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Pathogenic analysis of suspected COVID-19 patients in a SARS-CoV-2 non-epidemic area of China

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Abstract. – OBJECTIVE: The aim of this study is to find the distributions of pathogens in 164 suspected COVID-19 patients from the outpatient clinic of Shenjing Hospital of China Medical University from 24th January, 2020, to 29th February of 2020.

PATIENTS AND METHODS: 164 COVID-19 suspected patients were from the Shengjing Hospital of China Medical University. Oropharyngeal swab specimens were acquired by respiratory doctors under standardized conditions. Specific nucleic acids of SARS-CoV-2, influenza A and B, respiratory syncytial virus A and B, adenovirus, parainfluenza virus, along with pneumonic mycoplasma were detected by real-time fluorescence PCR. Symptomatic, epidemiologic, laboratory and radiological data of the patients were obtained from the electronic medical record system of our hospital.

RESULTS: Among the 164 patients, 3 were positive for SARS-CoV-2, 15 were positive for other respiratory viruses and 16 were positive for pneumonic mycoplasma. Of the positive patients above, 1 patient was co-infected with SARS-CoV-2 and adenovirus, and 1 was co-infected with influenza B and pneumonic mycoplasma. The 3 SARS-CoV-2 infected patients were clinically diagnosed as COVID-19 because they meet the diagnostic criteria listed in "Chinese Clinical Guidance for COVID-19 Pneumonia diagnosis and treatment", including epidemic history, symptom and pathogenic detection, as well as abnormalities of the laboratory and radiological data. However, the clinical characteristics of COVID-19 patients were non-specific compared to those of the patients infected with other respiratory viruses.

CONCLUSIONS: The endemic common respiratory pathogens are more prevalent than SARS-CoV-2 in the SARS-CoV-2 non-epidemic areas of this research. Detection of the pathogen is the unique means for definite COVID-19 diagnosis.

Key Words:
Respiratory pathogens, Real-time fluorescence PCR, COVID-19.

Introduction

A large global outbreak of the Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been declared a worldwide pandemic by World Health Organization (WHO)[1,2] on March 11th, 2020. On May 11th, 2020, data from the WHO showed that more than 4 million confirmed cases have been identified in more than 200 countries/regions. However, the infection rates of SARS-CoV-2 are great different among different countries or regions, which is affected by complex medical or social factors, as well as the epidemic prevention and control to the disease. In the authors' city with about 8 million permanent residents, only 28 COVID-19 cases were confirmed totally from January 24th, 2020, to February 29th, 2020, which was the worst time of epidemic situation of SARS-CoV-2 in China. Viral infections are a major cause of respiratory diseases, especially in winter. In the outpatient clinic, especially for COVID-19, all the patients were preliminarily screened according to the clues of symptoms, epidemiologic surveys, and routine blood tests. However, a large number of patients infected with endemic common respiratory pathogens should be mixed in the suspected SARS-CoV-2 infected patients. It is important to distinguish COVID-19 from other respiratory infectious diseases for accurately judging the epidemic situation and avoiding public panic. Furthermore, the identification of pathogens should provide the premise for a precise treatment.

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In the present study, the oropharyngeal swab specimens of 164 COVID-19 suspected patients were collected from Shengjing Hospital of China Medical University from 24th January to 29th February of 2020. These specimens were tested synchronously for the nucleic acids of SARS-CoV-2, influenza A and B viruses (flu A, flu B), respiratory syncytial virus A and B (RSV A, RSV B), adenovirus (ADV), parainfluenza virus (PIV) and pneumonic mycoplasma (MP) using real-time fluorescence PCR. The results of pathogenic detection were analyzed by synthesis with the symptoms, clinical blood examinations and lung imaging features to provide a more credible diagnosis basis for the suspected patient.

Patients and Methods

Patients

A total of 164 suspected SARS-CoV-2 infected patients enrolled in this investigation were from the outpatient department of Shengjing Hospital of China Medical University between January 24th, 2020, to February 29th, 2020. This outpatient department was responsible for the screening of COVID-19 patients as assigned by the government. The definition of suspected cases of COVID-19 was based on "Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and treatment" (Trial Version Third to Fifth) issued by the National Health Commission of China[3]. The clinic manifestations included fever, respiratory symptom, imageology characteristics of viral pneumonia and normal or reduced leukocytes with or without reduced lymphocyte. The epidemiologic requirements comprised traveling or living in Wuhan city or Hubei province within 14 days, and/or in the event of close contact with a confirmed COVID-19 case. The standards of suspected cases of COVID-19 changed during the study period from meeting any two clinic evidence and one of epidemiologic data to meeting all the clinic data above with or without epidemiologic data.

