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# Serum level of testosterone predicts disease severity of male COVID-19 patients and is related to T-cell immune modulation by transcriptome analysis

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ABSTRACT

*Background:* Severe disease of COVID-19 and mortality occur more frequently in male patients than that in female patients may be related to testosterone level. However, the diagnostic value of changes in the level of testosterone in predicting severe disease of male COVID-19 patients has not been determined yet.
*Methods:* Sixty-one male COVID-19 patients admitted to the First Affiliated Hospital of Zhejiang University School of Medicine were enrolled. Serum samples at different stages of the patients after admission were collected and testosterone levels were detected to analyze the correlation between testosterone level and disease severity. Transcriptome analysis of PBMC was performed in 34 patients.
*Results:* Testosterone levels at admission in male non-ICU COVID-19 patients (3.7 nmol/L, IQR: 1.5 ~ 4.7) were significantly lower than those in male ICU COVID-19 patients (6.7 nmol/L, IQR: 4.2 ~ 8.7). Testosterone levels in the non-ICU group increased gradually during the progression of the disease, while those in the ICU group remained low. In addition, testosterone level of enrolled patients in the second week after onset was significantly correlated with the severity of pneumonia, and ROC curve showed that testosterone level in the second week after onset was highly effective in predicting the severity of COVID-19. Transcriptome studies have found that testosterone levels of COVID-19 patients were associated with immune response, including T cell activation and regulation of lymphocyte activation. In addition, CD28 and Inositol Polyphosphate-4-Phosphatase Type II B (INPP4B) were found positively correlated with testosterone.
*Conclusions:* Serum testosterone is an independent risk factor for predicting the severity of COVID-19 in male patients, and the level of serum testosterone in the second week after onset is valuable for evaluating the severity of COVID-19. Testosterone level is associated with T cell immune activation. The monitoring of serum testosterone should be highlighted in clinical treatment and the related mechanism should be further studied.

*Abbreviations:* COVID-19, Coronavirus Disease 2019; SARS, Severe Acute Respiratory Syndrome; MERS, Middle East Respiratory Syndrome; ARDS, Acute Respiratory Distress Syndrome; ICU, Intensive Care Unit; PBMC, Peripheral Blood Mononuclear Cell; PSI, Pneumonia Severity Index; IL-6, Interleukin-6; IL-10, Interleukin-10.

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## 1. Background

Multiple studies have shown that disease severity and mortality of COVID-19 are significantly higher in males than those in females. Scully et al. analyzed COVID-19 data from 38 countries and found that the fatality rate of male patients was 1.7 times of that in female patients [1]. Other previous studies have reported that the fatality rate is higher in men than that in women with SARS and MERS [2,3]. In addition to gender-related differences in social behaviors, such as smoking, medical-seeking behavior and underlying diseases [4,5], biological risk factors such as sex steroid hormones, immune response status, sex chromosomes, and genomic differences are also associated with an increased risk of mortality in males [6,7].

As one of the most important sex hormones in males, testosterone has been reported to play an important role in maintaining the normal physiological and mental activities of human tissues and organs [8]. In recent years, a growing body of research has shown that testosterone also plays an important role in the occurrence, progress and outcome of viral infectious diseases. Tuku et al. found that the testosterone level of mice infected with influenza virus significantly decreased, and the condition improved after testosterone treatment, confirming that testosterone has a protective effect on the process of influenza infection [9]. Vom et al. further found that testosterone regulated androgen receptor signaling pathway promoted the down-regulation of harmful inflammatory immune responses in mice infected with influenza virus, slowing down the occurrence of severe disease [10]. Other study reported that testosterone levels were directly related to the risk of admission to ICU and the severity of the disease. A latest study found that male COVID-19 patients with low testosterone levels who were admitted to hospital had an increased risk of ARDS progression, longer stay in the ICU and higher risk of death [11].

However, the diagnostic value of testosterone in predicting severe disease and the regulatory mechanism of testosterone in the severity of COVID-19 in male patients remains unclear. The purpose of this study is to analyze the relationship between testosterone changes and disease severity in male patients with COVID-19 and to compare the differences in transcriptome expression in patients with different testosterone levels. Taken together, this study clarifies the diagnostic value of testosterone in predicting disease severity in male patients with COVID-19 and the possible regulatory pathways.

