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# Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19



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**Background:** The outbreak of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 was first reported in Wuhan, December 2019, and continuously poses a serious threat to public health, highlighting the urgent need of identifying biomarkers for disease severity and progression.

**Objective:** We sought to identify biomarkers for disease severity and progression of COVID-19.

**Methods:** Forty-eight cytokines in the plasma samples from 50 COVID-19 cases including 11 critically ill, 25 severe, and 14 moderate patients were measured and analyzed in combination with clinical data.

**Results:** Levels of 14 cytokines were found to be significantly elevated in COVID-19 cases and showed different expression profiles in patients with different disease severity. Moreover, expression levels of IFN- $\gamma$ -induced protein 10, monocyte chemotactic protein-3, hepatocyte growth factor, monokine-

induced gamma IFN, and macrophage inflammatory protein 1 alpha, which were shown to be highly associated with disease severity during disease progression, were remarkably higher in critically ill patients, followed by severe and then the moderate patients. Serial detection of the 5 cytokines in 16 cases showed that continuously high levels were associated with deteriorated progression of disease and fatal outcome. Furthermore, IFN- $\gamma$ -induced protein 10 and monocyte chemotactic protein-3 were excellent predictors for the progression of COVID-19, and the combination of the 2 cytokines showed the biggest area under the curve of the receiver-operating characteristics calculations with a value of 0.99.

**Conclusions:** In this study, we report biomarkers that are highly associated with disease severity and progression of COVID-19. These findings add to our understanding of the immunopathologic mechanisms of severe acute respiratory syndrome coronavirus 2 infection, and provide potential therapeutic targets and strategies. (*J Allergy Clin Immunol* 2020;146:119-27.)

**Key words:** COVID-19, SARS-CoV-2, biomarkers, disease progression, prediction

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Coronaviruses are the largest known viruses with a single positive-sense genome of about 31 Kb that could infect a wide range of different species causing a wide range of symptoms.<sup>1</sup> Six members of coronaviruses have been previously found to infect human beings: HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV).<sup>1,2</sup> Human infection with the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in late December 2019 in Wuhan, China.<sup>3,4</sup> As of March 6, 2020, a total of 80,860 coronavirus disease 2019 (COVID-19) cases with 3213 fatal cases were reported in China.<sup>5</sup> Furthermore, another 145 countries, areas, or territories have reported COVID-19 cases, causing a pandemic around the world.<sup>6</sup>

Studies have revealed that pneumonia is the most common complication following SARS-CoV-2 infection, followed by acute respiratory distress syndrome (ARDS).<sup>7,8</sup> Inflammation is the body's first coordinated line of defense against tissue damage caused by either injury or infection, involving activating both the innate and adaptive immune responses.<sup>9</sup> However, exuberant immune responses following infection, also termed cytokine storm, have been found to be associated with excessive levels of proinflammatory cytokines and widespread tissue damage.<sup>10</sup> Cytokine

**Abbreviations used**

ARDS:	Acute respiratory distress syndrome
AUC:	Area under the curve
COVID-19:	Coronavirus disease 2019
dao:	Days after onset
FiO <sub>2</sub> :	Fraction of inspired oxygen
HGF:	Hepatocyte growth factor
IP-10:	IFN- $\gamma$ -induced protein 10
MCP-3:	Monocyte chemoattractant protein-3
MIG:	Monokine-induced gamma IFN
MIP-1 $\alpha$ :	Macrophage inflammatory protein 1 alpha
MERS-CoV:	Middle East respiratory syndrome coronavirus
PaO <sub>2</sub> :	Arterial oxygen partial pressure
ROC:	Receiver-operating characteristic
SARS-CoV-2:	Severe acute respiratory syndrome coronavirus 2

storm has been found during the infection of influenza viruses<sup>11-16</sup> as well as coronaviruses,<sup>17-24</sup> and contributes to acute lung injury and the development of ARDS.<sup>2</sup> Preliminary studies have shown that SARS-CoV-2 infection triggers cytokine storm, and results in an increase in various cytokines.<sup>25,26</sup> However, identification of biomarkers for disease severity and progression is still in urgent need.

In this study, we recruited 50 COVID-19 cases including 11 critically ill, 25 severe, and 14 moderate patients defined according to China National Health Commission Guidelines for Diagnosis and Treatment of SARS-CoV-2 infection, and analyzed the expression profiles of plasma cytokines in combination with clinical data in detail.

**METHODS****Subject information and data collection**

Subjects presented in this study were hospitalized patients with COVID-19 (N = 50), including 11 critically ill, 25 severe, and 14 moderate patients defined according to China National Health Commission Guidelines for Diagnosis and Treatment of 2019-nCoV infection. Healthy controls (N = 8) were also included. Clinical information and laboratory results were collected at the earliest time point after hospital admission. The study protocol was approved by the ethics committees of Shenzhen Third People's Hospital (2020-010). Verbal informed consents were obtained from all patients with 2019-nCoV and H7N9 infections or patients' family members because of the special circumstances in which pens and papers were not allowed to be brought into containment facilities. The study was conducted in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice and the Declaration of Helsinki and institutional ethics guidelines.

**Disease severity classification**

Disease severity classification and Murray score calculation were evaluated as previously reported.<sup>26</sup> Severity of 2019-novel CoV infection was graded according to China National Health Commission Guidelines for Diagnosis and Treatment of SARS-CoV-2 infection (seventh version). Laboratory-confirmed patients with fever, respiratory manifestations, and radiological findings indicative of pneumonia were considered moderate cases. Laboratory-confirmed patients who met any of the following were considered to have severe COVID-19: (1) respiratory distress (respiration rate,  $\geq 30$ /min), (2) resting oxygen saturation less than or equal to 93%, or (3) arterial oxygen partial pressure (PaO<sub>2</sub>)/fraction of inspired oxygen (FiO<sub>2</sub>) less than or equal to 300 mm Hg (1 mm Hg = 0.133 kPa). Laboratory-confirmed patients who had any of the following were considered critically ill: (1) respiratory failure requiring mechanical ventilation, (2) shock, or (3) failure of other organs requiring intensive care unit admission.

**Quantification of hypoxia and Murray score**

PaO<sub>2</sub> in arterial blood taken from the patients at various time points after hospitalization was measured by the ABL90 blood gas analyzer (Radiometer, Copenhagen, Denmark). FiO<sub>2</sub> was calculated by using the following formula:  $FiO_2 = (21 + \text{oxygen flow [in units of L/min]} \times 4)/100$ . The PaO<sub>2</sub>/FiO<sub>2</sub> ratio (in units of mm Hg) was calculated by dividing the PaO<sub>2</sub> value with the FiO<sub>2</sub> value. A PaO<sub>2</sub>/FiO<sub>2</sub> ratio of less than or equal to 100 mm Hg is considered one of the criteria for severe ARDS. Murray score was calculated as reported.<sup>27</sup>

**Measurement of plasma cytokines**

The plasma of patients with laboratory-confirmed COVID-19 (N = 50) was collected at the earliest possible time point after hospitalization and thereafter. The plasma of healthy subjects (N = 8) was included as the negative control group. The concentrations of 48 cytokines were measured using Bio-Plex Pro Human Cytokine Screening Panel (Bio-Rad, Berkeley, Calif) as previously reported.<sup>26</sup>

**Statistical analysis**

Chi-square and ANOVA tests were used to compare the indicated rates and the plasma cytokine levels among the moderate, severe, and critical groups. The Spearman rank correlation coefficient was used for linear correlation analysis between the expression level of plasma cytokine and Murray score. The area under the receiver-operating characteristic (ROC) curve (AUC) of plasma cytokine levels was estimated for the patients developing ARDS or not. Moreover, the combined values for the prediction of developing ARDS was calculated using binary logistic regression. All statistical tests were calculated using SPSS 20.0 for Windows (IBM, Chicago, Ill). *P* value of less than .05 was considered statistically significant.

