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Research Article

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Effects of angiotensin II receptor blocker usage on viral load, antibody dynamics, and transcriptional characteristics among COVID-19 patients with hypertension

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Abstract: Epidemiological evidence suggests that patients with hypertension infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are at increased risk of acute lung injury. However, it is still not clear whether this increased risk is related to the usage of renin-angiotensin system (RAS) blockers. We collected medical records of coronavirus disease 2019 (COVID-19) patients from the First Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China), and evaluated the potential impact of an angiotensin II receptor blocker (ARB) on the clinical outcomes of COVID-19 patients with hypertension. A total of 30 hypertensive COVID-19 patients were enrolled, of which 17 were classified as non-ARB group and the remaining 13 as ARB group based on the antihypertensive therapies they received. Compared with the non-ARB group, patients in the ARB group had a lower proportion of severe cases and intensive care unit (ICU) admission as well as shortened length of hospital stay, and manifested favorable results in most of the laboratory testing. Viral loads in the ARB group were lower than those in the non-ARB group throughout the disease course. No significant difference in the time of seroconversion or antibody levels was observed between the two groups. The median levels of soluble angiotensin-converting enzyme 2 (sACE2) in serum and urine samples were similar in both groups, and there were no significant correlations between serum sACE2 and biomarkers of disease severity. Transcriptional analysis showed 125 differentially expressed genes which mainly were enriched in oxygen transport, bicarbonate transport, and blood coagulation. Our results suggest that ARB usage is not associated with aggravation of COVID-19. These findings support the maintenance of ARB treatment in hypertensive patients diagnosed with COVID-19.

Key words: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); Coronavirus disease 2019 (COVID-19); Renin-angiotensin system (RAS); Angiotensin-converting enzyme 2 (ACE2); Hypertension

1 Introduction

Since first emerging in late 2019, coronavirus disease 2019 (COVID-19) has caused a pandemic, with more than 100 million confirmed cases and two million deaths as of Mar. 16, 2021 (World Health Organization, 2020). Analysis of the clinical characteristics of COVID-19 patients consistently shows that patients with cardiovascular comorbidities like hypertension have a higher proportion of severe cases and even an increased risk of developing fatal cases (Grasselli et al., 2020; Zheng SF et al., 2020). However, the pathophysiological mechanisms underlying this phenomenon remain largely unknown.

Angiotensin-converting enzyme 2 (ACE2) plays a key role in the infection process of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as with SARS-CoV (Lan et al., 2020; Shang et al., 2020).

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SARS-CoV-2 gains entry into host cells by binding its surface spike protein to the membrane-bound form of ACE2 following priming by transmembrane serine protease 2 (TMPRSS2) (Romagnoli et al., 2020). Previous studies have suggested that taking renin-angiotensin system (RAS) blockers, including angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB), may contribute to the expression of ACE2, although most of the results originated from animal studies (Zhang JS et al., 2020). As such, concerns have been raised regarding whether taking ACEI or ARB would result in an increased susceptibility to infection or predisposition to more severe illness (Fang et al., 2020; Zheng YY et al., 2020). Controversially, inhibiting RAS activity with losartan has been shown to protect mice from acute lung injury induced by injection of SARS-CoV spike protein (Kuba et al., 2005), and similar benefits were also observed with increased levels of ACE2 (Imai et al., 2005).

RAS blockers are often prescribed as first-line medications for cardiovascular disease management, and discontinuation of these drugs may promote disease progression. Therefore, whether there is an association between RAS blocker usage and clinical outcomes in COVID-19 patients with hypertension requires further elucidation. We designed this retrospective study with the aim of evaluating the potential impact of RAS blockers on COVID-19 patients with pre-existing hypertension. To that end, we collected medical records of hypertensive COVID-19 patients from the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, and evaluated the impacts of ARB usage on viral load, antibody dynamics, and soluble ACE2 (sACE2) levels in serum and urine samples. In addition, we explored the effects of ARB usage on gene expression using transcriptome sequencing of the RNA isolated from whole blood samples.

2 Subjects and methods

2.1 Study design and participants

Patients with laboratory-confirmed SARS-CoV-2 infection admitted consecutively to the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, from Jan. 19 to Mar. 16, 2020, were included for the initial screen. COVID-19 patients with pre-existing hypertension were divided into two subgroups, non-ARB and an ARB groups, based on the antihypertensive therapies they received.

2.2 Data collection

Demographics, medical history, and laboratory findings of patients were extracted from electronic medical records. Data on the usage of antihypertensive drugs prior to admission and during the hospital stay were collected. The medical history included the date of symptom onset, symptoms and signs, antiviral treatment, and progression and resolution of clinical illness. Laboratory findings included hemogram, coagulation profile, infection-related biomarkers, blood gas analysis, and other blood biochemistry tests. The severity of illness was evaluated according to the 7th edition of the Guideline for Diagnosis and Treatment of SARS-CoV-2 issued by the National Health Commission of the People's Republic of China (2020). All data were reviewed by a trained team of physicians.

