Letters

RESEARCH LETTER

Cytokine Levels in Critically Ill Patients With COVID-19 and Other Conditions

An abnormally strong proinflammatory response known as "cytokine storm" may play an important role in the pathophysiology of coronavirus disease 2019 (COVID-19), although cytokine storm remains ill defined.[1] Sinha and colleagues[2] reported that although IL-6 levels are elevated in severe COVID-19, they are lower than levels usually observed in (non-COVID-19) acute respiratory distress syndrome (ARDS). However, this comparison is limited by the use of different assays, which are not well standardized.[3] We compared cytokine levels in critically ill patients with COVID-19 vs levels in patients with other critical illnesses.

Methods | All patients in this study were admitted to the intensive care unit (ICU) of Radboud University Medical Center. Plasma concentrations of the proinflammatory cytokines tumor necrosis factor (TNF), IL-6, and IL-8 were determined in consecutive mechanically ventilated patients with COVID-19 with ARDS (partial pressure of oxygen/fraction of inspired oxygen ratio <300; sampled within 48 hours after ICU admission), bacterial septic shock with or without ARDS (sampled within 24 hours after septic shock diagnosis), out-of-hospital cardiac arrest (OHCA; sampled within 24 hours after ICU admission), and multiple traumas (sampled within 24 hours after trauma). The patients with sepsis and trauma are part of larger published cohorts,[4,5] whereas data of 14 patients with OHCA were previously published.[6] Sampling occurred between 2010 and 2020 (**Table**). Patients with immunological insufficiencies were excluded, defined as chronic/concomitant use of immunosuppressive medication, chemotherapy/radiotherapy in the last year or in the past for (non-)Hodgkin lymphoma, or humoral/cellular deficiencies. Cytokines in all cohorts were determined using the same methodology (Milliplex assay, Millipore, on a MAGPIX instrument, Luminex Corporation) by the same technician using the same protocol.

Patient characteristics were analyzed using Fisher exact or Kruskal-Wallis tests followed by Dunn post hoc tests. Cytokine data are presented as geometric means (95% CIs) and analyzed using 1-way analysis of variance on log-transformed data followed by Dunnett post hoc tests.

Table. Patient Characteristics[a]

Characteristic	COVID-19 with ARDS, March 11 to April 27, 2020 (n = 46)	Septic shock, March 15, 2013, to March 28, 2017		Out-of-hospital cardiac arrest, February 5, 2010, to December 12, 2013 (n = 30)	Trauma, March 19, 2011, to May 30, 2013 (n = 62)
		With ARDS (n = 51)	Without ARDS (n = 15)		
Sex, No. (%)					
Male	34 (74)	36 (71)	6 (40)	22 (73)	44 (71)
Female	12 (26)	15 (29)	9 (60)	8 (27)	18 (29)
Age, median (IQR), y	67 (57-71)	62 (53-72)	73 (64-78)	65 (52-75)	58 (37-72)
BMI, median (IQR)	27.5 (25.0-29.3)	26.4 (23.8-30.5)	25.0 (21.5-30.3)	25.1 (23.4-26.9)[b]	24.7 (23.2-27.4)[c]
Medical history, No. (%)					
Cardiovascular insufficiency	12 (26)	2 (4)[c]	2 (13)	1 (3)[b]	1 (2)[d]
Respiratory insufficiency	3 (7)	1 (2)	0	0	0
COPD	3 (7)	5 (10)	0	0	0
Kidney insufficiency	0	5 (10)	0	0	0
Metastatic neoplasm	4 (9)	1 (2)	2 (13)	1 (3)	0[b]
Diabetes	13 (28)	8 (16)	1 (7)	1 (3)[c]	4 (6)[c]
Hematologic malignancy	0	0	0	0	0
APACHE II score, median (IQR)[e]	14 (12-18)	21 (17-26)[d]	24 (18-31)[d]	27 (20-34)[d]	20 (14-25)[c]
Pao_2/Fio_2 ratio, median (IQR)	139 (107-171)	206 (162-260)[d]	354 (328-424)[d]	246 (159-370)[d]	253 (201-361)[d]
Leukocytes, median (IQR), $\times 10^9$/L	8.2 (6.4-11.1)	14.0 (9.8-20.8)[d]	15.4 (7.2-24.4)[c]	12.9 (10.0-16.7)[d]	11.8 (8.9-14.0)[c]

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; ARDS, acute respiratory distress syndrome; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; Fio_2, fraction of inspired oxygen; IQR, interquartile range; Pao_2, partial pressure of oxygen.

[a] Data were obtained on the same day that blood was obtained for cytokine determination.

