Lower Serum Angiotensin-Converting Enzyme Level in Relation to Hyperinflammation and Impaired Antiviral Immune Response Contributes to Progression of COVID-19 Infection

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ABSTRACT

Introduction: As a homologue of the angiotensin-converting enzyme (ACE), angiotensin-converting enzyme 2 (ACE2) has been identified as the main receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) invasion. We aimed to investigate the role of serum ACE in predicting the coronavirus disease 2019 (COVID-19) disease progression and the underlying mechanisms.

Methods: We retrospectively enrolled 120 patients with confirmed COVID-19 who underwent serum ACE detection on admission. The clinical characteristics and laboratory findings during hospitalization were evaluated dynamically to identify the potential risk factors for disease progression.

Results: ACE level was demonstrated as one of the independent risk factors. Patients with ACE level ≤ 33.5 U/L showed a higher cumulative virus RNA detection rate, elevated pro-inflammatory mediators levels, declined lymphocyte count, and decreased SARS-CoV-2-specific antibodies than those with ACE level > 33.5 U/L.

Conclusion: Lower serum ACE levels in relation to delayed virus elimination, hyperinflammatory condition, and impaired host antiviral immune responses contribute to disease progression of COVID-19.

Keywords: COVID-19; Serum angiotensin-converting enzyme; Disease progression; Immune response; Virus clearance
Key Summary Points

Why carry out this study?
COVID-19 is still spreading all over the world and risk factors for disease progression warrant further investigation.

As a homologue of the angiotensin-converting enzyme (ACE), angiotensin-converting enzyme 2 (ACE2) has been identified as the main receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) invasion.

What was learned from the study?
Serum ACE level is predictive of COVID-19 disease progression.
Serum ACE level is related to poor clinical outcome, delayed virus clearance, hyperinflammation, and immunosuppression during the course of COVID-19.

INTRODUCTION

The coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an ongoing global pandemic that has resulted in significant social and economic losses. As reported previously, patients with COVID-19 disease progression had significantly poorer clinical outcome and higher mortality rates than those without [1]. Therefore, identifying risk factors for COVID-19 disease progression has important implications in developing treatment options andrationally allocating medical resources.

Angiotensin-converting enzyme (ACE) is a type I transmembrane monomeric glycoprotein that could be classified as a peptidyl dipeptidase belonging to the M2 family of zinc metallopeptidases. ACE is abundant in the lungs, intestine, kidneys, brain, and aorta and is mostly expressed in the luminal membrane of vascular endothelial cells, particularly the pulmonary endothelium [2]. Membrane-bound ACE can be cleaved and transferred into circulation; this process is reportedly regulated by ACE secretase, which has not been identified yet but is believed to be a membrane-bound metalloprotease [3]. ACE plays a key role in the renin–angiotensin system (RAS) by catalyzing conversion of angiotensin I (Ang I) to angiotensin II (Ang II), which then exerts its effects including vasoconstriction, blood pressure elevation, and induction of inflammatory and pro-fibrotic pathways, and vasoconstriction via type 1 receptors (AT1R). The ACE–Ang I–AT1R axis was demonstrated to participate in the progression of acute respiratory distress syndrome (ARDS)/acute lung injury (ALI) [3]. In 2000, angiotensin-converting enzyme 2 (ACE2) was discovered as a close homologue of ACE and could be detected mainly in the intestine, lungs, heart, kidneys, and pancreas [4, 5]. By acting as an endogenous counter-regulatory axis to the ACE axis, ACE2 converts Ang II into angiotensin 1–7 and exerts vasodilatory, anti-inflammatory, and anti-fibrotic effects via the Mas receptor [2, 4]. Besides being an RAS modulator, ACE2 was identified as the functional receptor for SARS-CoV-2 following the COVID-19 outbreak [6].

It has been indicated in recent years that serum ACE level correlates with the diagnosis and prognosis of a variety of respiratory disorders [7]; however, the potential relationship between ACE level and clinical outcomes of SARS-CoV-2 infection is not adequately understood. The latest research based on 136 patients with confirmed COVID-19 suggested that serum ACE level could serve as a marker to reflect the clinical condition of COVID-19 rather than an independent risk factor for disease severity [8]. In our study, 120 patients with confirmed COVID-19 who underwent measurement of serum ACE levels on admission were retrospectively enrolled, to evaluate the potential role of serum ACE level in combination with other risk factors in predicting disease progression of COVID-19 and further investigate the possible mechanisms involved.
METHODS

Participants and Study Design

Between January 13, 2020 and March 1, 2020, 120 patients with confirmed COVID-19 based on detection of SARS-CoV-2 RNA who were hospitalized at Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology and underwent detection of serum ACE levels on admission were enrolled in this retrospective study cohort. We collected data on demographics, comorbidities, symptoms and signs, and serum ACE levels and other laboratory findings during the hospitalization from the inpatient electronic medical recording system from Tongji Hospital.

