2019-novel coronavirus (2019-nCoV) infections trigger an exaggerated cytokine response aggravating lung injury

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Date: 2020-02-12T00:00:00+00:00

Abstract

Recently, an outbreak of pneumonia cases occurred in Wuhan, China, caused by a novel coronavirus named 2019-CoV. In our previous study, based on the clinical characteristics of 12 patients infected with 2019-nCoV in Shenzhen, China, all cases developed pneumonia and half progressed to acute respiratory distress syndrome (ARDS). In this article, we investigated the plasma factor expression profiles of these 12 patients. We examined the expression of 48 factors in the plasma of 2019-nCoV-infected patients, of which 38 were significantly elevated compared with healthy individuals; the hypercytokinemia levels in plasma of severe 2019-nCoV-infected patients were significantly lower than those in patients infected with influenza A virus H7N9, while slightly higher than those in patients with bacterial infections. Among these 38 cytokines, 17 were correlated with 2019-CoV viral load, and 15 of them (M-CSF, IL-10, IFN- 2, IL-17, IL-4, IP-10, IL-7, IL-1 receptor antagonist, G-CSF, IL-12 (p40), IFN- , IL-1 , IL-2, HGF, and PDGF-BB) were highly correlated with the Murray score for lung injury and could be used to predict disease severity in 2019-nCoV-infected patients. Our findings suggest that these 15 cytokines may serve as potential biomarkers for assessing disease severity in 2019-nCoV-infected patients, and factors that influence these signaling mediators may represent potential therapeutic agents for the novel 2019-nCoV pandemic.

Full Text

Preamble

2019-nCoV Infections Trigger an Exaggerated Cytokine Response Aggravating Lung Injury

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Abstract

A recent outbreak of pneumonia in Wuhan, China was caused by the 2019 novel coronavirus (2019-nCoV). Here we report on 12 patients with 2019-nCoV infections in Shenzhen, China; all developed pneumonia and half developed acute respiratory distress syndrome (ARDS). We characterized the plasma cytokine profiles of these 12 patients and found that 38 out of 48 cytokines measured were significantly elevated compared to healthy individuals. Seventeen cytokines were correlated with 2019-nCoV viral load. Fifteen cytokines—M-CSF, IL-10, IFN- 2, IL-17, IL-4, IP-10, IL-7, IL-1ra, G-CSF, IL-12, IFN- , IL-1 , IL-2, HGF, and PDGF-BB—showed strong associations with lung injury as measured by Murray score and could predict disease severity according to area under the curve (AUC) of receiver operating characteristic (ROC) calculations. Our results demonstrate that 2019-nCoV infections trigger extensive changes across a wide array of cytokines, some of which may serve as potential biomarkers of disease severity. These findings improve our understanding of the immunopathologic mechanisms of this emerging and still evolving disease and suggest that modulators of cytokine responses could play a therapeutic role in combating it.

Introduction

Since the outbreak of 2019-nCoV infections in late December 2019 in Wuhan, Hubei Province, China, the number of infected persons has exceeded 10,000, surpassing the previous severe acute respiratory syndrome (SARS) epidemic. Reflecting the pandemic potential of coronaviruses, 2019-nCoV cases have now been reported in Thailand, Germany, the USA, and other parts of the world. The mortality rate is 15% among a case series of 41 hospitalized patients in Wuhan, compared with 10% for SARS-CoV and 37% for Middle East respiratory syndrome coronavirus (MERS-CoV). Although 2019-nCoV—the seventh reported human-infecting member of the family Coronaviridae, which includes SARS-CoV and MERS-CoV—has been identified as the causative agent, the immunopathologic mechanisms of 2019-nCoV-associated diseases remain poorly understood.

