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Coronavirus Pandemic

A prediction model for lung involvement using circulating angiotensin converting enzyme-2 and renin levels in COVID-19 patients

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Abstract

Introduction: The potential role of the renin-angiotensin-aldosterone system (RAAS) in the pathogenesis of coronavirus disease 2019 (COVID-19) is controversial, with concerns mainly about the part RAAS peptides play in the prediction of progression to more severe disease. Given the importance of COVID-19 prognostication at early disease stages, we established and validated a multivariable risk stratification tool for COVID-19 associated lung involvement by utilizing a combination of RAAS peptides.

Methodology: In this prospective study, circulating renin and angiotensin converting enzyme-2 (ACE-2) levels were measured in 116 COVID-19 patients who were admitted to our hospital from March 30, 2021 to January 24, 2022 and underwent a lung computed tomography (CT) scan. Clinical severity was measured with a national early warning score (NEWS). Associations among RAAS peptides, inflammation-dependent biomarkers, demographic variables, and clinical outcomes were studied using logistic regression and Cox proportional-hazards models.

Results: We assessed 116 COVID-19 patients (mean age 45.1 ± 12.6 years; 51.7% male), of whom 66 (56.9%) had COVID-19 associated pneumonia. Baseline circulating ACE-2 (2.63 ± 0.12 ng/mL) and renin levels (85.04 ± 6.8 ng/L) were lower in patients with COVID-19 related pneumonia compared to patients without pneumonia (6.4 ± 0.7 ng/mL and 211.6 ± 21.9 ng/L, respectively) ($p < 0.001$ for both). Both RAAS components were found to be significantly related to adverse outcomes, including COVID-19 associated pneumonia and intensive care unit (ICU) admission, in both crude and adjusted multivariable logistic regression analyses.

Conclusions: Circulating ACE-2 and renin levels can predict lung involvement in COVID-19 patients, and they display good correlation and agreement with NEWS.

Key words: ACE-2; COVID-19; emergency medicine; NEWS; pneumonia; renin.

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Introduction

The novel coronavirus disease (COVID-19), which is caused by severe acute respiratory syndrome virus 2 (SARS-CoV-2) was first identified in Wuhan, China, as an outbreak of unusual viral pneumonia resulting in increased mortality rates [1]. During the pandemic, the tasks of patient triage, management, and infection containment have largely fallen on emergency department (ED) physicians, who have become the first line of defense against this serious outbreak. Therefore, the appraisal of disease severity and the prognostication of clinical consequences with the help of validated clinical, laboratory, and radiological risk assessment tools at the early stage of the disease, especially on admission to the ED, is crucial. In order to achieve this goal, there have been extensive investigations of several prognostication systems, including the quick sequential (sepsis-related) organ failure assessment (qSOFA) score, national early warning score (NEWS), lung ultrasound score (LUS), and computed tomography severity score (CT-SS) [2–4]. Among these standardized assessment tools, NEWS is one of the best-known and validated risk stratification tools that has proven effective in ED settings and shown merit in predicting COVID-19 associated adverse outcomes [4].

The renin-angiotensin-aldosterone system (RAAS) is a vital component of the human body involved in homeostasis, as well as blood pressure and fluid volume regulation, with a cascade of several vasoactive peptides, including angiotensin-converting enzyme (ACE), angiotensin-2, ACE-2, angiotensin-(1–7), renin, angiotensin-2 type 1 receptor (AT1R) and angiotensin-2 type 2 receptor (AT2R). Importantly, SARS-CoV-2 utilizes and disrupts this system by direct modulation of ACE-2 [5,6]. ACE-2 degrades angiotensin-1 into inactive angiotensin-(1–9) and