Definitions

Patients were categorized into the progressive group if they fulfilled any of the following three criteria: use of mechanical ventilation, admission to intensive care unit (ICU), or death; otherwise, they were categorized into the non-progressive group. Hypoxemia was defined as a PaO₂/FiO₂ ratio of less than 300. Acute kidney injury was diagnosed according to at least one of the three following definitions: (1) increase in serum creatinine (Scr) of 0.3 mg/dL (≥ 26.5 μmol/L) within 48 h; (2) increase in Scr to > 1.5 times the basal value within 7 days of known or assumed renal impairment; (3) urine output < 0.5 mL/(kg h) for 6 h. Acute liver injury was defined as jaundice with a total bilirubin level of ≥ 3 mg/dL and an acute increase in alanine transaminase (ALT) by at least five times the upper limit of the normal range and/or an increase in alkaline phosphatase by at least twice the upper limit of the normal range. Cardiac injury was diagnosed if serum levels of cardiac biomarkers (e.g., troponin I) were greater than the 99th percentile of the upper reference limit, or if new abnormalities were detected in electrocardiography and echocardiography.

Detection of SARS-CoV-2 Viral RNA

Throat or nasopharyngeal swabs were obtained for laboratory testing of SARS-CoV-2. Viral RNA was extracted from clinical samples and further used for real-time reverse transcription polymerase chain reaction (RT-PCR) assay of SARS-CoV-2 by using a commercial TaqMan one-step real-time RT-PCR kit from Shanghai BioGerm Medical Biotechnology Co. Ltd. (Shanghai, China). Primers and probes involved were designed to target the open reading frame 1ab (ORF1ab) and nucleocapsid (N) gene of SARS-CoV-2, whose sequences have been described in a previous study [9]. Real-time RT-PCR was performed using the following conditions: 50 °C for 10 min, 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 55 °C for 40 s. A cycle-count threshold value of < 37 was defined as positive for SARS-CoV-2 RNA.

Detection of Angiotensin-Converting Enzyme

ACE activity level in the serum was quantified on the basis of a microplate fluorescent reader-based assay using a commercial ACE assay kit according to the manufacturer’s instructions. The ACE Assay Kit (Angiotensin I Converting Enzyme, #AD309AC) was purchased from Glenbio (UK).

Laboratory Measurements

Routine laboratory measurements including complete blood count, serum biochemical test (including liver and renal function, creatine kinase, LDH, and electrolytes), blood gas analysis, measurement of serum inflammatory mediators (CRP, ferritin, ESR, and PCT), myocardial enzyme spectrum analysis, and coagulation profile study were performed in the Department of Laboratory Medicine in Tongji Hospital according to the clinical requirement. Serum cytokine profile was determined by chemiluminescence immunoassay (CLIA) performed on a fully automated analyzer (Siemens Immulite 1000, DiaSorin Liaison, or Roche Diagnostics Cobas e602) according to the
manufacturers’ instructions. CLIA kits for interleukin-1beta (IL-1β) (LKL11), IL-2R (LKIP1), IL-8 (LK8P1), IL-10 (LKP1), and tumor necrosis factor alpha (TNFα) (LKNF1) were purchased from DiaSorin. An IL-6 kit (05,109,442,190) was purchased from Roche Diagnostics.

Peripheral lymphocyte subsets, including the proportions and numbers of NK cells, B cells, total T cells, CD4+ T cells, and CD8+ T cells were investigated in all patients. The following reagents were purchased from Becton Dickinson (BD) Biosciences and used according to the manufacturer’s instructions: BD Multitest 6-Color TBNK reagent (#644,611) and BD Tru-count Tubes (#340,334). BD Multitest 6-Color TBNK reagent contains anti-CD3(SK7, FITC), anti-CD16(B73.1, PE), anti-CD56(NCAM16.2, PE), anti-CD45(2D1, PerCP-Cy5.5), anti-CD4(SK3, PE-Cy7), anti-CD19(SJ25C1, APC), and anti-CD8(SK1All, APC-Cy7). All samples were detected by a BD FACS Canto II flow cytometry system and analyzed with the BD FACSDiva software.

Detection of specific antibodies against SARS-CoV-2 was based on an automated paramagnetic particle chemiluminescent immunoassay (CLIA) kit supplied by Yhlo Biotech Co. Ltd. (Shenzhen, China). A test result ≥ 10 AU/mL was considered reactive (positive).