Data Collection

Laboratory and radiological characteristics data were obtained from the electronic medical record system (HIS) of our hospital. Laboratory data included a complete blood count, serum biochemical tests for liver function (AST, ALT) and myocardial injury (CK, CK-MB), a coagulation marker of D-dimer, and C-reactive protein (CRP), all of which had been enrolled in the "Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and treatment" published by China National Health Commission.

Nucleic Acid Detection of SARS-CoV-2 and Other Pathogens by Real-Time PCR Method

Oropharyngeal swab specimen was acquired by respiratory doctors under standardized conditions. The swab specimens were placed in viral transport medium and were sent to be tested immediately or kept at -20°C less than 24 hours before testing. Total nucleic acid was extracted from 200 μl of the viral transport medium by total nucleic acid paramagnetic particle extraction kit (Jiangsu Mole Biotechnology Co., Ltd. Taizhou, Jiangsu, China, Registration number: 20140015) using NP988 automatic nucleic acid extractor (Xian Tianlong Biotechnology Co., Ltd. Xian, Shanxi, China). Three genetic fragments, including open reading frame 1ab (ORF1ab), nucleocapsid protein gene (N) and envelope protein gene (E), or two of them (ORF1ab and N), were detected simultaneously for SARS-CoV-2 using two one-step reverse transcription real-time PCR (RT-PCR) kits, respectively. Six other common respiratory viruses, including flu A, flu B, RSV A, RSV B, ADV, PIV, along with MP, were detected using the same nucleic acid samples as detection of SARS-CoV-2 by real-time PCR method. The PCR kits, reaction systems and PCR procedures are listed in Table I.

Statistical Analysis

Enumeration data were compared with the chi-squared test. The continuous variables were compared using independent group *t*-tests when the data were normally distributed. Differences were considered significant at $p<0.05$ with a two-tailed test. All analysis was performed using the statistical software GraphPad prism Vision 5.0 (La Jolla, CA, USA).

Results

Detection of Respiratory Pathogens

Among the total 164 patients, 3 were confirmed positive for SARS-CoV-2 by testing simultaneously with two different RT-PCR kits, and 15 were positive for other respiratory viruses, including 7 for ADV, 4 for flu B, 2 for flu A and 2 for RSV A. In addition, 16 patients were

Table I. Information of RT-PCR used in this study.

Pathogens	RT-PCR reaction condition	RT-PCR kit
SARS-Cov-2 (ORF1ab/ N/E)	45°C 10 min, 95°C 3 min, 95°C 15 sec→58°C 30 sec 45 cycle, 58°C data collection	SARS-Cov-2 real-time PCR kit (Shanghai Zhijiang Biotechnology Co., Ltd. Shanghai, China, Registration number: 20203400057)
SARS-Cov-2 (ORF1ab/N)	50°C 10 min,95°C 5 min, 95°C 10 sec→55°C 40 sec 40 cycle, 55°C data collection	SARS-Cov-2 real-time PCR kit (Shanghai Bojie Biotechnology Co., Ltd. Shanghai, China, Registration number: 20203400058)
fluA and flu B	50°C 30 min, 95°C 5 min, 95°C 10 sec→55°C 40sec 45 cycle,55°C data collection	Influenza A and B virus real-time PCR Kit. (Jiangsu Bioperfectus Technologies Co., Ltd. Taizhou, Jiangsu, China, Registration number: 20163401432)
RSV A and RSV B	50°C 30 min, 95°C 5 min, 95°C 10 sec→55°C 40 sec 45 cycle, 55°C data collection	Respiratory syncytial A and B virus real-time PCR Kit. (Jiangsu Bioperfectus Technologies Co., Ltd. Taizhou, Jiangsu, China, Registration number: 20163400799)
ADV	50°C 2 min, 94°C 2 min, 94°C 10 sec→55°C 30 sec→72°C 15 sec 40 cycle, 55°C data collection	Adenovirus real-time PCR Kit. (Shenzhen purekang Biotechnology Co., Ltd. Shenzhen, Guangdong, China, Registration number: 20163400197)
PIV	48°C 5 min, 94°C 2 min, 94°C 10 sec→55°C 35 sec 40 cycle, 55°C data collection	Parainfluenza virus type 1&3 nucleic acid detection kit. (Guangdong Hexin Health Technology Co., Ltd. Dongguan, Guangdong, China, Registration number: 20183400585)
MP	50°C 2 min, 94°C 5 min, 94°C 10 sec→60°C 45 sec 40 cycle, 60°C data collection	Pneumonic mycoplasma nucleic acid detection kit. (Shanghai Fosun Long March Meical Science Co., Ltd. Shanghai, China, Registration number: 20153401899)

positive for MP. Among the infected patients, 1 was positive both for SARS-CoV-2 and ADV, and 1 was positive both for flu B and MP. While, RSV B and PIV infections were not found in the studied population. The detection rate of the pathogens in the studied population is 18.90% (31/164) totally.

