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Clinical Features and Dynamics of Viral Load in Imported and Non-imported Patients with COVID-19

Running title: Comparison of imported and non-imported COVID-19

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The manuscript contains 2 tables and 1 figure.

Highlights

1. Characteristics of COVID-19 in imported and non-imported patients were analyzed.

2. Fever was the most common symptom at the onset of illness.

3. Half of patients had a low-grade temperature with a duration of fever <7 days.

4. Viral load was undetectable for all patients on day 14 in the tertiary group.

5. Virus was detectable on day 14 for 1/3rd of the imported and secondary patients.

Abstract

Objectives: To compare the clinical characteristics and the dynamics of viral load between the imported and non-imported patients with COVID-19.

Design and methods: Data from 51 laboratory-confirmed patients were retrospectively analyzed.

Results: The incubation period in the tertiary group was longer than that in the imported and secondary groups (both P<0.05). Fever was the most common symptom at the onset of illness (73.33%, 58.82%, and 68.42%, respectively), and half of the patients had a low-grade temperature (<38.0℃) with a short duration of fever (<7 days). The CT scan showed that most patients had bilateral pneumonia in the three groups (80.00%, 76.47%, and 73.68%, respectively). Ct values detected from the tertiary patients were similar to those from the imported and secondary groups at the time of admission (both P>0.05). For the tertiary group, the viral load was undetectable for half of the patients (52.63%) on day 7, and all patients on day 14. For 1/3rd of the patients in the imported and secondary groups, the viral load remained positive on day 14 after the admission.

Conclusions: COVID-19 can present as pneumonia with less onset of symptoms, and the infectivity of SARS-CoV2 may gradually decrease in the tertiary patients.

Keywords: COVID-19; SARS-CoV2; Ct value; imported

Introduction

2019 novel coronavirus (nCoV-2019, now known as SARS-CoV-2) infection, which can cause respiratory failure, poses a global threat to public health [1, 2]. The coming 2-3 weeks are crucial in monitoring the situation of community transmission [3, 4].

The imported patients with COVID-19 were those who visited or originated from Wuhan city, and were detected at the end of January 2020; subsequently, secondary and tertiary transmissions occurred nationwide [5]. Person-to-person transmission of SARS-CoV-2 in the hospital and family settings was confirmed in other geographical regions [6]. Although the speed of transmission declined in China, the concerns about the outbreak of pneumonia in other countries are constantly rising [4, 7, 8]. To date, the differences between the cases of imported, secondary, and tertiary transmission remain largely unknown.

Herein, we analyzed the characteristics of COVID-19 in Changzhou city, Jiangsu province. The clinical, laboratory, and radiological characteristics, as well as the dynamics of viral load, were compared between the imported, secondary, and tertiary patients.

METHODS

Patients

A cohort of 51 patients in Changzhou was confirmed with COVID-19 according to the Chinese guideline for diagnosis and treatment of COVID-19 [9] and admitted to the Third Hospital of Changzhou, a designated hospital. These patients were included from January 23 to February 18, 2020. The outcomes were followed up until February 27,

4

2020. The epidemiological history and symptoms data were confirmed by two doctors in a negative-pressure ward. Viral RNA was detected in throat swabs obtained from the patients, and a computer tomography (CT) scan was acquired immediately after symptom onset.

The 51 patients were divided into three groups, including imported (15 cases), secondary (17 cases), and tertiary (19 cases) according to the epidemiological history. The imported patients with COVID-19 were those who visited or originated from Wuhan city, Hubei Province. The secondary patients were defined as those who did not visit or come from Wuhan, but closely contacted with the imported patients. The tertiary cases were those who did not visit Wuhan or contact with the imported patients, but acquired through contact with the secondary cases [10, 11, 12].

All the data of the included individuals were reported to the Chinese Center for Disease Control and Prevention (CDC).

Reverse transcriptase-polymerase chain reaction (RT-PCR) assay

COVID-19 was confirmed according to the cycle threshold (Ct) values of *Orf1ab* and *N* genes by RT-PCR assay. The assay was performed by Changzhou CDC and the Clinical Laboratory of the Third People's Hospital of Changzhou using a commercial kit (Biogerm Medical Biotechnology Co., Shanghai, China). Ct values were inversely related to viral RNA copy numbers [13], and Ct value <40 was considered positive.

