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LETTER TO EDITOR

Alteration of serum markers in COVID-19 and implications on mortality

Dear editor,

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has emerged as a global pandemic since its initial outbreak in Wuhan, China.[1] Among the many unanswered questions for COVID-19, how to reduce mortality and improve survival of patients is the most critical. Evidence indicates that increased cytokine levels (also known as cytokine storm) might be a major contributor of disease deterioration in COVID-19 leading to death.[2–4] Previous reports focused on cytokine storm in COVID-19 have been limited by shorter follow ups, small sample sizes, and evaluation of subgroups of patients.[5–8] Longitudinal profile of cytokine variations in large cohort has not been comprehensively evaluated.

Here, we present an analysis of laboratory indices and cytokines, along with risk factors and mortality in patients with COVID-19 using a closed, multicenter retrospective cohort study. Overall, 2044 COVID-19 patients, who hospitalized in the Optical Valley Campus and Sino-French New City Campus of Tongji Hospital in Wuhan, China, between January 27 and March 21 and had definite outcome (discharge or death), were included in our analysis (Figure S1).

Overall, 235 patients died during hospitalization, while 1809 patients were discharged from the hospital (Table S1). The median (IQR) age of all patients was 62.0 (IQR 51.0-70.0) years, and 48.92% were men. Over half of the patients (1175, 57.63%) had at least one comorbidity. Patients who died were significantly older (median (IQR) age, 70 [63-78] vs 61 [49-69], $P < .0001$), more likely to be male (156 [66.38%] vs 844 [46.66%], $P < .001$) and to have comorbidities (189 [81.12%] vs 986 [54.60%], $P < .001$).

Cytokine profiles of patients are shown in Table S2. Over half of COVID-19 patients (1110, 57.33%) had increased C reactive protein (CRP) on admission. Elevated ferritin and tumor necrosis factor-α (TNF-α) occurred in 651 (56.17%) and 762 (46.15%) patients, respectively. Elevation of cytokines such as IL-2R and IL-6 occurred in 589 (35.61%) and 547 (32.89%) patients, respectively. Patients who died had significantly higher median levels of CRP (103.2 [61.2-169.1)] vs 11.4 [2.0-51.1], $P < .0001$) and ferritin (1427.6 [848.2-2395.4)] vs 494.4 [288.0-840.8], $P < .0001$) compared to the population who recovered. They also had significantly higher proportion of increased TNF-α (75.54% vs 42.47%, $P < .001$) and multiple cytokines compared to survivors, such as IL-2R (1174.0 [828.5-1611.0] vs 529.0 [344.0-771.0], $P < .0001$) and IL-6 (62.67 [30.17-157.2] vs 4.31 [1.78-15.06], $P < .0001$). The details of treatments and outcomes are described in Table S3.

To depict the dynamic course of COVID-19 and further explore the risk factors associated with poor prognosis, cytokines and other significant indices were tracked over 6 weeks (shown in Figures S2 and S3). The mortality reached its peak approximately around the third week of illness from the onset. Similarly, elevations of D-dimer and cTnI were also observed around 3 weeks from the illness onset among patients with higher mortality. Lastly, in week 5-6, elevated levels of cytokines (including IL-2R, IL-6, IL-8, and IL-10, Ferritin, and TNF-α) were seen among non-survivors compared with patients who recovered. Other laboratory indices, such as LDH, procalcitonin (PCT), and NT-proBNP, also peaked in the corresponding period.

To further understand the role of cytokine storm and other risk factors in COVID-19-related mortality, univariable logistic regression was performed as summarized in Table 1. Older patients were associated with increased odds of death than the younger. The risk of death increased proportionately with the number of comorbidities. The presence of severe respiratory symptoms and unstable vital signs on admission also predicted poor outcomes.

Surprisingly, we found that some risk factors of mortality disproportionately affected males compared to females. Hypertension (OR 2.88, 95% CI 2.02 to 4.11, $P < .001$) and coronary heart disease (CHD) (2.96, 1.88 to 4.67, $P < .001$) increased the odds of death in males but had no

Abbreviations: CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CRP, C reactive protein; cTnI, high-sensitivity cardiac troponin I; IL-2R, interleukin-2R; IL-6, interleukin-6; IL-8, interleukin-8; LDH, lactate dehydrogenase; PCT, procalcitonin

