Correlation Between Early Plasma Interleukin 37 Responses With Low Inflammatory Cytokine Levels and Benign Clinical Outcomes in Severe Acute Respiratory Syndrome Coronavirus 2 Infection

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Background. The immune protective mechanisms during severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection remain to be deciphered for the development of an effective intervention approach.

Methods. We examined early responses of interleukin 37 (IL-37), a powerful anti-inflammatory cytokine, in 254 SARS-CoV-2–infected patients before any clinical intervention and determined its correlation with clinical prognosis.

Results. Our results demonstrated that SARS-CoV-2 infection causes elevation of plasma IL-37. Higher early IL-37 responses were correlated with earlier viral RNA negative conversion, chest computed tomographic improvement, and cough relief, consequently resulted in earlier hospital discharge. Further assays showed that higher IL-37 was associated with lower interleukin 6 and interleukin 8 (IL-8) and higher interferon α responses and facilitated biochemical homeostasis. Low IL-37 responses predicted severe clinical prognosis in combination with IL-8 and C-reactive protein. In addition, we observed that IL-37 administration was able to attenuate lung inflammation and alleviate respiratory tissue damage in human angiotensin-converting enzyme 2–transgenic mice infected with SARS-CoV-2.

Conclusions. Overall, we found that IL-37 plays a protective role by antagonizing inflammatory responses while retaining type I interferon, thereby maintaining the functionalities of vital organs. IL-37, IL-8, and C-reactive protein might be formulated as a precise prediction model for screening severe clinical cases and have good value in clinical practice.

Keywords. IL-37; SARS-CoV-2; COVID-19; inflammation; clinical outcomes.
Shanghai, China, between 6 February 2020 and 27 May 2020. Plasma samples from the 254 patients were collected on admission, before treatment. According to the median level of IL-37, 127 patients with lower IL-37 levels (75 male and 52 female) and 127 (65 male and 62 female) with higher IL-37 levels were enrolled. Table 1 shows the distributions by age, sex, other demographic characteristics, and biochemical and immunologic indexes in the low- and high-IL-37 groups, along with P values. All patients were treated according to the COVID-19 Prevention and Control Plan [10].

Quantification of Plasma IL-37 and Other Cytokines
Plasma IL-37 from the 254 patients with COVID-19 was assayed using a human IL-37/interleukin 1F7 enzyme-linked immunosorbent assay kit (Beijing 4A; Biotech); 10 cytokines, including interferon (IFN) γ, interleukin 1β, 4, 5, 10, 12P70, and 22, interleukin 6 and 8 (IL-6 and IL-8), and tumor necrosis factor α, were measured using the Simoa CorPlex human cytokine detection kit (catalog no. 85–0329; Quanterix). All biosafety regulations were followed during the assays.

Patient Information Collection
Clinical data for the 254 patients were collected from clinical test reports (routine blood and urine studies, coagulation indexes), biochemical reports (blood gas analysis, liver function, kidney function), immune reports (cytokine determination, absolute cell number determination, complement measurement) computed tomographic (CT) reports, and vital sign monitoring (body temperature, respiration, blood oxygen saturation, blood pressure), among other records (Supplementary Figures 1 and 2).

Animal Experiments
The human angiotensin-converting enzyme 2 (hACE2)–transgenic mice used were 5–6-week-old Institute of Cancer Research female mice, which were purchased from Hua-Fu-Kang and split into 2 groups, with 3 mice in each group. All mice were inoculated intranasally with 50 µL (10^5 median tissue culture infective dose) of SARS-CoV-2 virus (provided by Z. Shi). Mice in the experimental group were then intravenously administered 12.5 µg/kg of recombinant human IL-37 [11] (Novoprotein Technology) at 12 and 48 hours after virus inoculation, and those in the control group were injected with phosphate-buffered saline (PBS). The protocol was approved by Institutional Animal Care and Use Committee at the Wuhan Institute of Virology, China Academy of Sciences, and implemented in an animal biosafety level 3 laboratory. All animal experiments were carried out at the Wuhan Institute of Virology, in