A prominent feature of 2019-nCoV infections is the development of acute respiratory distress syndrome (ARDS) in a substantial proportion of cases: 29% (12/41) in the series by Huang et al. and 17% in a series of 99 cases of 2019-nCoV pneumonia. Elevated production of proinflammatory cytokines/chemokines—or even hypercytokinemia, also known as cytokine storm—occurs in SARS-CoV and MERS-CoV infections and contributes to acute lung injury and ARDS development. In this study, we examined the plasma cytokine/chemokine profile of 12 patients with laboratory-confirmed 2019-nCoV infections in Shenzhen, China, compared with 8 healthy subjects, 8 bacterial pneumonia patients, and 8 patients with influenza A H7N9 infections (see Extended Data Table 1).

Results

Analysis using the Bio-Plex Pro Human Cytokine Screening Panel revealed extensive and significant elevations in 38 out of 48 plasma cytokines/chemokines in patients with severe 2019-nCoV pneumonia (2019-nCoV-S) compared to healthy individuals, suggesting hypercytokinemia in these patients (see Extended Data Table 2). Overall, plasma cytokine/chemokine levels in 2019-nCoV-S patients were markedly lower than in H7N9 influenza virus-infected patients but moderately higher than in patients with bacterial pneumonia (see Extended Data Table 2). Furthermore, plasma cytokine/chemokine levels were higher at week 2 than at week 1 following symptomatic onset of 2019-nCoV infections (see Extended Data Table 3). Levels were comparable between days 8–14 and day 15+ after illness onset, indicating persistent elevations at later disease stages (see Extended Data Table 3), consistent with clinical features of 2019-nCoV infections.

We quantified viral RNA loads in throat swabs, sputum, and bronchoalveolar lavage fluid (BALF) samples by quantitative reverse transcription polymerase chain reaction (qRT-PCR) (see Extended Data Table 4). Spearman correlation analysis revealed that 2019-nCoV viral load was highly positively associated with plasma levels of 16 cytokines (M-CSF, IL-10, IFN- 2, IL-13, IL-17, IL-4,

IP-10, IL-1 , IL-7, IL-1ra, G-CSF, IL-12, IFN- , IL-1 , IL-2, and HGF) and negatively associated with PDGF-BB (Table 1). These findings suggest that 2019-nCoV infection is associated with elevated production of a wide array of cytokines/chemokines in patient plasma.

Using Spearman rank correlation analysis, we discovered strong positive linear associations between plasma levels of 15 cytokines (IL-12, IFN- , IL-2, HGF, IFN- 2, IL-4, IL-17, IP-10, G-CSF, IL-10, IL-1ra, M-CSF, IL-1 , and IL-7) and lung injury Murray score, and a negative association between PDGF-BB and Murray score (Fig. 1 [Figure 1: see original paper]). The area under the curve (AUC) of the receiver operating characteristic (ROC) exceeded 0.8 for each of these 15 cytokines, indicating their potential to predict disease severity in 2019-nCoV infections (Fig. 2 [Figure 2: see original paper]). Additionally, plasma cytokine/chemokine levels differed significantly between 2019-nCoV-S and mild 2019-nCoV pneumonia (2019-nCoV-M) patients (Fig. 3 [Figure 3: see original paper]). Our data suggest these 15 cytokines may serve as biomarkers for disease severity in 2019-nCoV-infected patients.

Discussion

In summary, we demonstrated that hypercytokinemia occurs in 2019-nCoV-infected patients and identified 15 cytokines linearly associated with lung injury (Murray score) that may serve as potential biomarkers for disease severity. These include antiviral cytokines IFN- 2 and IFN- , IL-1ra, IL-2, IL-4, IL-7, IL-10, IL-12, and IL-17, chemokine IP-10, and growth factors G-CSF and M-CSF. Notably, levels of proinflammatory Th1, Th2, and Th17 cytokines were all increased. Previous studies have demonstrated marked elevation of IP-10, IL-6, IL-8, MCP1, and MIP-1 in sera from SARS-CoV-infected patients and significant increases in IL-10, IL-15, and IL-17 as well as IFN- 2 and IFN- in plasma from MERS-CoV-infected patients. Although plasma cytokine measurements were not performed simultaneously, cytokine levels in blood samples from SARS-CoV and MERS-CoV-infected patients appeared considerably higher than those in 2019-nCoV-infected patients. The most extreme hypercytokinemia has been reported in serum samples from patients infected with avian influenza A viruses, including H5N1-associated hypercytokinemia factors (IP-10, MCP-1, MIG, and IL-8) and MIF, SCF, MCP-1, HGF, SCGF- , IP-10, IL-18, and IFN- in H7N9-infected patients.