**RESULTS****Epidemiological and clinical characteristics**

A total of 50 patients with 29 males (58%) were included in our study. These patients were further divided into 3 groups of critically ill, severe, and moderate on the basis of disease severity according to the China National Health Commission Guidelines for Diagnosis and Treatment of SARS-CoV-2 infection. The median age of these patients was 62 years, and ranged from 22 to 78 years (Table I). Most of the critically ill (81.8%) and severe cases (68%) were 60 years or older, whereas the moderate cases were mainly in the age group of 16 to 59 years (64.3%) (Table I). Initial symptoms of fever and myalgia, complications including ARDS, respiratory failure, and hepatic and renal insufficiency, occurred more frequently in critically ill and severe patients (Table I). The median duration of hospitalization was 16, 21, and 39 days for the moderate, severe, and critically ill patients, respectively. A complete blood cell count with differential was assessed for each patient either on the date of hospital admission or at the earliest time point (see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The percentage of lymphocyte as well as the CD4 and CD8 counts were significantly lower in the critically ill and severe patients, whereas the percentage of neutrophils was higher in critically ill patients. Indexes for the evaluation of liver, kidney, and heart injuries showed no differences among the 3 groups on admission (Table E1).

**Differential expression profile of cytokines in patients with different disease severity**

First, we analyzed the expression profile of the patients with COVID-19 upon admission. Results showed that a total of 30 cytokines including both proinflammatory and anti-inflammatory

**TABLE I.** Epidemiologic and clinical features of patients with COVID-19 in this study

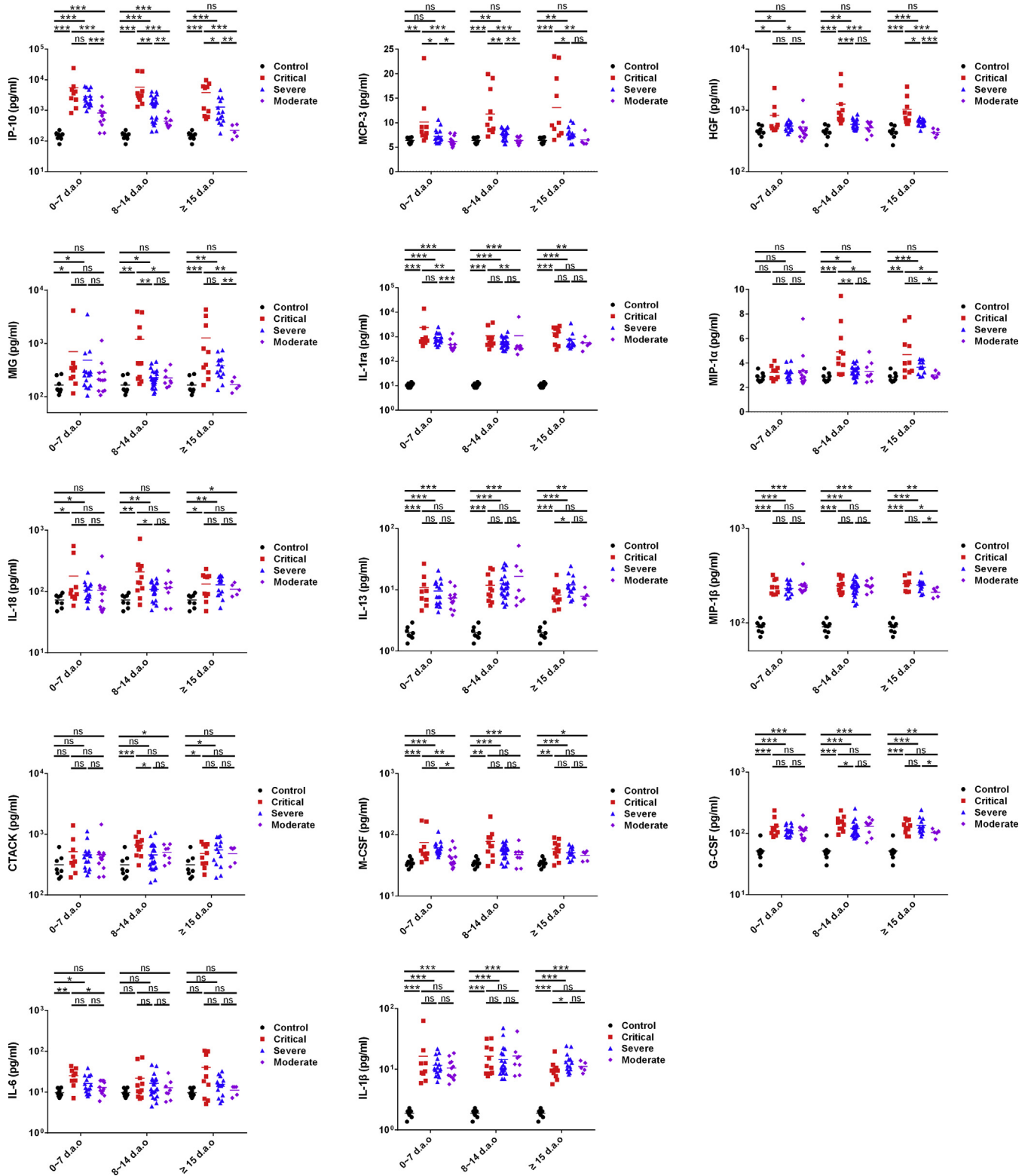
Characteristic	Total (N = 50)	Moderate (N = 14)	Severe (N = 25)	Critical (N = 11)
Age (y), median (range)	62 (22-78)	51.5 (22-78)	63 (38-74)	65 (46-73)
Age subgroups (y)				
0-15	0 of 50 (0%)	0 of 14 (0%)	0 of 25 (0%)	0 of 11 (0%)
16-59	18 of 50 (36%)	9 of 14 (64.3%)	8 of 25 (32%)	2 of 11 (18.2%)
≥60	32 of 50 (64%)	5 of 14 (35.7%)	17 of 25 (68%)	9 of 11 (81.8%)
Sex: male	29 of 50 (58%)	7 of 14 (50%)	14 of 25 (56%)	8 of 11 (72.7%)
Initial symptoms				
Fever	42 of 50 (84%)	9 of 14 (64.3%)	23 of 25 (92%)	10 of 11 (90.9%)
Cough	35 of 50 (70%)	11 of 14 (78.6%)	17 of 25 (68%)	7 of 11 (63.6%)
Headache	6 of 50 (12%)	1 of 14 (7.1%)	3 of 25 (12%)	2 of 11 (18.2%)
Myalgia	27 of 50 (54%)	5 of 14 (35.7%)	14 of 25 (56%)	8 of 11 (72.7%)
Chill	6 of 50 (12%)	0 of 14 (0%)	4 of 25 (16%)	2 of 11 (18.2%)
Nausea or vomiting	0 of 50 (0%)	0 of 14 (0%)	0 of 25 (0%)	0 of 11 (0%)
Diarrhea	4 of 50 (8%)	0 of 14 (0%)	3 of 25 (12%)	1 of 11 (9.1%)
Coexisting chronic medical conditions	30 of 50 (60%)	4 of 14 (28.6%)	20 of 25 (80%)	6 of 11 (54.55%)
Chronic heart disease	19 of 30 (63.3%)	2 of 4 (50%)	14 of 20 (70%)	3 of 6 (50%)
Chronic lung disease	2 of 30 (6.7%)	0 of 4 (0%)	1 of 20 (5%)	1 of 6 (16.7%)
Chronic renal disease	2 of 30 (6.7%)	0 of 4 (0%)	2 of 20 (10%)	0 of 6 (0%)
Chronic liver disease	2 of 30 (6.7%)	0 of 4 (0%)	2 of 20 (10%)	0 of 6 (0%)
Diabetes	7 of 30 (23.3%)	0 of 4 (0%)	2 of 20 (10%)	4 of 6 (66.67%)
Cancer	4 of 30 (13.3%)	1 of 4 (25%)	2 of 20 (10%)	1 of 6 (16.67%)
Bacterial coinfections	11 of 50 (22%)	3 of 14 (21.4%)	4 of 25 (16%)	4 of 11 (36.4%)
Interval, median days (interquartile range)				
Onset to admission	4 (2-6)	3.5 (2-5)	4 (2-6)	5 (3.5-7)
Onset to starting antiviral treatment	4.5 (3.25-7)	5 (2.5-6.75)	4 (2-6)	5 (2.5-6.5)
Complications				
Pneumonia	50 of 50 (100%)	14 of 14 (100%)	25 of 25 (100%)	11 of 11 (100%)
ARDS	37 of 50 (74%)	0 of 14 (0%)	24 of 25 (96%)	11 of 11 (100%)
Hepatic insufficiency	14 of 50 (28%)	1 of 14 (7.1%)	5 of 25 (20%)	8 of 11 (72.7%)
Renal insufficiency	10 of 50 (20%)	0 of 14 (0%)	4 of 25 (16%)	6 of 11 (54.5%)
Cardiac failure	3 of 50 (6%)	0 of 14 (0%)	0 of 25 (0%)	3 of 11 (27.3%)
Shock	2 of 50 (4%)	0 of 14 (0%)	0 of 25 (0%)	2 of 11 (18.2%)
Treatment				
Received antivirals ≤2 d after illness onset	8 of 50 (16%)	5 of 14 (35.7%)	1 of 25 (4%)	3 of 11 (27.3%)
Received antivirals 3-5 d after illness onset	22 of 50 (44%)	4 of 14 (28.6%)	14 of 25 (56%)	4 of 11 (36.4%)
Received antivirals ≥6 d after illness onset	20 of 50 (40%)	6 of 14 (42.9%)	10 of 25 (40%)	4 of 11 (36.4%)
Corticosteroid	15 of 50 (30%)	3 of 14 (21.4%)	6 of 25 (24%)	6 of 11 (54.5%)
Mechanical ventilation	24 of 50 (48%)	2 of 14 (14.3%)	13 of 25 (52%)	9 of 11 (81.8%)
Invasive mechanical ventilation	9 of 50 (18%)	0 of 14 (0%)	1 of 25 (4%)	8 of 11 (72.7%)
Median days of hospitalization (interquartile range)	19 (15-25)	16 (12.25-17.5)	21 (15-25)	39 (30-44)