Statistical analysis

Continuous variables are expressed as median (IQR) or mean ± standard deviation and compared using Kruskal–Wallis test or one-way ANOVA, followed by post hoc tests to compare the differences between the three groups. Categorical values were expressed as frequencies, and the differences were analyzed using Fisher's exact test. All analyses

were performed using SPSS 23.0 software (Chicago, IL, USA). A two-sided P<0.05 was considered statistically significant.

RESULTS

Demographics and clinical characteristics of patients with COVID-19

The demographic and clinical characteristics of the patients are shown in Table 1. Twelve family clusters were found in the present study. Six patients without any symptoms were diagnosed according to the chest CT scans, and the Ct values detected in throat swabs. 10/15 (66.67%) patients in the imported group were males, while almost half of the patients in the secondary and tertiary groups were females. The patients in the tertiary group were significantly older than those in the secondary group (P=0.03) and had more comorbidities than those in the imported group (χ^2=8.259, P<0.01).

Since none of the patients developed severe pneumonia or ARDS, all patients survived, and no mechanical ventilation was given in the present study. Interestingly, the incubation period for the tertiary group was longer than that for the imported and secondary groups (P<0.01 and P=0.03, respectively).

Fever was the most common symptom at the onset of illness (73.33%, 58.82%, and 68.42%, respectively), and about half of the patients (45.45%, 50.00%, and 61.54%, respectively) had a low-grade temperature (<38.0 °C) with a short duration of fever (<7 days). However, no significant difference was detected in the development of fever and other symptoms, including cough, sputum production, pharyngalgia, fatigue, myalgia, dyspnea, and diarrhea between the three groups (all P>0.05).

Laboratory and radiology findings of patients with COVID-19

Four patients in the tertiary group showed an elevated level (<85 U/L) of alanine transaminase (ALT) and aspartate aminotransferase (AST) at admission. The level of D-

6

dimer, troponina and creatine kinase was normal for all patients.

Leucopenia (white blood cell count $<4\times10^9$/L) was more common in the tertiary group than that in the imported patients (χ^2=8.295, P=0.01) (Table 2). Moreover, B lymphocyte counts were lower in the tertiary patients as compared to those in the imported group (P<0.01).

CT scan showed that most patients had bilateral pneumonia in the three groups (80.00%, 76.47%, and 73.68%, respectively).

Dynamic changes of Ct value in patients with COVID-19

Of the 51 patients, 49 (96.08%) showed positive RNA tests in the throat swabs, while one patient had a positive RNA test in the bronchoalveolar lavage fluid, and another tested positive in the anal swab. As shown in Figure 1, the viral load in throat swabs with respect to days was analyzed after admission.

The Ct values detected from the tertiary patients were similar to those from the imported and secondary groups on admission (both P>0.05). For the tertiary group, the viral load was undetectable for half of the patients (52.63%) on day 7 and all patients on day 14 but was positive for 1/3rd of the patients in the imported and secondary groups on day 14 after the admission.

Discussion

In the present study, we analyzed the clinical, laboratory, and radiological characteristics, as well as the dynamics of viral load in patients with COVID-19. Half of the patients had low-grade (<38.0 ℃) and a short duration of fever (<7 days), while the incubation period was long, and the viral load was undetectable in the early stage in the tertiary group than that in the imported and secondary groups.

Different from SARS-CoV infection, COVID-19 shows less onset of symptoms [14,]

7

[15]. Gao *et al.* [16] reported that the majority of the patients in Wuhan presented a moderate or high temperature (>38.0 ℃), while low-grade fever with or without mild respiratory symptoms was common in the present study because no patients with severe pneumonia were included. Moreover, SARS-CoV-2 causes lower respiratory tract lesions even in patients without any clinical symptoms [1, 17]. Interestingly, six patients in the present study had radiological evidence of pneumonia, but no fever or cough during infection. These findings were in agreement with those from previous studies [16, 18]. Thus, the chest CT scan needs to be monitored routinely in clinical practice.

Although none of the patients developed a severe infection in the present study, the comorbidities were common in tertiary patients, demanding focus on elderly patients. Moreover, low B lymphocyte counts in the tertiary patients may be related to the comorbidities, thereby necessitating additional studies to elucidate the immunological mechanisms during COVID-19 infection [19].