Table 1. Clinical Characteristics and Immunological and Biochemical Indexes in 254 Patients With Coronavirus Disease 2019

| Characteristic or Index | All Patients (N = 254) | Low IL-37 Response (n = 127) | High IL-37 Response (n = 127) | P Value |
|------------------------|-----------------------|-----------------------------|-----------------------------|--------|
| Age, mean (SEM), y     | 42.76 (0.951)         | 44.88 (1.484)               | 40.63 (1.167)               | .12    |
| Age, median (IQR), y   | 41 (32–53.25)         | 42 (32–61)                  | 41 (32–47)                  |        |
| Sex, no. (%)           |                       |                             |                             |        |
| Male                   | 140 (55.12)           | 75 (53.57)                  | 65 (46.43)                  | .66    |
| Female                 | 114 (44.88)           | 52 (45.61)                  | 62 (54.38)                  |        |
| Underlying disease, no. (%) |                   |                             |                             |        |
| Hypertension           | 30 (11.81)            | 20 (15.74)                  | 10 (7.87)                   | .05    |
| Diabetes               | 20 (7.87)             | 10 (7.87)                   | 10 (7.87)                   | .999   |
| Hepatitis              | 5 (1.97)              | 3 (2.36)                    | 2 (1.57)                    | .65    |
| Pharyngitis            | 3 (1.18)              | 2 (1.57)                    | 1 (0.78)                    | .57    |
| Rhinitis               | 12 (4.72)             | 2 (1.57)                    | 10 (7.87)                   | .02    |
| Asthma                 | 4 (1.57)              | 3 (2.36)                    | 1 (0.78)                    | .32    |
| Body temperature, mean (SEM), °C | 37.02 (0.037) | 37.1 (0.052)               | 36.94 (0.052)               | .02    |
| Body temperature, median (IQR), °C | 37 (36.5–37.5) | 37.2 (36.5–37.5)          | 36.8 (36.4–37.5)            |        |
| Fever, no. (%)         | 94 (37)               | 55 (43.3)                   | 39 (30.7)                   |        |
| Immunological indexes  |                       |                             |                             |        |
| Blood cells counts, x 10^3/L |                   |                             |                             |        |
| Leukocytes, mean (SEM) | 5.719 (0.14)          | 5.546 (0.12)                | 5.89 (0.23)                 | .49    |
| Leukocytes, median (IQR) | 5.29 (4.34–6.65)     | 5.23 (4.26–6.34)            | 5.38 (4.41–6.8)             |        |
| Lymphocytes, mean (SEM) | 1.51 (0.04)          | 1.49 (0.06)                 | 1.52 (0.06)                 | .54    |
| Lymphocytes, median (IQR) | 1.4 (1.1–1.83)      | 1.4 (1.05–1.83)             | 1.4 (1.14–1.83)             |        |
| Monocytes, mean (SEM)  | 0.47 (0.01)           | 0.45 (0.02)                 | 0.50 (0.02)                 | .09    |
| Monocytes, median (IQR) | 0.45 (0.35–0.59)    | 0.42 (0.31–0.58)            | 0.47 (0.35–0.59)            |        |
| Platelets, mean (SEM)  | 214 (4.52)            | 205 (6.15)                  | 222 (6.55)                  | .04    |
| Platelets, median (IQR) | 209 (161–256)        | 201 (155–244)               | 213 (167–267)               |        |
| Basophils, mean (SEM)  | 0.02 (0.00)           | 0.02 (0.00)                 | 0.02 (0.00)                 | .79    |
| Basophils, median (IQR) | 0.01 (0.01–0.02)     | 0.01 (0.01–0.03)            | 0.01 (0.01–0.02)            |        |
| Characteristic or Index | All Patients (N = 254) | Low IL-37 Response (n = 127) | High IL-37 Response (n = 127) | P Value |
|-------------------------|------------------------|-----------------------------|-----------------------------|--------|
| Eosinophils, mean (SEM)  | 0.05 (0.00)            | 0.04 (0.01)                 | 0.06 (0.01)                 | .12    |
| Eosinophils, median (IQR)| 0.02 (0.01–0.06)       | 0.02 (0.00–0.05)            | 0.03 (0.01–0.07)            |        |
| Neutrophils, mean (SEM)  | 3.70 (0.13)            | 3.60 (0.15)                 | 3.80 (0.21)                 | .84    |
| Neutrophils, median (IQR)| 3.3 (2.46–4.46)        | 3.28 (2.48–4.34)            | 3.46 (2.32–4.56)            |        |
| **Eosinophils, mean (SEM)** |                      |                             |                             |        |
| **Eosinophils, median (IQR)** |                    |                             |                             |        |
| **Neutrophils, mean (SEM)** |                      |                             |                             |        |
| **Neutrophils, median (IQR)** |                    |                             |                             |        |
| **T-cell count, cells/µL** |                      |                             |                             |        |
| **CD3+, mean (SEM)**     | 1084 (35.9)            | 1053 (54.75)                | 1116 (46.42)                | .18    |
| **CD3+, median (IQR)**   | 963 (729–1352)         | 963 (640–1334)              | 962 (762–1388)              |        |
| **CD4+, mean (SEM)**     | 602 (18.65)            | 583 (24.89)                 | 622 (27.83)                 | .51    |
| **CD4+, median (IQR)**   | 558 (397–775)          | 543 (388–775)               | 571 (412–779)               |        |
| **CD8+, mean (SEM)**     | 389 (12.5)             | 352 (17.3)                  | 406 (17.78)                 | .04    |