hydrolyzes angiotensin-2 into the effector peptide product angiotensin-(1–7), which has anti-fibrotic, anti-inflammatory, antiproliferative, and vasodilatory effects. Moreover, ACE-2 not only counters RAAS activation but also serves as a functional receptor for coronaviruses as a point of entry into human lung cells [7]. SARS-CoV-2 enters the target cell after binding to ACE-2 receptors. Subsequently, the shedding of host ACE-2 receptors occurs. This may disrupt RAAS tissue homeostasis, causing a significant adverse impact on COVID-19 progression [8]. Although it can be suggested that soluble ACE-2 levels may protect against severe COVID-19 by blocking viral entry into lung cells, data in the literature is still not in agreement for COVID-19 patients, as their results show elevated, normal, or even lowered circulating ACE-2 levels compared with healthy controls [8–12]. Therefore, in this study we first sought to determine ACE-2 and renin levels among COVID-19 patients. Then, we established and validated a multivariable risk stratification tool for COVID-19 associated lung involvement by utilizing a combination of RAAS peptides and NEWS.

Methodology

Study design and patient eligibility

This prospective randomized controlled study enrolled 116 patients who were positive for SARS-CoV-2 (ascertained by reverse transcription-polymerase chain reaction test of nasal and pharyngeal swab samples) and admitted to the ED at Canakkale Onsekiz Mart University (COMU) Training and Research Hospital from March 30, 2021 to January 24, 2022. There were also 51 healthy controls included in the study. The exclusion criteria were as follows: (1) non-adults (< 18 years); (2) patients who had been receiving ACE inhibitors, angiotensin II type 1 receptor (AT1R) blockers, and diuretics three weeks prior to ED admission; (3) patients with a previous history of cardiopulmonary and/or renal failure, decompensated cirrhosis, hypertension, and diabetes mellitus; (4) patients with electrolyte imbalance; (5) pregnant patients; and (6) patients on whom a computed tomography (CT) scan was not performed. We included age-matched and sex-matched healthy controls without any history of acute/chronic inflammatory disorders or drug use. The study was approved by the institutional review board of the COMU Medical Center (Approval No: 2011-KAEK-27/2021-E.2100041801). It was carried out in accordance with the guidelines of the Helsinki Declaration and written informed consent was obtained from all study participants.

The clinical severity of the patients was measured using NEWS [13]. This score (from 0–20; higher score = greater severity) consists of seven physiological parameters (body temperature; heart rate; respiratory rate; requirement of supplemental oxygen (O_2); blood O_2 saturation; systolic blood pressure; and consciousness level) and is used to identify high-risk patients in acute care conditions. High scores have been proven to predict intensive care unit (ICU) admission, intubation, and mortality.

Clinical and laboratory data assessment

Demographic, laboratory, and radiological data were collected from the COMU Hospital Information and Management System (HIMS) after permission was granted from the relevant authorities. The medical and demographic data acquired from HIMS allowed us to analyze a large number of variables, including age, gender, vital and biochemical parameters, date presented to the ED, mortality, treatments received, accompanying disorders, discharge status, and disposition at discharge (home, hospital admission, ICU admission, death). Laboratory parameters at admission included complete blood cell (CBC) count, serum C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), renal and liver function tests, ferritin, D-dimer, and lactate dehydrogenase (LDH). Blood samples of approximately 3 mL were collected into vacuum containers without anticoagulants, centrifuged at 4000 rpm for 15 minutes, and stored at −80 °C.

Assessment of circulating renin and ACE-2

Levels of circulating renin and ACE-2 were calculated using the enzyme-linked immunosorbent assay (ELISA) method. The tests were prepared with human ELISA kits (Human Renin ELISA Kit, Cat. No. E1016Hu; Human Angiotensin Converting Enzyme 2 ELISA Kit, Cat. No. E3169Hu) according to the manufacturer's instructions. A BioTek ELX800 absorbance reader (BioTek Instruments Inc., Winooski, VT, USA) was used to read the florescence of the ELISA sample plates.