2.3 Sample collection and analysis

After admission, respiratory, serum, stool, and urine samples were collected daily if possible. Respiratory samples were collected from the sputum or saliva after deep coughing. Blood samples were collected in special vacuum tubes, and urine and stool samples were collected in special sterile containers.

Viral RNA was extracted from respiratory and stool samples using MagNA Pure LC 2.0 (Roche, Basel, Switzerland). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using a China Food and Drug Administration (CFDA)-approved commercial kit specific for SARS-CoV-2 detection (Brotek, Shanghai, China) according to the manufacturer's protocol. The standard curve was generated by 10-fold serial dilutions of standard positive control, and viral load was calculated by plotting quantification cycle (C_q) values onto the standard curve. Enzyme-linked immunosorbent assay (ELISA) kits for the total antibody (Ab), immunoglobulin M (IgM) antibody, and immunoglobulin G (IgG) antibody against SARS-CoV-2 were purchased from Wantai Biological Pharmacy Enterprise Co., Ltd. (Beijing, China). ELISA kits for sACE2 levels in serum and urine samples were purchased from Donglin Sci & Tech Development Co., Ltd. (Wuxi, China).

Transcriptional sequencing of the RNA isolated from whole blood samples was carried out as described elsewhere (Liu et al., 2017). 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 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

Statistical comparisons between the non-ARB and ARB groups were evaluated by the Mann-Whitney *U*-test or Fisher's exact test when appropriate. To explore the dynamics of viral load and antibody levels against SARS-CoV-2 across the days after symptom onset, we calculated the daily median viral load in respiratory samples as well as the relative antibody-binding signal, and then fitted smooth lines using the locally weighted scatterplot smoothing (LOESS) method, as described previously (Zheng SF et al., 2020). The curves of the cumulative seroconversion rates for total antibody, IgM, and IgG detected by ELISA were plotted using the Kaplan-Meier method. Spearman's correlations between serum levels of sACE2 and laboratory findings were assessed. A *P*-value less than 0.05 was considered statistically significant. Statistical analysis was performed using R software, Version 3.5.3 (R Project for Statistical Computing).

3 Results

3.1 Participants

A total of 106 COVID-19 patients were admitted to the First Affiliated Hospital, Zhejiang University School of Medicine as of Mar. 16, 2020, of which 38 (35.8%) had pre-existing hypertension (Fig. S1). After excluding eight patients following our exclusion criteria, 30 COVID-19 patients with hypertension were included in subsequent analysis. Of the 30 patients, 17 were classified into the non-ARB group and the remaining 13 were classified into the ARB group based on the antihypertensive therapies they received.

3.2 Demographics and clinical characteristics

The demographics and clinical characteristics of COVID-19 patients with hypertension in the non-ARB and ARB groups are summarized in Table 1. The median age of the ARB group was 56 (interquartile range (IQR), 48−62) years, much younger than the median age of 70 (IQR, 62−80) years in the non-ARB group, and five (38.5% and 29.4%, respectively) were female in both the ARB and non-ARB groups. Compared with the non-ARB group, patients in the ARB group had a lower proportion of severe cases, γ-globulin or antibiotics treatments, invasive mechanical ventilation or artificial liver supportive therapies, and ICU admission, as well as shortened length of hospital stay. Although patients in the ARB group showed higher systolic blood pressure, blood pressure was well controlled in both groups. There was no significant difference in characteristics of disease symptoms, complications, or time from illness onset to antiviral treatment between the two groups.

3.3 Laboratory findings

The results of laboratory testing for the two groups are shown in Table 2. In general, patients in the ARB group manifested favorable results in most of the laboratory testing in comparison with the non-ARB group. The median absolute number of lymphocytes in the ARB group was significantly higher than that in the non-ARB group (0.7×10^9 (IQR, 0.5×10^9–0.9×10^9) cells/L vs. 0.5×10^9 (IQR, 0.3×10^9–0.6×10^9) cells/L). Of note, the median level of D-dimer in the ARB group was much lower than that in the non-ARB group (458.5 (IQR, 318.0–895.0) μg/L vs. 2722.5 (IQR, 1602.0−5204.0) μg/L). For blood oxygen index, patients in the ARB group showed better arterial partial pressure of oxygen, oxygen saturation, and arterial partial pressure of oxygen/fraction of inspire oxygen (PaO_2/FiO_2) ratio compared with the non-ARB group. For infection-related biomarkers, patients in the ARB group had lower levels of interleukin-6 (IL-6)

Table 1 Demographics and clinical characteristics of COVID-19 patients with hypertension in the non-ARB and ARB groups