To date, the epidemiological characteristics of COVID-19 are unclear. Notably, the long incubation period suggests the potential transmission route in tertiary, even asymptomatic, patients. Although lopinavir/ritonavir and arbidol have been administered in clinical practice, the effectiveness of inhibiting SARS-CoV2 replication is yet not confirmed [9]. Interestingly, the viral load could not be detected early in tertiary patients; thus, it could be speculated that the infectivity of SARS-CoV2 may gradually decrease.

Nevertheless, the present study has several limitations. First, the incubation period may be short for several patients, who lived in Wuhan, because they can be infected several days before coming to Changzhou. Second, the number of patients is limited, and hence, a multicenter study with a large sample size is essential for further substantiation of these findings.

In conclusion, COVID-19 can present as pneumonia with the onset of fewer symptoms, and the infectivity of SARS-CoV2 may gradually decrease in tertiary patients.

Authors' contributions:

Study design: Yuan Xue and Hong Dai. Data collection: Zhen Zhu, Manman Cui, Chunhua Chen, Cong Chen, and Yuan Xue. Data analysis: Yuan Xue, Tianmin Xu, and Cong Chen. Writing: Yuan Xue, Tianmin Xu, and Cong Chen. All authors read and approved the final manuscript. Tianmin Xu and Cong Chen contributed equally to this work.

Ethical Approval

The study was anonymous, and the protocol was approved by the Ethics Committee of the Third People's Hospital of Changzhou, according to the Declaration of Helsinki, 2013. Written informed consent was obtained from all participants.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgment