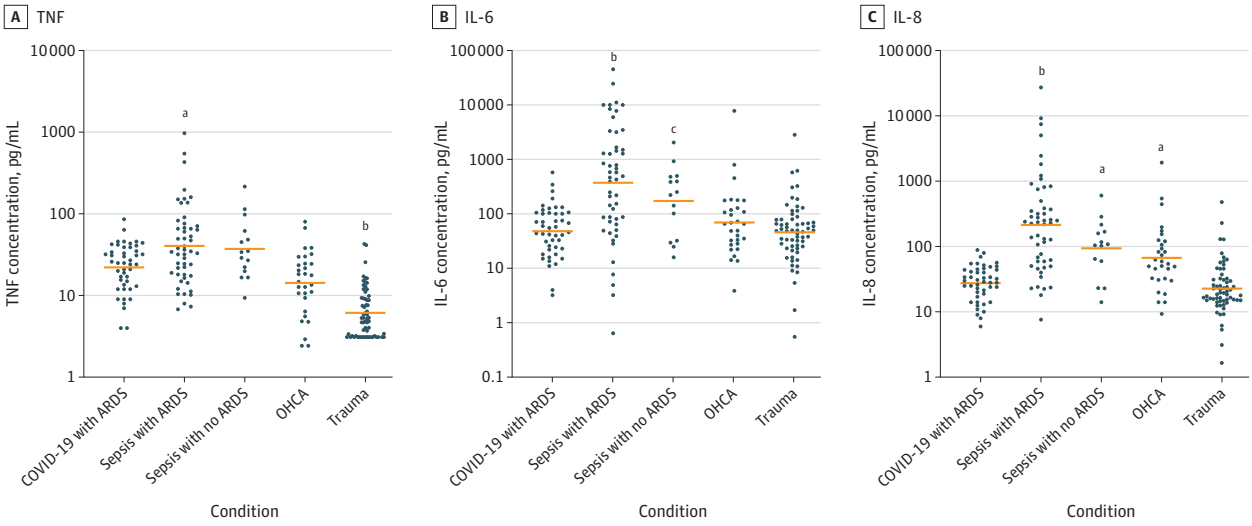
[b] $P < .05$ vs COVID-19 with ARDS.

[c] $P < .01$ vs COVID-19 with ARDS.

[d] $P < .001$ vs COVID-19 with ARDS.

[e] Intensive care unit score of overall disease severity ranging from 0-71; a higher score indicates more severe disease.

Figure. Cytokine Levels in Critically Ill Patients With Coronavirus Disease 2019 (COVID-19) and Other Conditions



Plasma concentrations of tumor necrosis factor (TNF) (A), IL-6 (B), and IL-8 (C) in patients with COVID-19 and acute respiratory distress syndrome (ARDS) (n = 46), septic shock with ARDS (n = 51), septic shock without ARDS (n = 15), out-of-hospital cardiac arrest (OHCA; n = 30), and multiple traumas (n = 62). Data are presented as scatter plots with red horizontal bars indicating the geometric mean levels.

[a] $P < .01$ vs COVID-19 with ARDS.

[b] $P < .001$ vs COVID-19 with ARDS.

[c] $P < .05$ vs COVID-19 with ARDS.

Data were analyzed using Graphpad Prism version 8.3.0 (Graphpad Software). A 2-sided $P < .05$ was considered statistically significant. The study was carried out in accordance with the applicable rules concerning the review of research ethics committees and informed consent in the Netherlands. All patients or legal representatives were informed about the study details and allowed to abstain from participation. Patients who consented to participate or their next of kin provided oral consent.

Results | There were 46 patients with COVID-19 with ARDS, 51 with septic shock with ARDS, 15 with septic shock without ARDS, 30 with OHCA, and 62 with multiple traumas. There were no significant differences in sex or age between patients with COVID-19 and other patient groups (Table). Patients with COVID-19 had a higher body mass index and prevalence of diabetes than patients with OHCA and trauma. In COVID-19, cardiovascular insufficiency was more common, overall disease severity and leukocyte counts were lower, and lung injury was more severe compared with the other groups.

Levels of all 3 cytokines were significantly lower in patients with COVID-19 than in patients with septic shock with ARDS; the geometric means were 22 pg/mL (95% CI, 18-27) vs 40 pg/mL (95% CI, 30-55) ($P < .01$) for TNF; 48 pg/mL (95% CI, 35-66) vs 376 pg/mL (95% CI, 190-744) ($P < .001$) for IL-6; and 27 pg/mL (95% CI, 23-33) vs 215 pg/mL (95% CI, 133-347) ($P < .001$) for IL-8 (depicted in the **Figure** on a log scale). Patients with COVID-19 also displayed significantly lower IL-6 and IL-8 concentrations compared with patients with septic shock without ARDS (Figure). TNF levels in patients with COVID-19 were higher than those in trauma patients, whereas no differences between patients with COVID-19 and OHCA or trauma were present for IL-6. For IL-8, lower concentrations were found in patients with COVID-19 compared with patients with OHCA, while no differences vs the trauma group were observed.

Discussion | In this study, critically ill patients with COVID-19 with ARDS had circulating cytokine levels that were lower compared with patients with bacterial sepsis and similar to other critically ill patients. These findings are in line with lower leukocyte counts observed in patients with COVID-19, and are possibly due to lower overall disease severity, despite the presence of severe pulmonary injury. The findings of this preliminary analysis suggest COVID-19 may not be characterized by cytokine storm. Whether anticytokine therapies will benefit patients with COVID-19 remains to be determined. Limitations of the study include the small sample sizes, single center involved, and the use of different lots of the same assays without data on lot-to-lot variability.

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Concept and design: Kox, Pickkers.
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Critical revision of the manuscript for important intellectual content: Kooistra, Gerretsen, Pickkers.
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