| **CD8+, median (IQR)**   | 339 (241–500)          | 308 (212–485)               | 357 (258–504)               |        |
| **NK cell count, cells/µL** |                      |                             |                             |        |
| **CD16+CD56+, mean (SEM)** |                    |                             |                             |        |
| **CD16+CD56+, median (IQR)** |                   |                             |                             |        |
| **Biochemical indexes**  |                        |                             |                             |        |
| **ALT, mean (SEM), U/L** | 29.39 (1.53)           | 29.34 (1.92)                | 39.44 (2.37)                | .40    |
| **ALT, median (IQR) (U/L)** |                   |                             |                             |        |
| **AST, mean (SEM) (U/L)** | 26.02 (0.95)           | 27.12 (1.48)                | 24.95 (1.20)                | .38    |
| **AST, median (IQR) (U/L)** |                   |                             |                             |        |
| **LDH, mean (SEM) (U/L)** | 225 (4.75)             | 239 (8.06)                  | 212 (4.88)                  | .049   |
| **LDH, median (IQR) (U/L)** |                   |                             |                             |        |
| **CHE, mean (SEM) (U/L)** | 9029 (132.8)           | 8839 (174.1)                | 9216 (199.5)                | .27    |
| **CHE, median (IQR) (U/L)** |                   |                             |                             |        |
| **CK, mean (SEM) (U/L)**  | 108.3 (7.17)           | 120.1 (11.15)               | 96.4 (8.923)                | .01    |
| **CK, median (IQR) (U/L)** |                   |                             |                             |        |
| **Myoglobin, mean (SEM) (U/L)** |                |                             |                             | <.001   |
| **Myoglobin, median (IQR) (U/L)** |             |                             |                             |        |
| **Creatinine, mean (SEM) (µmol/L)** | 66.61 (1.21) | 69.57 (2.12)                | 63.72 (1.15)                | .17    |
| **Creatinine, median (IQR) (µmol/L)** |               |                             |                             |        |
| **ATTP, mean (SEM) (s)** | 38.27 (0.47)           | 38.54 (0.89)                | 38.01 (0.37)                | .59    |
| **ATTP, Median (IQR) (s)** |                   |                             |                             |        |
| **ESR, mean (SEM) (mm/h)** | 375 (35–40.2)          | 373 (35.03–40.18)           | 3785 (34.98–40.2)           | .03    |
| **ESR, median (IQR), mm/h** |                   |                             |                             |        |
| **Albumin, mean (SEM), g/L** | 42.82 (0.27)           | 42.4 (0.40)                 | 43.22 (0.37)                | .20    |
| **Albumin, median (IQR), g/L** |                   |                             |                             |        |
| **DHDLC, mean (SEM), mmol/L** | 1.11 (0.02)           | 1.09 (0.03)                 | 1.13 (0.03)                 | .42    |
| **DHDLC, median (IQR), mmol/L** |                |                             |                             |        |
| **LDLC, mean (SEM), mmol/L** | 2.88 (0.06)           | 2.82 (0.09)                 | 2.94 (0.08)                 | .28    |
| **LDLC, median (IQR), mmol/L** |                |                             |                             |        |
| **D-dimer, mean (SEM), µg/mL** | 0.58 (0.11)           | 0.78 (0.22)                 | 0.38 (0.02)                 | .42    |
| **D-dimer, median (IQR), µg/mL** |                |                             |                             |        |
| **pH, mean (SEM)**       | 7.39 (0.00)            | 7.39 (0.00)                 | 7.39 (0.00)                 | .66    |
| **pH, median (IQR)**      | 7.38 (736–741)         | 7.38 (736–741)              | 7.39 (737–741)              |        |
| **PCO₂, mean (SEM), kPa** | 5.44 (0.04)            | 5.40 (0.06)                 | 5.49 (0.06)                 | .49    |
| **PCO₂, median (IQR), kPa** |                   |                             |                             |        |
| **PO₂, mean (SEM), kPa**  | 13.97 (0.27)           | 13.86 (0.41)                | 14.08 (0.34)                | .36    |
| **PO₂, median (IQR), kPa** | 13.06 (11.68–15.13)    | 12.85 (11.33–15.38)         | 13.3 (11.78–15.13)          |        |
| **Arterial lactate, mean (SEM), mmol/L** | 2.07 (0.18)     | 1.98 (0.11)                 | 2.16 (0.35)                 | .03    |
| **Arterial lactate, median (IQR), mmol/L** |                |                             |                             |        |
| **Lactate, mean (SEM), mmol/L** | 2.57 (0.05)           | 2.65 (0.07)                 | 2.50 (0.07)                 | .049   |
| **Lactate, median (IQR), mmol/L** |                |                             |                             |        |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATTP, activated partial thromboplastin time; CHE, cholinesterase; CK, creatine kinase; ESR, erythrocyte sedimentation rate; HDLC, high-density lipoprotein cholesterol; IL-37, interleukin 37; IQR, interquartile range; LDH, lactic dehydrogenase; LDLC, low-density lipoprotein cholesterol; NK, natural killer; SEM, standard error of the mean.
strict compliance with the Guide for the Care and Use of Laboratory Animals, to ensure the personnel safety and animal welfare.