Characteristics	Non-ARB (n=17)	ARB (n=13)	P-value
Age (year)	70 (62–80)	56 (48–62)	**0.01**
Female	5 (29.4%)	5 (38.5%)	0.71
Body mass index (kg/m^2)	25.7 (24.0–26.7)	26.0 (24.3–28.0)	0.54
Blood pressure (mmHg)			
Diastolic blood pressure	135 (123–141)	125 (120–141)	0.81
Systolic blood pressure	77 (68–83)	87 (79–90)	**0.03**
Disease severity			
Mild	0	4 (30.8%)	
Severe	17 (100.0%)	9 (69.2%)	**0.03**
Symptoms			
Fever	16 (94.1%)	13 (100.0%)	1.00
Cough	7 (41.2%)	10 (76.9%)	0.07
Sputum	5 (29.4%)	4 (30.8%)	1.00
Chest distress	3 (17.6%)	4 (30.8%)	0.67
Dizziness	1 (5.9%)	0	1.00
Headache	0	0	NA
Nausea	1 (5.9%)	0	1.00
Vomiting	1 (5.9%)	0	1.00
Diarrhoea	3 (17.6%)	1 (7.7%)	0.61
Myalgia	3 (17.6%)	2 (15.4%)	1.00
Fatigue	2 (11.8%)	2 (15.4%)	1.00
Underlying diseases in addition to hypertension			
Diabetes mellitus	5 (29.4%)	2 (15.4%)	0.43
Heart disease	3 (17.6%)	0	0.24
Respiratory disease	3 (17.6%)	0	0.24
Liver disease	1 (5.9%)	0	1.00
Malignancy	1 (5.9%)	0	1.00
Treatment			
γ-Globulin	16 (94.1%)	6 (46.2%)	**0.01**
Glucocorticoids	17 (100.0%)	12 (92.3%)	0.43
Antibiotics	15 (88.2%)	3 (23.1%)	**0.01**
Antivirals	17 (100.0%)	13 (100.0%)	NA
Time from illness onset to antiviral treatment			
≤5 d	12 (70.6%)	4 (30.8%)	
>5 d	5 (29.4%)	9 (69.2%)	0.06
Supportive therapy			
Invasive mechanical ventilation	10 (58.8%)	1 (7.7%)	**0.01**
Extracorporeal membrane oxygenation	5 (29.4%)	1 (7.7%)	0.20
Artificial liver	7 (41.2%)	0	**0.01**
Length of hospital stay (d)	37 (16–92)	18 (16–22)	**0.04**
ICU admission	16 (94.1%)	4 (30.8%)	**<0.001**

Data expressed as median (IQR) or number (percentage) of patients. ARB: angiotensin II receptor blocker; ICU: intensive care unit; IQR: interquartile range; NA: not available. 1 mmHg=0.133 kPa. P values in bold are considered statistically significant (P<0.05).

and IL-10. For other blood biochemistry testing, patients in the ARB group showed improved levels of urea, creatinine, direct bilirubin, aspartate transferase, high-sensitive cardiac troponin I, B-type natriuretic peptide, creatine kinase, creatine kinase MB, and lactate dehydrogenase. In contrast, patients in the ARB group had higher blood platelet counts and shorter prothrombin time.

Table 2 Laboratory findings of COVID-19 patients with hypertension in the non-ARB and ARB groups

Biomarker*	Non-ARB (*n*=17)	ARB (*n*=13)	*P*-value
Blood cell count (×10^9 cells/L)			
White blood cells	9.9 (8.5–11.0)	9.1 (8.5–10.9)	0.97
Neutrophils	8.6 (7.7–10.2)	7.5 (6.8–10.3)	0.74
Lymphocytes	0.5 (0.3–0.6)	0.7 (0.5–0.9)	**0.04**
Blood oxygen index			
Arterial partial pressure of oxygen (mmHg)	70.2 (65.8–81.2)	103.0 (86.9–123.0)	**0.01**
Oxygen saturation (%)	94.2 (93.9–96.8)	97.4 (96.9–98.1)	**0.01**
PaO_2/FiO_2	198.6 (151.6–230)	315.2 (199.7–379.0)	**0.02**
Coagulation			
Blood platelet count (×10^9 cells/L)	121.0 (92.0–172.5)	234.5 (170.0–305.0)	**0.01**
Prothrombin time (s)	11.7 (11.1–12.6)	10.8 (10.3–11.1)	**0.01**
Activated partial thromboplastin time (s)	28.3 (26.8–35.1)	27.0 (24.5–28.5)	0.13
D-dimer (μg/L)	2722.5 (1602.0–5204.0)	458.5 (318.0–895.0)	**0.01**
Infection related			
Hypersensitive C-reactive protein (mg/L)	7.4 (2.2–42.5)	3.5 (2.0–11.0)	0.26
Procalcitonin (ng/mL)	0.05 (0.04–0.16)	0.05 (0.03–0.07)	0.17
Erythrocyte sedimentation rate (mm/h)	48.0 (31.5–70.0)	28.0 (15.5–49.0)	0.07
IL-6 (pg/mL)	17.0 (9.4–42.5)	4.7 (3.5–13.4)	**0.02**
IL-10 (pg/mL)	3.9 (2.6–6.7)	2.3 (2.1–3.0)	**0.02**
IFN-γ (pg/mL)	3.3 (2.0–6.5)	6.0 (3.3–12.5)	0.11
Blood biochemistry			
Urea (mmol/L)	8.7 (7.2–11.8)	7.3 (6.2–8.2)	**0.04**
Creatinine (μmol/L)	73.0 (65.5–84.5)	64.5 (54.0–76.0)	**0.04**
Estimated glomerular filtration rate (mL/min per 1.73 m^2)	79.4 (74.5–95.2)	101.3 (93.7–104.6)	**0.01**
Direct bilirubin (μmol/L)	8.2 (5.1–13.0)	4.6 (4.0–6.2)	**0.01**
Indirect bilirubin (μmol/L)	7.1 (4.9–8.6)	5.8 (4.2–6.9)	0.34
Alanine transaminase (U/L)	32.0 (20.0–42.0)	29.0 (22.0–48.5)	0.68
Aspartate transferase (U/L)	24.0 (19.0–30.0)	17.0 (14.0–19.5)	**0.03**
B-type natriuretic peptide (pg/mL)	89.0 (59.4–147.5)	51.0 (37.0–67.0)	**0.04**
Creatine kinase (U/L)	70.0 (52.0–86.0)	33.0 (27.5–61.5)	**0.01**
Creatine kinase MB (U/L)	22.0 (20.0–23.0)	19.0 (16.0–22.0)	**0.02**
Lactate dehydrogenase (U/L)	327.0 (277.0–379.0)	246.5 (231.0–310.0)	**0.01**