While the mechanisms of cytokine-mediated immunopathology remain largely unknown, therapeutic use of cytokines or cytokine inhibitors has shown increasing success. Interferon and have been used clinically despite side effects, and interferon is officially recommended for 2019-nCoV treatment in China' s National Health Commission Guidelines. Numerous antagonistic antibodies against cytokines have been used clinically or in trials for autoimmune/autoinflammatory diseases, including monoclonal antibodies targeting IL-1, IL-10, IL-12, IL-17, and IP-10. These antibodies could be repurposed to attenuate hypercytokinemia in 2019-nCoV-infected patients and may provide

potential treatments for the current outbreak.

Methods

Experimental Ethics Policy

The study protocol was approved by the Ethics Committees of Shenzhen Third People' s Hospital (SZTHEC2016001). Verbal informed consent was obtained from all patients or their family members. The study was conducted in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice, the Declaration of Helsinki, and institutional ethics guidelines.

Acquisition of Clinical Specimens

Throat swabs, rectal swabs, sputum, and BALF specimens were collected within 24 hours of blood sample collection from laboratory-confirmed 2019-nCoV cases upon admission in January 2020 and at various time points thereafter. Plasma samples were also collected from 8 healthy subjects undergoing wellness examinations. Additionally, we obtained archived plasma samples from 8 patients with laboratory-confirmed H7N9 infections hospitalized between January 2015 and March 2017, and from 8 bacterial pneumonia patients hospitalized between August and December 2019.

qRT-PCR

Viral RNAs were extracted from clinical specimens using the QIAamp RNA Viral Kit (Qiagen, Heiden, Germany) according to manufacturer instructions. Samples were amplified by quantitative reverse transcription polymerase chain reaction (qRT-PCR) using primers and probes recommended by the Chinese Center for Disease Control and Prevention (China CDC) and a commercially available 2019-nCoV detection kit (GeneoDX Co., Shanghai, China). Samples with cycle threshold (Ct) values 38.0 were considered putatively positive. Samples with Ct >38 were retested and considered positive if Ct was 38 but 40 on the second test, and negative if viral RNAs were undetectable.

Disease Severity Classification and Murray Score Calculation

Severity of 2019-nCoV infection was graded according to China' s National Health Commission Guidelines for Diagnosis and Treatment of 2019-nCoV infection. Briefly, patients with fever, respiratory symptoms, and radiological findings indicative of pneumonia were classified as mild 2019-nCoV pneumonia (2019-nCoV-M). Patients meeting any of the following criteria were classified as severe 2019-nCoV pneumonia (2019-nCoV-S): respiratory distress (respiration rate 30/min), resting oxygen saturation 93%, or arterial oxygen partial pressure (PaO)/fraction of inspired oxygen (FiO) 300 mmHg (1 mmHg = 0.133 kPa). Patients with respiratory failure requiring mechanical ventilation, shock, or other organ failure requiring ICU care were classified as critical 2019-nCoV

pneumonia (2019-nCoV-C). Clinicopathological variables were collected at admission for 12 patients (4 mild, 5 severe, and 3 critical), and disease severity was assessed using Murray scores.

Cytokine and Chemokine Measurements

Plasma concentrations of 48 cytokines and chemokines were measured in duplicate using the Bio-Plex Pro Human Cytokine Screening Panel (48-Plex #12007283, Bio-Rad) according to manufacturer instructions. Plasma samples were fixed in 2% paraformaldehyde before analysis and measured in a biosafety level III laboratory.

