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LETTER TO THE EDITOR

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Circulating levels of IL-2, IL-4, TNF-α, IFN-γ, and C-reactive protein are not associated with severity of COVID-19 symptoms

To the Editor,

As of 5 May 2020, the total number of coronavirus disease 2019 (COVID-19) cases had reached over 3.5 million worldwide. The outbreak of COVID-19 has been officially declared as a pandemic by the World Health Organization because of global spread and severity. Accumulating evidence has been showing that patients with severe COVID-19 have cytokine storm syndrome. However, we found cytokine storm of interleukin (IL)-2, IL-4, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and C-reactive protein (CRP) is absent in 25 patients who were admitted to the intensive care unit (ICU) with confirmed infection of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). All of these indicate the severity of COVID-19 symptoms is not directly associated with circulating levels of IL-2, IL-4, TNF-α, IFN-γ, and CRP. Anti-inflammatory agents were believed to combat against severe COVID-19 patients, we suggest the anti-inflammatory drugs should be used very carefully based on our observation. At least, each patient should be tested circulating levels of inflammatory cytokines before launching anti-inflammatory treatment. In summary, our study will benefit to the guidance of the COVID-19 clinical treatment strategy.

The pandemic outbreak of COVID-19 is sharply spreading all over the world. The severity of a viral disease usually is a positive association with immune-mediated inflammatory responses. The aggressive and persistent inflammatory response leads to a high risk of multiorgan failure and death.[1] Antiviral drugs are a generally effective approach to treat severe viral infections. However, resistant viruses may arise upon administration of the antiviral drugs.[2] Thus, anti-inflammation agents are critical for releasing the severity of disease but not for viral clearance. The overproduction inflammatory cytokines result in a cytokine storm, cytokine storm indicates the excessive release of proinflammatory cytokines including CRP and proinflammatory cytokines (TNF-α, IL-8).[3] The extraordinary body of evidences suggest that severe COVID-19 patients have cytokine storm.[4-8] The high levels of cytokines manifest the destructive process by leading to pneumonia, vascular endotheliitis, coagulopathy, and other life-threatening respiratory symptoms in COVID-19 patients.[9,10] Moreover, coagulopathy was recently showed to be associated with COVID-19 severity in Caucasian patients.[11] However, the relationship between cytokines storm and COVID-19 pathology still keeps elusive.

Here, we analyzed the cytokines level in COVID-19 patients admitted to the ICU with hypoxemic respiratory failure. We found cytokine storm of IL-2, IL-4, TNF-α, IFN-γ, and CRP is absent in these 25 patients. Our observation suggests anti-inflammation agents can not apply to each patient although they are in ICU. We highlight that circulating levels of inflammatory cytokines should be tested before any anti-inflammatory treatment, in case inhibition of essential but not excessive cytokines for priming immune responses against SARS-CoV-2. The nonappropriate anti-inflammatory treatment might be harmful in the context of the COVID-19 pandemic.

A total of 25 patients from 39 to 85 years old were confirmed to be SARS-CoV-2 positive in nasopharyngeal swabs. The clinical characteristics and chest computed tomography scans indicate these patients are severe COVID-19 patients who were admitted to ICU. The inflammatory cytokines and immune cells in the peripheral blood of these patients were detected. All the patients were admitted to ICU with hypoxemic respiratory failure. The comorbidities are hypertension (20%; 5/25) and diabetes (20%; 5/25). All of these 25 surviving patients were discharged from the ICU to the hospital ward before being discharged home. As showed in the Table 1, inflammatory cytokines including CRP, IL-2, IL-4, IL-10, TNF-α, and IFN-γ were in the normal value range compared to the reference value. These cases showed that IL-2, IL-4, TNF-α, IFN-γ, and CRP level is not associated with severe COVID-19 pathology. However, IL-6 and IL-10 level of some severe COVID-19 patients is over to the reference value. This indicates that COVID-19 patients have severe clinical characteristics independent of circulating levels of inflammatory cytokines in peripheral blood including IL-2, IL-4, TNF-α, IFN-γ, and CRP.

A recent study reported that CRP is an important indicator for COVID-19 prognostic prediction based on machine learning tools.[12] In our study, CRP is in the normal range in all patients who were admitted to ICU and all of these patients are surviving. Our observation is consistent with reports indicating CRP as biomarkers COVID-19 mortality.[12] Dissertation of the level and role of proinflammatory cytokines in the pathophysiology of COVID-19 are critical for evaluation anticytokine therapy. At present, very limited experience of cytokine inhibitors affects COVID-19 patients. SARS-CoV-2 might well adapt for humans and the viral genomic

Abbreviations: CRP, C-reactive protein; ICU, intensive care unit.