were significantly elevated in patients with COVID-19 upon admission, when compared with the healthy control (see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Then, the differential expression profiles of patients with different disease severity at different phases of disease were further analyzed (Fig 1; see Fig E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Time points of sample collection were stratified into 3 groups according to the disease progression as previously reported,<sup>28</sup> the first 7 days after illness onset (0-7 days after onset [dao]), between 8 and 14 days following illness onset (8-14 dao), and during the recovery phase from 15 days after disease onset (≥15 dao). Fourteen cytokines including IL-1β, IL-1ra, IL-6, IL-13, IL-18, HGF (hepatocyte growth factor), MCP-3 (monocyte chemotactic protein-3), MIG (monokine-induced gamma IFN), M-CSF, G-CSF, MIP-1α (macrophage inflammatory protein 1 alpha), MIP-1β, CTACK (cutaneous T-cell-attracting chemokine), and IP-10 (IFN-γ-induced protein 10) showed differential expression levels among the patients with different disease

severity (Fig 1). Expression levels of IP-10, HGF, and MCP-3 were highest in critically ill patients, followed by severe patients, and then the moderate in all groups of sample collection time points. Differential expressions of IL-1ra and M-CSF were mainly found in the early stage of disease progression (0-7 dao), whereas differential expressions of MIG and MIP-1α were found in the later stages of disease progression (8-14 and ≥15 dao). However, little differences were observed in IL-6, IL-13, IL-18, IL-1β, cutaneous T-cell-attracting chemokine, MIP-1β, and G-CSF (Fig 1).

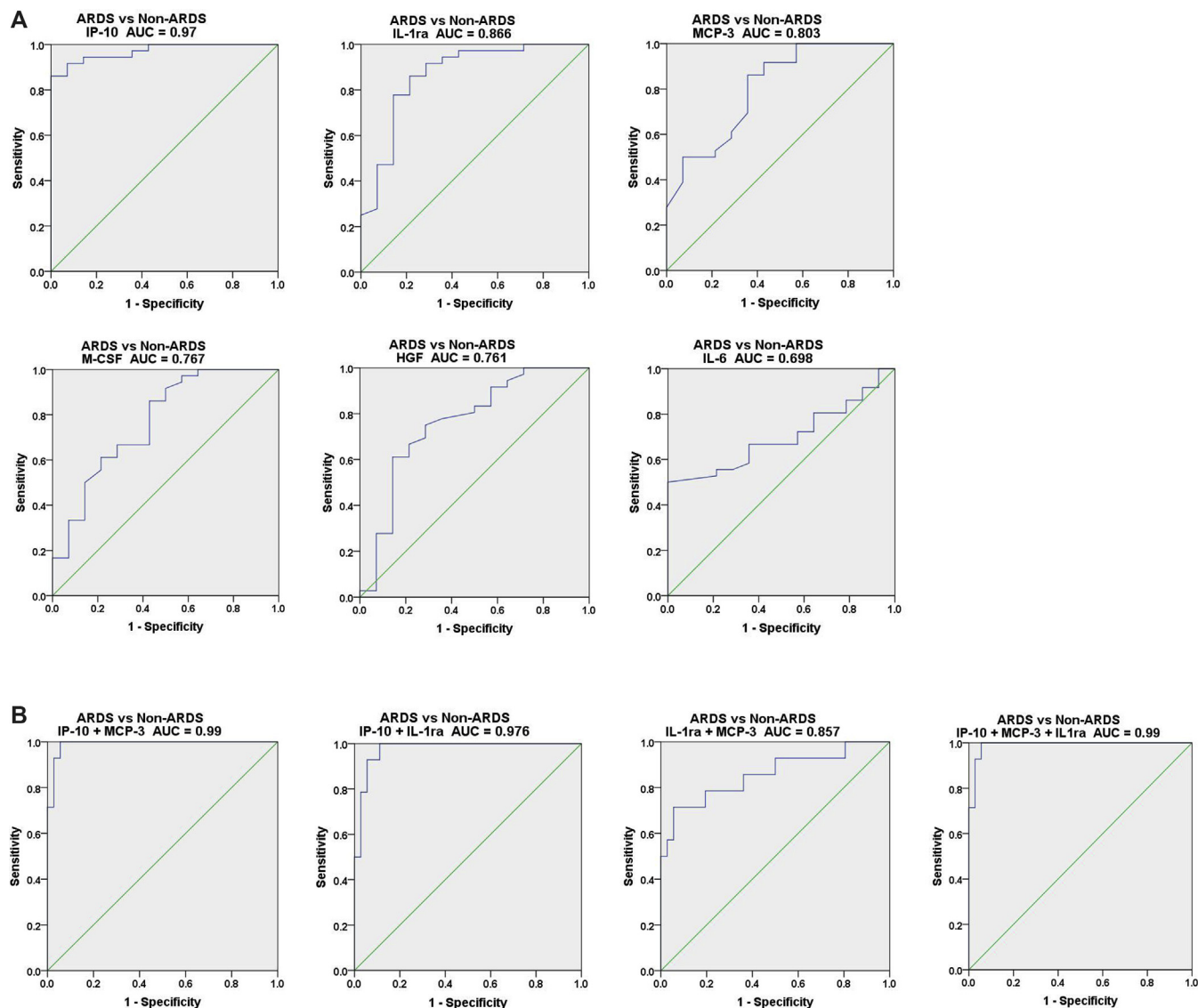
### IP-10, MCP-3, and IL-1ra are independent predictors for the progression of COVID-19