Data expressed as median (IQR). *Biomarkers of each patient were aggregated as medians during hospitalization for further comparison. ARB: angiotensin II receptor blocker; IL-6: interleukin-6; IFN-γ: interferon-γ. PaO_2/FiO_2: arterial partial pressure of oxygen/fraction of inspire oxygen. 1 mmHg=0.133 kPa. *P* values in bold are considered statistically significant (*P*<0.05).

3.4 Antibody and viral load dynamics

As shown in Fig. 1a, there was a significant difference in the duration of detection of SARS-CoV-2 in respiratory samples between the two groups. The median viral duration in the ARB group was 16.0 (IQR, 14.0–25.0) d, significantly shorter than that in the non-ARB group (28.0 (IQR, 16.0–34.0) d). The median viral duration in stool samples in the ARB group was 21.0 (IQR, 18.5–28.0) d, similar to the 28.0-d duration (IQR, 20.0–34.5 d) in the non-ARB group. Fig. 1b shows the LOESS regression analysis of viral load across the days after symptom onset in respiratory samples. Both groups showed a similar pattern of viral load dynamics, i.e., escalating during the initial stage of the disease and reaching a peak in the third week from disease onset, followed by lower loads in the late stage. However, viral loads in the ARB group were lower than those in the non-ARB group throughout the disease course.

As shown in Fig. 2, there was no significant difference in the time of seroconversion or antibody levels throughout the disease course between the two groups. The antibody response profiles of both groups were largely the same, and seroconversion appeared