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Figure legend

Figure.1 Dynamic changes of cycle threshold (Ct) values in patients with imported, secondary and tertiary COVID-19. Ct, cycle threshold.
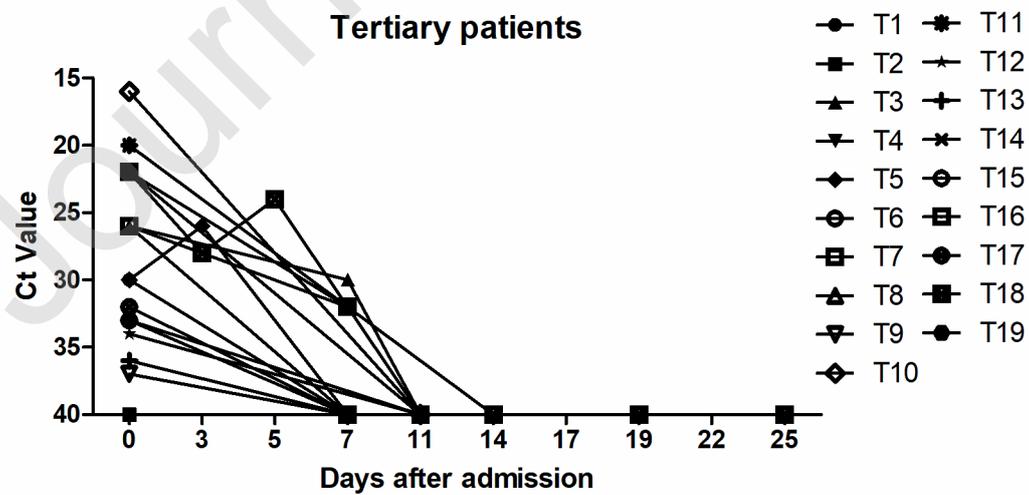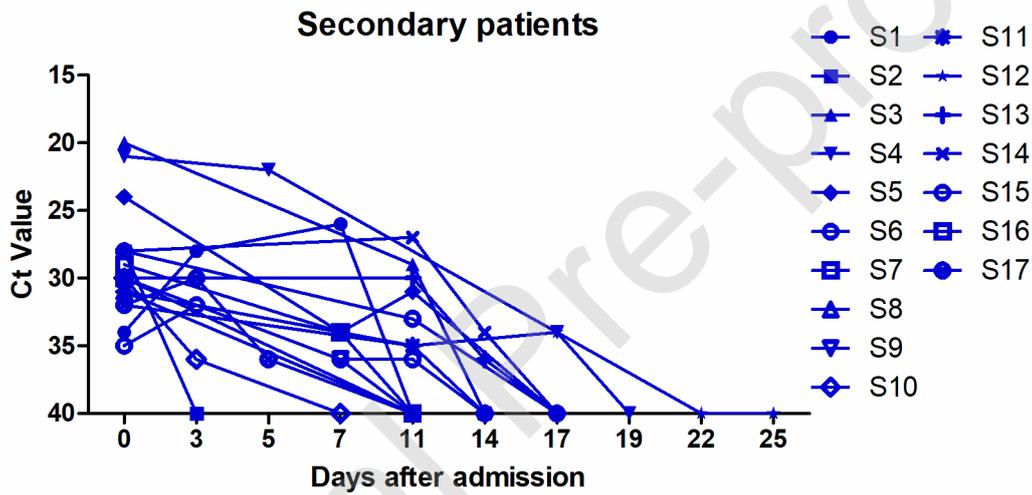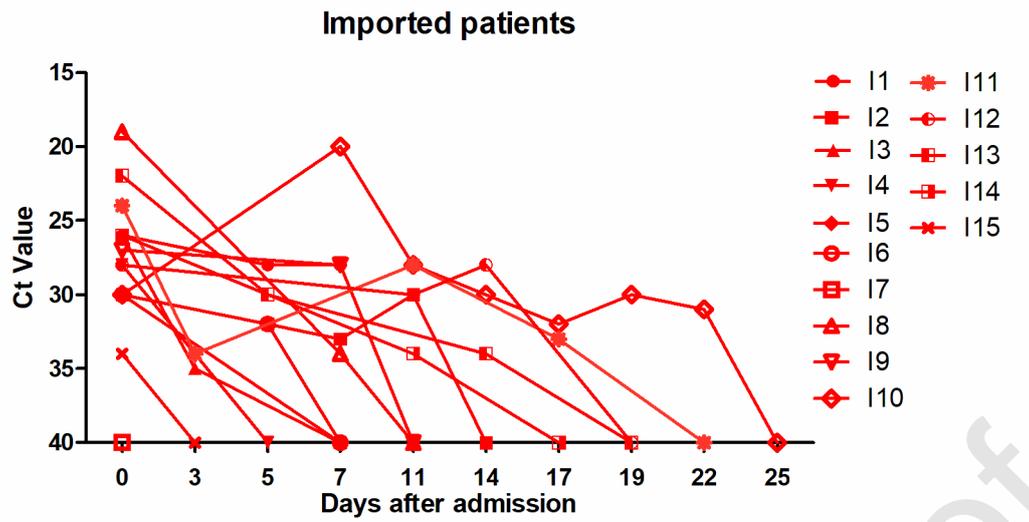
Table 1. Demographics and clinical characteristics of patients with COVID-19

Variables	Imported(n=15)	Secondary(n=17)	Tertiary(n=19)	Z or χ^2	P value
Age, years	35.0(29.0-51.0)	37.0(24.0-47.5)	53.0(35.0-65.0) [*]	5.614	0.06
Male, n (%)	10(66.7)	7(41.2)	8(42.1)	2.601	0.28
Comorbidity, n (%)	0(0)	4(23.5)	8(42.1) [**]	8.766	0.01
Pulmonary disease	0(0)	0(0)	1(5.3)	1.648	0.99
Cardiovascular disease	0(0)	2(11.8)	3(15.8)	2.383	0.36
Diabetes	0(0)	1(5.9)	3(15.8)	2.463	0.37
Chronic liver disease	0(0)	0(0)	1(5.3)	1.648	0.99
Chronic kidney disease	0(0)	1(5.9)	0(0)	1.871	0.63
Incubation period, days	8.0(4.0-10.0)	8.0(4.0-11.0)	12.0(9.0-14.0) [*,**]	10.943	＜0.01
Fever, N (%)	11(73.3)	10(58.8)	13(68.4)	0.820	0.75
Highest temperature＜38℃, n(n/N,%)	5(45.5)	5(50.0)	8(61.5)	0.742	0.76
Duration, days	2.0(0-3.0)	2.0(0-4.0)	2.0(0-5.0)	0.573	0.75
Duration＜7days, n(n/N,%)	11(100.0)	8(80.0)	11(84.6)	2.278	0.43
Cough, n (%)	5(33.3)	9(52.9)	9(47.4)	1.309	0.53
Sputum production, n (%)	3(20.0)	6(35.3)	4(21.1)	1.258	0.56
Pharyngalgia, n (%)	1(6.7)	0(0)	2(10.5)	1.746	0.50
Fatigue, n (%)	1(6.7)	1(5.9)	0(0)	1.547	0.52
Myalgia, n (%)	4(26.7)	2(11.8)	2(10.5)	1.814	0.44
Dyspnea, n (%)	2(13.3)	1(5.9)	1(5.3)	0.982	0.67
Diarrhea, n (%)	2(13.3)	1(5.9)	2(10.5)	0.623	0.86