**Statistics**

Continuous variables were expressed as mean and standard error of the mean (SEM) and compared with the unpaired two-sided Student’s t test; categorical variables were expressed as number (%) and compared using the chi-squared test or Fisher’s exact test between the two study groups. Prediction value was appraised by the receiver operating characteristic (ROC) curves. The 95% confidence interval (CI) was utilized to calculate the sensitivity and specificity; the cutoff value was selected when the Jordan index was at its maximum. Kaplan–Meier analysis was conducted to value the survival rate and cumulative RNA positive rate. Statistical analyses were carried out using SPSS (version 19.0, IBM). a two-sided P value < 0.05 was considered to indicate statistical significance.

**Study Approval**

The study was performed in accordance with the tenets of the Declaration of Helsinki and the Good Clinical Practice principles. The case series was approved by the Institutional Review Board of Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology (TJ-C20200101). Written informed consent was waived because of the rapid emergence of this infectious disease.

**RESULTS**

**Baseline Demographics and Disease Characteristics**

As summarized in Table 1, the cohort comprised 120 enrolled patients: 24 showed disease progression during hospitalization (progressive group) whereas the remaining 96 patients did not (non-progressive group). The distribution of sex between the two groups was comparable, while the mean age of the progressive group was significantly higher than that of the non-progressive group (63.7 vs. 56.8 years, P = 0.034).

Patients in the progressive group were more likely to have at least one underlying comorbidity (62.5% vs. 37.5%, P = 0.037), especially a possible comorbidity of diabetes (45.8% vs. 14.6%, P = 0.002) than those in the non-progressive group. Although serum level of ACE has seldom been discussed as a risk factor for COVID-19 disease progression in the existing literature, significantly lower ACE levels were found in patients in the progressive than non-progressive group (mean 18.5 vs. 28.0 U/L, P = 0.002), suggesting its potential use as a biomarker of disease progression. Much more frequent fever and higher peak temperature were found in the progressive than non-progressive group [23 (95.8%) vs. 74 (77.1%), P = 0.042 and 39.0 °C vs. 38.1 °C, P = 0.000]. Dyspnea was significantly more frequent in the progressive group than in the non-progressive...
| Characteristics                      | All patients (<i>n = 120</i>) | Progression (<i>n = 24</i>) | Non-progression (<i>n = 96</i>) | <i>P</i> value |
|--------------------------------------|---------------------------------|-----------------------------|---------------------------------|---------------|
| Male, <i>n</i> (%)                   | 56 (46.7%)                      | 11 (45.8%)                  | 45 (46.9%)                      | 1             |
| Age, years                           | 58.1 (1.3)                      | 63.7 (2.6)                  | 56.8 (1.5)                      | 0.034*        |
| Any comorbidity, <i>n</i> (%)        | 51 (42.5%)                      | 15 (62.5%)                  | 36 (37.5%)                      | 0.037*        |
| Hypertension, <i>n</i> (%)           | 35 (29.2%)                      | 9 (37.5%)                   | 26 (27.1%)                      | 0.325         |
| Diabetes, <i>n</i> (%)               | 25 (20.8%)                      | 11 (45.8%)                  | 14 (14.6%)                      | 0.002*        |
| ACE, U/L                             | 26.1 (1.4)                      | 18.5 (1.7)                  | 28.0 (1.6)                      | 0.002*        |
| Fever, <i>n</i> (%)                  | 97 (80.8%)                      | 23 (95.8%)                  | 74 (77.1%)                      | 0.042*        |
| Highest temperature, °C              | 38.3 (0.1)                      | 39.0 (0.2)                  | 38.1 (0.1)                      | 0.000*        |
| Cough, <i>n</i> (%)                  | 94 (78.3%)                      | 20 (83.3%)                  | 74 (77.1%)                      | 0.591         |
| Sputum production, <i>n</i> (%)      | 54 (45.0%)                      | 12 (50.0%)                  | 42 (43.8%)                      | 0.65          |
| Chest tightness, <i>n</i> (%)        | 62 (51.7%)                      | 15 (62.5%)                  | 47 (49.0%)                      | 0.261         |
| Dyspnea, <i>n</i> (%)                | 44 (36.7%)                      | 16 (66.7%)                  | 28 (29.2%)                      | 0.002*        |
| Fatigue, <i>n</i> (%)                | 42 (35.0%)                      | 12 (50.0%)                  | 30 (31.3%)                      | 0.098         |
| Diarrhea, <i>n</i> (%)               | 32 (26.7%)                      | 8 (33.3%)                   | 24 (25.0%)                      | 0.444         |
| Myalgia, <i>n</i> (%)                | 24 (20.0%)                      | 8 (33.3%)                   | 16 (16.7%)                      | 0.087         |
| Headache, <i>n</i> (%)               | 15 (12.5%)                      | 4 (16.7%)                   | 11 (11.5%)                      | 0.497         |
| Heart rate, bpm                      | 92.3 (1.4)                      | 91.4 (3.2)                  | 92.5 (1.6)                      | 0.76          |
| Respiratory rate, per min            | 21.6 (0.5)                      | 24.9 (2.0)                  | 20.8 (0.4)                      | 0.001*        |
| ≥ 30, <i>n</i> (%)                   | 9 (7.5%)                        | 4 (16.7%)                   | 5 (5.2%)                        | 0.078         |
| Percutaneous oxygen saturation       | 94.9 (0.6)                      | 90.4 (2.1)                  | 96.0 (0.5)                      | 0.002*        |
| ≤ 93%, <i>n/N</i> (%)                | 30/118 (25.4%)                  | 10/23 (43.5%)               | 20/95 (21.1%)                   | 0.035*        |
| PaO<sub>2</sub>/FiO<sub>2</sub>, mmHg | 234.3 (20.9)                    | 169.0 (27.2)                | 262.3 (24.2)                    | 0.036*        |
| < 300 mmHg, <i>n/N</i> (%)           | 14/20 (70.0%)                   | 6/6 (100.0%)                | 8/14 (57.1%)                    | 0.115         |
| Laboratory examinations              |                                 |                             |                                 |               |
| White blood cell count, × 10<sup>9</sup>/L | 6.5 (0.3)                      | 8.9 (1.0)                   | 5.9 (0.2)                       | < 0.001*      |
| > 10, <i>n</i> (%)                   | 9 (7.5%)                        | 7 (29.2%)                   | 2 (2.1%)                        | < 0.001*      |
| Neutrophil count, × 10<sup>9</sup>/L  | 4.7 (0.3)                       | 7.7 (0.9)                   | 3.9 (0.2)                       | < 0.001*      |
| > 63, <i>n</i> (%)                   | 16 (13.3%)                      | 12 (50.0%)                  | 4 (4.2%)                        | < 0.001*      |
| Lymphocyte count, × 10<sup>9</sup>/L  | 1.2 (0.1)                       | 0.7 (0.1)                   | 1.3 (0.1)                       | < 0.001*      |
| < 1, <i>n</i> (%)                    | 58 (48.3%)                      | 19 (79.2%)                  | 39 (40.6%)                      | 0.001         |
The frequencies of other prevalent symptoms at onset of illness including cough, sputum production, and chest tightness, and frequencies of less common symptoms including diarrhea, myalgia, and headache were comparable between the two groups. According to vital signs recorded on the day of hospital admission, significantly higher respiratory rate and lower oxygen saturation were found in the progressive patients than the non-progressive patients (mean 24.9/min vs. 20.8/min, \( P = 0.001 \); and...
mean 90.4% vs. 96.0%, \( P = 0.002 \). Further, 43.5% (10/23) of progressive patients but only 21.1% (20/95) of non-progressive patients had percutaneous oxygen saturation of \( \geq 93.0\% \) on admission (\( P = 0.035 \)).

