Early risk factors for the duration of SARS-CoV-2 viral positivity in COVID-19 patients

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Summary: Our findings firstly provided early laboratory parameters such as count of CD8+ T cells, as risk factors for the duration of SARS-CoV-2 viral positivity, which have significance in control and prevention of the disease.

Abstract

Background

Pneumonia COVID-19 has became a pandemic. However, information on early risk factors for the duration of SARS-CoV-2 viral positivity is unavailable yet.

Methods In this prospective study, a cohort of 137 patients with confirmed SARS-CoV-2 infection were enrolled. Clinical information and laboratory data were retrieved from electronic medical records. Viral positivity duration was calculated by an interval from the day SARS-CoV-2 positive confirmed to the day SARS-CoV-2 returned to negative in these 137 COVID-19 patients. Early risk factors for the duration of SARS-CoV-2 viral positivity were evaluated.

Findings The median SARS-CoV-2 viral positivity duration is 12 days (range: 4 days ~ 45 days) for this cohort. Cox regression results showed a significantly shorter viral positivity duration was related to younger [hazard ratio (HR) = 0.658, p = 0.017], not severe patient (HR = 0.653, p = 0.076), higher count of lymphocytes (HR = 1.464, p = 0.033), eosinophils (HR = 1.514, p = 0.020) and CD8+ T cells (HR=1.745, p=0.033), and lower IL-6 (HR = 0.664, p = 0.036) and IL-10 (HR = 0.631, p = 0.021). Multivariate analysis with covariables adjusted results showed that the count of CD8+ T cells (HR=2.376, p=0.114) was a predominant risk factor for the SARS-CoV-2 viral positivity duration.

Interpretation Our findings firstly provided early laboratory parameters such as count of CD8+ T cells, as risk factors for the duration of SARS-CoV-2 viral positivity, which have significance in control and prevention of the disease.

Introduction

The outbreak of pneumonia COVID-19 caused by the novel betacoronavirus SARS-CoV-2 has now became a global emergency. Laboratory-confirmed cases of COVID-19 have been reported over 100 countries world-wide. Since the first case occurred in China in late December, there were more than 100 thousands confirmed cases and caused over 3500 deaths accumulated by March 8, 2020 [1].

Thanks to physicians and scientists' hard work, more and more detail information on the clinical and epidemiological characteristics of patients with COVID-19 have been released [2, 3, 4]. In addition to the severe acute respiratory syndrome coronavirus (SARS-Cov) in 2003 and middle east respiratory syndrome coronavirus (MERS-CoV) in 2012, SARS-CoV-2 becoming the third major lethal coronavirus which can cause severe respiratory diseases [5]. Symptoms such as cough and/or fever, ground-glass opacity radiologic image and lymphocytopenia have been commonly observed. SARS-CoV-2 has been found in respiratory tract, blood, feces, and in tears and conjunctival secretions [2, 6]. The human-to-human transmission route for SARS-CoV-2 can be sporadic, healthcare- and intra-familial-associated [7]. However, few information on the risk factors for the duration of the SARS-CoV-2 viral positivity is available yet.

In this prospective study, early risk factors including clinical and laboratory data for the duration of SARS-CoV-2 viral positivity were evaluated in 137 COVID-19 patients.

Methods

Patients and data collection

In this study, a cohort of 137 laboratory confirmed COVID-19 patients (73 male and 64 female) were prospectively enrolled from January 19th 2020 to March 9th 2020. This study was approved by the institutional ethics committee (#K20200111).

Confirmation of the SARS-CoV-2 infection was determined by real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR). Consecutive virus screening (sampling interval at least 1 day) was performed with RT-PCR during the period of hospitalization. SARS-CoV-2 returned to negative (sampling interval at least 1 day) were determined with at least consecutive two times of the RT-PCR negative results.