## 2. Materials and methods

### 2.1. Clinical specimens

Male COVID-19 patients were recruited at the First Affiliated Hospital, School of Medicine, Zhejiang University (n = 61). All patients were confirmed by SARS-CoV-2 nucleic acid detection. Clinical information was collected on the same day as serum sampling. Blood samples were collected using VACUETTE serum clot activator tubes and were centrifuged to obtain the serum. Serum was collected at least twice during the hospitalization, including one during the first week and one during the second week after onset.

### 2.2. Measurement of testosterone in patients' serum

The concentration of serum testosterone was measured using Abbott i2000 automatic chemiluminescence immunoassay analyzer (Abbott, Chicago, USA). Reagents included the Abbott 2nd Generation Testosterone Reagent Kit, which is based on chemiluminescent particle immunoassay (Abbott, Chicago, USA). The medical reference range of testosterone levels is 4.94 to 32 nmol/L for males.

### 2.3. Transcriptome sequencing in patients' PBMCs

We collected PBMC samples from 34 male COVID-19 patients, including 15 non-ICU patients and 19 ICU patients, and 11 healthy males for transcriptome analysis. Transcriptome sequencing of the RNA isolated from whole blood samples was carried out as previously described [12]. Briefly, RNA was isolated from whole blood samples using the QIAamp RNA Blood Mini kit (Qiagen, Valencia, CA, USA). The RNA was then reverse-transcribed to generate complementary DNA (cDNA) and was used to construct sequencing libraries using the NEB Next Ultra II Library Prep Kit (New England Biolabs, MA, USA). We used the Illumina HiSeq 2500 for sequencing, and generated 2 × 125 base-read paired-end reads according to the manufacturer's instructions. The reads were further trimmed to remove low-quality bases. Sequencing reads were mapped against human reference genome GRCh38, and per gene read counts were calculated with TopHat2 (Version 2.1.1) and RSEM (Version 1.2.31). The obtained read counts were normalized using trimmed mean normalization, and differentially expressed genes (DEGs) were estimated using the likelihood ratio test. Functional enrichment analysis was performed based on the list of DEGs using Database for Annotation, Visualization and Integrated Discovery (DAVID) Bioinformatics Resources (https:// david.ncifcrf.gov).

### 2.4. Statistical analysis

For most variables, we calculated descriptive statistics, such as medians with interquartile ranges (for data with skewed distribution) and proportions (percentages). Statistical comparisons between the ICU and non-ICU groups were evaluated by *t* test, analysis of variance, Mann-Whitney U tests, and Kruskal-Wallis tests when appropriate. Risk factors for severe COVID-19 were analyzed by Logistic regression. Loess regression was used to analyze the dynamic change trend of testosterone levels. Spearman correlation analysis was performed, and receiver operating characteristic (ROC) curve was used to analyze the efficacy of serum testosterone level in predicting COVID-19 severity. Statistical analyses were performed using the R software package, v3.6.2. A P value of $< 0.05$ was considered significant.

## 3. Results

### 3.1. Characteristics of patients

The 61 male patients with COVID-19 were divided into non-ICU group (38 cases) and ICU group (23 cases) according to the need for intensive care, and the demographic and clinical characteristics of COVID-19 patients are shown in Table 1. The median age of patients in the non-ICU group was 52 years old (IQR: 36.8–61.3) while that in the ICU group was 64 years old (IQR: 51.8–74). The difference between the two groups was statistically significant ($P = 0.001$). Hypertension and diabetes were the most common underlying diseases in enrolled patients. The proportion of hypertension and diabetes in the ICU group was significantly higher than that in the non-ICU group ($P < 0.05$). Fever and cough are the most common clinical manifestations. The proportion of sputum in ICU group was significantly higher than that in non-ICU group ($P = 0.017$). Compared with the non-ICU group, neutrophils count, C-reactive protein, interleukin-6, interleukin-10 levels and PSI index were increased in the ICU group, while lymphocyte count, hemoglobin, albumin and testosterone levels were decreased, with significant differences between the two groups ($P < 0.05$).

### 3.2. Dynamic changes of serum testosterone levels in COVID-19 patients

Loess regression analysis was performed on the dynamic changes of serum testosterone levels in male COVID-19 patients in the non-ICU group and the ICU group. Serum testosterone levels in the non-ICU group gradually increased during the progression of the disease, while serum testosterone levels in the ICU group remained low (Fig. 1A). Further stratified analysis of age showed that in patients younger than 50 years old, serum testosterone levels in either the non-ICU group or