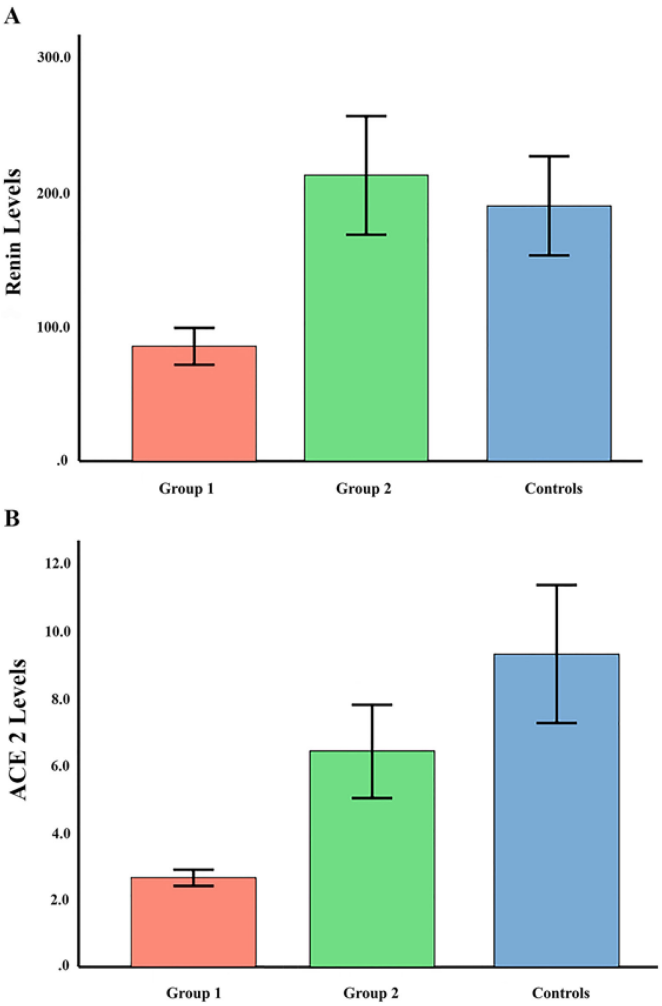
Thin-section CT imaging

All COVID-19 patients underwent a dedicated CT scan in the radiology department of our hospital. CT imaging was performed using a CT scanner (Asteion TSX-021B; Toshiba Corporation, Tokyo, Japan). The tube voltage was 120 kVp, and the tube current was set to 150 mA. The scans were first assessed as negative or positive for typical findings of COVID-19 pneumonia [14].

Statistical analysis

Descriptive statistics were expressed as mean and standard deviation or median and interquartile range for numerical variables. Categorical variables were expressed as numbers (n) and percentages (%). The normality test for numerical variables was done with the Shapiro–Wilk test. Student's T-test was used for normally distributed data in paired group comparisons, the variance analysis test was used in triple group comparisons. Pearson's chi-square test was used to compare categorical variables. Receiver operating characteristics (ROC) analysis was used to determine the best cut-off values for renin, ACE-2, and other COVID-19-related parameters that could identify COVID-19 associated lung involvement. Univariate and multivariate logistic regression analyses were used to calculate the odds ratios of independent clinical parameters to predict ICU admission. Multivariate logistic regression analysis was created by performing step-by-step variable selection on parameters with $p < 0.25$ in univariate logistic regression analysis. Renin- and ACE-2-adjusted ROC curve analysis was performed using multiple logistic regression to predict COVID-19 associated lung involvement. The R package pROC was used to perform the ROC analysis, calculate the differences between the areas under the curve, and construct the ROC curves via the bootstrap method [15]. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows version 19.0 (IBM Corp., Armonk, NY, USA) and R software version 3.6.2. For all analyses, $p < 0.05$ was considered significant.

Figure 1. Renin (A) and ACE-2 (B) levels of COVID-19 patients and controls.



Results

CT imaging was completed on a total of 116 COVID-19 patients, who were included in the present study between March 30, 2021 and January 24, 2022. Overall, the mean age was 45.1 ± 12.6 years for COVID-19 patients [M/F: 60 (51.7%)/56 (48.3%)] and 42.6 ± 11.8 years for healthy controls (M/F: 29/22). No significant difference was observed between the patients and controls with respect to age ($p = 0.234$) or gender ($p = 0.540$). The patients were further divided into two groups with respect to the presence of pneumonia on the basis of CT scans. Serum renin levels were significantly lower in patients with COVID-19 pneumonia (85.04 ± 6.8 ng/L) compared to patients without pneumonia (211.6 ± 21.9 ng/L) and healthy controls (188.9 ± 18.3 ng/L) ($p < 0.001$). Similarly, serum ACE-2 levels were significantly lower in patients with COVID-19 pneumonia (2.63 ± 0.12 ng/mL) compared to patients without pneumonia (6.4 ± 0.7ng/mL) and healthy controls (9.3 ± 1.01 ng/mL) (Figure 1). The clinical, demographic, and laboratory characteristics of all COVID-19 patients according to the presence or absence of pneumonia and healthy controls are presented in Table 1.

