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CORRESPONDENCE

Next-generation sequencing revealed influenza and *Chlamydia* infection in recurrent pneumonia in a patient who had recovered from COVID-19

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Dear Editor,

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the cause of an outbreak of a global pandemic of coronavirus disease 2019 (COVID-19) in December 2019.[1] COVID-19 often starts with non-specific upper respiratory tract symptoms, making it difficult to distinguish from other diseases that present similar symptoms, such as fever, lung infection, and asthenia.[2,3] At present, clinically, patients with fever or respiratory tract symptoms are primarily considered as having COVID-19. Here, we report a unique case of recurrence of suspected COVID-19 ground-glass opacity-like pneumonia in a 53-year-old patient 82 days after recovery from COVID-19. The patient was subsequently diagnosed with influenza virus co-infection and *Chlamydia pneumoniae*, based on next-generation sequencing (NGS).

The patient presented to Anqing Municipal Hospital (Anhui province) with a positive result on nasopharyngeal swabs for SARS-CoV-2, and was discharged in good clinical condition after consecutive negative results on 9 February 2020. On 1 May 2020, a new ground-glass opacity (GGO) was found in the patient's right lung during a random chest CT scan (Fig. 1A). The patient's vital signs were as follows: temperature 37.4 °C; leukocyte count 2.89 × 10^9/L; and white blood

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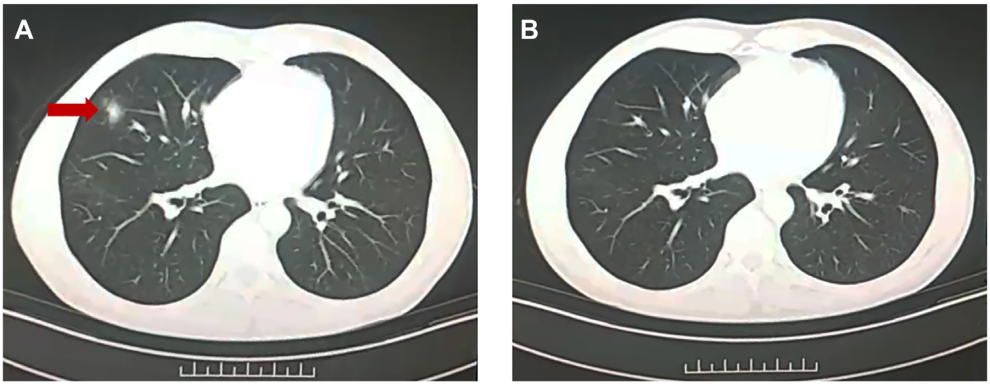

Figure 1. CT scan of the chest. (A) Chest computed tomography demonstrated a new ground-glass opacity (GGO) in the right lung upon readmission on 1 May 2020. (B) Chest computed tomography showed completely absorbed lesion when he was discharged on 28 May 2020.

Table 1. Main pathogens of alveolar lavage fluid sequenced by next-generation sequencing.

No.	%	Reads	Genus	No.	%	Reads	Genus
1	72.1	28 366 988	Unclassified	15	0.233	91 578	*Listeria*
2	6.948	2 733 676	*Chlamydia*	16	0.205	80 778	*Idiomarina*
3	4.764	1 874 455	Cannot be assigned to a genus	17	0.197	77 399	*Klebsiella*
4	2.387	939 292	*Enterococcus*	18	0.195	76 888	*Salmonella*
5	1.956	769 431	*Lingulodinium*	19	0.18	70 859	*Epulopiscium*
6	1.381	543 303	*Bacillus*	20	0.162	63 717	*Curvibacter*
7	1.278	502 795	*Acinetobacter*	21	0.142	55 903	*Clostridioides*
8	1.152	453 243	*Plasmodium*	22	0.111	43 483	*Sarcocystis*
9	0.55	216 421	*Pseudomonas*	23	0.109	42 722	*Kangiella*
10	0.491	193 240	*Clostridium*	24	0.107	41 987	*Neisseria*
11	0.384	151 225	*Streptococcus*	25	0.096	37 916	*Enterobacter*
12	0.362	142 335	*Escherichia*	26	0.095	37 467	*Burkholderia*
13	0.34	133 773	*Mycobacterium*	27	0.095	37 280	Viruses
14	0.322	126 861	*Staphylococcus*				

cell 1.18 $\times$ 10^9/L. Relapsed COVID-19 was suggested based on his disease history, clinical symptoms, and CT findings.[4] Therefore, we performed nucleic acid testing for COVID-19 and antibody tests for other viruses including adenovirus, Coxsackie B virus, influenza virus type A, influenza virus type B, parainfluenza virus, respiratory syncytial virus, *Mycoplasma pneumoniae* antibody, and *Chlamydia pneumoniae* antibody. All test results were negative. Next, we collected a deep respiratory alveolar lavage fluid specimen by bedside bronchoscopy for nucleic acid testing and NGS. The NGS result was also negative for COVID-19 but positive for influenza and *Chlamydia* virus and other pathogenic bacteria. As shown in Table 1, 0.095% viruses and 6.948% *Chlamydia*, which were presumed to be the main cause of pneumonia, were sequenced in the alveolar lavage fluid. Influenza A and Influenza B of different subtypes and origins were found at the same time, including Influenza B virus (Connecticut/Flu110/2013), Influenza A virus H1N1 subtype (Brazil/RS-3335/2009), Influenza A virus H3N2 subtype (Brazil/RS-3335/2009), and Influenza A virus H10N6 subtype (American green-winged teal/Alaska/11508/2006). Based on the clinical symptoms and sequencing results, he was started on oseltamivir and antibiotics to treat virus infection, *Chlamydia* infection, and other bacterial infections. On 28 May 2020, the patient was discharged from the hospital without any fever, cough, or chest tightness; chest CT at discharge showed that the lesion in the right lung was completely absorbed (Fig. 1B).