**Table 1**
Clinical Characteristics of 61 male patients with SARS-CoV-2 infection.

| Characteristics | Non-ICU (n = 38) | ICU (n = 23) | *P* value |
|---|---|---|---|
| Demographics | | | |
| Median (interquartile range) age (years) | 52 (36.8 ~ 61.3) | 64 (51.8 ~ 74) | **0.001** |
| Underlying conditions, n (%) | | | |
| Any | 16 (42.1) | 17 (73.9) | **0.016** |
| Hypertension | 10 (26.3) | 15 (65.2) | **0.003** |
| Diabetes mellitus | 5 (13.2) | 5 (21.7) | 0.603 |
| Heart disease | 3 (7.9) | 2 (8.7) | 1.000 |
| Lung disease | 0 (0) | 3 (13.0) | **0.049** |
| Liver disease | 1 (2.6) | 2 (8.7) | 0.551 |
| Renal disease | 0 (0) | 1 (4.3) | 0.377 |
| Immune compromise | 0 (0) | 1 (4.3) | 0.377 |
| Symptoms, n (%) | | | |
| Fever | 36 (94.7) | 21 (91.3) | 0.628 |
| Cough | 16 (42.1) | 13 (56.5) | 0.275 |
| Sputum | 6 (15.8) | 10 (43.5) | **0.017** |
| Diarrhoea | 8 (21.1) | 7 (30.4) | 0.410 |
| Fatigue | 3 (7.9) | 6 (26.1) | 0.117 |
| Chest distress | 6 (15.8) | 2 (8.7) | 0.698 |
| Nausea | 1 (2.6) | 1 (4.3) | 1.000 |
| Vomiting | 2 (5.3) | 0 (0) | 0.522 |
| Treatment, n (%) | | | |
| Gammaglobulin | 18 (47.4) | 20 (87.0) | **0.002** |
| Glucocorticoids | 27 (71.1) | 23 (100) | **0.012** |
| Antivirals | 38 (100) | 23 (100) | NA |
| Invasive mechanical ventilation | 0 (0) | 11 (47.8) | **<0.001** |
| ECMO | 0 (0) | 8 (34.8) | **<0.001** |
| Laboratory Indicators, median [IQR] | | | |
| Neutrophils count, $\times 10^9$/L | 3.6 (2.5 ~ 6.6) | 7.9 (3.6 ~ 12) | **0.004** |
| Lymphocyte count, $\times 10^9$/L | 0.9 (0.6 ~ 1.3) | 0.6 (0.5 ~ 0.9) | **0.006** |
| Hemoglobin, g/L | 148 (137 ~ 155) | 131 (121 ~ 151) | **0.006** |
| Platelet count, $\times 10^9$/L | 184 (138.5 ~ 234.5) | 176 (148 ~ 193) | 0.401 |
| $PaO_2$, mmHg | 87.0 (80.2 ~ 102.8) | 84.9 (69.3 ~ 93.6) | 0.297 |
| Albumin, g/L | 41.5 (36.4 ~ 44.6) | 34.8 (31.3 ~ 40.9) | **0.004** |
| Aspartate aminotransferase, IU/L | 22.5(18 ~ 34) | 27 (19 ~ 42) | 0.284 |
| Creatinine, μmol/L | 83 (70.5 ~ 91.3) | 90 (72 ~ 107) | 0.094 |
| Creatine kinase, IU/L | 77 (62.5 ~ 115) | 105 (60 ~ 296) | 0.134 |
| Lactate dehydrogenase, IU/L | 247 (214 ~ 317) | 301 (242 ~ 386) | 0.067 |
| Fibrinogen, g/L | 4.4 (3.4 ~ 5.6) | 4.5 (4.1 ~ 5.5) | 0.553 |
| D-dimer, ug/IFEU | 320 (176.5 ~ 544) | 440.5 (281.8 ~ 797) | 0.063 |
| C-reactive protein, mg/L | 24.3 (10.9 ~ 45.2) | 46.4 (26.8 ~ 88.1) | **0.012** |
| IL-6, pg/mL | 22 (8.7 ~ 44.5) | 47.2 (24 ~ 84.5) | **0.008** |
| IL-10, pg/mL | 3.9 (2.7 ~ 7.5) | 6.7 (4.8 ~ 8.6) | **0.024** |
| Testosterone, nmol/L | 6.7 (4.2 ~ 8.7) | 3.7 (1.5 ~ 4.7) | **0.012** |
| PSI index | 56.5 (51 ~ 66) | 72 (60 ~ 86) | **0.002** |

the ICU group gradually recovered during the progression of the disease, and serum testosterone levels in the ICU group were always lower than those in the non-ICU group (Fig. 1B). In patients aged 50 years or older, serum testosterone levels gradually recovered in the non-ICU group, while remained low in the ICU group (Fig. 1C).