TABLE 1 Analysis of risk factors associated with fatal outcome in COVID-19

	Univariable Analysis				Effect modification	Multivariable Analysis			
	Male		Female			Male		Female	
	OR (95% CI)	*P*-value[†]	OR (95% CI)	*P*-value[†]	*P*-value[‡]	OR (95% CI)	*P*-value[†]	OR (95% CI)	*P*-value[†]
Risk Factors with Differences between Males and Females									
Hypertension	2.88 (2.02-4.11)	<.001	1.07 (0.67-1.71)	.779	<.001	2.04 (1.17-3.54)	.012		
CHD	2.96 (1.88-4.67)	<.001	1.50 (0.74-3.02)	.257	.044				
Tumor	2.87 (1.36-6.04)	.006	4.82 (2.07-11.22)	<.001	.076				
COPD	3.50 (1.13-10.84)	.030	18.99 (3.12-115.37)	.001	.064				
Platelet count, 10^9/L <100 versus ≥100	7.58 (4.37-13.13)	<.001	13.93 (7.00-27.70)	<.001	.082				
PT, s ≥17 versus <17	20.02 (10.34-38.78)	<.001	49.61 (19.11-128.82)	<.001	.094				
NT-proBNP, pg/mL >241 versus ≤241	9.79 (6.49-14.77)	<.001	26.80 (14.34-50.09)	<.001	.006				
C reactive protein, mg/L	1.02 (1.01-1.02)	<.001	1.02 (1.02-1.03)	<.001	.018				
Procalcitonin, ng/mL	10.73 (5.68-20.29)	<.001	11.88 (5.71-24.72)	<.001	.058				
IL-2R, U/mL >710 versus ≤710	6.08 (3.82-9.69)	<.001	14.56 (7.58-27.95)	<.001	.041			3.02 (1.27-7.19)	.012
IL-6, pg/mL	1.02 (1.02-1.03)	<.001	1.04 (1.03-1.05)	<.001	.005				
IL-8, pg/mL ≥62 versus <62	6.47 (3.77-11.11)	<.001	11.88 (6.15-22.96)	<.001	.060			5.61 (1.61-19.47)	.007
IL-10, pg/mL	1.03 (1.01-1.05)	.016	1.22 (1.16-1.29)	<.001	<.001				
TNFα, pg/mL ≥8.1 versus <8.1	2.75 (1.78-4.27)	<.001	6.23 (3.42-11.35)	<.001	.020				
Characteristics									
Age, years	1.07 (1.05-1.08)	<.001	1.07 (1.05-1.09)	<.001					
≥50 years	20.03 (6.33-63.41)	<.001	7.49 (2.34-23.98)	.001		5.90 (1.33-26.11)	.019		
Presence of comorbidities	4.46 (2.82-7.03)	<.001	2.48 (1.47-4.18)	.001					
Number of comorbidities	1.86 (1.58-2.19)	<.001	1.53 (1.24-1.88)	<.001					
Respiratory rate, per min ≥24 versus <24	8.75 (5.97-12.82)	<.001	11.97 (7.03-20.39)	<.001					
SpO2, % ≤93 versus >93	13.37 (8.71-20.54)	<.001	17.83 (10.06-31.62)	<.001					
SOFA	4.24 (3.40-5.27)	<.001	3.61 (2.88-4.53)	<.001					

(Continues)

TABLE 1 (Continued)

	Univariable Analysis				Effect modification	Multivariable Analysis			
	Male		Female			Male		Female	
	OR (95% CI)	*P*-value[†]	OR (95% CI)	*P*-value[†]	*P*-value[‡]	OR (95% CI)	*P*-value[†]	OR (95% CI)	*P*-value[†]
Laboratory findings									
WBC count, 10^9/L >10 versus ≤10	11.96 (7.77-18.43)	<.001	24.35 (14.12-42.00)	<.001		5.64 (2.83-11.24)	<.001	6.87 (2.68-17.63)	<.001
Lymphocyte count, 10^9/L <0.8 versus ≥0.8	8.21 (5.56-12.12)	<.001	10.49 (6.32-17.42)	<.001		3.47 (1.96-6.12)	<.001	3.65 (1.60-8.29)	.002
Hemoglobin, g/L <120 versus ≥120	1.54 (1.01-2.34)	.043	1.91 (1.17-3.14)	.010					
BUN, mmol/L ≥10 versus <10	15.25 (9.56-24.30)	<.001	27.34 (14.31-52.21)	<.001					
Creatinine, μmol/L ≥110 versus <110	4.42 (2.90-6.72)	<.001	10.31 (5.29-20.10)	<.001					
APTT, s ≥52 versus <52	5.99 (3.22-11.12)	<.001	3.82 (1.59-9.20)	.003					
D-dimer, μg/mL >1 versus ≤1	9.02 (5.65-14.39)	<.001	17.94 (8.51-37.79)	<.001		2.91 (1.56-5.44)	.001		
High-sensitivity cardiac troponin I, pg/mL Male >34.2 versus ≤34.2[§]; Female >15.6 versus ≤15.6[§]	39.06 (23.10-66.05)	<.001	22.44 (13.02-38.68)	<.001				6.70 (3.02-14.89)	<.001
Procalcitonin, ng/mL ≥0.25 versus <0.25	11.53 (7.67-17.32)	<.001	19.21 (10.86-34.00)	<.001		2.42 (1.33-4.41)	.004		
C reactive protein, mg/L >10 versus ≤10	22.90 (8.40-62.48)	<.001	13.32 (5.72- 31.06)	<.001		4.99 (0.98-25.31)			
Ferritin, μg/L Male >800 versus ≤ 800[¶]; Female >300 versus ≤ 300[¶]	5.08 (3.08-8.40)	<.001	18.01 (4.34-74.78)	<.001					
IL-6, pg/mL ≥14 versus <14	16.63 (9.32-29.68)	<.001	35.51 (15.04-83.86)	<.001		5.21 (2.65-10.27)	<.001	12.89 (4.71-35.30)	<.001