Clinical Characteristics and Epidemiological Data

The common symptoms of all the patients included fever (90.24%, 148/164), cough (66.46%, 109/164) and expectoration (28.65%, 47/164). The patients infected with the detected pathogens had higher maximum body temperature than the other patients (38.46±0.14 *vs.* 37.97±0.08°C t=2.76, p<0.01). All of the 3 SARS-CoV-2 positive patients had body temperature higher than 38.0°C. Two of them had the symptom of cough, and none of them had expectoration. Other respiratory symptoms were sore throat (6.10%, 10/164), chest tight (10.36%, 17/164) and dyspnea (5.49%, 9/164). Nearly half of all patients enrolled in this research had nonspecial symptoms, including hypodynamia and muscle or joint pain.

Among the 3 COVID-19 patients, one had a travel history of Hubei province within five days before the onset of symptoms. The second patient took a journey five days before by train, where he contacted with a confirmed COVID-19 patient. And the last one is a taxi driver, who had taken the same confirmed COVID-19 patient as the second patient has contacted with.

The Features of CT Images

Among the 164 patients, 160 have undergone chest computed tomography (CT). Pneumonia related abnormalities in chest CT images were found in 137 patients (85.63%, 137/164). The common abnormalities included ground-glass opacities (GGOs), consolidation and interlobular thickening. Most of the radiological findings presented in these patients are generic and can be seen in many viral or bacterial pneumonia. Of the 137 patients, 62 (45.26%) had bilateral involvements. The 3 COVID-19 patients showed bilateral GGOs and subsegmental areas of con-

solidation. Of the total 31 patients infected with the known pathogens, 13 (41.94%) were bilateral, 15 (48.39%) were unilateral and 3 (9.68%) were normal images. Of the other 133 patients, which were RT-PCR negative, 49 (36.57%) were bilateral, 60 (44.78%) were unilateral and 24 (17.91%) were normal images. Although the percentages of the patients with abnormal and bilateral chest CT images in RT-PCR positive patients were higher than those in negative patients, no significant difference was found between them (p=0.419; 1.000).

Laboratory Data

More than 70% of patients (71.34%, 117 of 164) had normal or decreased white blood cell (WBC) counts. Seven patients infected with the known pathogens (5 MP positive, 1 ADV positive and 1 fluA positive) and 40 RT-PCR negative patients had increased WBC counts. Of the total 164 patients, 59 (35.98%) had lymphocytopenia. All the 3 COVID-19 patients showed normal WBC counts with lymphocytopenia. However, neither the ratios of lymphocyte to neutrophil nor the lymphocyte counts showed significantly different between the positive and negative patients (t=0.16, p=0.29; t=0.90, p=0.37).

It was found that CRP increased in 92 (56.1%) of the 164 patients. The percentage of patients with CRP abnormally increased in the 31 RT-PCR positive patients was 77.41% (24/31), which was significantly higher than that (51.13%, 68/133) in negative patients (x^2=7.056, p=0.008). And the CRP median values in patients with bilateral pneumonia and unilateral pneumonia were of 63.62 mg/L and 33.52 mg/L respectively, which is statistically significant (t=2.652, p=0.009). Of the 3 COVID-19 patients, 2 showed abnormally increased D-dimer levels. However, the percentages of D-dimer increased patients in RT-PCR positive and negative patients were not significantly different (x^2=0.9716, p=0.3243).

Although the percentages of the patients with abnormally increased levels of AST, ALT, CK and CK-MB in RT-PCR positive patients were all higher than those in negative patients, but no statistically significant differences were found between the two groups. Of the 3 COVID-19 patients, 2 showed abnormal increasing levels of AST and ALT along with at least one of CK and CK-MB. The third one showed normal for all of the biochemical markers. The detailed data are listed in Table II.

Discussion

We reported the distributions of pathogens and clinical characteristics of 164 suspected SARS-CoV-2 infected patients from Shengjing Hospital of China Medical University in Shenyang, the biggest city in the northeast of China, during the worst time of COVID-19 outbreak in China. The 164 patients were screened rigorously as the suspected cases according to the "Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and treatment" issued by the National Health Commission of China. However, only 3 patients were identified as the COVID-19 confirmed cases. Nearly twenty percent of total patients were identified to be infected with other respiratory viruses or MP.