### 3.3. Correlation between serum testosterone level and PSI index

PSI index is an indicator to evaluate the severity of pneumonia. Spearman correlation analysis was used to evaluate the relationship between serum testosterone level and PSI index. The results showed that the serum testosterone level of COVID-19 patients was negatively

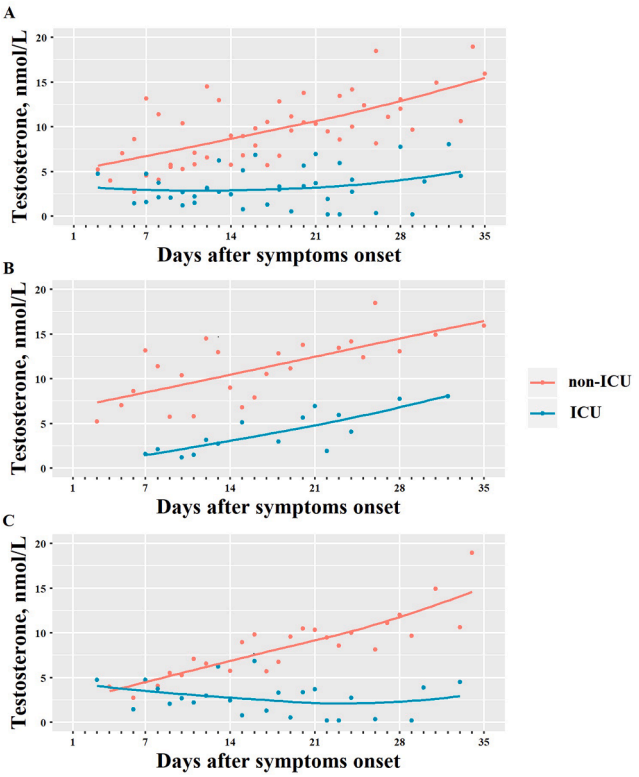


**Fig. 1.** Dynamic changes of testosterone levels in male patients with COVID-19 in non-ICU and ICU groups (A: All patients, B: Younger than 50 years old patients, C: Older than 50 years old patients).

correlated with PSI index in the first week after onset, with a correlation coefficient of −0.372 ($P = 0.043$) (Fig. 2A). In the second week after onset, serum testosterone level was negatively correlated with PSI index, and the correlation coefficient was −0.502 ($P < 0.001$) (Fig. 2B).

### 3.4. Correlation between testosterone level in week 2 and other laboratory indicators

In the second week after onset, serum testosterone level was positively correlated with lymphocyte count, hemoglobin, platelet count, $PaO_2$, and albumin, with correlation coefficients of 0.522, 0.375, 0.296, 0.461, and 0.352, respectively ($P < 0.05$). Meanwhile, serum testosterone level was negatively correlated with neutrophils count, aspartate aminotransferase, creatine kinase, lactate dehydrogenase, C-reactive protein, D-dimer, IL-6 and IL-10, and the correlation coefficients were −0.358, −0.297, −0.392, −0.519, −0.403, −0.533, −0.367 and −0.348, respectively ($P < 0.05$). (Table 2)

### 3.5. Testosterone levels in week 2 could predict disease severity

ROC curve analysis was performed for testosterone, neutrophils count, lymphocyte count, C-reactive protein and IL-6 of male patients with COVID-19 in the second week after onset. The results showed that the areas under the ROC curve of testosterone, neutrophil count, lymphocyte count, C-reactive protein and IL-6 were 0.876, 0.725, 0.756, 0.727 and 0.715, respectively, where serum testosterone had the highest efficacy in predicting the severity of disease. With the serum testosterone level of 3.26 nmol/L, the sensitivity and specificity for predicting severe disease was 65.0% and 97.1%, respectively (Table 3).