Statistical Analysis

Spearman rank correlation coefficient was used for linear correlation analysis between plasma cytokine levels and Murray scores in 2019-nCoV-infected patients. Area under the receiver operating characteristic (ROC) curve (AUC) of plasma cytokine levels was estimated for 2019-nCoV-M versus 2019-nCoV-S infections. ANOVA or Mann–Whitney U test was used to compare plasma cytokine levels among groups. All statistical tests were performed using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). A two-tailed P value <0.05 was considered statistically significant.

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Acknowledgements

The authors would like to thank a scientist who insisted on remaining anonymous. This work was supported by the National Natural Science Foundation of China (Grant 81788101) and the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (Grant 2017-12M-1-009).

Author Contributions

C. Jiang conceived the project. C. Jiang, L. Liu, and W.J. Liu guided the study. Y. Liu, Y. Yang, F. Wang, J. Yuan, Z. Zhang, Z. Wang, J. Li, C. Shen, J. Li, L. Peng, W. Wu, M. Cao, L. Xing, Z. Xu, and L. Chen collected clinical samples from 2019-nCoV-infected patients, H7N9-infected patients, bacterial pneumonia patients, and healthy subjects. C. Zhang, supervised by C. Zhou, measured cytokines/chemokines. F. Huang performed biostatistical analysis assisted by C. Zhang, Y. Qin, X. Li, D. Zhao, and S. Li. S. Tan provided helpful assistance. C. Jiang, F. Huang, C. Zhang, and Y. Qin wrote the manuscript. All authors have read and approved the manuscript.

Competing Interests

The authors declare no competing interests.

Tables

Table 1. Correlation between cytokine levels and viral Ct value of patients with laboratory-confirmed 2019-nCoV infection.

Cytokine	Spearman r	Ct value P value
M-CSF	0.848	$P < 0.001$
IL-10	0.695	$P < 0.001$
IFN- 2	0.733	$P < 0.001$
IL-13	0.786	$P < 0.001$
IL-17	0.690	$P < 0.001$
IP-10	0.685	$P < 0.001$
IL-1	0.675	$P < 0.001$
IL-1ra	0.606	$P = 0.001$
G-CSF	0.657	$P < 0.001$
IL-12 (p40)	0.513	$P = 0.009$
IFN-	0.656	$P < 0.001$
IL-1	0.695	$P < 0.001$
PDGF-BB	-0.428	$P = 0.033$
MCP-3	0.629	$P = 0.001$
MIP-1	0.680	$P < 0.001$
MCP-1 (MCAF)	0.656	$P < 0.001$
GM-CSF	0.695	$P < 0.001$
IL-18	0.629	$P = 0.001$
-NGF	0.513	$P = 0.009$
SCGF-	0.656	$P < 0.001$
IL-12 (p70)	0.680	$P < 0.001$
FGF basic	0.695	$P < 0.001$
TRAIL	0.629	$P = 0.001$
TNF-	0.513	$P = 0.009$

Cytokine	Spearman r	Ct value P value
IL-2R	0.656	$P < 0.001$
SDF-1	0.695	$P < 0.001$
TNF-	0.629	$P = 0.001$
CTACK	0.513	$P = 0.009$

The viral titers were measured in 18 samples from 10 patients infected with 2019-nCoV. #: P value [0.05,0.1), -: P value >0.05

Extended Data Table 1. Epidemiological and clinical features of subjects hospitalized with 2019-nCoV, H7N9 avian influenza virus and bacterial infections.