Community-acquired respiratory infections caused by various viruses and MP are very common during winter in northern China. The most popular pathogens are MP, flu A/B, ADV, RSV and PIV et al[4,5]. In this study, most of the suspected patients are non-SARS-Cov-2 infection identified by pathogenic tests. Among of the infected patients, more than half of them (16/31, 51.62%) were infected by MP, in which one patient showed multiple infections with SARS-Cov-2. The others of the infected patients were infected by ADV (6/31, 19.35%), fluB (4/31, 12.90%), fluA (2/31, 6.45%) and RSV A (2/31, 6.45%), respectively. It is very meaningful to identify MP and fluA infections from the patients because of efficient drugs being available for MP and fluA treatment. The percentages of the patients with abnormal laboratory data in the RT-PCR positive patients were higher than those in the negative patients, although no statistical difference was found for the limit cases. This result implied that the pathogens in severe patients are easy to be detected than those in mild patients. However, the total pathogenic detection rate in this study is less than 20%. As only 7 kinds of respiratory viruses and MP were included in this study, it should limit the screening of potential pathogens in the respiratory tract.

It has been reported that the detection rate of pathogens in the oropharyngeal swab specimen was usually lower than that in the specimens from deep lower respiratory tract[6]. However, some patients with viral pneumonia cough without sputum, and the bronchoalveolar lavage fluid specimen is usually difficult to be obtained. Repeated detection of oropharyngeal swab specimens in the early infection stage should be the most feasible way to improve the detection rate.

Table II. Laboratory data of the suspected COVID-19 patients.

	Infected by					Total	Non-infection	χ^2 and *p*-value
	SARS-CoV-2	Other viruses	MP	SARS-CoV2 &ADV co-infection	Flu B & MP co-infection			
White cell count, $\times 10^9$/L (3.5-9.5)								
< 3.5	0	0	0	0	0	0	7	$\chi^2 = 2.800$
3.5-9.5	2	10	10	1	1	24	85	$p = 0.247$
> 9.5	0	2	5	0	0	7	40	
Lymphocytes, $\times 10^9$/L (1.1-2.7)								
< 1.1	1	8	5	0	1	14	47	$\chi^2 = 1.353$
1.1-2.7	1	4	7	1	0	13	71	$p = 0.508$
>2.7	0	1	3	0	0	4	15	
CRP, mg/L (0-8)								
0-8	0	4	0	0	0	4	58	$\chi^2 = 10.081$
> 8	2	8	15	1	1	27	75	$p = 0.0015$
D-dimer, μg/L (0-252)								
0-252	1	8	14	1	0	24	91	$\chi^2 = 0.9716$
> 252	1	4	1	0	1	7	42	$p = 0.3243$
AST, U/L (5-34)								
5-34	0	8	11	1	0	20	107	$\chi^2 = 3.654$
> 34	2	4	4	0	1	11	26	$p = 0.0559$
ALT, U/L (0-40)								
0-40	0	11	11	1	0	23	103	$\chi^2 = 0.1492$
>40	2	1	4	0	1	8	30	$p = 0.6993$
CK, U/L (29-200)								
29-200	1	9	13	1	1	25	121	$\chi^2 = 2.747$
> 200	1	3	2	0	0	6	12	$p = 0.0975$
CK-MB, U/L (0-24)								
0-24	1	9	15	1	1	26	125	$\chi^2 = 2.524$
> 24	1	3	1	0	0	5	8	$p = 0.0605$

The common abnormalities of the laboratory data of COVID-19 patients had been listed in the "Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and treatment", which involving lymphocytopenia, increased CRP and D-dimer, and abnormal indexes related to liver function and myocardial injury. In this study, the 3 COVID-19 patients showed most of the abnormalities listed above. However, these abnormalities were also common for other patients. For example, the CK levels of two patients with ADV and fluA infections respectively were 5 times more than the upper limit of normal value, and the ADV infected patient died of fulminant myocarditis finally. The percentage of CRP increased patients in the RT-PCR positive patients was significantly higher than that in the negative patients, and the average value of CRP in bilateral lung involved patients was significantly higher than that in unilateral lung involved patients. Both of the above confirmed the correlation of CRP with intensity of infection[7].

Conclusions

Our investigation demonstrated that the endemic common respiratory pathogens are more prevalent than SARS-CoV-2 in non-epidemic areas of COVID-19. It is difficult to distinguish the infections between the endemic common respiratory pathogens and the SARS-CoV-2 because of their similar clinical features, CT images and laboratory data. The detection of pathogens is a unique means for a definite diagnosis. More sensitive detection methods with a more expanded scope of pathogens are expected in the future.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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