Of these 137 COVID-19 patients, there were 1 critical, 22 severe, and 114 ordinary cases with the median age of 47 years (range: 4~86 years). All patients were admitted and treated in Taizhou Hospital of Zhejiang Province, Taizhou Enze Hospital, Taizhou EnZe

Medical Group (Center), Zhejiang, China. All patients were discharged and no death case was occurred. Clinical classification of COVID-19 was according to the Diagnosis and Treatment Guidance of Corona Virus Diseases 2019 (Tentative Fifth Edition), National Health Commission (NHC) of the People's Republic of China [8]. Critical cases meet any of the following: respiratory failure requires mechanical ventilation; shock occurs; complicated with other organ failure requires intensive care unit treatment. Severe cases meet any of the following: respiratory distress with the respiratory rate more than 30/min; resting plus oxygen saturation ≤ 93%; arterial blood oxygen partial pressure/oxygen concentration ≤300mmHg. Ordinary cases are those who have symptoms such as fever and respiratory tract symptoms, and pneumonia manifestation in imaging.

Patient medical history and routinely laboratory test data including counts of peripheral white blood cells, immune cell subsets, and cytokines interleukin (IL)-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-α and interferon (IFN)-γ were collected from the electronic medical records.

For SARS-CoV-2 viral positivity duration, time cut-off was calculated by an interval from the day with SARS-CoV-2 positive confirmed to the first day SARS-CoV-2 returned to negative (at least consecutive two times of the RT-PCR negative results) during the period of hospitalization. Early risk factors for the duration of viral positivity were evaluated with the variables on the day when SARS-CoV-2 positive confirmed.

Statistical analysis

Groups with low and high levels for peripheral immune subsets were defined by the low-limit of the normal reference interval, and for patient age and cytokine levels were defined by the median. Kaplan-Meier method with Log-rank test was performed to evaluate the significance for duration of SARS-CoV-2 viral positivity between low and high level of variables in each group. Cox regression proportional-hazards model was applied to assess the hazard ratio (HR) of each variable for the duration of SARS-CoV-2 viral positivity, and significant risk factors whose HR was further adjusted with covariate analysis. Statistical analysis was performed performed with the SPSS v.13.0 (SPSS, Inc., Chicago, IL, USA). All statistical tests were two-sided and a $p<0.05$ was considered statistical significance.

Results

Early risk factors related to the duration of SARS-CoV-2 viral positivity

The median SARS-CoV-2 viral positivity duration is 12 days in this cohort (range: 4 days~ 45 days). The SARS-CoV-2 viral positivity duration for the severe and not severe COVID-19 patients were 11 days (range: 4 days~ 45 days) and 15 days (range: 5 days~ 38 days), respectively.

Early risk factors for the duration of SARS-CoV-2 viral positivity were evaluated with the clinical and laboratory parameters on the day SARS-CoV-2 positive confirmed. Kaplan-Meier with Log-rank method has been performed to evaluate the significance of peripheral cell subsets, cytokines, patient gender, age and disease severity to the SARS-CoV-2 viral positivity duration. Results showed that patient age, disease severity, count of CD3+ lymphocytes, CD8+ T cells and eosinophils, levels of cytokine IL-6 and IL-10 were markedly related to the viral positivity duration **(Figure 1).**

A significantly shorter SARS-CoV-2 viral positivity duration was related to younger (mean: 12.7 days *vs* 16.6 days; $p = 0.011$) with the median age of 47 years as a cut-off value, while a much shorter but not reach statistical significant when use the age above 60 years as a cut-off (14.2 days *vs* 16.9 days, $p = 0.190$). A markedly shorter viral positivity duration was also related to not severe patient (13.9 days *vs* 18.4 days; $p = 0.060$), higher count of lymphocytes (13.1 days *vs* 16.8 days; $p = 0.024$), eosinophils (12.3 days *vs* 16.3 days; $p = 0.014$), and CD8+ T cells (11.8 days *vs* 16.8 days; $p = 0.022$), and lower levels of IL-6 (11.9 days *vs* 16.2 days; $p = 0.026$) and IL-10 (11.7 days *vs* 16.2 days; $p = 0.014$), respectively (**Table 1)**. Similar results were observed for the duration from disease onset to SARS-CoV-2 returned to negative (data not shown).