Of the 116 patients, 8 (6.8%) were followed in the ICU, and of these patients, 6 (5.1%) ultimately died. Patients admitted to the ICU and those who died were older, with a mean age of 54 ± 16.8 years ($p = 0.038$). Initial presentation with high ESR, CRP, ferritin, D-dimer, NEWS, qSOFA, CT-SS, and low renin and ACE-2 levels ($p < 0.05$ for all) was more commonly present in patients admitted to the ICU. Table 2 presents the demographic, clinical, and laboratory characteristics of all patients with regard to ICU admission.

We established another univariate and multivariate logistic regression analysis to reveal the role of distinct factors with scoring systems to predict lung involvement in our COVID-19 patient group (Table 3).

Table 1. Comparison of Baseline Characteristics of COVID-19 Patients.

	COVID-19 with lung involvement (n = 66)	COVID-19 without lung involvement (n = 50)	Controls (n = 51)	*p*
Years, mean ± SD	48.12 ± 1.59	41.1 ± 1.56	42.6 ± 1.66	0.009[a,b]
Gender				
Female, n (%)	27 (36.5)	31 (60.8)	22 (43.1)	0.025
Male, n (%)	47 (63.5)	20 (39.2)	29 (56.9)	
Vital parameters				
SBP (mmHg)	125.7 ± 13.1	124.2 ± 10.9	127,6 ± 10,7	0,648
DBP (mmHg)	78.1 ± 9.5	75.7 ± 7.8	82,3 ± 8,9	0,063
Heart rate (/minute)	88.3 ± 12.7	85.1 ± 9.3	81,5 ± 8,3	0,141
Respiratory rate (/minute)	18.1 ± 3.9	15.8 ± 1.4	15,5 ± 0,5	< 0,001[a,b]
SpO_2 (%)	93.0 ± 7.5	98.6 ± 1.2	-	< 0.001
Laboratory Parameters				
WBC (× 10^3/µl)	6.7 ± 2.7	6.1 ± 1.7	7,9 ± 2,3	0,084
Hgb (g/dL)	13.7 ± 1.52	13.4 ± 1.81	13,6 ± 1,1	0,632
Plt (× 10^3/µl)	206.6 ± 80.4	226.6 ± 57.2	215,4 ± 70,5	0,149
Urea (mg/dL)	27.9 ± 10.3	24.4 ± 6.9	24,8 ± 4,7	0,045[a,b]
Creatinine (mg/dL)	0.9 ± 0.2	0.8 ± 0.2	0,8 ± 0,1	0,274
ALT (U/L)	36.6 ± 33.9	18.8 ± 11.9	7,9 ± 2,3	< 0,001[a,b]
AST (U/L)	44.8 ± 39.9	17.8 ± 8.1	5,5 ± 1,5	< 0,001[a,b]
LDH (U/L)	437.6 ± 286.9	188.9 ± 42.4	-	< 0.001
ESR (mm/s)	30.8 ± 20.2	14.3 ± 10.5	-	< 0.001
CRP (mg/dL)	6.6 ± 6.9	1.0 ± 1.7	-	< 0.001
Ferritin (ng/mL)	555.8 ± 568.2	106.7 ± 106.5	-	< 0.001
D-dimer (µg FEU/mL)	0.9 ± 1.5	0.3 ± 0.3	-	0.003
Renin (ng/L)	85.04 ± 6.8	211.6 ± 21.9	188.9 ± 18.3	< 0.001[a,b]
ACE-2 (ng/mL)	2.63 ± 0.12	6.4 ± 0.7	9.3 ± 1.01	< 0.001[a,b,c]
Illness Severity Assessment Tools				
NEWS	3.0 ± 3.1	0.4 ± 0.9	-	< 0.001
qSOFA	0.2 ± 0.4	0.0 ± 0.0	-	0.001