**Histopathological Examination**
Mice were euthanized at 5 days after infection, and lung tissues were isolated and fixed with 4% formalin. After sectioning with a microtome, slides were stained by hematoxylin-eosin (HE), following a standard protocol. The scanning procedures were conducted with TissueFAXS Confocal Plus 200 (TissueGnostics), and the images acquired were analyzed using Strata Quest 6.0X software to calculate pathological scores and quantify inflammatory cells.

**Statistical Analysis**
Student t tests were used for parametric continuous data, and unpaired 2-sided Mann-Whitney U tests for nonparametric data. Log-rank (Mantel-Cox) tests were used to evaluate significant differences between discharge rates, viral RNA negative conversion rates and cough relief rates in the 2 patient groups. The significant P value cutoff was set at .05. A predicted receiver operating characteristic curve of severely classified patients was examined, and the equation was calculated using SPSS 23 software and binary logistic regression analysis. The Python library “numpolyfit” was used to fit the smooth receiver operating characteristic curve. Quantitative indexes are reported as mean values (with standard errors of the mean [SEMs]), continuous features as medians (with interquartile ranges), and categorical features as numbers (with percentages).

**Ethical Considerations**
The cohort study was approved by the Ethics Committee of Shanghai Public Health Clinical Center (no. YJ-2020-S130-01), and all participants enrolled in this study signed informed consent forms. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Wuhan Institute of Virology, China Academy of Sciences (no. WIVAO5202009).

**RESULTS**

**Correlation Between Elevated Plasma IL-37 Responses in Patients With COVID-19 and Benign Clinical Outcome**
An enzyme-linked immunosorbent assay was performed to quantify IL-37 levels in 254 patients with COVID-19 at admission (before treatment intervention) and 36 healthy subjects (negative for SARS-CoV-2 RNA). We found that patients with COVID-19 presented significantly higher plasma IL-37 levels (mean [SEM], 196.2 [35.78] pg/mL; 95% confidence interval [CI], 125.7–266.6 pg/mL) than healthy individuals (19.72 [5.60] pg/mL; 8.36–31.09 pg/mL) (P < .001) (Figure 1A), suggesting that SARS-CoV-2 infection is likely to result in anti-inflammatory responses, such as those involving IL-37.

We next explored the possible role of increased plasma IL-37 levels. The 254 patients were split into high–IL-37 (n = 127) and low–IL-37 (n = 127) groups, according to the overall median plasma IL-37 value (19.01 pg/mL). Interestingly, the high–IL-37 group experienced significantly shorter hospitalizations than the low–IL-37 group (mean [SEM], 14.8 [0.42] vs 20.3 [0.87] days, respectively; P < .001) (Figure 1B); rates of discharge within 9 days were 15.75% (20 of 127) and 1.57% (2 of 127), respectively (Figure 1C). All patients were discharged within 25 days for the high–IL-37 group and within 69 days for the low–IL-37 group (P < .001; log-rank test) (Figure 1D).