Predictive value of risk factors for the duration of SARS-CoV-2 viral positivity

Cox regression proportional hazards model results showed a significantly shorter SARS-CoV-2 viral positivity duration was related to younger (HR = 0.658, $p = 0.017$), higher count of lymphocytes (HR = 1.464, $p = 0.033$), eosinophils (HR = 1.514, $p = 0.020$), and CD8+ T cells (HR = 1.745, $p = 0.033$), and lower levels of IL-6 (HR = 0.664, $p = 0.036$) and IL-10 (HR = 0.631, $p = 0.021$), respectively. Multivariate analysis after adjusted covariables, the count of CD8+ T cells (HR = 2.376, $p = 0.144$) was the predominant risk factor for the duration of SARS-CoV-2 viral positivity (**Table 2**). Similar results were observed for the duration from disease onset to SARS-CoV-2 returned to negative (data not shown).

Discussion

Virus shedding during the period with viral positivity is one of major features of virus spreading and transmission [9]. In our study, the median viral positivity duration (from the day SARS-CoV-2 positive confirmed to the day SARS-CoV-2 returned to negative) is 12 days (range: 4 days ~ 45 days). This is quite comparable to a recent study by Young et al [4]. In that study, in a cohort of 18 COVID-19 patients in Singapore, authors reported that the median duration of viral positivity from first to last positive nasopharyngeal swab collected was 12 days (range, 1-24), and most patients had viral shedding from the nasopharynx for 7 days or longer. In a previous study on 2134 samples obtained from 1041 SARS cases, Cheng et al. [10] reported that positive rates of the SARS coronavirus RNA peaked at 6 ~11 days after onset of illness for nasopharyngeal aspirates, and 9 ~ 14 days for samples from feces. In a cohort of 13 MERS-CoV cases, Memish and co-authors found that 76% of cases were still positive on day 12 of illness for MERS-CoV by RT-PCR [11]. In a recent study on 10 children with SARS-CoV-2 infection, duration of SARS-CoV-2 viral positivity in respiratory tract samples ranges from 6 ~ 22 days, while RNA detection in feces had a prolonged duration of viral positivity in feces for at least 2 weeks and even more than 1 month [12].

However, few information on risk factors for the duration of SARS-CoV-2 viral positivity is available yet. Importantly, dynamics of SARS-CoV-2 viral load in asymptomatic patients has been reported to be similar to that in the symptomatic patients [13]. In this scenario, risk factors for SARS-CoV-2 viral positivity duration could have importance for evaluation of transmission potential of SARS-CoV-2 among asymptomatic COVID-19 patients. In this study, our findings revealed that COVID-19 patient characteristics such as age, disease severity, count of total lymphocyte, CD8+ T cells and eosinophils, levels of cytokines IL-6 and IL-10 were markedly related to the viral positivity duration. Host anti-viral immune status including immune cells and cytokines can play critical roles in virus clearance and outcome of infectious diseases [14]. Immune cells CD8+ T cells and eosinophils have been reported to be critical for viral clearance in acute infections such as coronavirus [15, 16]. As our findings revealed, count of CD8+ T cells at early stage of the

COVID-19 is a predominant risk factor for the SARS-CoV-2 viral positivity duration, which is in line with a study by Shin et al. that extraordinarily high frequencies of MERS-CoV-reactive CD8+ T cells were observed in most of the MERS patients at the acute stage prior to the detection of humoral and CD4+ T cell responses [17].

Summary, our findings regarding early risk factors for the duration of SARS-CoV-2 viral positivity, such as counts of CD8+ T cells, is important for further understand the pathogenesis of the disease and for application of appropriate measurements for infection prevention and control.

Authors' contributions

AL and W-H Y had roles in the study design, data collection, data analysis, data interpretation, literature search, and writing of the manuscript. Z-B H and SZ had roles in patient recruitment, data collection, and clinical management. J-G Z and XZ had roles in data collection, and data interpretation.

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Declaration of interests

We declare no competing interests.