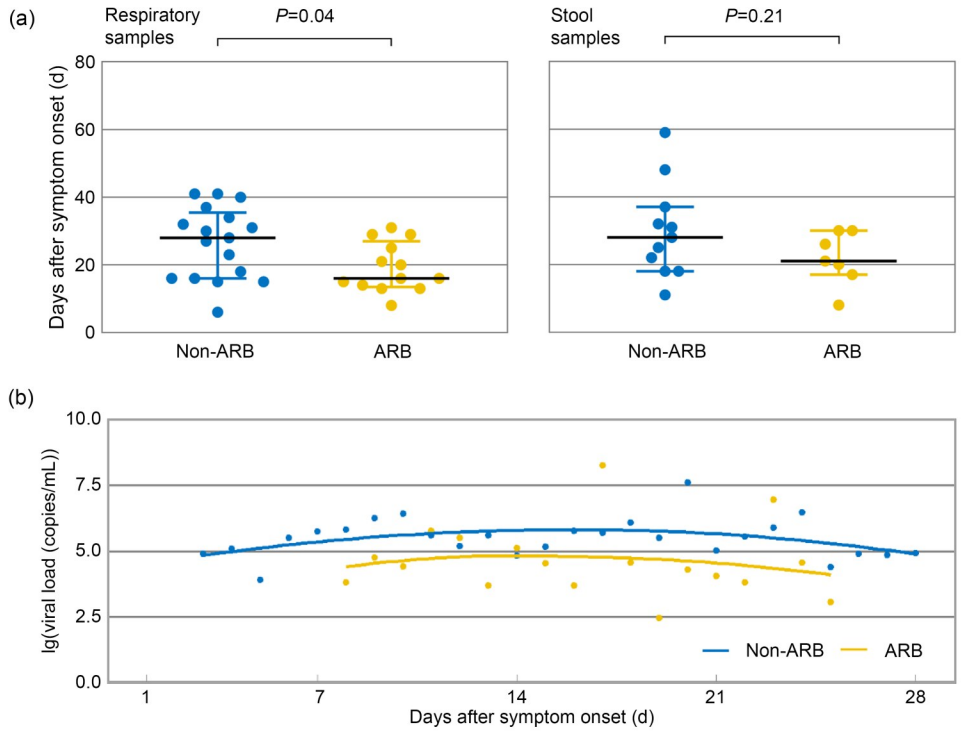

Fig. 1 Viral load dynamics in the non-ARB and ARB groups. (a) Duration of detection of SARS-CoV-2 in respiratory and stool samples. (b) Viral load variation across the days after symptom onset in respiratory samples. ARB: angiotensin II receptor blocker. Colored bars represent medians, and black bars represent interquartile ranges (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

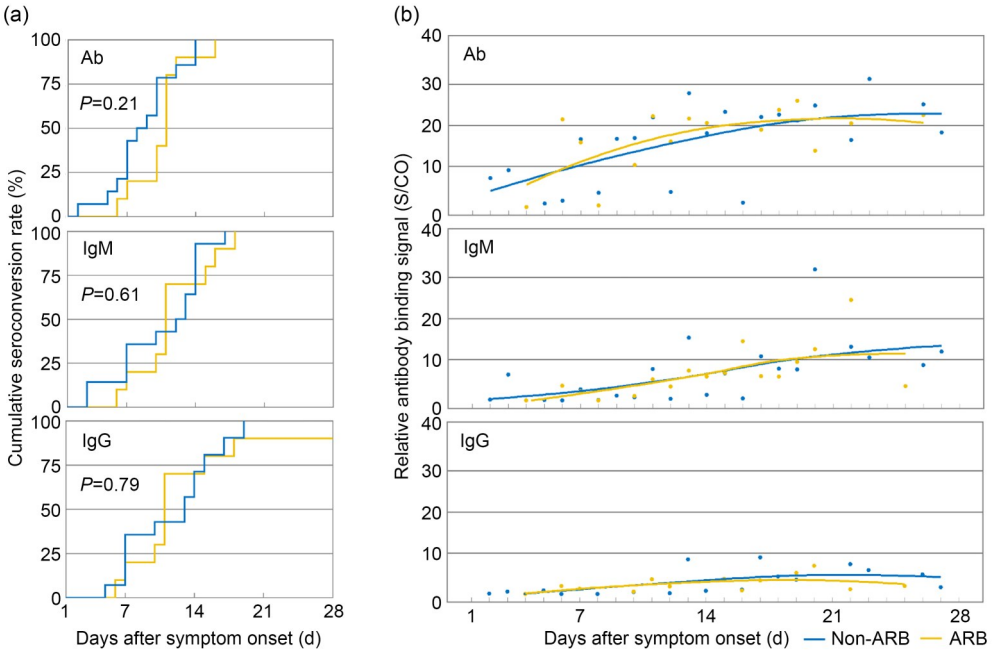

Fig. 2 Cumulative seroconversion rates and antibody dynamics across the days after symptom onset. (a) The curves of the cumulative seroconversion rates for Ab, IgM, and IgG were plotted using the Kaplan-Meier method. (b) The antibody levels were expressed as surrogates using the relative binding signals compared with the cutoff value (S/CO). ARB: angiotensin II receptor blocker; Ab: total antibody; IgM: immunoglobulin M; IgG: immunoglobulin G.

sequentially for Ab, IgM, and IgG. The seroconversion rates for Ab, IgM, and IgG in the ARB group were 100%, 100%, and 90%, respectively, which is comparable with those in the non-ARB group.

3.5 sACE2 levels and correlations with laboratory findings

The median level of sACE2 in serum samples in the ARB group was 1552.0 (IQR, 921.9–1685.5) pg/mL, trending higher than that in the non-ARB group (1124.3 (IQR, 947.2−1271.9) pg/mL) but with no statistical significance (Fig. 3a). The median levels of sACE2 in urine samples were similar between the two groups (Fig. 3b). Serum levels of sACE2 negatively correlated with viral duration, D-dimer, lactate dehydrogenase, and IL-10, and positively correlated with lymphocytes and estimated glomerular filtration rate, although these correlations failed to reach statistical significance (Figs. 3c–3h).