Among the 20 patients who underwent arterial blood gas (ABG) testing, the PaO\(_2\)/FiO\(_2\) ratio was obviously lower in the progressive than non-progressive patients (mean 169.0 vs. 262.3 mmHg, \( P = 0.036 \)). Moreover, all six progressive cases showed a PaO\(_2\)/FiO\(_2\) ratio < 300 mmHg, indicating the occurrence of acute respiratory distress syndrome in these patients.

Laboratory findings showed remarkably higher counts of white blood cells (WBCs) and neutrophils; higher levels of aspartate aminotransferase (AST), total bilirubin (TBIL), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), troponin, BNP, d-dimer, procalcitonin (PCT), C-reactive protein (CRP), ferritin, erythrocyte sedimentation rate (ESR), IL-2R, IL-6, IL-8, IL-10, and TNF\( \alpha \); but lower lymphocyte count and albumin level in the progressive than non-progressive patients. More patients with progressive disease developed lymphopenia and leukocytosis than those with non-progressive disease [19/24 (79.2%) vs. 39/96 (40.6%), \( P = 0.001 \); 7/24 (29.2%) vs. 2/96 (2.1%), \( P < 0.001 \)]. These findings suggest that impairment of multiple organ function and systemic inflammation are more prevalent among patients with COVID-19 disease progression.