Patients with COVID-19 often have symptoms such as fever, cough, and asthenia. Most importantly, their blood tests typically show leukopenia and lymphopenia, and their chest CT scans show increased lung texture and GGO. In most cases, chest CT offers a faster and more convenient evaluation of patients with suspected COVID-19 pneumonia than does nucleic acid testing.[5,6] However, because of overlapping clinical manifestations, it is difficult to distinguish COVID-19 from other causes of respiratory illness. Worldwide, increasing incidence has been reported of co-infection with SARS-CoV-2 and other respiratory viruses.[3] In our report, the patient recovered from COVID-19 and developed a lung infection with GGO 82 days later, after being discharged from hospital. However, we detected influenza virus and *Chlamydia* sequences by NGS. The diagnosis of co-infection with influenza and *Chlamydia* by sensitive means played an important role for subsequent treatment.

To date there are no effective treatments for the COVID-19 pandemic, and the race to develop more accurate diagnosis and treatments continues to be the focus of the global healthcare system. Recently, some scientists provided evidence of patients with COVID-19 being re-infected with SARS-CoV-2,[7,8] which raised concerns in terms of massive recurrent pneumonia in patients with COVID-19 caused by SARS-CoV-2 infection, and that SARS-CoV-2 may continue to circulate among human populations despite herd immunity from natural infection or vaccination. However, this case could reduce concerns about the recurrence of COVID-19 pneumonia, in that seasonal flu and other respiratory pathogens, but not SARS-CoV-2, might contribute to pneumonia after successful recovery from COVID-19. This case also highlights some challenges in diagnosis of COVID-19 and other respiratory tract infections. First, possible underestimation of COVID-19 because of a high number of false-negative tests for upper respiratory specimens has been emphasized by many experts.[3] In the present case, although the nasopharyngeal swabs were negative for SARS-CoV-2, the patient was still presumed to have COVID-19 infection at readmission based on the imaging findings and recent clinical history. Second, according to the current diagnostic rates of both SARS-CoV-2 and other respiratory infections, it will be necessary to comprehensively evaluate imaging and other clinical findings as well as consider possible co-infection with other respiratory viruses.[9] Early diagnosis of concurrent respiratory pathogens is important to improve preventive measures, clinical management, and outcome.[10] Lastly, more sensitive and accurate methods of diagnosis such as NGS should be considered, especially when the patient has a negative antibody test and nucleic acid test but shows signs of serious infection.

Author contributions

S.Z and J.W designed the research. W.W. collected data and prepared the manuscripts. G.Z. and W.T. performed the experiment. S.L., B.Z., J.S., and L.W. provided clinical input. S.Z. and J.W supervised the project.

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Conflict of interest statement

None declared.

References

1. Huang C, Wang Y, Li X, *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;**395**:497–506. doi:10.1016/S0140-6736(20)30566-3.
2. Zhang JJ, Dong X, Cao YY, *et al.* Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 2020;**75**:1730–17 41. doi:10.1016/S0140-6736(20)30183-5.
3. Wu X, Cai Y, Huang X, *et al.* Co-infection with SARS-CoV-2 and Influenza A Virus in Patient with Pneumonia, China. *Emerg Infect Dis* 2020;**26**:1324–132 6. doi:10.3201/eid2606.200299.
4. Li S, Zong Z, Sun X, *et al.* New evidence-based clinical practice guideline timely supports hospital infection control of coronavirus disease 2019. *Precis Clin Med* 2020;**3**:1–2. doi:10.1093/pcmedi/pbaa008.
5. Yang Z, Li Y, Pang T, *et al.* HRCT imaging features in representative imported cases of 2019 novel coronavirus pneumonia. *Precis Clin Med* 2020;**3**:9–13. doi:10.1093/pcmedi/pbaa004.
6. Ai T, Yang ZL, Hou HY, *et al.* Correlation of chest CT and RT-PCR testing for coronavirus disease 2019 (COVID-19) in China: A report of 1014 cases. *Radiology* 2020;**296**:E32–40. doi:10.1148/radiol.2020200642.
7. To KK, Hung IF, Ip JD, *et al.* COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing. *Clin Infect Dis* 2020; doi:10.1093/cid/ciaa1275.
8. Zhang K, Lau JY-N, Yang L, *et al.* SARS-CoV-2 reinfection in two patients who have recovered from COVID-19. *Precis Clin Med* 2020; doi:10.1093/pcmedi/pbaa031.
9. Zhang W, Zhao X, Wu W, *et al.* Recommended psychological crisis intervention response to the 2019 novel coronavirus pneumonia outbreak in China: a model of West China Hospital. *Precis Clin Med* 2020;**3**:3–8. doi:10.1093/pcmedi/pbaa031.
10. Guan WJ, Ni ZY, Hu Y, *et al.* Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020;**382**:1708–17 20. doi:10.1056/NEJMoa2002032.