**Correlation of Low Plasma IL-37 Responses With Severe Clinical Prognoses**
We further examined the clinical prognoses of patients in the high– and low–IL-37 groups, including the timing and rates of SARS-CoV-2 viral RNA negative conversion, chest CT improvement, and cough relief. As expected, viral RNA negative conversion was significantly later in the low–IL-37 group (mean [SEM], 8.1 [0.6] days; 95% CI, 6.88–9.28 days) than in the high–IL-37 group (4.5 [0.36] days; 3.82–5.26 days) (P < .001), with 46% negative viral RNA in the low–IL-37 group versus 74% in the high–IL-37 group within 6 days. In addition, 31 days was required for all 122 patients in the low–IL-37 group to become RNA negative, whereas only 20 days was needed for all 121 patients in the high–IL-37 group (P < .001; log-rank test) (Figure 2A).

We next determined chest CT improvement. Chest CT scans of patients with COVID-19 usually showed multiple small patch shadows and interstitial changes at the early stage and then gradually developed into multiple ground-glass opacities or infiltration shadows in both lungs [12, 13]. These lesions become absorbed and dissipate with the improvement of clinical symptoms and disease status [10]. Of 254 patients, 23 exhibited no manifestations at chest CT (10 in the low– and 13 in the high–IL-37 group), 17 had only a single chest CT report (10 in the low– and 7 in the high–IL-37 group), and 18 showed long-term unchanged chest CT observations (9 in the low– and 9 in the high–IL-37 group). After exclusion of those 58 patients, 196 patients remained, 98 patients in each group. Chest CT improvement took more time in the low–IL-37 group (mean [SEM], 9.44 [0.6] days; 95% CI, 8.24–10.64 days) than in the high–IL-37 group (6.43 [0.3] days; 95% CI, 6.15–7.32 days) (P < .001; Mann-Whitney U test), with 34 of 98 (35%) achieving chest CT improvement within 6 days in the low–IL-37 versus 56 of 98 (57%) in the high–IL-37 group. In addition, it took 27 days to achieve chest CT improvement for all 98 patients in the low–IL-37 group, compared with only 14 days for all 98 patients in the high–IL-37 group (P < .001; log-rank test) (Figure 2B).

After SARS-CoV-2 infection, patients often present clinical symptoms of cough [14–16]. Among all 254 patients, 84 presented with cough symptoms, with 37 cases in the low– and 47 in the high–IL-37 group. The low–IL-37 group required more time to achieve cough relief (mean [SEM], 11.67 [3.51]
days; 95% CI, 2.647–20.69 days) than the high–IL-37 group (8.72 [0.91] days; 6.855–10.58 days) (P = .002). Only 11 of 37 patients (29.7%) in the low–IL-37 group achieved relief within 5 days, compared with 29 of 47 (61.7%) in the high–IL-37 group. It took 9 days for half of the patients in the low–IL-37 group to achieve relief, compared with only 4 days in the high–IL-37 group (Figure 2C). Overall, these observations suggest that the absence of IL-37 responses in patients with COVID-19 is correlated with severe clinical prognoses, and IL-37 is likely to play a protective role during SARS-CoV-2 infection.

Effect of Low Plasma IL-37 Responses on the Capacity to Maintain Biochemical Homeostasis

We further determined the correlation of IL-37 and various biochemical indicators in the laboratory. We analyzed concentrations of lactate, lactate dehydrogenase, and arterial lactic acid in the groups with low and high IL-37 levels and observed that those metabolic indicators were not maintained in the normal range in many patients in both groups, suggesting the occurrence of hypoxemia and respiratory distress symptoms after SARS-CoV-2 infection. Overall, all 3 indicators were significantly higher in the low– than in the high–IL-37 group (P = .02 for arterial lactic acid; P = .049 for lactate and lactate dehydrogenase (Figure 3A). Thus, patients with low IL-37 responses are likely to have more severe respiratory distress and hypoxemia. Accordingly, the low–IL-37 group exhibited significantly higher myoglobin, creatine kinase, and total bilirubin levels than the high–IL-37 group (P < .001, .01, and .03, respectively), suggesting more damage to heart and liver functions. No significant differences were detected in D-dimer (P = .42), aspartate aminotransferase (P = .40), or alanine aminotransferase (P = .38) levels (Figure 3B and 3C).