**Table 2** Univariate analysis and multivariate analysis of disease progression risk factors

|                         | Univariate analysis | Multivariate analysis |
|-------------------------|---------------------|-----------------------|
|                         | \( P \) value       | OR (95% CI)           | \( P \) value       | OR (95% CI)           |
| ACE (U/L)               | 0.004               | 0.931 (0.887–0.978)   | 0.008               | 0.929 (0.880–0.981)   |
| Age (year)              | 0.038               | 1.040 (1.002–1.079)   |                      |                       |
| Diabetes (yes vs. no)   | 0.002               | 4.569 (1.726–12.099)  | 0.009               | 5.266 (1.501–18.475)  |
| Lymphocyte count \( \times 10^9 \)/L | 0                   | 0.079 (0.021–0.292)   | 0.001               | 0.075 (0.016–0.343)   |

**Serum ACE Level is Predictive of COVID-19 Disease Progression**

In the univariate analysis, ACE level, age, underlying diabetes, and lymphocyte count on admission were found to correlate with COVID-19 disease progression. Further multivariate logistic analysis indicated that ACE level, underlying diabetes, and lymphocyte count on admission were independent risk factors for COVID-19 disease progression (Table 2). As for their performance for predicting disease progression, the ROC curves displayed the area under the curve (AUC) of serum ACE, underlying diabetes, and lymphocyte count as 0.701 (95% CI 0.594–0.809), 0.651 (95% CI 0.518–0.784), and 0.794 (95% CI 0.705–0.883), respectively. Combination of serum ACE level and underlying diabetes or lymphocyte count exhibited a superior performance for predicting COVID-19 disease progression than any single predictor, with an increased AUC of 0.829 (95% CI 0.745–0.912) and 0.857 (95% CI 0.777–0.936), respectively. A composite predictor consisting of ACE level, underlying diabetes, and lymphocyte count further improved the predictive abilities of coupled predictors, although the increase in AUC was modest (0.860 [95% CI 0.786–0.933]) (Fig. 1a). We determined an optimal ACE cutoff value of 33.5 U/L to predict disease progression with a sensitivity specificity, positive predictive value, and negative predictive value of 100%, 30.2%, 26.4%, and 100%, respectively (Fig. 1b). These
results implied the relationship between serum ACE level and COVID-19 prognosis, and indicated the potential contribution of ACE level to the development of a predictive model for COVID-19 disease progression.

**Serum ACE Level in Relation to Poor Clinical Outcome**

We further compared the baseline characteristics and clinical outcomes between patients...
with COVID-19 with an ACE ≤ 33.5 U/L and those with an ACE > 33.5 U/L. As shown in Table 3, there was no significant difference in the relative distributions of sex, age, and comorbidities between the two groups. However, a significantly increased requirement of mechanical ventilation and higher incidence of complications including ARDS, acute kidney injury, acute cardiac injury, and acute liver injury were found in patients with ACE ≤ 33.5 U/L than in those with ACE > 33.5 U/L. Moreover, it is worth noting that all eight deaths occurred in those with ACE ≤ 33.5 U/L, whereas none of the patients with ACE > 33.5 U/L developed ARDS, acute kidney injury, acute liver injury, or were in need of mechanical ventilation. This provided us with an explanation for why lower ACE level could act as a predictor of disease progression in SARS-CoV-2 infection.

### Table 3 Baseline characteristics and clinical outcomes of patients with COVID-19 between ACE ≤ 33.5 and ACE > 33.5

| Characteristics                  | All patients (n = 120) | ACE ≤ 33.5 (n = 91) | ACE > 33.5 (n = 29) | P value |
|----------------------------------|------------------------|---------------------|---------------------|---------|
| Male, n (%) 56 (46.7%)           | 41 (45.1%)             | 15 (51.7%)          | 0.67                |
| Age, years 58.1 (55.6–60.7)      | 58.9 (56.3–61.7)       | 55.6 (48.8–62.4)    | 0.499               |
| Any comorbidity, n (%) 51 (42.5%)| 41 (45.1%)             | 10 (34.5%)          | 0.39                |
| Hypertension, n (%) 35 (29.2%)   | 28 (30.8%)             | 7 (24.1%)           | 0.64                |
| Diabetes, n (%) 25 (20.8%)       | 19 (20.9%)             | 6 (20.7%)           | 1                   |