Variance analysis, Bonferroni correction; [a]: Group 1 vs Group 2, [b]: Group 1 vs Control, [c]: Group 2 vs Control. SD: Standard Deviation; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SpO_2: Oxygen saturation; WBC: White Blood Cell; Hgb: Hemoglobin; Plt: Platelet; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; LDH: Lactate Dehydrogenase; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; ACE: Angiotensin Converting Enzyme; NEWS: National Early Warning Score; qSOFA: quick Sequential (Sepsis-related) Organ Failure Assessment.

Table 2. Comparison of COVID-19 patients according to illness severity.

	Non-ICU Admission* (n = 108)	ICU Admission (n = 8)	*p*
Years, mean ± sd	44.4 ± 12.1	54 ± 16.8	0.038
Gender			
Female, n (%)	54 (50.0)	2 (25.0)	0.172
Male, n (%)	54 (50.0)	6 (75.0)	
Laboratory Parameters			
WBC (× 10^3/µl)	6.3 ± 2.2	8.5 ± 2.9	0.009
Hgb (g/dL)	13.5 ± 1.7	13.5 ± 1.3	0.959
Plt (× 10^3/µl)	214.6 ± 72.5	223.1 ± 63.4	0.748
Urea (mg/dL)	25.6 ± 7.9	37.1 ± 16.3	< 0.001
Creatinine (mg/dL)	0.8 ± 0.2	0.9 ± 0.3	0.038
ESR (mm/s)	22.5 ± 17.5	39.6 ± 25.8	0.011
CRP (mg/dL)	3.5 ± 5.4	13.5 ± 6.8	< 0.001
Ferritin (ng/mL)	309.6 ± 410.4	1073.4 ± 837.8	< 0.001
D-dimer (µg FEU/mL)	0.5 ± 1.0	1.9 ± 2.8	0.001
Renin Level (ng/L)	143.4 ± 129.7	87.8 ± 23.1	< 0.001
ACE-2 Level (ng/mL)	4.0 ± 3.9	2.8 ± 1.7	0.010
Illness Severity Assessment Tools			
NEWS	1.6 ± 2.3	6.6 ± 3.2	< 0.001
qSOFA	0.1 ± 0.2	0.6 ± 0.5	< 0.001
CT-SS	4.2 ± 5.3	19.6 ± 4.5	< 0.001

*Admission to regular hospital ward or discharge. sd: standard deviation; WBC: White Blood Cell; Hgb: Hemoglobin; Plt: Platelet; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; ACE: Angiotensin Converting Enzyme; NEWS: National Early Warning Score; qSOFA: quick Sequential (Sepsis-related) Organ Failure Assessment; CT-SS: Computed Tomography Severity Score.

Table 3. Univariate and multivariate logistic regression analysis for the prediction of COVID-19 associated lung involvement.

	Lung Involvement (n:66)			
	Univariable		Multivariable	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Years	1.049 (1.015-1.083)	0.004		
Gender M[F (ref)]	2.308 (1.089-4.890)	0.029		
Vital parameters				
Temperature	2.971 (1.235-7.144)	0.015	6.141 (1.001-38.108)	0.049
Respiratory rate	1.454 (1.164-1.817)	0.001		
Laboratory Parameters				
Urea	1.049 (1.001-1.099)	0.047		
ALT	1.066 (1.029-1.104)	< 0.001	1.049 (0.984-1.118)	0.143
AST	1.167 (1.093-1.245)	< 0.001		
LDH	1.026 (1.015-1.037)	< 0.001	1.032 (1.014-1.052)	0.001
ESR	1.084 (1.046-1.123)	< 0.001		
CRP	1.640 (1.274-2.111)	< 0.001		
Ferritin	1.007 (1.003-1.010)	< 0.001		
Renin Level	0.980 (0.972-0.989)	< 0.001	0.981 (0.968-0.994)	0.005
ACE-2 Level	0.340 (0.209-0.554)	< 0.001	0.497 (0.216-1.146)	0.101
NEWS	2.140 (1.453-3.153)	< 0.001		

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; LDH: Lactate Dehydrogenase; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; ACE: Angiotensin Converting Enzyme; NEWS: National Early Warning Score.