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Legend:

Figure 1. Kaplan-Meier method with Log-rank test was performed to evaluate the significance of clinical variables for the duration of SARS-CoV-2 viral positivity. Comparison between the groups of (A) patients age ($p = 0.011$); (B) severe and non-severe cases ($p = 0.060$); (C) lymphocytes ($p = 0.024$); (D) CD8+ T cells ($p = 0.022$); (E) eosinophils counts ($p = 0.014$); and (F) IL-6 ($p = 0.026$) and (G) IL-10 levels ($p = 0.014$).

Table 1 Log-rank analysis of risk factors for the duration of viral positivity in COVID19 patients

Variables		Groups	No.	from virus positive to negative (days) Mean (95% CI)	*p* value
Whole cohort		/	137	14.6 (5.0-36.0)	/
Gender		male	73	14.8 (12.7-16.8)	0.891
		female	64	14.8 (12.3-17.3)	
Age	Cut-off (median) 47 years	<47 ys	67	12.7 (10.7-14.6)	0.011
		>47 ys	70	16.6 (14.4-18.9)	
	Cut-off (older) 60 years	<60 ys	111	14.2 (12.5-15.8)	0.190
		>60 ys	26	16.9 (13.1-20.7)	
Severity		no	114	13.9 (12.3-15.5)	0.060
		Yes	23	18.4 (13.7-23.0)	
WBC count (10^9/L)		<3.5	8	14.8 (9.69-19.8)	0.972
		>3.5	129	14.7 (13.1-16.4)	
Lymphocyte count (10^9/L)		<1.1	59	16.8 (14.2-19.5)	0.024
		>1.1	78	13.1 (11.4-14.8)	
CD3+ T cell (per μL)		<770	40	15.2 (12.5-17.8)	0.230
		>770	23	12.6 (9.40-15.7)	
CD4+ T cell (per μL)		<414	39	15.4 (12.7-18.1)	0.121
		>414	24	12.3 (9.26-15.4)	
CD8+ T cell (per μL)		<248	30	16.8 (13.6-20.0)	0.022
		>248	33	11.8 (9.40-14.3)	
CD19+ B cell (per μL)		<90	10	15.6 (10.7-20.5)	0.390
		>90	53	13.9 (11.7-16.1)	
CD56+ NK cell (per μL)		<150	21	14.6 (11.9-16.5)	0.773
		>150	42	14.1 (11.3-16.8)	
Eosinophil count (10^9/L)		<0.02	81	16.3 (14.2-18.5)	0.014
		>0.02	56	12.3 (10.4-14.3)	
IL-2 (median:1.35 pg/mL)		<1.35	57	13.1 (11.2-15.0)	0.275
		>1.35	58	15.0 (12.3-17.8)	
IL-4 (median:1.45 pg/mL)		<1.45	56	13.8 (11.5-16.1)	0.811
		>1.45	59	14.3 (11.9-16.7)	
IL-6 (median:6.75 pg/mL)		<6.75	57	11.9 (10.2-13.6)	0.026
		>6.75	58	16.2 (13.3-19.0)	
IL-10 (median:3.52 pg/mL)		<3.52	55	11.7 (10.1-13.4)	0.014
		>3.52	60	16.2 (13.4-19.0)	
TNF-α (median:1.25 pg/mL)		<1.25	56	14.7 (12.2-17.2)	0.414
		>1.25	59	13.5 (11.2-15.8)	
IFN-γ (median:1.89 pg/mL)		<1.89	57	14.5 (12.3-16.8)	0.464
		>1.89	58	13.6 (11.1-16.1)	

Table 2 Cox proportional hazards model analysis of risk factors for the duration of viral positivity in COVID19 patients