**Clinical outcomes**

- **Mechanical ventilation, n** 23 (19.2%) 23 (25.3%) 0 (0.0%) 0.001*
- **Death, n/N** 8/117 (6.8%) 8/88 (9.1%) 0/29 (0%) 0.093
- **Any complication, n (%)** 33 (27.5%) 31 (34.1%) 2 (6.9%) 0.004*
- **ARDS, n/N** 9/116 (7.8%) 9/87 (10.3%) 0/29 (0%) 0.071
- **Acute cardiac injury, n/N** 10/115 (8.7%) 10/87 (11.5%) 0/28 (0%) 0.061
- **Acute kidney injury, n/N** 22/117 (18.8%) 20/89 (22.5%) 2/28 (7.1%) 0.07
- **Acute liver injury, n/N** 10/118 (8.5%) 10/89 (11.2%) 0/29 (0%) 0.059

Data are median (IQR), n (%), or n/N (%), where N is the total number of patients with available data. P values comparing severe cases and moderate cases are from \( \chi^2 \) test, Fisher’s exact test, unpaired two-sided Student’s \( t \) test or Mann–Whitney \( U \) test.

ARDS acute respiratory distress syndrome

**Fig. 2** Kaplan–Meier survival curve for statistical analysis. Patients with ACE ≤ 33.5 U/L showed a higher cumulative detection rate of positive viral RNA (33.74% vs. 17.46%, 9.71% vs. 0% at days 20 and 30 post illness onset, respectively).
Serum ACE Level in Relation to Delayed Virus Clearance

The cumulative detection rate of positive viral RNA was also evaluated in patients with different levels of ACE. As shown in Fig. 2, when compared to patients with an ACE > 33.5 U/L, those with ACE ≤ 33.5 U/L exhibited a significantly elevated cumulative detection rate of positive viral RNA during hospitalization (33.7% vs. 17.5%, 9.7% vs. 0.0% at days 20 and 30 post illness onset, respectively), suggesting that ACE might participate in the prognosis of COVID-19 by affecting viral invasion and replication.

Serum ACE Level in Relation to Hyperinflammation and Immunosuppression During the Course of COVID-19

To further explore the possible mechanisms underlying the effect of ACE on COVID-19 disease progression, variations in the parameters reflecting inflammation and immune status were evaluated in patients with various levels of serum ACE. Overproduction of proinflammatory cytokines has been demonstrated as one of the most important factors in determining the severe deterioration of COVID-19. Among available key cytokines observed in this study, larger amounts of IL-2R, IL-6, and IL-10 were observed in patients who had an ACE ≤ 33.5 U/L than in those with ACE > 33.5 U/L during the first 3 weeks of hospitalization, with the most striking change being concentrated within 7 days after admission (Fig. 3a). Inflammatory mediators including CRP, ferritin, and ESR in patients with ACE ≤ 33.5 U/L were markedly higher than in those in patients with ACE > 33.5 U/L, with the most striking difference detected on admission and day 21 for CRP, on day 7 for ESR, and on admission for ferritin. Serum levels of PCT were comparable between the two groups (Fig. 3b). We next analyzed the dynamic changes in the peripheral lymphocyte count during hospitalization, lymphocyte subsets, and specific antibody responses against SARS-CoV-2 in a fraction of patients who underwent two consecutive detections with an interval of 7 ± 3 days. As shown in Fig. 4a, patients with ACE ≤ 33.5 U/L had a significantly lower lymphocyte count on admission, day 7, and day 28 than those with ACE > 33.5 U/L. With regard to peripheral lymphocyte subsets compared between the two detections, there were no statistically significant differences in the absolute numbers of lymphocyte subsets for patients with ACE > 33.5 U/L. In contrast, patients with ACE ≤ 33.5 U/L were characterized by a significant decline in total T, CD4+ T, B, and NK cell counts, and their NK cell counts during the second testing were notably lower than that of patients with ACE > 33.5 U/L. Our results also indicated that

Fig. 3 Comparisons of the proinflammatory cytokines and inflammatory mediators between patients with ACE ≤ 33.5 U/L (n = 91) and patients with ACE > 33.5 U/L (n = 29) during hospitalization. The comparison of IL-2R, IL-6, IL-10 (a); CRP, PCT, ESR, and ferritin (b) between patients with ACE ≤ 33.5 U/L and patients with ACE > 33.5 U/L on admission and days 7, 14, and 21.
Fig. 4 Comparisons of the immune status between patients. The comparison of lymphocyte count in patients during hospitalization. The change in total T lymphocytes and CD4$^+$ T, CD8$^+$ T, B, and NK cells (a), specific antibody IgM, and IgG against SARS-CoV-2 (b) between two consecutive detections with an interval of 7 ± 3 days in patients. ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$.
both IgM and IgG titers were significantly reduced in patients with ACE ≤ 33.5 U/L, and these patients also displayed dramatically less production of specific IgM antibody as well as a slightly lower level of IgG according to the results of their second round of testing than those with ACE > 33.5 U/L (Fig. 4b). These laboratory findings were indicative of a relatively hyperinflammatory but immunosuppressive status in patients with lower ACE levels than those with higher ACE levels.