A 10-unit decrease in renin was associated with a 20% increased risk of lung involvement among the COVID-19 patients, and a 1-unit decrease in ACE-2 was associated with 66% increased risk. Multivariate logistic regression analysis revealed a significant predictive value for LDH and renin for COVID-19-related lung involvement (Table 3).

Prediction of lung involvement in COVID-19 patients according to NEWS, renin and ACE-2 levels is demonstrated in Table 5. Lung involvement varied significantly within the renin and ACE-2 subgroups, depending on NEWS. Classifying patients by NEWS level significantly improved the prediction of lung involvement across the renin subgroups. When patients with NEWS scores < 2 and renin > 92.5 were taken as references, those with NEWS scores < 2 and renin ≤ 92.5 had a 10.81x (95% CI 3.8–30.8) increased risk of COVID-19-associated lung involvement. Patients with NEWS scores ≥ 2 and renin ≤ 92.5 had the highest odds ratio [113.4 (95% CI 13.6–928.7), $p < 0.001$].

Similar improvements were also observed in the age- and gender-adjusted model (Table 4). Classifying patients by NEWS level also significantly improved the prediction of lung involvement across the ACE-2 subgroups. When patients with NEWS scores < 2 and ACE-2 > 3 were used as references, those with NEWS scores < 2 and ACE-2 ≤ 3 had a 13.8x (95% CI 4.8-40.3) increased risk of COVID-19-associated lung involvement. Patients with NEWS scores ≥ 2 and ACE-2 ≤ 3 had a more than 74-fold increased risk of lung involvement (< 0.001). Similar findings were also detected in the age- and gender-adjusted models (Table 4).

The ROC analyses of renin, ACE-2, and other clinical and laboratory parameters in predicting COVID-19-associated lung involvement are depicted in Table 5. According to these analyses, the optimum renin level cut-off points for lung involvement were 92.5 ng/L, with a sensitivity, specificity, negative predictive value, and positive predictive value of 63.6%, 88.0%, 64.7%, and 87.5%, respectively [Area Under ROC Curve (AUROC): 0.851]. The optimum ACE-2 level cut-off points for lung involvement were 3 ng/mL, with a sensitivity, specificity, negative predictive value, and positive predictive value of 77.2%, 78.0%, 72.2%, and 82.2%, respectively

Table 4. Discrimination accuracy of different models on the prediction of COVID-19 associated lung involvement.

	Area under the ROC curve (95% CI)		Pairwise Analysis (95% CI)				
Prognostic Model	**Without ACE-2**	**With ACE-2**	**DBA**	**SE**	**LowerUpper**	**Z statistic**	***p***
Base Model = Years, Gender, Ferritin, D-Dimer	0.853 (0.783-0.923)	0.925 (0.879-0.970)	-0.072	0.241	(-0.120)- (-0.024)	-2.951	0.003
NEWS	0.784 (0.702-0.867)	0.921 (0.873-0.969)	-0.137	0.250	(-0.207)- (-0.066)	-3.807	< 0.001
Base Model with NEWS	0.861 (0.792-0.930)	0.937 (0.896-0.978)	-0.076	0.234	(-0.125)- (-0.027)	-3.039	0.002
Prognostic Model	**Without Renin**	**With Renin**	**DBA**	**SE**	**LowerUpper**	**Z statistic**	***p***
Base Model = Years, Gender, Ferritin, D-Dimer	0.853 (0.783-0.923)	0.911 (0.859-0.963)	-0.058	0.248	(-0.115)- (-0.001)	-1.991	0.047
NEWS	0.784 (0.702-0.867)	0.902 (0.850-0.955)	-0.118	0.254	(-0.183)- (-0.052)	-3.511	< 0.001
Base Model with NEWS	0.861 (0.792-0.930)	0.923 (0.876-0.970)	-0.062	0.241	(-0.117)- (-0.007)	-2.213	0.027

ROC: Receiver Operating Characteristics; DBA: Difference Between Areas; SE: Standard Error; ACE: Angiotensin Converting Enzyme; NEWS: National Early Warning Score.