3.6 Transcriptome analysis

Differential expression analysis performed by comparing transcriptional profiles of the ARB and non-ARB groups revealed 125 DEGs from the pool

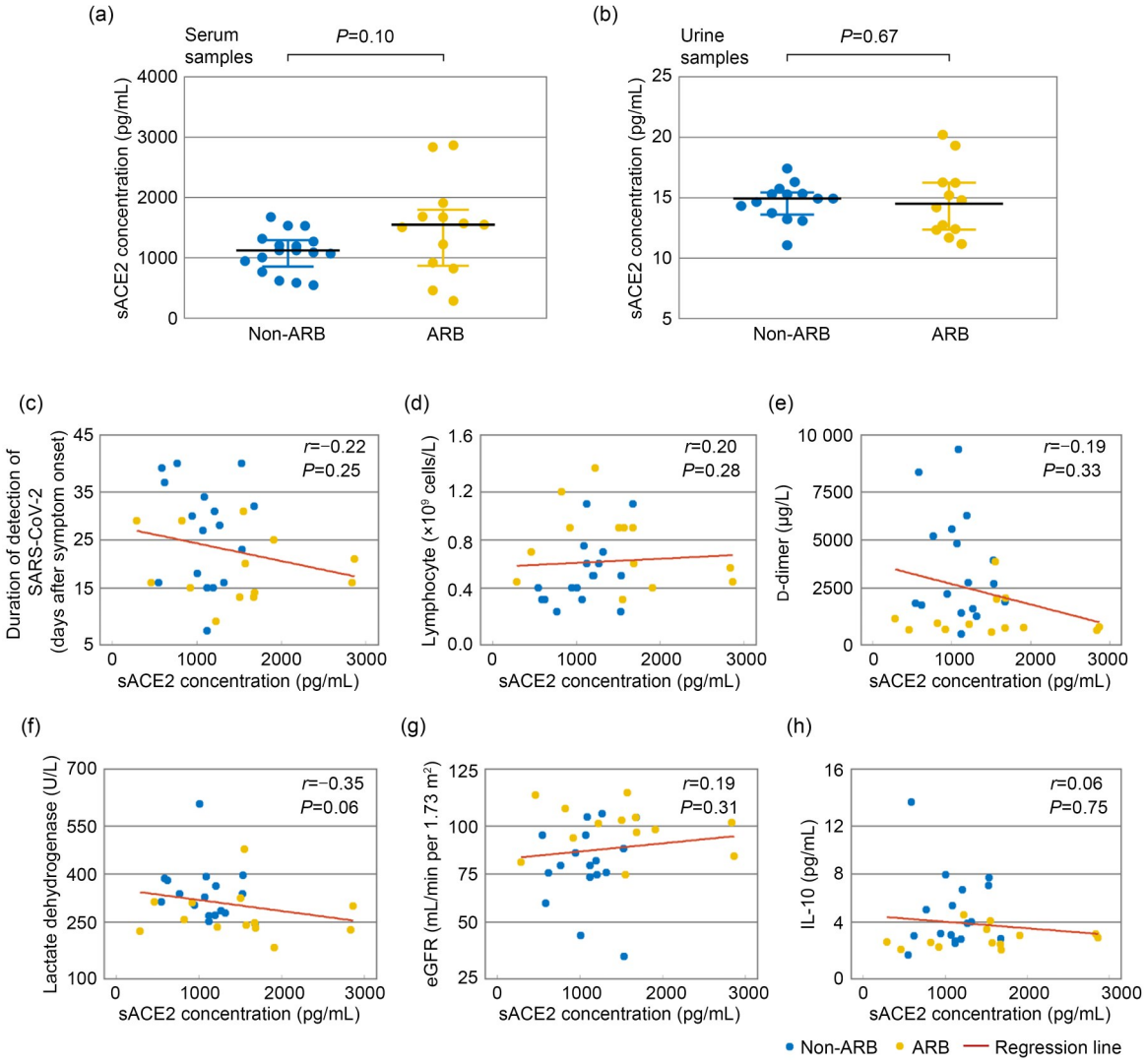

Fig. 3 sACE2 levels and correlations with laboratory findings. (a) sACE2 levels in serum; (b) sACE2 levels in urine sample; (c–h) Spearman's correlations between serum levels of sACE2 and selected laboratory findings, including duration of detection of SARS-CoV-2 (c), lymphocytes (d), D-dimer (e), lactate dehydrogenase (f), eGFR (g), and IL-10 (h). ARB: angiotensin II receptor blocker; sACE2: soluble angiotensin-converting enzyme 2; eGFR: estimated glomerular filtration rate; IL-10: interleukin-10.

of 19 944 transcripts identified in total. Of these, 114 genes were significantly downregulated and 11 upregulated. No viral reads were recorded in either group. The DEGs were represented in a volcano plot comparing the ARB group with the non-ARB group (Fig. 4a and Table S1). Functional enrichment analysis showed that the downregulated DEGs in the ARB group mainly were enriched in oxygen transport, bicarbonate transport, blood coagulation, platelet granulation, platelet aggregation, and negative regulation of the extrinsic apoptotic signaling pathway (Fig. 4b).

4 Discussion

In this study, we investigated the impacts of ARB usage on clinical outcomes among a cohort of COVID-19 patients with pre-existing hypertension. Patients receiving ARB treatment had favorable clinical outcomes as well as improved laboratory findings. Moreover, patients in the ARB group showed lower viral loads and shortened viral duration. No significant difference in sACE2 levels was observed between the two groups. Our results suggest that ARB usage is not associated with aggravation of COVID-19.