TABLE 1 Clinical characteristics of the 25 COVID-19 patients in ICU

Patient number	Gender/age	IL-2 pg/mL	IL-4 pg/L	IL-6 pg/L	IL-10 pg/L	TNF-α pg/L	IFN-γ pg/L	CRP mg/L	T4 cells/μL	T8 cells/μL	NK cells/μL	B cells/μL
1	F/49	1.19	0	3.4	6.45	0.05	0.86	0.499	238	1442	116	89
2	M/62	0.67	2.27	11.18	6	1.03	0.83	0.499	958	172	215	127
3	M/57	1.15	0.41	6.02	3.45	0.56	0.96	0.499	393	271	86	88
4	F/62	2.59	1.96	4.15	2.77	1.86	3.24	1	628	285	45	151
5	M/67	1.27	1.69	8.43	5.03	0.56	1.68	1.25	450	103	70	55
6	F/85	1.77	0.54	4.98	3.88	0.27	0.86	1.26	728	285	102	150
7	M/56	1.08	0.98	1.55	3.3	0.05	1.02	1.58	208	137	81	62
8	F/50	1.77	0.96	5.4	5.69	1.27	1.28	2.64	722	381	273	284
9	F/59	1.19	0.84	14.36	9.49	0.84	1.29	2.66	372	332	33	53
10	M/60	1.32	0	17.6	4.96	0.13	1.25	2.95	552	430	65	161
11	F/54	0.89	0.31	3.6	4.57	0.2	1.15	3.87	792	169	53	138
12	M/52	2.11	1.79	36.3	5.73	1.04	1.22	4.31	601	346	12	41
13	M/57	1.11	0.6	0	0.74	0	0.51	4.32	692	261	63	138
14	F/60	1.11	0	19.26	5.81	0.16	1.15	4.49	330	220	4	58
15	F/65	1.11	0	9.29	4.56	0	0.78	4.7	681	282	81	208
16	M/70	1.4	0	10.48	6.01	0.59	1.28	4.89	365	263	16	114
17	M/39	1.19	0.6	6.11	6.37	0	0.96	5.22	426	783	282	42
18	M/47	1.25	1.42	22.84	3.05	0.97	0.64	5.5	481	200	41	185
19	F/66	1.4	0.89	3.74	2.85	0	0.71	6.26	397	231	14	81
20	F/62	1.32	0.41	4.64	4.83	0	0.62	6.88	414	111	35	104
21	M/46	1.59	0	16.21	6.18	0.79	1.05	6.97	276	188	105	127
22	F/61	1.11	0.79	28.04	12.44	0.5	1.15	7.27	144	78	18	21
23	M/56	1.53	0.69	10.88	6.21	0.56	0.83	8.12	1069	574	280	175
24	F/67	1.44	0.93	7.75	6.61	0.32	1.39	9.36	444	371	70	79
25	M/44	0.96	0.55	3.95	3.35	0.38	0.9	9.75	405	308	2	52

Note: F indicates female, M indicates male. The reference value of serum IL-2 levels is 0.08 to 5.71 pg/mL. The reference value of serum IL-4 levels is 0.1 to 2.8 pg/L. The reference value of serum IL-6 levels is 1.18 to 5.3 pg/L. The reference value of serum IL-10 levels is 0.19 to 4.91 pg/L. The reference value of serum TNF-α levels is 0.1 to 2.31 pg/L. The reference value of serum IFN-γ levels is 0.16 to 7.42 pg/L. The reference value of serum C-reactive protein (CRP) levels is 0 to 10 mg/L. The reference value of T4 (CD4+ T cells) is 404 to 1612. The reference value of T8 (CD8+ T cells) is 220 to 1129. The reference value of NK cells is 150 to 1100. The reference value of B cells is 90 to 560. The cytokines and CRP were detected by automatic immunofluorescence analyzer (Jet-iStar 3000; JOINSTAR, China) and the reagent is a supporting product by the same company. To determine the immune cells count, ethylenediaminetetraacetic acid anticoagulated peripheral blood samples were tested on routinely calibrated FACSCanto II flow cytometer according to the instructions (Becton-Dickinson, Franklin Lakes, NJ).

Abbreviations: CRP, C-reactive protein; COVID-19, coronavirus disease 2019; ICU, intensive care unit; IFN-γ, interferon gamma; IL, interleukin; TNF-α, tumor necrosis factor alpha.

RNA or the intermediates can not be recognized by the immune system for activation downstream inflammation cascades. Our study emphasizes circulating levels of IL-2, IL-4, TNF-α, IFN-γ, and CRP are not associated with the severity of COVID-19 symptoms. To address why severe COVID-19 is independent of inflammation response that would be a fundamental question for us to understand COVID-19 pathophysiology.

COVID-19 has disparate features in terms of severity, mortality, and spread across countries. A striking variation in mortality rates has been observed in different countries.[13] Enormous differences in human leukocyte antigen haplotype might confer the different immune responses to SARS-CoV-2, which leads to the variation in severity, mortality, and spread rates of COVID-19.[14] However, the causation of COVID-19

severity and mortality requires more investigation. Some reports showed cytokine storm is correlated with the severity and mortality of COVID-19 patients.[5,7,8] But the correlation does not indicate causation. More viral replication also could drive the consequent severity of COVID-19. Janus kinase (JAK) inhibitors targeting cytokines with JAK-dependent signaling were thought to be the potential to restrain the excessive level of cytokine signaling.[15] Currently, IL-6R and IL-6 inhibitors were used in COVID-19 patients which have already been launched. Experts hope IL-6R and IL-6 inhibitors could inhibit hyperinflammatory response in COVID-19 patients independent on viral clearance.[16] The hypothesis that blocking cytokine storm eases COVID-19 severity needs to be a more careful investigation based on our observation. At least, each patient should be tested circulating levels of inflammatory cytokines before launching anti-inflammatory treatment.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

QW conceptualized the study. QW and WL analyzed the data. WL and JZ contributed to manuscript preparation. WL, WZ, and YL collected the patients information. QW wrote the first draft of the manuscript. All of the authors contributed to revising the manuscript, and read and approved the final version for publication.

ETHICS STATEMENT

Each patient consents to participate in this study.

Wen Luo[1]
Jia-Wen Zhang[1]
Wei Zhang[1]
Yuan-Long Lin[2]
Qi Wang[3]

[1]*Department of Respiratory and Critical Care, First Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang, China*
[2]*Department of Infection, Fourth Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China*
[3]*State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, Heilongjiang, China*

Correspondence
Qi Wang, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150069, Heilongjiang, China.
Email: qiwang@caas.cn

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Wen Luo and Jia-Wen Zhang contributed equally to this work.

ORCID

Qi Wang http://orcid.org/0000-0001-6942-2410

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