Characteristics	2019-nCoV	H7N9	Bacteria	Control
Median age (range)	62.5 (10-72)	56 (21-67)	41 (31.5-49)	28 (25-34)
Age subgroups				
0–15 years	1/12 (8.3%)	0/8 (0%)	1/8 (12.5%)	0/8 (0%)
16–59 years	4/12 (33.3%)	6/8 (75%)	5/8 (62.5%)	8/8 (100%)
60+ years	7/12 (58.3%)	2/8 (25%)	2/8 (25%)	0/8 (0%)
Male (%)	8/12 (66.7%)	5/8 (62.5%)	5/8 (62.5%)	4/8 (50%)
Initial symptoms				
Fever	9/12 (75%)	8/8 (100%)	5/8 (62.5%)	0/8 (0%)
Cough	12/12 (100%)	7/8 (87.5%)	3/8 (37.5%)	0/8 (0%)
Headache	0/12 (0%)	3/8 (37.5%)	1/8 (12.5%)	0/8 (0%)
Myalgia	4/12 (33.3%)	4/8 (50%)	0/8 (0%)	0/8 (0%)
Chill	5/12 (41.7%)	5/8 (62.5%)	0/8 (0%)	0/8 (0%)
Nausea or vomiting	2/12 (16.7%)	0/8 (0%)	1/8 (12.5%)	0/8 (0%)
Diarrhea	3/12 (25%)	2/8 (25%)	0/8 (0%)	0/8 (0%)
Co-existing chronic medical conditions				

Characteristics	2019-nCoV	H7N9	Bacteria	Control
Chronic heart disease	4/12 (33.3%)	2/8 (25%)	0/8 (0%)	0/8 (0%)
Chronic lung disease	1/12 (8.3%)	1/8 (12.5%)	0/8 (0%)	0/8 (0%)
Chronic renal disease	2/12 (16.7%)	0/8 (0%)	0/8 (0%)	0/8 (0%)
Chronic liver disease	1/12 (8.3%)	0/8 (0%)	0/8 (0%)	0/8 (0%)
Diabetes	2/12 (16.7%)	0/8 (0%)	0/8 (0%)	0/8 (0%)
Cancer	0/12 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)
Bacterial co-infections	6/12 (50%)	6/8 (75%)	8/8 (100%)	0/8 (0%)
Interval, median days (IQR)				
Onset to admission	6.5 (5, 9.25)	6 (4.75, 7)	6 (4.5, 8)	NA
Onset to starting antiviral treatment	6 (5, 9.25)	1 (0, 3.5)	5.5 (3.75, 6.25)	NA
Onset to laboratory confirmation	7 (4.5, 14.5)	5.5 (3.75, 6.25)	7 (5.75, 8.25)	NA
Complications				
Pneumonia	12/12 (100%)	8/8 (100%)	8/8 (100%)	0/8 (0%)
Severe ARDS	6/12 (50%)	7/8 (87.5%)	2/8 (25%)	0/8 (0%)
Respiratory failure	2/12 (16.7%)	2/8 (25%)	5/8 (62.5%)	0/8 (0%)
Hepatic insufficiency	3/12 (25%)	7/8 (87.5%)	4/8 (50%)	0/8 (0%)
Renal insufficiency	2/12 (16.7%)	2/8 (25%)	3/8 (37.5%)	0/8 (0%)
Cardiac failure	1/12 (8.3%)	1/8 (12.5%)	1/8 (12.5%)	0/8 (0%)
Shock	1/12 (8.3%)	0/8 (0%)	0/8 (0%)	0/8 (0%)
Treatment				
Received antivirals 2 days after illness onset	1/12 (8.3%)	1/8 (12.5%)	4/8 (50%)	NA

Characteristics	2019-nCoV	H7N9	Bacteria	Control
Received antivirals 3-5 days after illness onset	4/12 (33.3%)	3/8 (37.5%)	5/8 (62.5%)	NA
Received antivirals 6 days after illness onset	7/12 (58.3%)	4/8 (50%)	0/8 (0%)	NA
Corticosteroid	5/12 (41.7%)	5/8 (62.5%)	3/8 (37.5%)	NA
Mechanical ventilation	2/12 (16.7%)	5/8 (62.5%)	4/8 (50%)	NA

NA: Not applicable.