### 3.6. Transcriptome analysis between different testosterone levels

To dissect the genes related with the testosterone levels in COVID-19

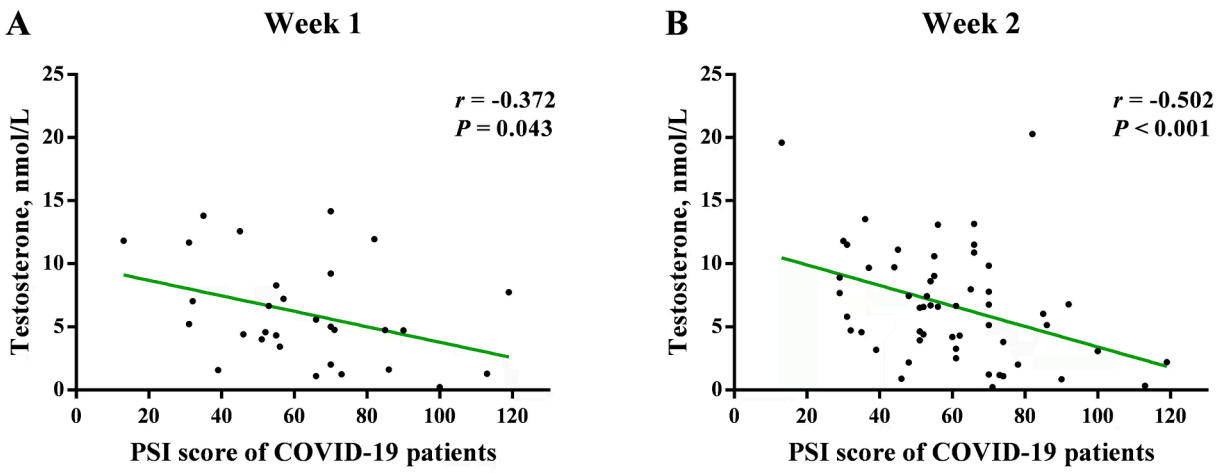


**Fig. 2.** Correlation analysis of testosterone levels and PSI index in different weeks after onset.

**Table 2**
Correlation analysis of serum testosterone level in the second week of onset with corresponding baseline laboratory indicators.

| Laboratory Indicators | r | *P* value |
|---|---|---|
| Lymphocyte count | 0.522 | **<0.001** |
| Neutrophils count | −0.358 | **0.007** |
| Hemoglobin | 0.375 | **0.005** |
| Platelet count | 0.296 | **0.028** |
| $PaO_2$ | 0.461 | **0.001** |
| Albumin | 0.352 | **0.009** |
| Aspartate aminotransferase | −0.297 | **0.029** |
| Creatine kinase | −0.392 | **0.004** |
| Lactate dehydrogenase | −0.519 | **<0.001** |
| Creatinine | −0.183 | 0.181 |
| Fibrinogen | −0.011 | 0.937 |
| D-dimer | −0.533 | **<0.001** |
| IL-6 | −0.367 | **0.010** |
| IL-10 | −0.348 | **0.015** |
| C-reactive protein | −0.403 | **0.003** |

patients, we performed correlation analysis between testosterone levels and gene expression levels from blood. 294 genes were found significantly correlated with testosterone levels in COVID-19 patients ($P < 0.01$). To further investigate the function of the genes, we performed GO enrichment analysis based on this gene set. Twenty-three GO function terms were identified significantly enriched in genes correlated with testosterone levels. Among the 23 GO function terms, most of the GO terms were related with immune response, such as T cell activation, regulation of lymphocyte activation and lymphocyte differentiation (Fig. 3).

Because we found the testosterone level was significantly correlated with the age of patients. To further identified the genes had significant changes between non-ICU and ICU groups but independent of age, we performed ANOVA (Analysis of Variance) based on patient age, severity and gene expression level. Among the 294 genes, 4 genes were found significantly correlated with testosterone levels but not correlated with patient age (Fig. 4). We found that CD28, inositol polyphosphate-4-phosphatase type II B (INPP4B) and poly (RC) binding protein 4 (PCBP4) were positively correlated with testosterone, independent of age, and negatively correlated with disease severity (Fig. 4A, B, C). Upstream transcription factor 2 (USF2) was positively correlated with testosterone, regardless of age, and was not expressed in the healthy group, while the expression was high in the non-ICU group and low in the ICU group (Fig. 4D). Transcriptional levels of IL-6 and IL-10 were significantly higher in COVID-19 patients than in healthy, but there was no statistical difference between the ICU and non-ICU (Supplementary Figure S1).

## 4. Discussion

Multiple studies have shown that disease severity and mortality of COVID-19 are significantly higher in males than those in females. Our previous study also found larger proportion of severe illness in male patients [13]. Additionally, in SARS, MERS, H1N1 and other infectious diseases, the death rate of male patients was higher [2,3,14]. Sex differences are related to many factors, including sex hormones, genetic background, immune status, lifestyle and so on. Studies have reported that testosterone, one of the male hormones, may play an important role. Decline in testosterone levels has been observed in male patients hospitalized for acute illness, and low testosterone levels are associated with the need for admission to the ICU [15]. In this study, we also found that the serum testosterone level of COVID-19 patients was significantly lower than that of healthy males, and the serum testosterone level of COVID-19 patients in the ICU group was lower than that of the non-ICU group. Schroeder et al. also found that low testosterone levels were significantly associated with admission to the respiratory intensive care unit for COVID-19 patients. When testosterone was < 5 nmol/L, the energy efficiency of patients who need to be admitted to the respiratory intensive care unit is higher [16].