Then, we further analyzed whether these cytokines could be used as predictors for the disease progression of COVID-19. These patients were divided into the ARDS group, which contained the critically ill and severe patients, and the



**FIG 1.** Comparison of the significantly elevated cytokines measured at different days after illness onset among critically ill, severe, and moderate COVID-19 patients. The expression levels of 14 cytokines measured at different disease severity days were shown and compared with the differential expression levels among patients with different disease severity days after illness onset with differential expression levels among patients with different disease severity days after illness onset with differential expression levels among patients with different disease severity days after illness onset. *P* values between .01 and .05, .001 and .01, and .0001 and .001 were considered statistically significant (\*), very significant (\*\*), and extremely significant (\*\*\*), respectively. *ns*, Not significant.



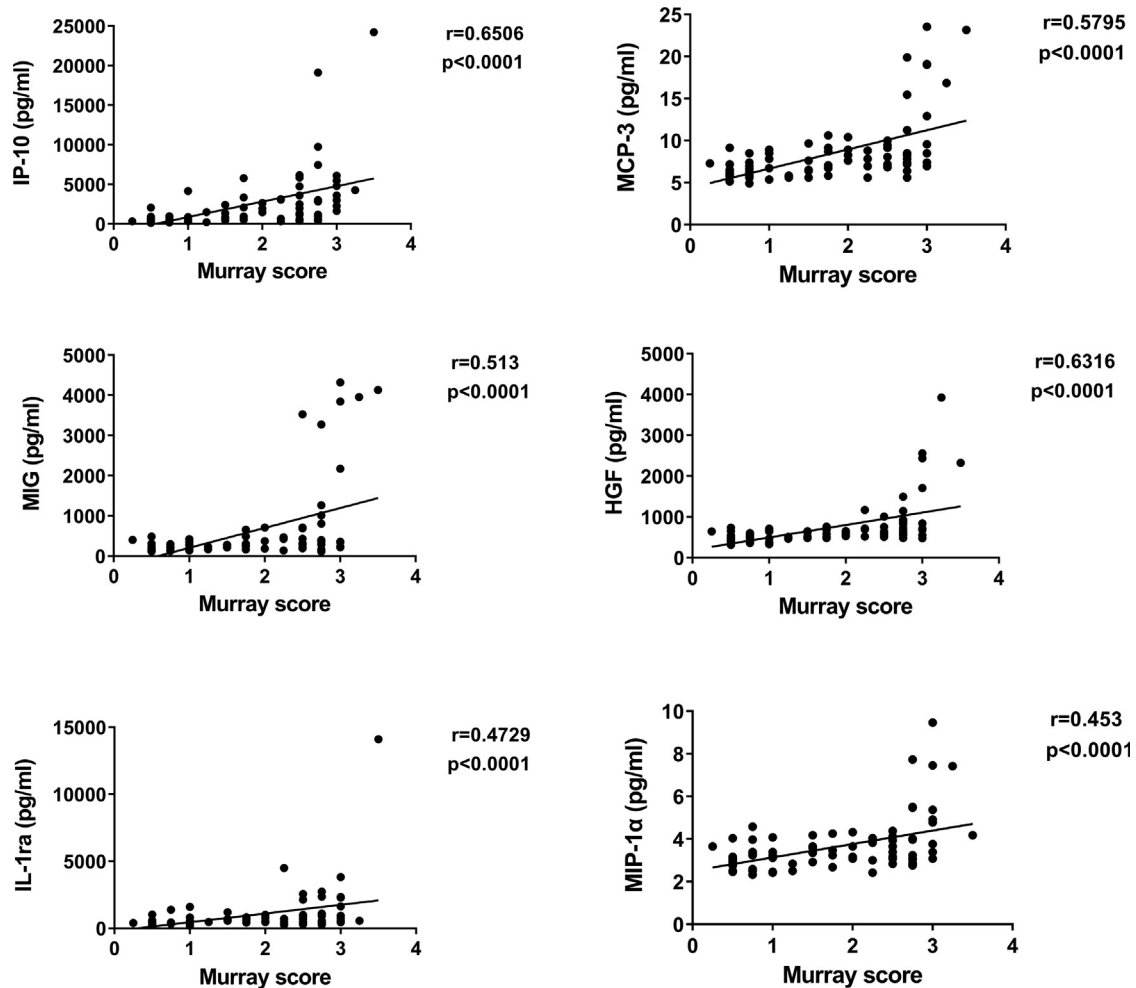


**FIG 2.** The ROC curve of plasma cytokine levels on admission for patients with and without ARDS during hospitalization. The ROC curve was calculated using SPSS. The AUC of the ROC curve for IL-1ra, IL-6, HGF, MCP-3, M-CSF, and IP-10 was estimated using the expression levels upon admission. **A**, The ROC curve of each cytokine. **B**, The ROC curves of different combination of IP-10, MCP-3, and IL-1ra. All the *P* values were less than .05.

non-ARDS group, which included the moderate patients. The ROC curve of each single cytokine was calculated using the expression levels upon admission (Fig 2; see Fig E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Results showed that the AUC of the ROC curve was 0.97 for IP-10, followed by 0.866 for IL-1ra and 0.803 for MCP-3 (Fig 2, A). The AUC of other cytokines varied from 0.565 to 0.767 (Fig 2, A). Then, we tested the different combination of the 3 cytokines for the prediction of disease progression (Fig 2, B). Combination of IP-10 and MCP-3 showed the highest AUC of 0.99, followed by the combination of IP-10 and IL-1ra (0.976) as well as the combination of MCP-3 and IL-1ra (0.857) (Fig 2, B). Combinations of the 3 cytokines showed the same AUC with combination of IP-10 and MCP-3 (Fig 2, B).