Extended Data Table 2. Cytokine comparison among healthy controls, bacteria-infected patients, H7N9-infected patients and 2019-nCoV-infected patients (disease severity classified)

Cytokine	Pa value	Pb value	Pc value	Pd value	Pe value	Pf value	Pg value	Ph value	Pi value	Pj value
IL-1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-1ra	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-10	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-12 (p70)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-13	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-15	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-17	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Eotaxin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FGF basic	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
G-CSF	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GM-CSF	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Cytokine	Pa value	Pb value	Pc value	Pd value	Pe value	Pf value	Pg value	Ph value	Pi value	Pj value
IFN-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IP-10	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MCP-1 (MCAF)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MIP-1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PDGF-BB	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MIP-1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RANTES	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TNF-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-1a	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-2R	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-12 (p40)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-16	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-18	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CTACK	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GRO-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IFN-2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MCP-3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
M-CSF	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
b-NGF	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SCGF-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SDF-1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Cytokine	Pa value	Pb value	Pc value	Pd value	Pe value	Pf value	Pg value	Ph value	Pi value	Pj value
TNF-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TRAIL	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Pa value: Healthy vs bacteria patients
Pb value: Healthy vs H7N9 patients
Pc value: Healthy vs 2019-nCoV-M patients
Pd value: Healthy vs 2019-nCoV-S patients
Pe value: Bacteria patients vs H7N9 patients
Pf value: Bacteria patients vs 2019-nCoV-M patients
Pg value: Bacteria patients vs 2019-nCoV-S patients
Ph value: H7N9 patients vs 2019-nCoV-M patients
Pi value: H7N9 patients vs 2019-nCoV-S patients
Pj value: 2019-nCoV-M patients vs 2019-nCoV-S patients

#: P value [0.05,0.1), -: P value >0.05

8 samples from 8 healthy controls, 8 samples from 8 bacteria-infected patients, 8 samples from 8 H7N9-infected patients, 8 samples from 4 mild 2019-nCoV-infected patients (2019-nCoV-M) and 17 samples from 8 severe 2019-nCoV-infected patients (2019-nCoV-S).

Extended Data Table 3. Cytokine comparison among healthy controls, bacteria-infected patients, H7N9-infected patients and 2019-nCoV-infected patients (Day0-7, Day8-14, Day15-)

Cytokine	Pa value	Pb value	Pc value	Pd value	Pe value	Pf value	Pg value	Ph value	Pi value	Pj value	Pk value	Pl value
IL-1	<0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001
IL-1ra	<0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001
IL-10	<0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001
IL-12 (p70)	<0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001
IL-13	<0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001
IL-15	<0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001	K0.001

Cytokine	Pa value	Pb value	Pc value	Pd value	Pe value	Pf value	Pg value	Ph value	Pi value	Pj value	Pk value	Pl value
IL-17	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Eotaxin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FGF basic	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
G-CSF	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GM-CSF	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IFN-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IP-10	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MCP-1 (MCAF)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MIP-1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PDGF-BB	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MIP-1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RANTES	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TNF-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-1a	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-2R	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-12 (p40)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-16	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IL-18	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CTACK	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GRO-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IFN-2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Cytokine	Pa value	Pb value	Pc value	Pd value	Pe value	Pf value	Pg value	Ph value	Pi value	Pj value	Pk value	Pl value
MCP-3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
M-CSF	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
b-NGF	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SCGF	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SDF-1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TNF-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TRAIL	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Pa value: Healthy vs 2019-nCoV patients (Day0-7)
Pb value: Healthy vs 2019-nCoV patients (Day8-14)
Pc value: Healthy vs 2019-nCoV patients (Day15-)
Pd value: Bacteria patients vs 2019-nCoV patients (Day0-7)
Pe value: Bacteria patients vs 2019-nCoV patients (Day8-14)
Pf value: Bacteria patients vs 2019-nCoV patients (Day15-)
Pg value: H7N9 patients vs 2019-nCoV patients (Day0-7)
Ph value: H7N9 patients vs 2019-nCoV patients (Day8-14)
Pi value: H7N9 patients vs 2019-nCoV patients (Day15-)
Pj value: 2019-nCoV patients (Day0-7) vs 2019-nCoV patients (Day8-14)
Pk value: 2019-nCoV patients (Day0-7) vs 2019-nCoV patients (Day15-)
Pl value: 2019-nCoV patients (Day8-14) vs 2019-nCoV patients (Day15-)