By analyzing the dynamic level of testosterone, it was found that the

**Table 3**
Receiver operating characteristic (ROC) curve parameters for the serum testosterone level and other biomarkers on the second week of admission in COVID-19 male patients.

| Variable | Cutoff value | Youden index | Sensitivity (%) | Specificity (%) | AUC | 95% CI | *P value* |
|---|---|---|---|---|---|---|---|
| Testosterone | 3.26 | 0.621 | 65.0 | 97.1 | 0.876 | 0.759 ~ 0.949 | **<0.001** |
| Neutrophils count | 5.25 | 0.514 | 100.0 | 51.4 | 0.725 | 0.590 ~ 0.836 | **<0.001** |
| Lymphocyte count | 0.56 | 0.429 | 71.4 | 71.4 | 0.756 | 0.623 ~ 0.861 | **<0.001** |
| C-reactive protein | 32.55 | 0.406 | 50.0 | 90.6 | 0.727 | 0.585 ~ 0.841 | **0.002** |
| IL-6 | 22.21 | 0.389 | 72.2 | 66.7 | 0.715 | 0.566 ~ 0.836 | **0.006** |

AUC: Area under the ROC curve; CI: Confidence Interval.

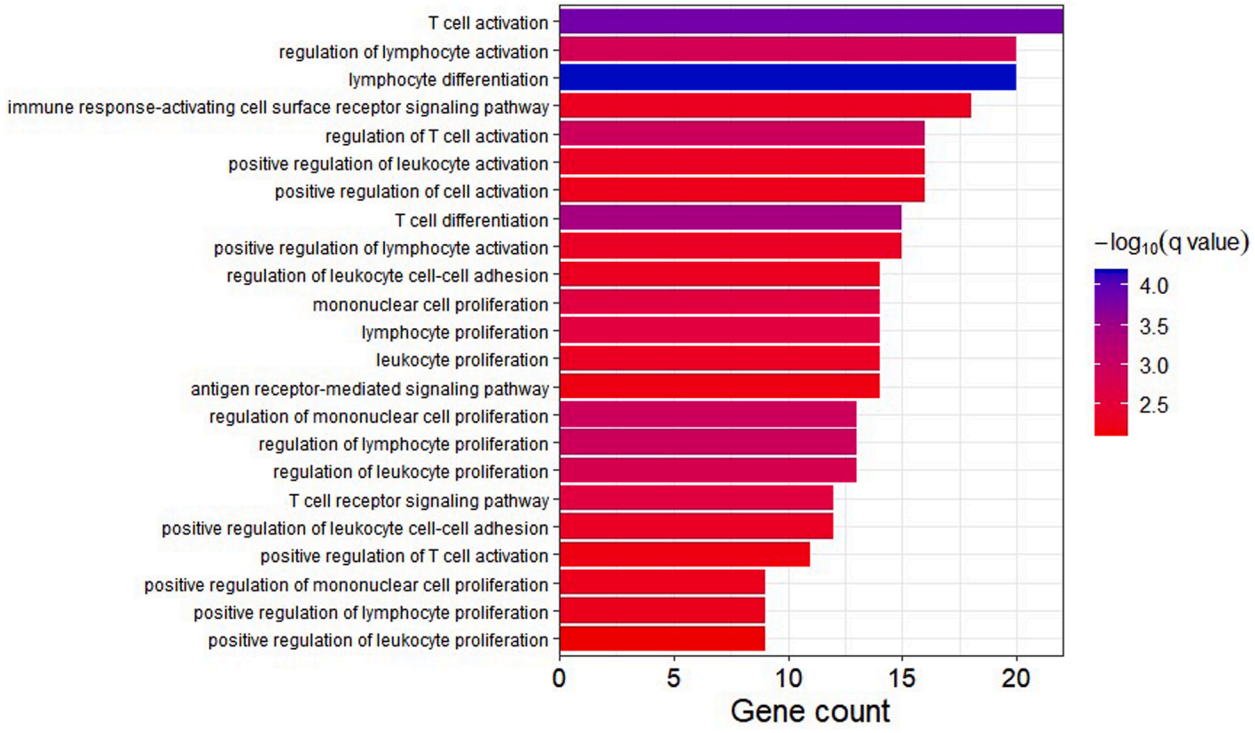


**Fig. 3.** Significantly enriched GO function terms between different levels of testosterone in COVID-19 male patients.

testosterone level in the non-ICU group gradually recovered, while that in the ICU group remained low. Moreover, the testosterone level in the second week after onset was significantly different between the non-ICU group and the ICU group, and the correlation with PSI index was more significant. Therefore, continued monitoring of testosterone levels in males with COVID-19 is warranted, with particular attention to those patients whose testosterone levels are still low in the second week after onset. In addition, low levels of testosterone in critically ill patients suggest that testosterone supplementation may be appropriate in these patients. A study has found that in influenza infected mice with low testosterone levels, testosterone replacement therapy has been observed to alter viral replication levels, improve pulmonary inflammation and reduce mouse mortality [17]. However, further research is needed to determine whether testosterone supplementation can improve disease in patients with low testosterone levels of COVID-19.

At present, studies have suggested that the occurrence of severe disease in COVID-19 patients is related to the dysregulation of immune inflammatory response in the body. Studies have found that massive viral replication leads to epithelial and endothelial cell apoptosis and vascular leakage, followed by strong release of IL-6, IP-10 and other pro-inflammatory cytokines [18]. As an important steroid hormone in male body, testosterone plays a certain role in regulating the release of pro-inflammatory cytokines and chemokines, and is an important regulatory factor of immune function. Freeman et al. found that IL-2, IL-6, IL-10, IL-12, and IL-13 were elevated in animals with reduced testosterone levels; and decreased in animals treated with physiological levels of testosterone [19]. In vitro studies showed that testosterone treatment diminished the production of TNF-α, IL-1β, and IL-6 in human