Variables	Groups	Univariate analysis		Multivariate analysis	
		HR (95% CI)	*p value*	HR (95% CI)	*p value*
Gender	Male *vs.* Female	1.023 (0.725-1.444)	0.896	/	
Age (years)	≤47 *vs.* >47	0.658 (0.468-0.927)	0.017	0.905 (0.495-1.653)	0.745
Severity	No vs Yes	0.653 (0.407-1.046)	0.076	0.521 (0.232-1.172)	0.115
WBC count (10^9/L)	<3.5 vs >3.5	1.012 (0.494-2.074)	0.974	/	
Lymphocyte count (10^9/L)	<1.1 vs >1.1	1.464 (1.032-2.077)	0.033	0.986 (0.409-2.377)	0.986
CD3+ T cell (per μL)	<770 vs >770	1.356 (0.798-2.303)	0.260	0.656 (0.248-1.736)	0.656
CD4+ T cell (per μL)	<414 vs >414	1.480 (0.873-2.509)	0.146	1.036 (0.459-2.341)	0.932
CD8+ T cell (per μL)	<248 vs >248	1.745 (1.046-2.912)	0.033	2.376 (0.812-6.953)	0.114
CD19+ B cell (per μL)	<90 vs >90	1.338 (0.655-2.733)	0.425	/	
CD56+ NK cell (per μL)	<150 vs >150	1.076 (0.631-1.837)	0.787	/	
Eosinophil count (10^9/L)	<0.02 vs >0.02	1.514 (1.067-2.148)	0.020	0.653 (0.300-1.422)	0.653
IL-2 (pg/mL)	<1.35 vs >1.35	0.819 (0.560-1.196)	0.301	/	
IL-4 (pg/mL)	<1.45 vs >1.45	0.958 (0.658-1.394)	0.821	/	
IL-6 (pg/mL)	<6.75 vs >6.75	0.664 (0.452-0.973)	0.036	0.859 (0.394-1.874)	0.703
IL-10 (pg/mL)	<3.52 vs >3.52	0.631 (0.427-0.933)	0.021	0.973 (0.436-2.169)	0.946
TNF-α (pg/mL)	<1.25 vs >1.25	1.160 (0.797-1.686)	0.439	/	
IFN-γ (pg/mL)	<1.89 vs >1.89	1.142 (0.785-1.663)	0.487	/	

Figure 1

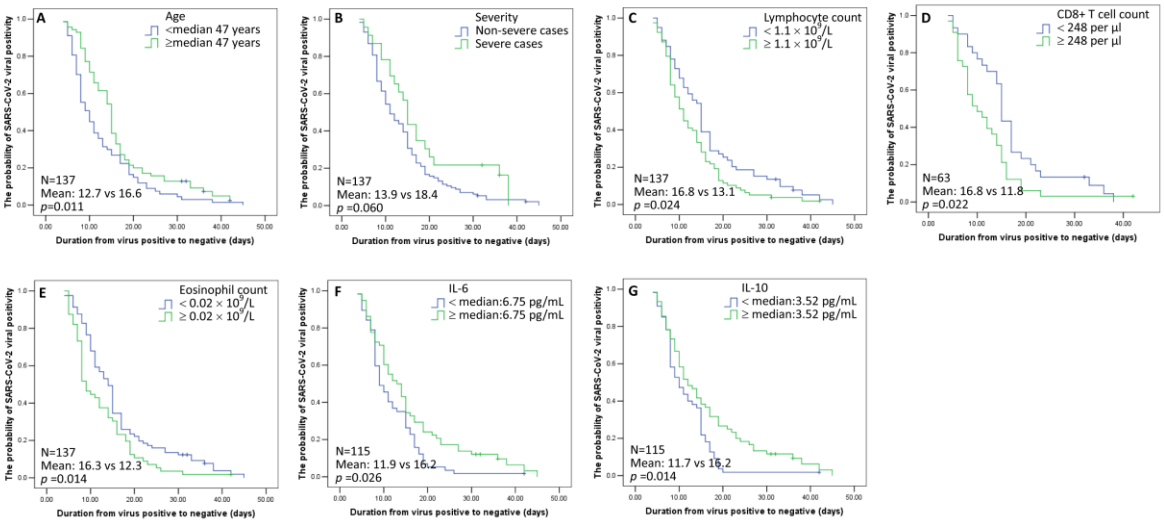

Figure 1