Data are expressed as median (IQR) and n (%). Comparison was conducted by Kruskal-Wallis test for continuous variables, and Fisher's exact test for categorical values. *, Tertiary *Vs* Secondary, P＜0.05; **, Tertiary *Vs* Imported, P＜0.05.

Table 2. Laboratory and radiology findings of patients with COVID-19

Variables	Imported(n=15)	Secondary(n=17)	Tertiary(n=19)	Z or χ^2	P value
Laboratory findings					
ALT, U/L	22.9(16.6-31.0)	13.5(11.6-21.1)	25.2(11.7-36.2)[*]	7.785	0.02
AST, U/L	19.0(17.0-31.0)	17.0(12.5-19.5)	22.0(17.0-34.0)[*]	9.382	＜0.01
C-reactive protein, mg/L	4.4(1.3-11.2)	2.2(1.0-16.2)	7.0(0.8-20.0)	0.930	0.63
WBC, E+09/L	5.2(4.6-6.3)	4.8(3.4-6.2)	3.9(3.1-5.7)[**]	5.747	0.06
WBC <4 E+09/L, n(%)	1(6.67)	6(35.29)	10(52.63)[**]	8.295	0.01
Lymphocytes, E+09/L	1.5(0.9-2.1)	1.1(1.0-1.9)	1.3(0.9-1.6)	1.077	0.58
CD3+ T lymphocyte, %	69.9(62.9-78.2)	65.9(60.0-72.9)	72.2(65.8-76.1)	2.297	0.32
CD4+ T lymphocyte, %	39.9(38.2-45.7)	37.5(32.2-43.9)	37.4(32.6-40.4)	3.266	0.20
CD8+ T lymphocyte, %	28.7(22.7-33.9)	26.7(20.8-31.9)	30.3(26.0-34.0)	1.592	0.45
B lymphocyte, %	13.9(9.8-18.1)	11.4(8.4-16.1)	9.1(7.5-10.9)[**]	7.425	0.02
CT findings					
Unilateral pneumonia	2(13.3)	3(17.7)	3(15.8)	0.256	0.99
Bilateral pneumonia	12(80.0)	13(76.5)	14(73.7)	0.269	0.99
Multiple mottling and ground-glass opacity	1(6.7)	1(5.9)	2(10.5)	0.517	0.99
Ct value on admission					
ORF1ab gene	28.0(26.0-30.0)	30.0(28.0-31.5)	30.0(22.0-34.0)	1.301	0.52
N gene	30.0(26.0-32.0)	30.0(27.5-32.0)	32.0(26.0-34.0)	0.778	0.68
Ct <40 on day 7, n(%)					
ORF1ab gene	9(60.0)	15(88.2)	9(47.4)[*]	6.767	0.03
N gene	7(46.7)	14(82.4)[***]	7(36.8)[*]	8.088	0.02
Ct <40 on day 14, n(%)					

16

ORF1ab gene	5(33.3)	6(35.3)	0(0) *,**	8.345	0.02
N gene	4(26.7)	5(29.4)	0(0) *,**	6.530	0.04

Data are expressed as median (IQR) and n (%). Comparison was conducted by Kruskal-Wallis test for continuous variables, and Fisher's exact test for categorical values. ALT, alanine aminotransferase; AST, aspartate aminotransferase; WBC, white blood cells counts; CT, computer tomography; Ct, cycle threshold. *, Tertiary *Vs* Secondary, P＜0.05; **, Tertiary *Vs* Imported, P＜0.05; ***, Imported *Vs* Secondary, P＜0.05.