### Expression levels of IP-10, MCP-3, HGF, MIG, MIP-1 $\alpha$ and IL-1ra were highly associated with disease severity and progression

Murray scores for evaluation of disease severity of COVID-19 from the corresponding date of sample collection were recorded,<sup>26,27</sup> and Spearman rank coefficient correlation analysis were done to analyze the association between the expression levels of cytokines and the Murray scores. The results showed that IP-10, MCP-3, HGF, MIG, IL-1ra, and MIP-1 $\alpha$  expression levels were highly and positively correlated with Murray scores, with *r* values greater than 0.45 and *P* values less than .0001 (Fig 3), whereas IL-1ra, IL-2ra, IL-6, IL-10, IL-18, M-CSF, and IFN- $\gamma$  showed weaker, albeit significant, positive associations with Murray scores (Fig 3).

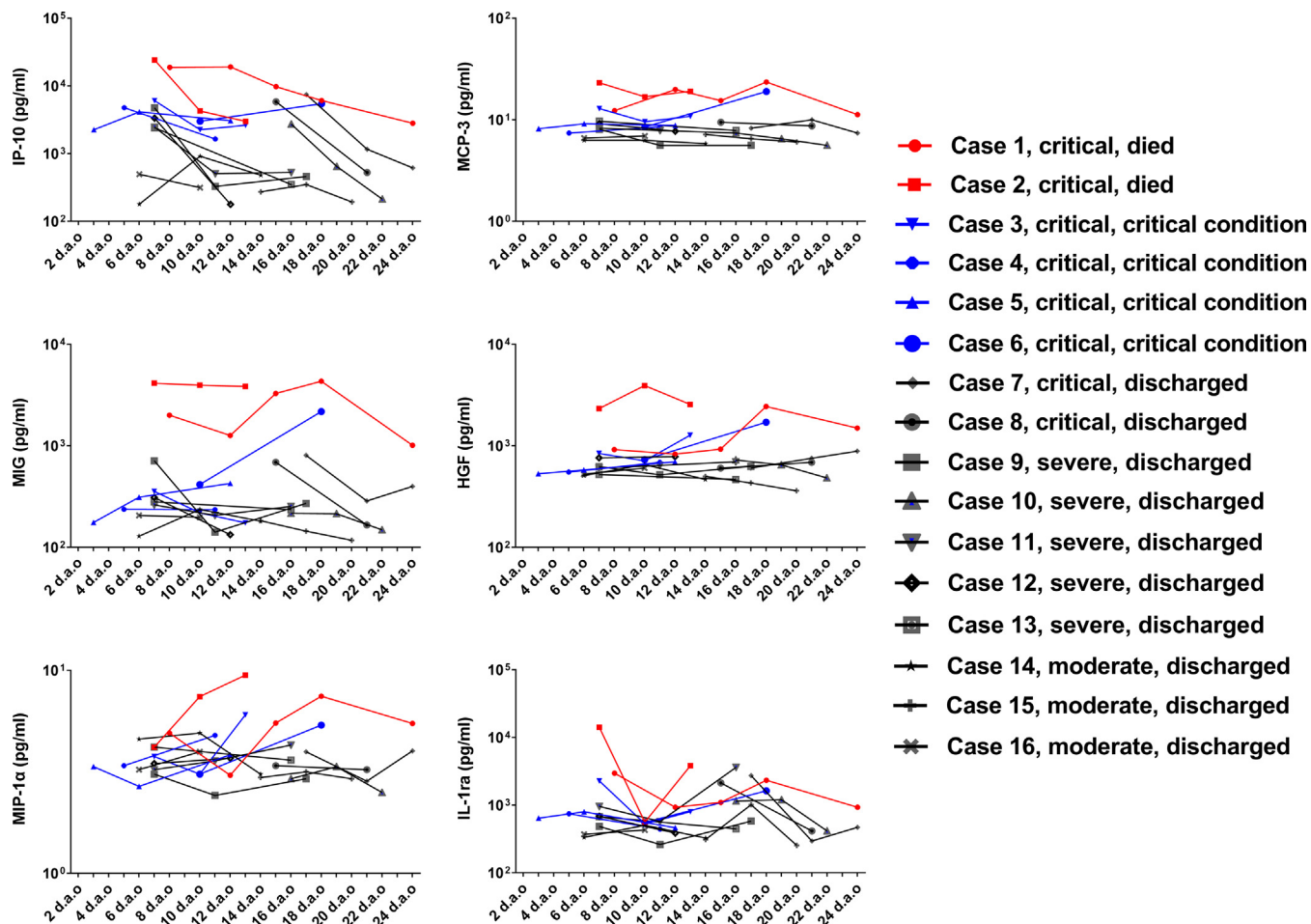


**FIG 3.** IP-10, MCP-3, HGF, MIG, MIP-1 $\alpha$ , and IL-1ra expression levels were highly correlated with Murray score. The expression levels of IL-1 $\beta$ , IL-1ra, IL-6, IL-13, IL-18, HGF, MCP-3, MIG, M-CSF, G-CSF, MIP-1 $\alpha$ , MIP-1 $\beta$ , CTACK, and IP-10 measured from plasma samples collected on admission and thereafter and the corresponding Murray scores at the same day were analyzed using Spearman rank correlation analysis, and the results of IP-10, MCP-3, HGF, MIG, MIP-1 $\alpha$ , and IL-1ra, which showed high association, were shown.

Furthermore, we analyzed the dynamic change of the 14 cytokines in 16 COVID-19 cases with different disease severity, including 8 critically ill patients (cases 1-8), 5 severe patients (cases 9-13), and 3 moderate patients (cases 14-16) (Fig 4). Of the 8 critically ill patients, 2 cases (cases 1 and 2) passed away, 4 cases were in critical condition (cases 3-6), and the remaining 2 cases (cases 7 and 8) were discharged from hospital. All the severe and moderate cases have been discharged from hospital. In the 2 fatal cases (cases 1 and 2), the expression levels of IP-10, MCP-3, HGF, MIG, MIP-1 $\alpha$  and IL-1ra were significantly high upon admission and maintained high expression levels during the disease progression (Fig 4). Meanwhile, the 4 cases still in critical condition (cases 7-9, 11, and 13) also showed continuously high levels of these 6 cytokines, although lower than the fatal cases (Fig 4). On the contrary, the 2 critically ill patients (cases 7 and 8) and all the severe and mild cases (cases 9-16) who were discharged from hospital showed lower levels or significantly decreased levels of the 6 cytokines during disease progression (Fig 4).

## DISCUSSION

Recent studies mainly focused on the clinical and epidemiological characteristics of patients with COVID-19,<sup>7,8,25,29-32</sup> and some preliminary studies have descriptively reported the immunologic characteristics with a limited number of cases.<sup>25,26</sup> In this study, a total of 50 patients were included and the relationships between expression levels of plasma cytokines and the disease severity as well as progression were analyzed in detail. Consistent with previous studies that have shown age to be a risk factor for developing severe disease following SARS-CoV-2 infection,<sup>8,30</sup> most of the severe and critically ill patients in our study were older than 60 years (Table I). CD4 and CD8 cells play vital roles in immune responses, including immune regulation, cytokines secretion, virus-specific antibody production, and cytolytic activities against target cells.<sup>33</sup> Similar with SARS-CoV and MERS-CoV infections,<sup>34,35</sup> significantly lower CD4 and CD8 counts were observed in severe and critically ill patients with COVID-19 (Table E1), which indicated that a possible dysfunction of immune responses happened following



**FIG 4.** Dynamic changes of the IP-10, MCP-3, HGF, MIG, MIP-1 $\alpha$ , and IL-1ra in 16 patients with COVID-19. The expression levels measured at the indicated time points of 8 critically ill (cases 1-8), 5 severe (cases 9-13), and 3 moderate patients (cases 14-16) were shown. The fatal cases (cases 1 and 2) were marked in red. Cases in critical condition at present (cases 3-6) were marked in blue, and the other patients were discharged from hospital.