Abbreviations: CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; CLD, chronic liver disease; HBV, hepatitis B virus; CKD, chronic kidney disease; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell; BUN, blood urea nitrogen; PT, prothrombin time; APTT, activated partial thromboplastin time; IL-2R, interleukin-2R; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; TNF-α, tumor necrosis factor-α.

[†] $P < .05$ was considered statistically significant.

[‡] The statistical significance of effect modification between gender and other factors were tested using logistic regression models containing the interaction terms (gender and hypertension, gender, CHD, etc.).

[§] Upper limit of normal value (ULN) for males and females, separately.

[¶] Two times of upper limit of normal value (ULN) for males and females, separately.

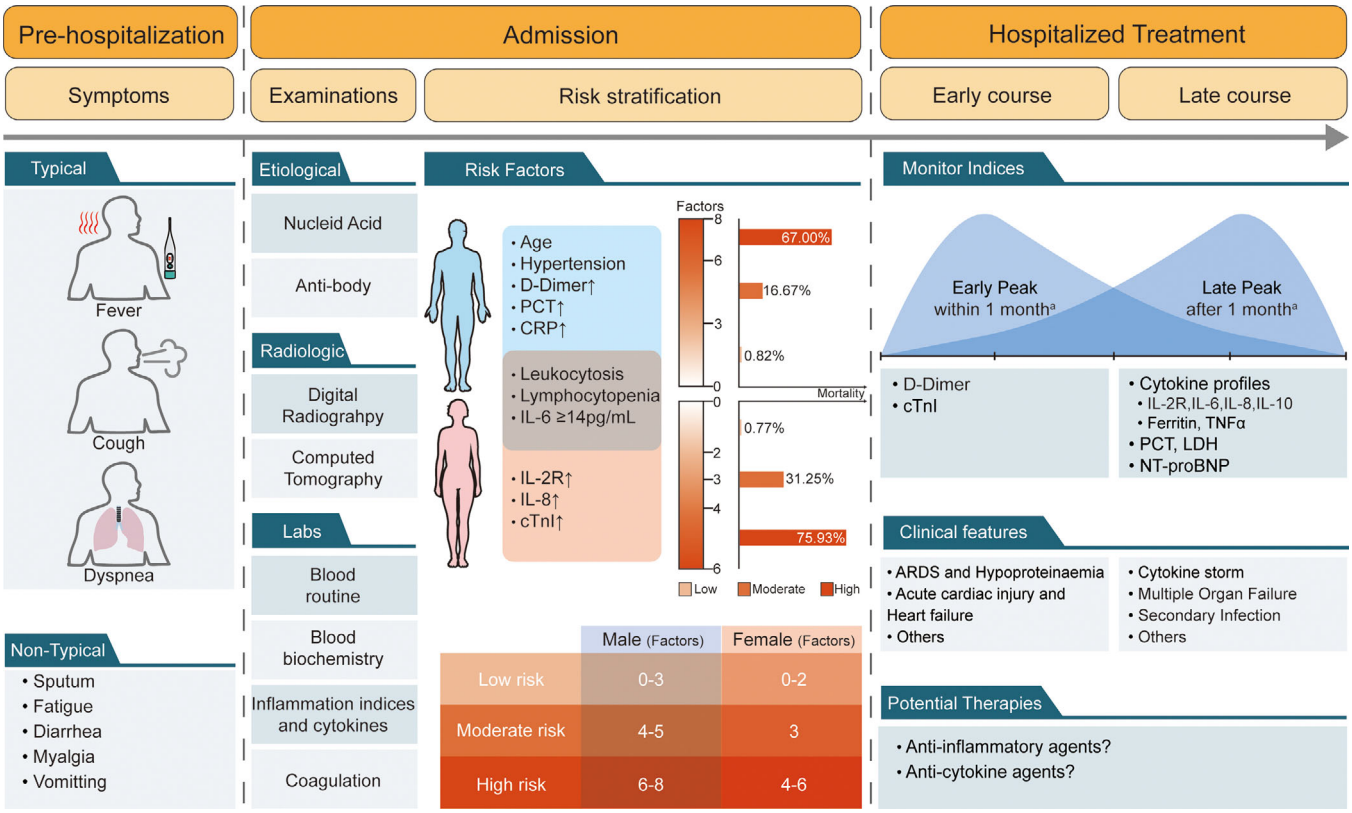

FIGURE 1 Risk Management during the Clinical Course of COVID-19. Different strategies of risk management in different periods of COVID-19. Leukocytosis was defined as white blood cells count greater than 10×10^9/L. Lymphocytopenia was defined as lymphocyte count less than 0.8×10^9/L. Risk factors for male: age ≥ 50 years, with hypertension, D-dimer > 1 μg/mL, PCT ≥ 0.25 ng/mL, CRP > 10 mg/L, leukocytosis, lymphocytopenia, and IL-6 ≥ 14 pg/mL. Risk factors for female: IL-8 ≥ 62 pg/mL, IL-2R > 710 U/mL, cTnI > 15.6 pg/mL, leukocytosis, lymphocytopenia, and IL-6 ≥ 14 pg/mL. [a]Time from illness onset

adverse influence on females (Figures S4 and S5). However, women with cancer and COPD had a higher risk of death compared to men. Females were also found to have increased odds of death in the cases of cytokine storm, cardiac injury, and coagulopathy compared to their male counterparts.

In the multivariable logistic regression model, we analyzed the factors with significant impact on mortality separately for males and females. Overall, IL-6 ≥ 14 pg/mL, leukocytosis, and lymphocytopenia were independent risk factors of death for both sexes. For male patients, advanced age (≥50 years), hypertension, the elevated PCT, CRP, and D-dimer were associated with increased odds of mortality. Meanwhile, IL-2R, IL-8, and cTnI were independent risks for higher mortality in females.

To further investigate the influence of risk factors, we plotted Kaplan-Meier curves to study the prognoses of patients based on the numbers of independent risk factors they had (Figure S4C,D). Males (females) who had 0-3 (0-2), 4-6 (3), and 7-8 (4-6) factors were regarded as low-risk, moderate-risk, and high-risk patients, respectively, and had diverse prognoses. 67.00% (75.93%) of the high-risk male (female) patients died while only 0.82% (0.77%) of the low-risk ones had fatal outcome. A summary of these findings is shown in Figure 1.