DISCUSSION

In this real-world retrospective cohort of 120 patients with confirmed SARS-CoV-2 infection, comparison of the clinical and laboratory findings between patients with and without disease progression revealed that lower ACE level on admission correlated with higher incidence of disease progression. To our knowledge, this is the first study to display the potential role of serum ACE as an independent risk factor for COVID-19 disease progression, which might be attributed to multiple mechanisms including delayed elimination of virus, hyperinflammation, and impairment of host immune response (Fig. 5).

At the time of generating this report (May 11, 2020), SARS-CoV-2 infection was a recognized global pandemic, threatening the health of more than 150 million human beings [10]. There is an urgent need to improve our understanding of the pathogenesis, risk factors, and clinical management of this novel disease. Currently, risk factors including older age, underlying comorbidities, lymphopenia, and cytokine storm have been suggested to contribute to COVID-19 disease progression [1, 11, 12]. A retrospective, multicenter, Chinese study of 150 patients with confirmed SARS-CoV-2 infection has shown that patients who died from COVID-19 had significantly lower lymphocyte counts than survivors, and elevated ferritin, ESR, and IL-6 could be used as predictors of fatality [13]. In accordance with these findings, an older age predominance, more frequent occurrence of underlying comorbidities, high fever, low oxygen saturation, impairment of multiple organ function, and systemic inflammation on admission were evidenced in the progressive cases in our study. At present, there are only few studies on the role of ACE in SARS-CoV-2 infection. In a recently published research article, significantly decreased baseline serum ACE level was observed in patients with COVID-19, which gradually increased to the

![Schematic outline of low serum ACE level in relation to inflammation, immune status, and clinical outcome](image-url)
normal level with disease remission. In that study, although the lowest ACE level was noted in the group with more severe infection, baseline ACE failed to serve as an independent risk factor for disease severity of COVID-19 [8]. To the best of our knowledge, this is the first investigation into whether, and to what extent, a relationship exists between ACE level and COVID-19 disease progression. Our data suggest that lower ACE levels on admission, observed in the progressive cases, might be an efficient predictor and independent risk factor of COVID-19 disease progression, and the predictive performance of which would be improved when combined with underlying diabetes and decreased lymphocyte count.

Since ACE2 has been demonstrated as the SARS-CoV-2 receptor, the scientific community rejected concerns about whether to continue the administration of ACE inhibitor (ACEI) and angiotensin receptor blockers (ARBs) in patients with cardiovascular disease (CVD) complicated with COVID-19. Although many researchers discourage the discontinuation of ACEI and ARBs [14], controversies still exist. A Korean clinical trial concluded that ACEI or ARBs treatment in severe patients with COVID-19 was correlated with the incidence of complications and increased in-hospital mortality. Patients who received higher dose of ACEI or ARBs may experience stronger potentially harmful adverse effects [15]. Additionally, administration of ACEI was reported to potentially decrease serum ACE level [16], which was in favor of our finding that lower serum ACE level was related to worse outcome in patients infected with SARS-CoV-2.

Changes in serum ACE have been previously investigated in multiple respiratory diseases including intrinsic and extrinsic asthma, squamous cell carcinoma of the lung, small cell carcinoma of the lung, sarcoidosis, and emphysema [7, 17]. Despite failing to confirm the prognostic value of serum ACE level regarding clinical outcome in patients with community-acquired pneumonia, researchers from the Netherlands showed a significantly decreased serum ACE level during the acute phase of pneumonia, and further indicated high incidence of bacteremia in patients with low serum ACE level at the time of hospital admission [3]. In line with the aforementioned findings, we found that patients with COVID-19 who developed progressive disease had significantly lower baseline ACE levels than those without disease progression. First, the impaired shedding of ACE from inflamed pulmonary vascular endothelium might be a reasonable explanation because respiratory symptoms had been documented in most of our enrolled patients. In addition, more severe inflammation on admission, manifested by significantly higher levels of WBC count, PCT, CRP, ferritin, and overproduction of various proinflammatory cytokines, was observed in the progressive cases, indicating that the entire vascular endothelium might be injured and hence responsible for reduced ACE release. Second, elevated reactive oxygen species (ROS) due to hyperinflammation in COVID-19 has been suggested to have an inhibitory effect on the activity of ACE [18, 19]. Finally, the overproduction of Ang II documented in SARS-CoV-2-infected patients might lead to a considerable consumption of the ACE pool [20].