In general, infection and inflammation result in an increase in the erythrocyte sedimentation rate, which was significantly higher in the low– than in the high–IL-37 group (P = .03), indicating that IL-37 may help induce inflammatory responses. No significant differences in creatinine or albumin were observed (P = .17 and .20, respectively) (Figure 3D).
Other biochemical indicators, such as Pco₂, Po₂, and blood pH, are shown in Supplementary Figure 1. Overall, IL-37 responses may facilitate the retention of organ functions by antagonizing inflammation and thereby maintaining biochemical homeostasis.

**Effect of Low Plasma IL-37 Responses on Innate Immune Balance Maintenance**

We also assessed a panel of cytokines in the initial plasma at patient admission, including IL-6, IL-8, and IFN-α. Surprisingly, an imbalance of innate immune responses was noted with significantly higher IL-6 and IL-8 levels and lower IFN-α levels in the low– than in the high–IL-37 group (P < .001 for both IL-6 and IL-8; P = .02 for IFN-α). IL-6 and IL-8 are inflammatory cytokines and prone to causing immune pathogenesis, whereas IFN-α exerts inhibitory activities against viral replication. Thus, the imbalanced innate responses in the low–IL-37 group conferred a tendency of failure to restrain SARS-CoV-2 replication. Accordingly, high-sensitivity C-reactive protein (HS-CRP) was also significantly higher in the low– than in the high–IL-37 group (P = .049) (Figure 4A).

We next examined immune cell numbers in peripheral blood at patient admission. Both myeloid and lymphoid-derived cells generally were in normal ranges, with abnormal levels in only a few patients, indicating that the majority of patients were at an early phase of the disease. Regardless, the low–IL-37 group presented significantly lower CD8+ T-cell and platelet counts than the high–IL-37 group (P = .04 for CD8+ T cells and .04 for
platelets, respectively), whereas no significant differences were observed for all other cells, including white blood cells (total cells of both myeloid and lymphoid-derived cells), monocytes, lymph cells, natural killer cells, and CD3+ and CD4+ T cells (Figure 4B and C). Other immunological indicators are shown in Supplementary Figure 2.

Overall, a low IL-37 response seems to attenuate host capacity to suppress inflammation and results in elevated inflammatory...
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cytokine levels. Importantly, elevated IFN-α levels was noted in the high–IL-37 group, suggesting that IL-37 is unlikely to interfere with IFN responses.

Combination of Low IL-37 Levels and Inflammatory Indicators in Predicting Severe COVID-19

Among the 254 cases, 20 were clinically sorted into the severe category. Interestingly, all 20 severe cases presented very low initial plasma IL-37 and elevated HS-CRP and IL-8 levels on admission before any treatment (Figure 5A). Furthermore, significant reverse correlations were identified between IL-37 and both HS-CRP (P = .04) and IL-8 (P = .01) (Figure 5B). The sensitivity of IL-37 alone to differentiate severe from moderate cases was 100%, with an area under the curve (AUC) of 0.747 and a specificity of 55.7%. To improve the prediction value, we added HS-CRP to IL-37 and observed that the AUC increased to 0.933 and that the specificity reached 91.2%, but the sensitivity decreased to 92.9% (Figure 5C and Supplementary Table 1).

Because omission of severe cases might result in loss of life, we further explored a more sensitive model by incorporating IL-8. Importantly, the sensitivity of the combination of IL-37, HS-CRP, and IL-8 reached 100%, with an AUC of 0.959 (95% CI, .932–.986) and a specificity of 91.2%. The final regression equation for this joint prediction model was as follows:

\[
\text{Logit}(P) = -2.77 - 0.0072 \cdot \text{IL-37 (pg/mL)} + 0.009 \cdot \text{IL-8 (pg/mL)} + 0.048 \cdot \text{HS-CRP (mg/L)},
\]