SARS-CoV-2 infection in these patients. Furthermore, virus-specific CD4 response played vital roles in the control of SARS-CoV and MERS-CoV infection.<sup>36,37</sup> However, little is known about the role of T cells in the control of SARS-CoV-2 infection currently, which merits further investigation.

Thirty cytokines including both proinflammatory and anti-inflammatory cytokines were found to be significantly elevated upon admission, most of which were consistent with previous studies,<sup>25,26</sup> indicating that cytokine storm occurred in COVID-19 patients following SARS-CoV-2 infection (Fig E1). A similar phenomenon was also observed in patients infected with SARS-CoV<sup>17-21</sup> and MERS-CoV,<sup>22-24</sup> despite the fact that the expression profiles were virus specific. Of note, MCP-3, HGF, MIG, and MIP-1 $\alpha$  showed no statistical differences between healthy controls and patients with moderate disease at different stages of disease, whereas significant elevation of these cytokines was observed in both the severe and critically ill patients (Fig 1). Moreover, expression levels of IL-1ra, IL-6, M-CSF, HGF, IP-10, MCP-3, MIP-1 $\alpha$ , and MIG were highest in critically ill patients, followed by severe patients and then the moderate ones. This indicated that cytokine storm was obviously

stronger in the severe and critically ill patients. Using the expression levels of the 14 cytokines measured on admission, we further tested whether some cytokines could be used as biomarkers to predict the disease progression of COVID-19 (Fig 2). AUC of ROC for IP-10 was 0.97, and the combination of IP-10 and MCP-3 showed a slightly higher value of 0.99. The results suggested that IP-10 and MCP-3 could serve as excellent biomarkers for the prediction of COVID-19 progression, and further study with a separate cohort of patients is needed to validate the association of elevated IP-10 and MCP-3 with critical disease.

Comparison of the expression levels of the 14 cytokines measured from samples collected at different time points of disease progression showed that differential expressions of IP-10, MCP-3, and HGF were found in all the 3 groups at all the stages of disease progression, whereas differential expressions of MIG and MIP-1 $\alpha$  were found mainly in the later stages (8-14 and  $\geq 15$  dao) (Fig 1). Meanwhile, the evident trend that highest expression levels of the 5 cytokines in critically ill patients, followed by severe patients and then the moderate ones, were also found. Using Spearman rank coefficient correlation analysis, we found that the 5 cytokines showed high correlation with disease severity (Fig 3).



Furthermore, dynamic change of the 5 cytokines in 16 COVID-19 cases with different disease severity and outcome also showed a high association between the expression levels and the disease severity as well as outcome, as continuously high expression levels of the 5 cytokines were found in patients with adverse progression and fatal outcome (Fig 4). These results suggested that these significantly elevated cytokines might play important roles in the immunopathogenesis of SARS-CoV-2 infection and were associated with disease severity and outcome, although there was a lot of overlap in cytokines levels between moderate and severe cases for most cytokines measured.

Cytokine storm with uncontrolled proinflammatory responses induces significant immunopathology and severe disease outcomes during some viral infections, indicating an important role in the pathogenesis of these viruses.<sup>2,38</sup> Accordingly, not only the pathogens but also the pathogen-induced cytokine storm should be considered during the treatment. Therapy strategy with a combination of antimicrobial and immunotherapy may produce a more favorable outcome.<sup>9</sup> Corticosteroids, which could downregulate proinflammatory cytokine transcription and subsequently inhibit the cytokine storm,<sup>39</sup> and the antiviral cytokine IFN- $\alpha$  were widely used in severe and critically ill patients during the treatment of COVID-19.<sup>8,30</sup> However, the use of high-dose corticosteroids has been shown to be associated with an increase in mortality and significantly longer durations of viral shedding in H7N9-infected patients,<sup>40</sup> which is of concern. Therefore, short courses of corticosteroids at low to moderate dose were recommended.<sup>41</sup> Moreover, the results of our study suggested a crucial role of specific cytokines such as IP-10 in the pathogenesis of SARS-CoV-2, which has also been shown to be associated with disease severity of H5N1, H1N1, SARS-CoV, and MERS-CoV.<sup>16,24,42,43</sup> A recent study has found that CXCL10 (IP-10)-CXCR3 signaling appears to be a critical factor for the exacerbation of the pathology of ARDS.<sup>44</sup> Thus, modulators such as antibody-targeting IP-10 might be a promising therapeutic strategy in the treatment of the acute phase of ARDS to ameliorate acute lung injury as shown in H1N1 mouse model.<sup>44,45</sup>

## Conclusions

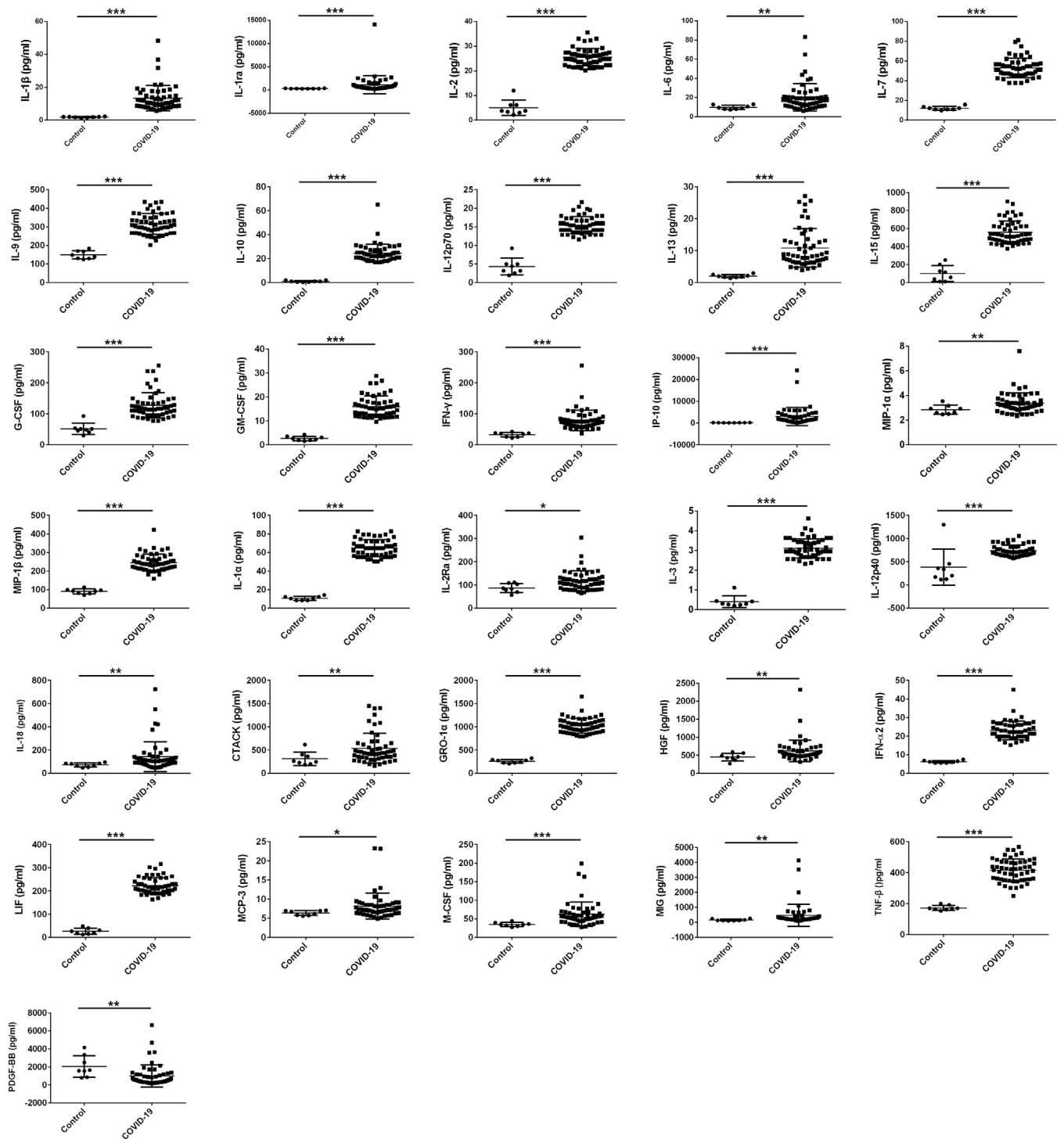
We compared the differences in cytokine expression profiles among critically ill, severe, and moderate COVID-19 cases in detail, and found that IP-10 and MCP-3 were highly associated with disease severity and could be used as excellent predictors of COVID-19 progression. Therefore, detecting the expression levels of IP-10 and MCP-3 in patients with COVID-19 in the early stage of the disease may provide useful information for the formulating of specific strategy of treatment. Our results added to our understanding of the immunopathologic mechanisms of SARS-CoV-2 infection, and provide potential therapeutic targets and strategies.