macrophages and human monocytes. Furthermore, testosterone treatment stimulated the expression of IL-10 [20,21]. Consistent with other studies, in this study, we also found that testosterone was negatively correlated with IL-6, CRP and other inflammatory indicators [16]. Thus, it is hypothesized that low levels of testosterone promote the expression of inflammatory cytokines such as IL-6, which exacerbate the pulmonary immune inflammatory response and make lung injury more severe. Other studies have found that testosterone is related to respiratory muscle metabolism, and hypogonadism is common in patients with respiratory failure requiring mechanical ventilation [22,23]. Further studies have found that testosterone supplementation can improve the prognosis of patients with COPD [24]. The relationship between low testosterone levels and pulmonary respiratory muscle metabolism in patients with severe COVID-19 requires further investigation. In addition, some scholars believe that the SARS-CoV-2 continues to attack the testes and affects testosterone production, thus reducing testosterone levels [11,25].

In this study, through transcriptome study, we found that the expression level of testosterone in COVID-19 patients may be mainly affected by T cell activation, regulation of lymphocyte activation and other signaling pathways. Previous studies have found that testosterone could act on T cells, regulating the corresponding immune function [26,27]. Page et al. found that testosterone and/or its metabolites may help maintain the physiological balance of autoimmunity and protective immunity by preserving the number of regulatory T cells and the activation of CD8 + T cells [28]. Our previous study also found that CD8 + T cells in severe patients were significantly lower than those in mild patients [13]. Therefore, high levels of testosterone may reduce the incidence of severe disease by activating CD8 + T cells. CD28 is a costimulatory molecule expressed on the surface of T lymphocytes, which mediates the costimulatory action of T cells and promotes their survival, proliferation and production of cytokines [29]. Through transcriptome studies, we found that the expression level of testosterone was positively correlated with the level of CD28, which was not affected by age factors, suggesting that the action of testosterone on T cells may be related to CD28. In addition, we also found that the level of testosterone expression is related to INPP4B, PCBP4, etc., but the correlation effect is not clear. Further research is needed to determine the exact mechanism of the decrease in testosterone levels and the role in affecting the severity of COVID-19 infection.

There are several limitations to our study. First, this study is a single-center retrospective study, testosterone levels are affected by a variety of confounders, especially age. It is difficult to completely rule out the effect of age on testosterone, although we stratified patients by age.