Because IL-6 has been implicated to be involved in disease pathogenesis caused by SARS-CoV-2 infection, we examined its relationship with disease prognosis and with IL-37. As expected, plasma IL-6 levels in the group with severe disease were significantly higher than in the moderate-disease group (Supplementary Figure 3A). IL-6 levels were inversely correlated with serum IL-37 levels, and high IL-37 levels were accompanied by low IL-6 levels (Supplementary Figure 3B). We also assessed the predictive value of IL-6, alone or in combination with IL-37 or both IL-37 and HS-CRP, for clinical outcomes of SARS-CoV-2 infection. As shown in Supplementary Figure 3C, the algorithm of a triple combination of IL-6, IL-37, and HS-CRP was most predictive, followed by IL-6 plus IL-37 and then IL-6 alone. However, the predictive power of combined
Figure 5. Prediction value of interleukin 37 (IL-37) in severe classification of coronavirus disease 2019. A, Significant differences in IL-37, high-sensitivity C-reactive protein (HS-CRP) and interleukin 8 (IL-8) levels between moderate and severe infections. B, Significant reverse correlations between IL-37 and HS-CRP and between IL-37 and IL-8. C, Left, Receiver operating characteristic (ROC) curves for different combinations: IL-37 alone; IL-37 and HS-CRP; and IL-37, HS-CRP, and IL-8. Right, ROC curve for IL-37, HS-CRP, and IL-8, along with sensitivity, specificity, and area under the curve [AUC, with 95% confidence interval]. The smooth ROC curves above were fitted by the Python library “numpy-polyfit.” Dotted lines represent a curve when the occurrence of an event is completely random, the farther the prediction curve deviates from the reference line, the stronger the prediction ability is. P values are represented as *P < .05, **P < .01, ***P < .001, ****P < .0001.
IL-6, IL-37, and HS-CRP was still much less than that of combined IL-8, IL-37, and HS-CRP (Figure 5C) Taking these findings together, we identified the triple-marker of IL-8, IL-37, and HS-CRP as remarkably predictive of the clinical severity of diseases caused by SARS-CoV-2. Because all 3 evaluations can be performed on patient admission, before treatment, this prediction model may have great value in clinical practice for patients with COVID-19.

**IL-37 and Pulmonary Inflammation in hACE2-Transgenic Mice Infected With SARS-CoV-2**

To further validate the anti-inflammatory ability of IL-37 during SARS-CoV-2 infection, we used a transgenic mice model that expressed hACE2 and was infected with SARS-CoV-2. After infection, the mice were split into 2 groups. The first group received intravenous IL-37 (dissolved in PBS) at 2 time points, 12 and 48 hours after infection, and the other (control) group was administered PBS only. As expected, lung tissue histopathological examination revealed more severe inflammatory cell infiltration and tissue injuries in control group, with more interstitial thickening and edema, blood capillary congestion and hemorrhage, and exfoliation of pulmonary alveoli epithelium on day 5 after infection than in the experimental group (Figure 6A and 6B). To quantify the lung damage overall, we adopted a pathological score and inflammatory cell quantification algorithm. The control group was scored significantly higher than the IL-37 treatment group ($P = .006$) (Figure 6C), accordingly, more inflammatory cells per unit tissue area were found in the control group ($P = .02$) (Figure 6D).

**DISCUSSION**

The COVID-19 pandemic due to SARS-CoV-2 infection has caused mass mortality worldwide and has become the most severe public health challenge; moreover, effective treatment for patients with COVID-19 remains unavailable. Because hypercytokinemia and respiratory inflammation are known as critical pathogenesis mechanisms during respiratory viral infection [17–19] and IL-37 is a powerful suppressor against inflammation, we rationalized that IL-37 may play a protective role during SARS-CoV-2 infection and should therefore be explored for treatment. Here, we report that among 254 patients with COVID-19, early IL-37 responses before any treatment correlate with clinical outcomes. Elevated IL-37 responses probably favor balancing innate responses and thus efficiently restrain viral replication to maintain the functionalities of host vital organs and thereby maintain internal homeostasis. In contrast, failure to mount IL-37 responses is likely to indicate a severe clinical prognosis.

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Interleukin 37 (IL-37) alleviates inflammation in human angiotensin-converting enzyme 2-transgenic mice infected with severe acute respiratory syndrome coronavirus 2. A, B, Representative hematoxylin-eosin (HE)-stained sections on day 5 after infection (scale bars represent 1 mm [left] and 50 µm [right]). A, HE-stained lung sections from control group treated with phosphate-buffered saline (PBS) ($n = 3$), with increased hemorrhage and inflammatory cell influx. B, HE-stained lung sections from experimental group treated with IL-37 ($n = 3$), with milder tissue injuries. C, Pathological scores were calculated on the basis of lung histochemical data ($P = .006$). D, Inflammatory cells were quantified in each lung tissue section ($P = .02$). Quantification data were analyzed as means with standard deviations, using unpaired t tests; the $P$ value cutoff was .05. $P$ values are represented as *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$.
Several studies have confirmed that the high inflammatory responses resulting from SARS-CoV-2 infection, presented as cytokine release syndrome [20], are among the preliminary pathogeneses for disease and death in patients with COVID-19 [21–23]. In severe COVID-19 cases, dynamic IL-6 and IL-8 levels are associated with disease progression [24]. Similarly, we identified elevation in IL-6 and IL-8 levels and their association with clinical pathogenesis. To antagonize inflammatory responses, corticosteroids are usually administered clinically; however, their efficacy in COVID-19–induced cytokine release syndrome is controversial, because it may increase viral replication and stimulate additional inflammatory responses. An ideal intervention is to retain intact innate immune responses but restrain inflammatory responses, and a novel treatment strategy with fine-tuning of the immune system is urgently needed [25].