#: P value [0.05,0.1), -: P value >0.05

8 samples from 8 healthy controls, 8 samples from 8 bacteria-infected patients, 8 samples from 8 H7N9-infected patients, 7 samples from 7 2019-nCoV-infected patients (Day0-7), 13 samples from 9 2019-nCoV-infected patients (Day8-14) and 5 samples from 5 2019-nCoV-infected patients (Day15-).

Extended Data Table 4. Detection of 2019-nCoV at respiratory and non-respiratory sites.

Site	Detectable RNA (n/N)	Ct Values (Median; range)
Nasopharynx		
2019-nCoV Total		30 (23-36)
Severe		29.2 (25-36)
Throat		

Site	Detectable RNA (n/N)	Ct Values (Median; range)
Detectable RNA (n/N)	24	
Ct Values (Median; range)		24 (19-26)
Sputum		
Detectable RNA (n/N)	24	
Ct Values (Median; range)		24 (19-26)
Plasma		
Detectable RNA (n/N)	27.5	
Ct Values (Median; range)		(27-28)
Rectum		
Detectable RNA (n/N)	U	
Ct Values (Median; range)		Undetected

U: Undetected.

Throat swabs were obtained after 4–18 days (median 10) of illness.
Sputum samples were obtained after 4–17 days (median 9.5) of illness.
Plasma samples were obtained after 7–10 days (median 9) of illness.
Rectal swabs were obtained after 4–16.5 days (median 9.5) of illness.

Figure Captions

Fig. 1. Murray Score highly correlates with plasma cytokine levels in patients with 2019-nCoV infections.

The plasma levels of cytokines (IL-12, IFN- , IL-2, HGF, IFN- 2, IL-4, IL-17, IP-10, G-CSF, IL-10, IL-1ra, M-CSF, IL-1 , IL-7 and PDGF-BB) were measured in a total of 25 blood samples from 12 patients with 2019-nCoV infections. Clinical indicators from the same day of blood sample collection were used to calculate Murray Score (an indicator of lung injury severity). Spearman rank correlation analysis (r) was used for linear correlation analysis.

Fig. 2. The ROC curve of plasma cytokine levels for patients with mild and severe 2019-nCoV infections.

The area under the receiver operating characteristic (ROC) curve (AUC) of the plasma cytokine levels (IL-12, IL-1ra, IP-10, PDGF-BB, IFN- 2, IFN- , M-CSF, IL-17, HGF, G-CSF, IL-2, IL-4, IL-10, IL-1 and IL-7) was estimated in eight samples from four mildly infected 2019-nCoV patients and 17 samples from eight severely infected patients. The P values of all AUC for plasma cytokine levels were less than 0.05.

Fig. 3. Plasma cytokine levels in healthy controls, bacterial pneumonia patients, H7N9-infected patients and 2019-nCoV-infected patients.

The plasma levels of cytokines (M-CSF, IL-10, IFN- 2, IL-17, IL-4, IP-10, IL-7, IL-1ra, G-CSF, IL-12 (p40), IFN- , IL-1 , IL-2, HGF, and PDGF-BB) were measured in eight samples from eight healthy controls, eight samples from eight bacterial pneumonia patients, eight samples from eight H7N9-infected patients, eight samples from four 2019-nCoV-infected patients with mild illness (2019-nCoV-M) and 17 samples from eight patients with severe 2019-nCoV infection (2019-nCoV-S). Detailed information is shown in Extended Data Table 2. *$P < 0.05$,* ***$P < 0.01$,*** $P < 0.001$.

Note: Figure translations are in progress. See original paper for figures.