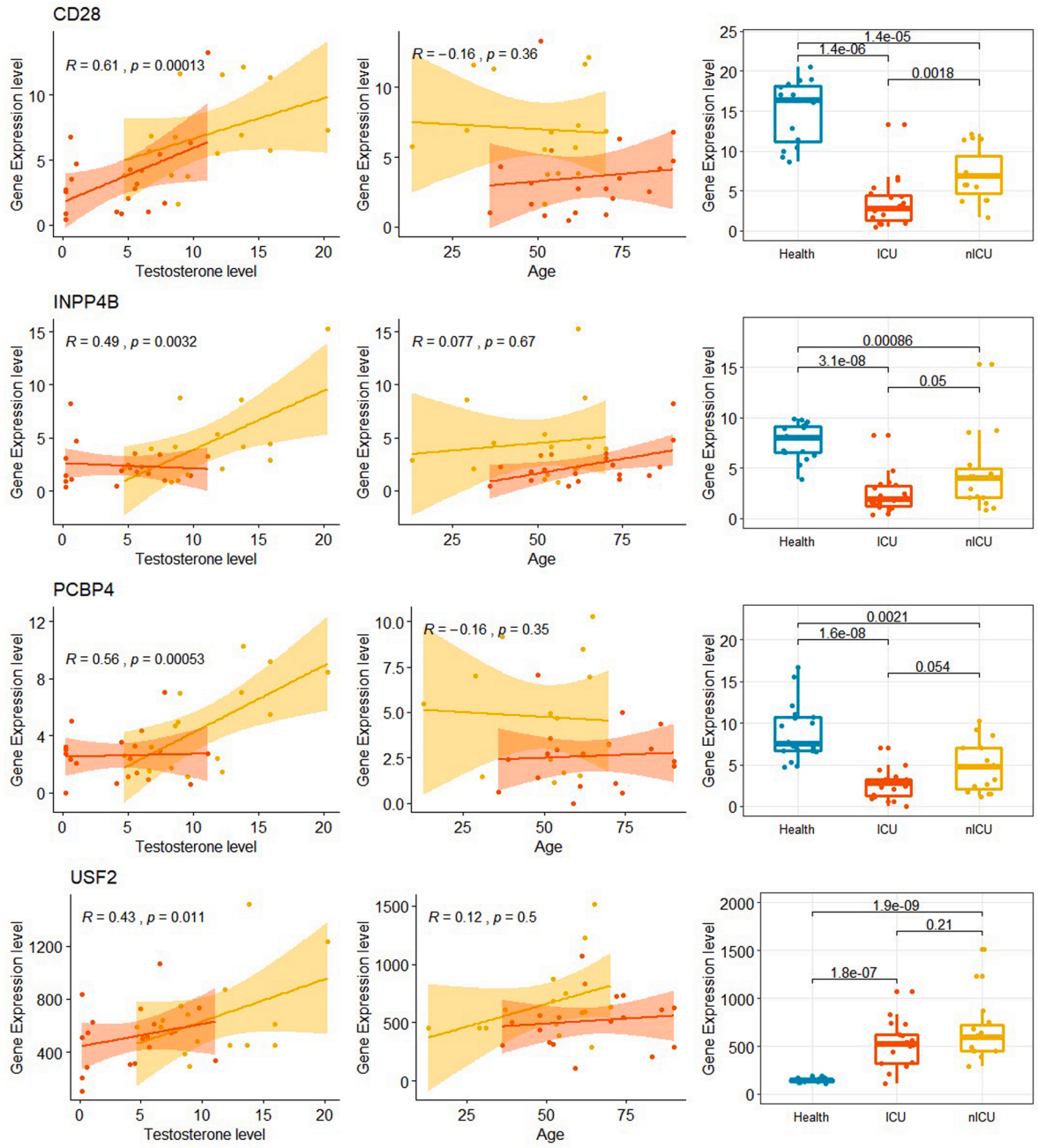


**Fig. 4.** Association of testosterone, age, COVID-19 severity with gene expression level in COVID-19 male patients. (A) CD28. (B) INPP4B. (C) PCBP4. (D) USF2.

Second, due to the relatively good control of COVID-19 in China, the number of COVID-19 cases included in this study was limited. Finally, this is an observational study that evaluated associations of sex hormones and transcriptome with COVID-19. Hence, we could not make interpretations of causality.

In summary, our findings suggest that serum testosterone is an independent risk factor for predicting the severity of COVID-19 in male patients, and the level of serum testosterone in the second week after onset is valuable for evaluating the severity of COVID-19. Testosterone level is associated with T cell immune activation and positively correlated with the level of CD28, INPP4B and PCBP4. The monitoring of serum testosterone should be highlighted in clinical treatment and the related mechanism should be further studied.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2021.11.006.

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