            | 1.91                                     | 24           | 439                                | 3.90                         | 120             | 1.50          |

Note: WBC, white blood cell; NEUT, neutrophil; LYM, lymphocyte; PLT, platelet; RBC, red blood cell; Hb, haemoglobin; CRP, C-response protein.

Fig. 3. CT scans of the chest of Patient 2. Chest CT images showed bilateral multiple lobular and subsegmental areas of ground-glass opacity four days after symptoms onset.

Patient 2, a 62-year-old woman (mother of Patient 1) had a history of diabetes for 10 years. She had fatigue, and no fever, no dry cough and diarrhea on Feb 3, 2020. She was admitted to Longgang Central Hospital of Shenzhen on Feb 7. Blood routine tests (on Feb 7) showed that white blood cells were normal, and lymphocyte counts decreased (Table 1). Chest CT images (on Feb 7) showed bilateral multiple lobular and subsegmental areas of ground-glass opacity (Fig. 3). Three times of nasopharyngeal swabs were collected (on Feb 8, 10, 12, respectively), and one (on Feb 10) was suspiciously positive, and one (on Feb 12) was positive for SARS-CoV-2 tested by qRT-PCR. Then she was diagnosed with COVID-19, and transferred to the Infectious Diseases Unit of the Third People’s Hospital of Shenzhen for isolation and treatment.

Patient 3, a 71-year-old man (father of Patient 1), had dry cough on Feb 9, 2020. He had no fever, fatigue, and diarrhea. Then he was admitted to Longgang Central Hospital of Shenzhen on Feb 10. Chest CT images showed bilateral multiple lobular and subsegmental areas of ground-glass opacity (Fig. 4). Furthermore, blood routine tests (on Feb 11) showed that white blood cell counts were normal and lymphocyte counts did not decrease (Table 1). However, seven times of nasopharyngeal swabs were collected (on Feb 11, 12, 13, 14, 15, 16, 17, respectively) and none was positive for SARS-CoV-2 tested by qRT-PCR. Then the patient was diagnosed as community-acquired pneumonia (probably caused by virus), and was isolated in the hospital. He was administrated with oseltamivir and supportive therapy. On Feb 16, 2020, chest CT images were same as that of CT images on Feb 11. On Feb 20, 2020, CT scans of the chest was tested again and showed that chest CT images became better than that of chest CT tested on Feb 16, 2020 (Fig. 4). Then he was excluded from COVID-19.

Discussion

Although SARS-CoV-2 mainly enters into lung cells and replicates in lung cells, it firstly has to pass nasopharyngeal tract, and then enters into the lung. Furthermore, the upper respiratory tract expresses angiotensin-converting enzyme 2 (ACE2) which is the best-known receptor for SARS-CoV-2 [5,6]. Viral particles of SARS-CoV-2 also exhibit “enhanced binding” to the olfactory epithelium [7]. The transmission route of SARS-CoV-2 is mainly...
through airborne transmission of virus-containing saliva droplets produced by speaking, sneezing or coughing [8–10]. Moreover, collecting nasopharyngeal swab sample is more convenient method for patients with dry cough. Taken together, PCR of nasopharyngeal samples is regarded as a vital method to detect nucleic acid of SARS-CoV-2 because of collecting nasopharyngeal samples simply and fast [7,11–13], which is recommended by WHO (https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected).

Recently, it is reported [14] that after symptoms onset of COVID-19, higher RNA loads of SARS-CoV-2 are discovered and viral RNA loads in the nose are higher than that in the throat. Moreover, RNA loads of SARS-CoV-2 in the asymptomatic virus carrier are comparable to that in the patient with symptoms. In the present report, in order to diagnose COVID-19, four times of nasopharyngeal swabs were collected from Patient 1 and three times of nasopharyngeal swabs were collected from Patient 2. Because Patient 3 had closely contacted with Patient 1 and Patient 2, because he had dry cough, and because his white blood cell counts were normal and chest CT images showed ground-glass opacity, which were clinical features of COVID-19 [1,2], he was highly suspected of SARS-CoV-2 infection. However, seven times of nasopharyngeal swabs were collected from Patient 3, and none of them was positive for SARS-CoV-2 assayed by qRT-PCR. So we believe that repeat laboratory testing of SARS-CoV-2 is necessary to diagnose COVID-19 or exclude the diagnosis of COVID-19.