In conclusions, IL-6 ≥ 14 pg/mL, leukocytosis, and lymphocytopenia were found to be risk factors for mortality in both sexes. Advanced age, presence of hypertension, elevated D-dimer, CRP, and PCT were independent risk factors of death only in males, while elevated IL-2R, IL-8, and cTnI increased the risk of mortality in females. Increase in D-dimer and cTnI were observed in the second and third weeks of illness onset while multiple cytokines were found to be increased in the fifth and sixth weeks among those with high mortality. Cytokine storm was a major concern throughout the clinical course, especially in later stages of COVID-19 and among females. Whether early intervention with potential anti-inflammatory or anti-cytokine agents can improve the prognosis in COVID-19 remains to be seen. Risk stratification based on cytokine profile and other risk factors might be considerable in the management of COVID-19.

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ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Research Ethics Commission of Tongji Hospital of Huazhong University of Science and Technology (TJ-IRB20200406) in view of the retrospective nature of the study and all the procedures being performed were part of the routine care. The trial has been registered in the Chinese Clinical Trial Registry (ChiCTR2000032161). The informed consents were waived by the Ethics Commission of Tongji Hospital of Huazhong University of Science and Technology.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTORS

QG had full access to all data in the study and take responsibility for the integrity of data and the accuracy of the data analysis. SZ, SW, YG, SX, RY, YW, and YY collected the clinical data. XJ, JC, YaY, CS, NJ, PC, JL, XZ, WG, XL, and GC double-checked and entered the data into database. DL, RL, XF, CL, and QG analyzed the clinical records. RL, RY, YW, XF, YY, HL, and AD drafted the manuscript. DL, QG, RL, RY, YW, XF, and YY analyzed and interpreted the data. CL, JS, and SK advised on the conception and design of the study. DL, CL, QG, and AD conceptualized and designed the study, supervised the project, and revised the manuscript. All authors vouch for the respective data and analysis, revised, approved the final version, and agreed to publish the manuscript. DL, RL, RY, YW, XF, and YY share first authorship, the order in which they are listed was determined by workload.

AVAILABILITY OF DATA AND MATERIAL

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

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