IL-37 is a newly discovered cytokine with strong anti-inflammatory activities. To date, researchers have focused more on its protective role and application in autoimmune diseases. Studies showed that increased plasma IL-37 correlates significantly with ameliorated clinical manifestations in systemic lupus erythematosus [26], rheumatoid arthritis [27] and inflammatory bowel disease [28].

The unique anti-inflammatory mechanism may confer IL-37 with a wide range of anti-inflammatory activities. Early researchers found that IL-37 can enter the nucleus and form a functional complex with Smad3 [9], thereby reducing anti-inflammatory function by regulating gene transcription [29]. In recent years, a new anti-inflammatory mechanism involving binding to interleukin 18Ra was discovered. IL-37 is able to inhibit downstream proinflammatory signal kinases, such as mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK). IL-37 also activates the anti-inflammatory signaling molecules Mer and PTEN [7, 30, 31].

The detailed mechanism of IL-37 against respiratory diseases has also been explored. Huang et al [32] found that IL-37 may reduce airway inflammation by suppressing activation of NF-κB and signal transducer and activator of transcription 1 type 3 in asthma. Kim et al [33] identified that IL-37 can inhibit transforming growth factor β–induced lung fibroblast proliferation, promoting the progression of idiopathic pulmonary fibrosis disease. Qi et al [11] observed that IL-37 attenuates secretion of inflammatory cytokines by down-regulating the MAPK signal transduction pathway in an H1N1 infection model, protecting mice from lung damage and improving survival. Zhou et al [34] found that IL-37 induced by H3N2 infection can directly restrain the replication of influenza virus.

In the current study, we demonstrated that IL-37 is likely to play a protective role during SARS-CoV-2 infection. Early elevated plasma IL-37 before treatment correlates with low inflammatory cytokines, high IFN-α, a high number of immune cells, and reduced hypoxemia. Furthermore, we demonstrated that IL-37 can effectively antagonize inflammatory responses in hACE2-transgenic mice after SARS-CoV-2 infection, the early administration of IL-37 reduced inflammatory cell infiltration and alleviated lung tissue damage, thereby facilitating disease remission. Recently, Hadjadj et al [35] found that exacerbated inflammatory responses and impaired type I IFN activity are hallmarks of severe illness, implicating the protective role of type I IFN. An important observation in our study is that the level of IFN-α in patients with high plasma IL-37 levels was significantly higher than in the low–IL-37 group, suggesting that IL-37 is unlikely to suppress and even might enhance production of IFN-α. These findings highlight the potential of IL-37 in the development of a novel therapeutic regimen, not only against coronaviruses such as SARS-CoV-2 and MERS, but also against influenza and other respiratory viruses.

It is crucial to identify severe cases early before development of clinical complications and thus gain time to prevent severe illness and death. We used IL-37 alone or in combination with other factors to formulate an effective prediction model. With incorporation of IL-37, IL-8, and HS-CRP, sensitivity reached 100%, and specificity reached 91.2%. Because all data for this model were collected from samples obtained at patient admission and before any clinical intervention, the prediction may be applicable at the moment of admission, and we believe the model is likely to prove valuable in the future, after validation in a large-scale longitudinal cohort.

Supplementary Data
Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
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Author contributions. J. X. configured the project, and X. Z. and J. X. designed the project and supervised the experiments. T. Z. and H. L. coordinated the study. A. L. performed the experiments, analyzed the data, and drafted the manuscript. Z. Song implemented the viral RNA assay. R. J. performed animal experiments. C. Z. scanned the lung tissue sections. All authors collected the blood samples and clinical data. J. X. revised the manuscript. X. Z. and J. X. acquired the funding.

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**Potential conflicts of interest.** A. L., X. Z., and J. X. have 2 pending patents: “A quantifiable, interleukin-37-based respiratory virus infection early warning model for severe (critical) cases” and “Application of interleukin 37 and interferon in the treatment of viral infections.” All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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