Table 5. Predictive value of renin, ACE-2 and other study variables for COVID-19 lung involvement prediction according to Receiver Operating Characteristics (ROC) curve analysis.

	Cut-Off	AUROC	Sensitivity (%)	Specificity (%)	NPV	PPV	Accuracy
Lung Involvement							
Renin	≤ 92.5	0.851	63.64	88	64.71	87.50	74.14
ACE-2	≤ 3	0.852	77.27	78	72.22	82.26	77.59
Ferritin	≥ 104	0.813	80.30	62	70.45	73.61	72.41
D-Dimer	≥ 0.2	0.767	81.82	62	72.09	73.97	73.28
WBC	≥ 6.4	0.534	45.45	70	49.3	66.67	56.03
CRP	≥ 0.93	0.839	80.3	70	72.92	77.94	75.86
LYM	≤ 1.3	0.710	54.55	76	55.88	75.00	63.79
SpO_2	≤ 96	0.825	53.03	98	61.25	97.22	72.41
NEWS	≥ 2	0.784	51.52	90	58.44	87.18	68.10

AUROC: area under the receiver operating characteristic; NPV: negative predictive value; PPV: positive predictive value; ACE-2: angiotensin-converting enzyme 2; WBC: white blood cell; CRP: C-reactive protein; LYM: lymphocyte; SpO_2: peripheral capillary oxygen saturation; NEWS: national early warning score.

(AUROC: 0.852). The same ROC curve analysis for other clinical and laboratory parameters is shown in Table 5.

The impact of renin and ACE-2 on the discriminating accuracy of different COVID-19-associated lung involvement models is given in Table 6. First, we designed a base model including age, gender, ferritin and d-dimer to identify patients at high risk of lung involvement. Pairwise analysis demonstrated that, after adjusting the base model with renin, significantly higher accuracy was observed in predicting lung involvement [Difference Between Areas (DBA): −0.058, $p = 0.047$]. After demonstrating that NEWS is an important factor in predicting lung involvement (AUROC: 0.784), we combined the base model with NEWS, which yielded a more accurate lung involvement prediction (AUROC: 0.861). The further combing of renin with the base model + NEWS significantly increased the accuracy of identifying patients at high risk for lung involvement (AUROC: 0.923). Pairwise analysis revealed that, after adjusting the base model + NEWS with renin, significantly higher accuracy was detected in predicting lung involvement (DBA: −0.062, $p = 0.027$). Similar improvements were also detected in ACE-2-based modeling. The combing of ACE-2 with the base model + NEWS improved the accuracy of recognizing patients at high risk for lung involvement (AUROC: 0.937). Pairwise analysis revealed that, after adjusting the base model + NEWS with ACE-2, significantly higher accuracy was detected in predicting lung involvement (DBA: −0.076, $p = 0.002$) (Table 6).

Discussion

The use of baseline clinical observations and laboratory and radiologic parameters of COVID-19 patients as predictors of adverse outcomes is generally accepted. This kind of prognostication allows clinicians to stratify their patients into distinct risk categories and permits early interventions and specific care where appropriate. In this regard, the present study aimed to assess the predictive performance of renin and ACE-2 in combination with NEWS to predict COVID-19-associated lung involvement in COVID-19 patients. Our findings suggested that lower renin and ACE-2 levels are significantly related to adverse outcomes, including COVID-19-associated pneumonia and ICU admission, in both crude and adjusted multivariable logistic regression analysis. Moreover, predictive models incorporating renin and ACE-2 were more precise than those that did not include these parameters. Therefore, the present study emphasizes the significance of serum ACE-2 and renin levels in combination with a generally used prognostication tool such as NEWS when appraising the possibility of progression to severe disease in hospitalized COVID-

Table 6. ACE-2 on the prediction of associated COVID-19 lung involvement.