**Clinical implications: Continuously high levels of IP-10, MCP-3, HGF, MIG, MIP-1 $\alpha$  and IL-1ra contribute to the disease deterioration and adverse outcome of COVID-19. IP-10 and MCP-3 could predict the progression of COVID-19.**

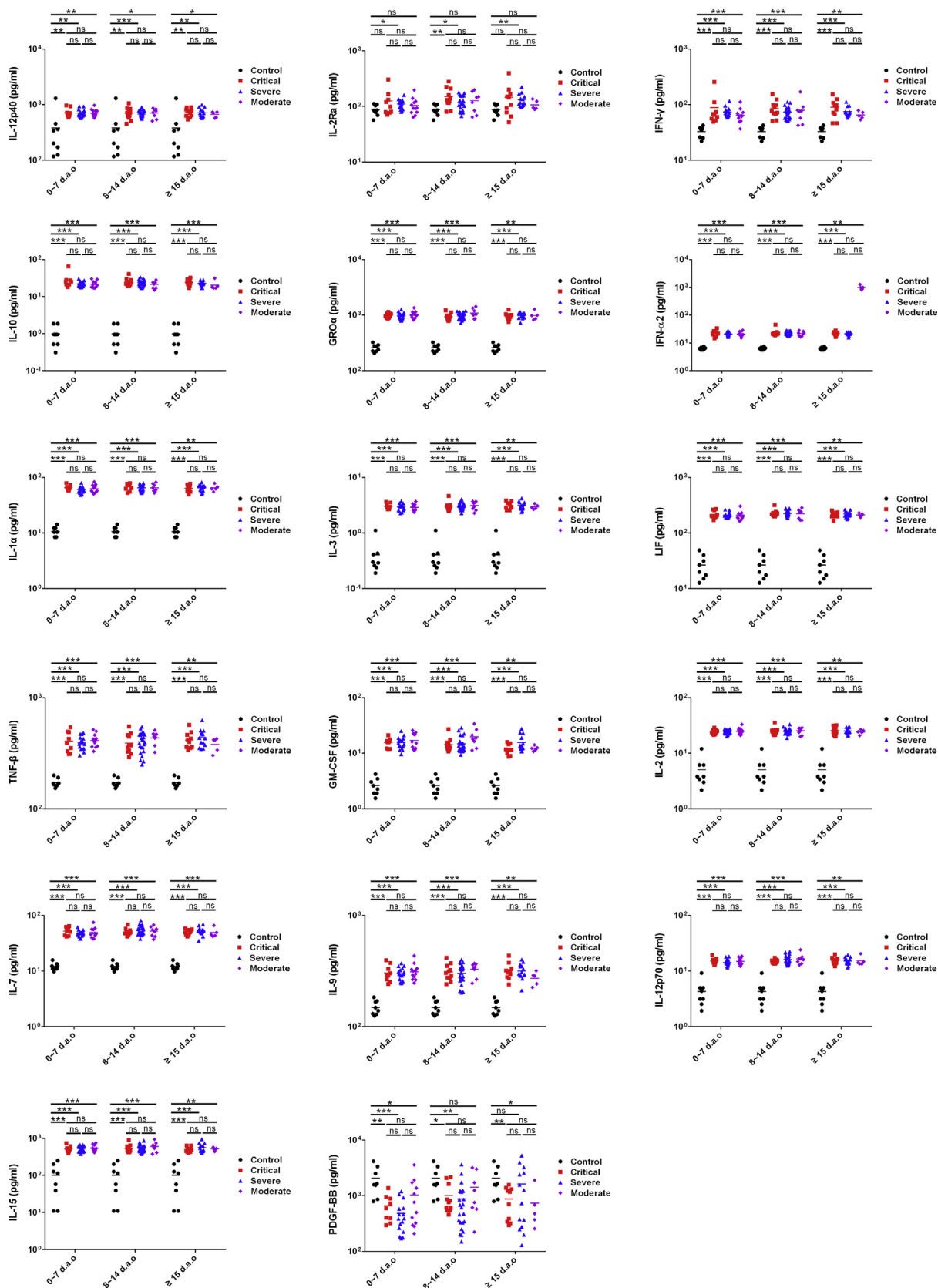
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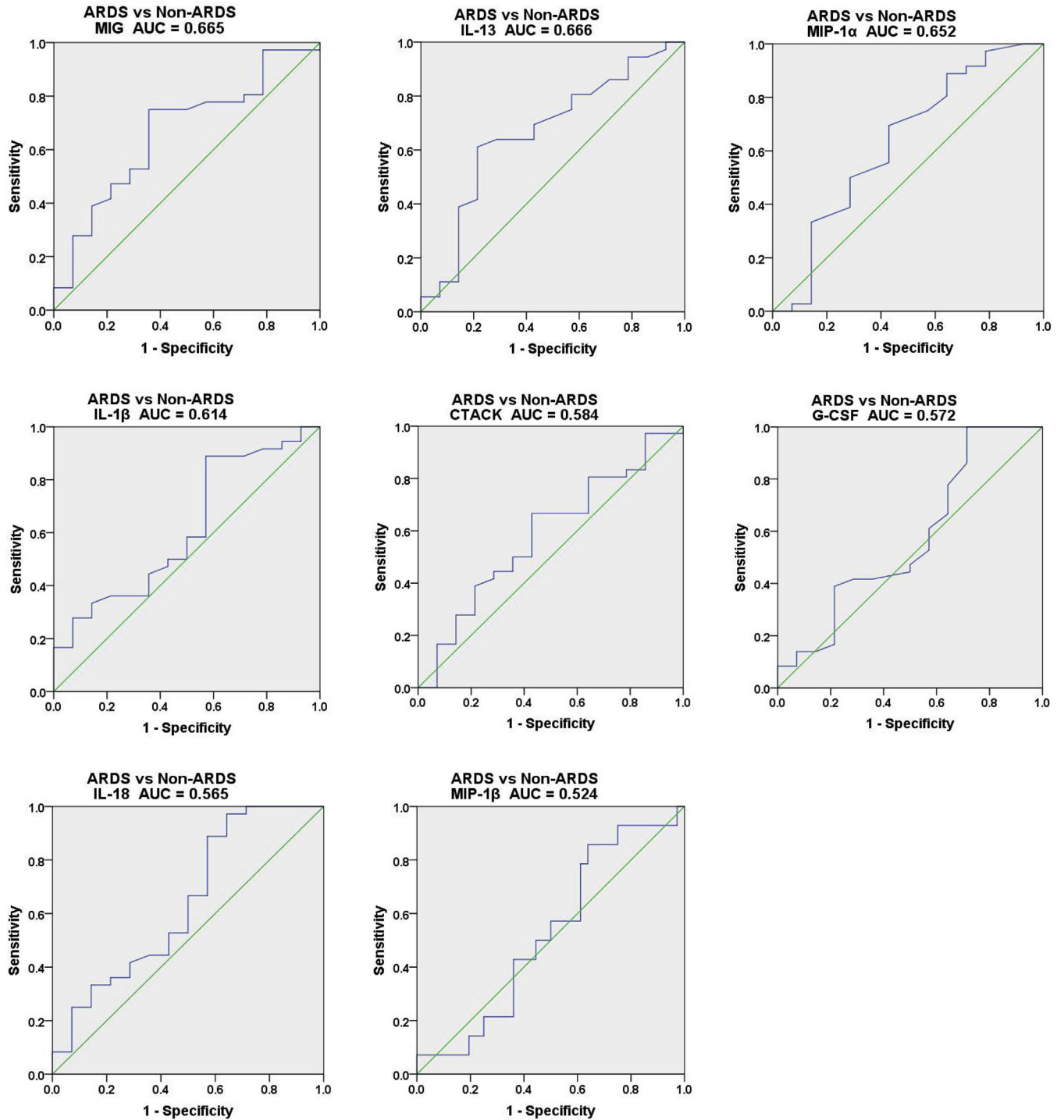
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**FIG E1.** Comparison of plasma cytokine concentrations between healthy volunteers and COVID-19 cases. Plasma samples from critically ill (N = 11), severe (N = 25), and moderate (N = 14) COVID-19 patients were collected at the earliest possible time point after hospitalization for assays measuring the concentrations of 48 cytokines. Eight healthy subjects were involved as control. Values were graphed on a logarithmic scale and presented in units of pg/mL. *P* values between .01 and .05, .001 and .01, and .0001 and .001 were considered statistically significant (\*), very significant (\*\*), and extremely significant (\*\*\*), respectively, whereas ns represents not significant.