More importantly, we further assessed the viral persistence and host responses underlying different levels of ACE, which might assist in better understanding of the role of ACE as a predictor of COVID-19 disease progression. The dynamic detection of viral RNA in clinical specimens revealed that patients with lower ACE level had a significantly higher cumulative detection rate of viral RNA positivity. It is well known that ACE and ACE2 counteract each other to control the metabolism of Ang peptides and play a crucial part in homeostasis [21, 22]. ACEI was widely used to reduce Ang II levels and treat cardiovascular diseases, and is capable of increasing ACE2 mRNA expression in the heart [21, 23]. Although the regulatory mechanism underlying the switch from ACE to ACE2 is still limited, Yang et al. demonstrated in a cardiac-stressed mouse model that endothelial Brg1–FoxM1 complex could tip the ACE/ACE2 balance to ACE2 expression at the transcription level [24]. Therefore, we speculate that as the critical functional receptor for SARS-CoV-2, ACE2 might be more abundant in patients with COVID-19 with lower ACE levels, resulting in
an increased susceptibility to SARS-CoV-2 and higher viral load. Furthermore, as shown in our results, an obviously diminished immune response to SARS-CoV-2 infection, as manifested by early significantly lower lymphocyte count, a marked decline in the total T cell, CD4⁺ T cell, B cell, and NK cell counts as well as decreased IgG and IgM titers between two detections, was documented for patients with ACE ≤ 33.5 U/L. Such altered immunity was insufficient to eliminate the virus and has been considered a risk factor associated with COVID-19 disease progression [1, 25, 26]. In line with our findings, a recent study showed that asymptomatic individuals with a prolonged duration of viral shedding displayed a weaker immune response to SARS-CoV-2 infection than those with symptoms [9]. Therefore, for patients with COVID-19 with lower ACE level, the potential ACE2 accumulation mediated virus entry and the impaired host antiviral immune responses may concomitantly contribute to the delayed virus clearance.

It is well recognized that ACE catalyzes the conversion of Ang I to Ang II, which subsequently exerts its effects including vasoconstriction, blood pressure elevation, activation of pro-inflammatory, and pro-fibrotic pathways via type 1 receptors. This axis was demonstrated to participate in the progression of ARDS and ALI [3]. Thus, theoretically, upregulation of ACE2 levels due to low ACE levels may contribute to vasodilation and alleviation of inflammation. However, we found in the present study that patients with lower ACE levels exhibited overproduction of cytokines as well as higher levels of inflammatory mediators. This may be partly related to a virus-induced cytopathic effect via ACE2 receptors which may play an important role in the development of inflammation. This process is characterized by the activation of the immune system and heavy infiltration of a variety of immune cells and non-immune cells, leading to production of large amounts of proinflammatory cytokines that cause damage to the host [27]. In addition, the hyperinflammation related to lower ACE level might also be attributed to increased serum level of Ang II. ACE2 elevation may enhance susceptibility to SARS-CoV-2. In the case of SARS-CoV-2, the spike protein binds to ACE2 on host cells and is then endocytosed, resulting in the fusion of viral and endosomal membranes, release of the viral genome into the cytoplasm, as well as the reduced and inoperable ACE2 on cells. The process is followed by an increase of serum Ang II and amplification of inflammatory reaction [28]. Moreover, Ang II can be formed through non-ACE pathways. When ACE is blocked, the increased bradykinin could activate the chymase pathway in tissues, allowing production of Ang II, Ang III, and Ang IV [29]. Therefore, the immune-mediated injury during SARS-CoV-2 infection, together with increased Ang II levels due to virus–host interactions and alternative formation pathways might contribute to the hyperinflammation in patients with COVID-19 with lower ACE level.

This study has some limitations. First, the correction of ACE genetic polymorphism is absent in this retrospective study, although the ACE I/D polymorphism showed no association with the poor outcome of pneumonia, including the need for ICU admittance and both in-hospital and 28-day mortality [3]. Second, the dynamic changes in serum ACE level as well as its relationship to ACE2 expression in lung tissue were not tested. Last but not least, this cohort size was not large enough to validate the role of ACE in predicting disease progression.

CONCLUSION

In this retrospective real-world study, we identified that lower level of serum ACE on admission was an independent risk factor for higher incidence of COVID-19 disease progression, which might correlate with prolonged duration of viral shedding, excessive inflammation, and depressed immune response. This study may provide new insights into the clinical management of COVID-19 and enhance the rational allocation of medical resources.
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Compliance with Ethics Guidelines. The study was performed in accordance with the tenets of the Declaration of Helsinki and the Good Clinical Practice principles. The case series was approved by the Institutional Review Board of Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology (TJ-C20200101). Written informed consent was waived because of the rapid emergence of this infectious disease.

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

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