To date, several studies focusing on the impact of RAS blockers on the susceptibility of SARS-CoV-2 infection have been published (Mackey et al., 2020). In a retrospective cohort study comprising 18 472 patients who attended the Cleveland Clinic Health System for COVID-19 testing, the authors found no association between ACEI/ARB usage and COVID-19 test positivity using the overlap propensity score weighting method (Mehta et al., 2020). In another population-based case-control study in the Lombardy region of Italy, Mancia et al. (2020) also found no evidence that ACEI/ARB affected the risk of contracting COVID-19. Moreover, ACEI/ARB usage has even shown potential benefit in improving clinical outcomes and reducing mortality of COVID-19 patients. In a multi-center study from Wuhan, China, ACEI/ARB usage was found to be associated with lower risk of all-cause mortality after adjusting for confounders (Zhang P et al., 2020). Similar results were also reported in studies conducted in certain regions of the UK and in Denmark (Bean et al., 2020; Fosbol et al., 2020). More recently, Lam et al. (2020) investigated continued or discontinued use of ACEI/ARB during hospitalization of 614 COVID-19 patients with hypertension and found that patients who continued ACEI/ARB in the hospital had markedly lower ICU admission rates and mortality rates. In the present study, we observed a significantly lower proportion of severe cases and ICU admission, as well as shortened length of

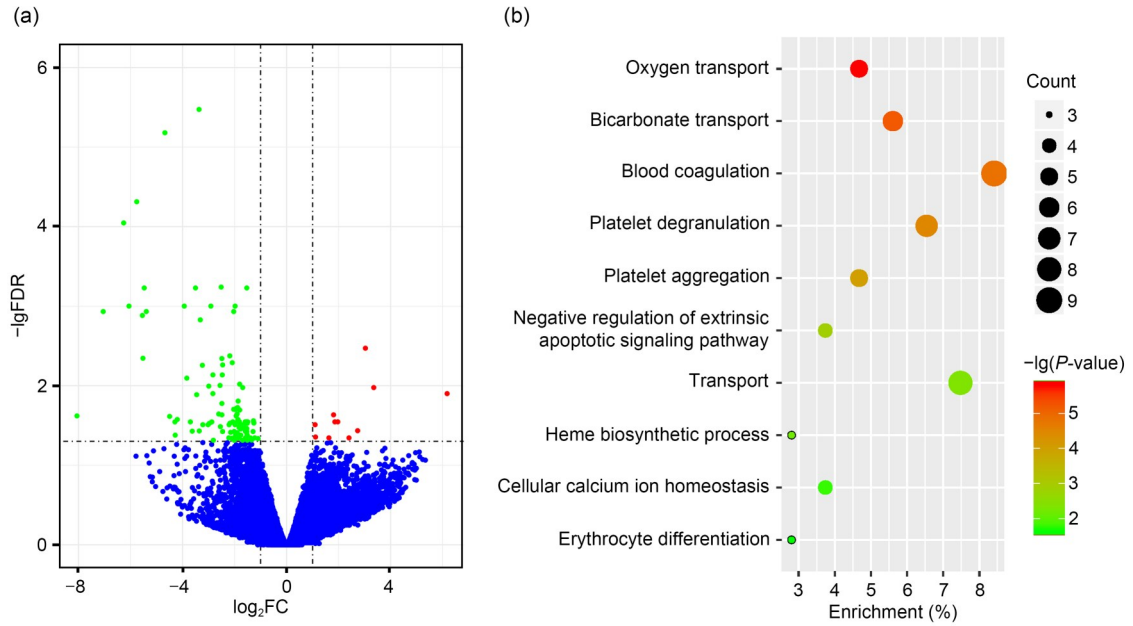

Fig. 4 Volcano plot (a) and pathway analysis (b) for differentially expressed genes. Differentially expressed genes were represented by comparing the ARB and non-ARB groups. ARB: angiotensin II receptor blocker; FDR: false discovery rate; FC: fold change.

hospital stay in the ARB group. Collectively, our results are in line with emerging evidence that support the continuation of ACEI/ARB.

We observed that patients in the non-ARB group showed lower blood platelet levels and prolonged prothrombin time, which have been reported to be associated with increased risk of mortality (Liao et al., 2020). Hematological characteristics manifested as lower blood platelet count, higher D-dimer, prolonged prothrombin time, and activated partial thromboplastin time. These indicators are suggestive of disseminated intravascular coagulation and have been observed in severe cases of COVID-19 (Levi et al., 2020). Consistently, the transcriptional analysis of the present study showed that the DEGs up-regulated in the non-ARB group mainly affected oxygen transport, bicarbonate transport, blood coagulation, platelet granulation, and platelet aggregation. These findings suggest worse coagulopathy in the non-ARB group.