**FIG E2.** Comparison of the significantly elevated cytokines measured at different days after illness onset among critically ill, severe, and moderate COVID-19 patients. The expression levels of 17 cytokines measured at different days after illness onset without differential expression levels among patients with different disease severity were shown and compared among the critically ill, severe, and moderate COVID-19 patients. *P* values between .01 and .05, .001 and .01, and .0001 and .001 were considered statistically significant (\*), very significant (\*\*), and extremely significant (\*\*\*), respectively. *ns*, Not significant.



**FIG E3.** The ROC curve of MIG, IL-13, MIP-1 $\alpha$ , IL-1 $\beta$ , CTACK, G-CSF, IL-18, and MIP-1 $\beta$  expression levels upon admission for patients with and without ARDS during hospitalization. All the *P* values were above .05. CTACK, Cutaneous T-cell-attracting chemokine.



**TABLE E1.** Laboratory results of patients with COVID-19 in this study

Variables*	COVID-19 cases (N = 50)			P values		
	Moderate (N = 14)	Severe (N = 25)	Critical (N = 11)	M vs S	M vs C	S vs C
WBC ( $\times 10^9/L$ )	4.48 (3.75-6.19)	4.31 (3.44-4.91)	5.59 (4.16-6.65)	.4347	.5023	.0785
LYM (%)	31.1 (18.75-35.05)	21.6 (16.4-32.1)	15.5 (11.45-23.95)	.2354	.0122	.1253
LYM ( $\times 10^9/L$ )	1.09 (0.82-1.4)	1.13 (0.89-1.52)	1.21 (1.06-1.31)	.694	.7987	.8194
NEU (%)	58.8 (52.75-71.4)	69.3 (57-75.3)	69.3 (66.65-82.1)	.209	.0283	.2797
NEU ( $\times 10^9/L$ )	2.8 (2.17-4.43)	2.74 (1.81-3.13)	3.53 (2.29-5.56)	.3795	.6736	.0874
PLT ( $\times 10^9/L$ )	151 (130-188.5)	171 (143-189)	173 (134-214)	.4427	.4201	.9394
AST (U/L)	35 (25-45)	28 (24-40)	33 (21.5-39.5)	.3425	.4971	.9528
ALT (U/L)	28 (18-33)	23 (18-26)	27 (20-36.5)	.2183	.9289	.1853
TB (umol/L)	9.8 (6.8-13.2)	9.5 (7.8-11.6)	8.7 (8.15-12)	.7456	.9405	.8325
CRE ( $\mu\text{mol/L}$ )	76 (59-88.5)	71 (54-86)	75 (54.5-79.5)	.7666	.7114	.9259
BUN (mmol/L)	4.38 (3.57-5.29)	4.68 (3.63-5.59)	5.05 (4.03-6.84)	.5252	.264	.435
CK (U/L)	100 (57.5-109)	105 (56.75-158.75)	84.5 (48.25-136.75)	.4408	.9778	.461
CK-MB (ng/mL)	0.86 (0.38-4.55)	0.7 (0.23-1.02)	0.93 (0.73-3.78)	.3199	.0945	.4667
LDH (U/L)	432 (270.5-586)	414.5 (204-533.25)	227 (185-450.5)	.507	.9806	.5632
CRP (mg/L)	15.2 (11.37-32.46)	13.9 (5-28.4)	14.23 (6.35-40.75)	.1733	.5539	.4259
PCT (ng/mL)	0.04 (0.03-0.06)	0.04 (0.03-0.08)	0.05 (0.04-0.08)	.2409	.3109	.8458
CD4 (count/ $\mu\text{L}$ )	561 (367-826)	377 (200.75-492.5)	246 (176-315.5)	.0327	.0006	.0474
CD8 (count/ $\mu\text{L}$ )	453.5 (232.8-586)	146 (97-225.25)	139 (107-171.5)	.0009	.0002	.7993

ALT, Alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C, critical; CK, creatine kinase; CK-MB, creatine kinase myocardial band; CRE, creatinine; CRP, C-reactive protein; LDH, lactate dehydrogenase; LYM, lymphocyte; M, moderate; NEU, neutrophil; PCT, procalcitonin; PLT, platelet; S, severe; TB, total bilirubin; WBC, white blood cell.

\*Values shown represent the mean and interquartile range.