Eighty percent of nucleotide of SARS-CoV-2 is identity to the original SARS virus. The N protein of SARS-CoV-2 is with about 90% of amino acid identity to the N protein of SARS-CoV [15]. The RBD sequences of SARS-CoV-2 are more closely related to those of SARS-CoVs (73.8–74.9% amino acid identity). Moreover, the RBD of the spike protein from SARS-CoV-2 is only one amino acid longer than the RBD of the spike protein from SARS-CoV [16]. As a result, SARS-CoV-2 appears to be optimized for binding to the human receptor ACE2 [17]. SARS-CoV-2 seems to have a receptor-binding domain (RBD) that binds with high affinity to ACE2 from humans [17]. Furthermore, relative to SARS-CoV, SARS-CoV-2 S harbors a furin cleavage site at the S1/S2 boundary cleavage by furin-like proteases, which could take part in expanding cell and tissue tropism of SARS-CoV-2, as well as increasing its transmissibility and/or altering its pathogenicity [18]. The two highly pathogenic CoVs could be transmitted from human to human. It is reported that compared to transmission between relatives occurring in 22–30% of SARS-CoV cases [19], the transmission route occurs in 57.6% of the total COVID-19 confirmed cases [20,21]. Taken together, we think that SARS-CoV-2 could be very “crafty” relative to SARS-CoV.

Fever, dry cough and fatigue are the most common symptoms of COVID-19 according to previous reports [1,2,22,23], whereas none of the three patients had fever and the earliest symptoms of Patient 1 were dizziness and diarrhea in the present report. Furthermore, there were some differences in symptoms of the three patients from a family cluster. Recently, we had already reported several COVID-19 cases with different symptoms from two family clusters. One was a family with three members, of whom one had fever, sore throat, arthralgia, and myalgia, and the other two cases did not show any symptoms [24]. The other was a family with six members, of whom three cases did not show any symptoms, and one case only had a fever, and the other two cases were diagnosed as severe.

![CT scans of the chest of Patient 3. Chest CT images showed bilateral multiple lobular and subsegmental areas of ground-glass opacity and consolidation two days after symptoms onset. Compared with Chest CT images on Feb 16, Chest CT images on Feb 20 were better.](image-url)
COVID-19 [25]. Taken together, all of these make the diagnosis of COVID difficult.

Any method could have some defects, and PCR also has some defects, such as false negative or false positive results [26,27]. As a result, a suspect could be diagnosed as a confirmed COVID-19 case after the suspect is positive for nucleic acids of SARS-CoV-2 at least twice. Moreover, there might be errors in the process of collecting nasopharyngeal samples and sample preparation. It should be taken into account that a false negative result is possibly obtained in the early stages of infection. Only after about 7 days from the human contact with the virus, molecular tests can show maximum sensitivity [12]. Taken together, we think that repeat laboratory testing of SARS-CoV-2 is necessary to diagnose COVID-19, especially for suspects, in order to avoid missing diagnosis.

It is accepted that a technique allowing confirmation of COVID-19 incidence is a CT (computed tomography) scan [12]. The resulting image corresponds to pneumonia. In a lot of cases, CT scans of the chest are decisive for excluding or confirming the diagnosis, but pathologies are not often found in patients. The image of ground-glass opacity which approximately 56% to over 76% of patients occurs, is the typical element of COVID-19. Bilateral patchy shadowing and segmental consolidation are also common [28,29]. Serological tests which allow the detection of antibodies in the patient's serum or blood or antigens in specimens from the respiratory tract are performed to diagnose COVID-19 [7,12,29]. According to the last WHO recommendation (https://apps.who.int/iris/handle/10665/334254. accessed on 18 March 2021), antigen-based tests could be implemented in the COVID-19 diagnostic algorithm if the performance of the test is acceptable.

In sum, PCR of nasopharyngeal samples, CT scans of the chest, and serological tests should be implemented in the COVID-19 diagnostic algorithm in order to confirm COVID-19 cases as soon as possible.

Conclusion

It is easily to miss diagnosis of COVID-19 especially for patients with uncommon symptoms, because the virus might be very “crafty”. More attention should be paid to observe the clinical characteristics of it and invent more accurate and convenient methods to detect SARS-CoV-2 as soon as possible.

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Competing interests

None declared.

Ethical approval

None declared.

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