Although ARB does not inhibit viral entrance or replication directly, the ARB group showed a lower viral load throughout the disease course as well as notably shortened viral duration in the present study. COVID-19 patients generally displayed immune dysfunction characterized by depletion of T cells and uncontrolled release of cytokines, which were more evident in severe cases (Cox and Brokstad, 2020). It has been hypothesized that RAS blockers play an indirect antiviral role by regulating immune function and inhibiting inflammatory responses (Meng et al., 2020). However, we did not observe a significant difference in the time of seroconversion or in antibody levels throughout the disease course between the two groups. Hence, ARB may contribute to viral clearance mainly by controlling cell-mediated immunity.

ACE2 has been identified as the SARS-CoV-2 receptor which mediates the process of entry into host cells. Epidemiological evidence suggests that patients with hypertension who are infected with SARS-CoV-2 are at an increased risk of acute lung injury and death (Gao et al., 2020; Grasselli et al., 2020). Up-regulated expression of ACE2 has been observed in the lungs of patients with comorbidities associated with severe COVID-19 (Pinto et al., 2020). However, it is still unclear whether this increased risk among hypertensive COVID-19 patients is related to RAS blocker usage, which is shown to promote the expression of ACE2 in animal models. In the present study, although we observed that the median level of ACE2 in serum samples in the ARB group tended to be higher than that in the non-ARB group, our results suggest that ARB usage does not increase SARS-CoV-2 virulence, instead appearing to shorten the duration of viral infection and decrease viral load throughout the disease course. Analysis of spike structure revealed that SARS-CoV-2 binds to ACE2 with affinity approximately 10- to 20-fold higher than that of SARS-CoV (Wrapp et al., 2020). Therefore, existing physiological expression of ACE2 may be sufficient for SARS-CoV-2, and immune dysregulation in hypertension perse may potentially explain the more severe course of COVID-19 (Kreutz et al., 2020; Yang et al., 2020).

Although studies including the present one have reported beneficial effects of RAS blocker usage among COVID-19 patients with hypertension, the mechanism underlying these effects is still not clear. ACE2 cleaves angiotensin II (AngII) to Ang(1−7), and with a low efficiency, AngI to Ang(1−9), which is further converted to Ang(1−7) by ACE. Ang(1−7) counterbalance the effects of AngII through activation of the Mas receptor (MasR) (Kreutz et al., 2020). A previous study demonstrated that injection of SARS-CoV spike protein in mice down-regulated ACE2 expression, and the induced acute lung failure could be attenuated by blocking the renin-angiotensin pathway (Kuba et al., 2005). In addition to modifying ACE2 expression, ARB simultaneously increases AngII levels, which act on AngII receptor type 2 (AT2R) and provide increased substrate for ACE2 to form Ang(1−7) (Vistisen et al., 2020). The activation of AngII/AT2R and ACE2/Ang(1−7)/MasR axes induces vasodilation and anti-inflammatory effects, and thereby plays a protective role. This was partially confirmed in the present study by our finding that serum levels of ACE2 negatively correlated with viral duration, D-dimer, lactate dehydrogenase, and IL-10, and positively correlated with lymphocytes and estimated glomerular filtration rate.

Our study has several limitations. Firstly, as the sample size was small, we were unable to further control for potential confounders. ARB users may have imposed particularly strict quarantine measures on themselves, because the hypothesis that using these drugs will increase risk was published in medical journals early in the spread of the epidemic (Fang et al., 2020; Zheng YY et al., 2020). This would lead to confounding by indication, which would influence the results.

Secondly, we only measured sACE2 in serum and urine samples. Since there is no compelling evidence as to whether sACE2 correlates with the membrane-bound form of ACE2, we were unable to assess the impact of ARB on the expression of ACE2 in tissues.

In summary, the results presented in this study suggest that ARB usage is associated with favorable clinical outcomes among COVID-19 patients with hypertension, and these findings support the maintenance of ACEI/ARB treatment for patients diagnosed with COVID-19. Further studies elucidating the mechanisms through which ARB regulates SARS-CoV-2 clearance may contribute to the development of effective therapy.

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Author contributions

Baihuan FENG performed the clinical laboratory testing and data analysis, and wrote and edited the manuscript. Dan ZHANG performed the clinical laboratory testing and edited the manuscript. Qi WANG, Fei YU, Qianda ZOU, Guoliang XIE, Ruonan WANG, Xianzhi YANG, Weizhen CHEN, and Bin LOU performed the data collection. Shufa ZHENG contributed to the study design, data analysis, and writing and editing of the manuscript. Yu CHEN contributed to the study design. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Baihuan FENG, Dan ZHANG, Qi WANG, Fei YU, Qianda ZOU, Guoliang XIE, Ruonan WANG, Xianzhi YANG, Weizhen CHEN, Bin LOU, Shufa ZHENG, and Yu CHEN declare that they have no conflict of interest.

This study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China (2020IIT A0107). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

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Supplementary information

Fig. S1; Table S1