	Area under the ROC curve (95% CI)		Pairwise Analysis (95% CI)				
Prognostic Model	**Without ACE-2**	**With ACE-2**	**DBA**	**SE**	**LowerUpper**	**Z statistic**	***p***
Base Model = Age, Gender, Ferritin, D-Dimer	0.853 (0.783-0.923)	0.925 (0.879-0.970)	-0.072	0.241	(-0.120)- (-0.024)	-2.951	0.003
NEWS	0.784 (0.702-0.867)	0.921 (0.873-0.969)	-0.137	0.250	(-0.207)- (-0.066)	-3.807	< 0.001
Base Model with NEWS	0.861 (0.792-0.930)	0.937 (0.896-0.978)	-0.076	0.234	(-0.125)- (-0.027)	-3.039	0.002
Prognostic Model	**Without Renin**	**With Renin**					
Base Model = Age, Gender, Ferritin, D-Dimer	0.853 (0.783-0.923)	0.911 (0.859-0.963)	-0.058	0.248	(-0.115)- (-0.001)	-1.991	0.047
NEWS	0.784 (0.702-0.867)	0.902 (0.850-0.955)	-0.118	0.254	(-0.183)- (-0.052)	-3.511	< 0.001
Base Model with NEWS	0.861 (0.792-0.930)	0.923 (0.876-0.970)	-0.062	0.241	(-0.117)- (-0.007)	-2.213	0.027

ACE-2: angiotensin converting enzyme 2; ROC: receiver operating characteristic; CI: confidence interval; DBA: difference between areas; SE: standard error; NEWS: national early warning score.

19 patients.

Estimating adverse outcomes in COVID-19 patients, especially in ED settings, is of paramount importance for not only patient management but also patient prognostication to prioritize those patients at the highest risk of hospitalization and deliver timely treatment [16]. Hence, given that COVID-19 strained hospital capacity to the breaking point in hard-hit epicenters worldwide, alternative methods are required to recognize patients with COVID-19 who are at the highest risk of progressing to severe disease, ICU admission and even death. In this context, it is not surprising to see an increasing number of studies that focus on the development and validation of several scoring models to predict hospitalization and adverse outcomes in large cohorts of ED and ambulatory patients with COVID-19 [17-20]. Unfortunately, a great number of these scoring systems propose prognostic models that are developed as simplified scoring systems or nomograms, and the validity of these models has not been demonstrated across independent research studies. Thus, most of these models propose male gender, older age, dyslipidemia, hypertension, O_2 saturation, neutrophil to lymphocytic ratio, platelet to lymphocyte ratio, elevated body mass index, interleukin-6 (IL-6), ferritin, CRP, LDH and d-dimer levels as prognostic factors for adverse events; there is scarce evidence for the association between serum ACE-2 and renin levels and adverse outcomes [21-25]. Considering that the early diagnosis and effective clinical monitoring of COVID-19 is indispensable for preventing severe outcomes or even death, the recognition and validation of novel blood-based biomarkers in COVID-19 are necessary. Therefore, this study aimed to examine the single and combined predictive performance of serum ACE-2 and renin levels in combination with NEWS on the discriminating accuracy of different mortality models to discover an alternative prognostication tool for COVID-19 patients.

ACE-2 was originally recognized as a functional receptor for SARS-CoV in 2003 by Li *et al.* [26]. It is a functional receptor for the fusion and endocytosis of SARS-CoV-2 into the pulmonary endothelium via the interaction of the viral S (spike) protein with membrane-bound ACE-2 [27,28]. The imbalance between the ACE/angiotensin II/AT1R pathway and ACE-2/angiotensin (1-7)/Mas receptor pathway in the RAAS could trigger a multisystemic inflammation [29]. Circulating ACE-2 levels are relatively low in normal circumstances, but they have been shown to be elevated in distinct disease conditions, including various cardiovascular diseases, such as hypertension, heart failure, aortic stenosis, atrial fibrillation and coronary artery disease [30-34]. In view of these data, it is reasonable to suggest that SARS-CoV-2-associated lung involvement can further increase circulating ACE-2 activity; therefore, an elevation of circulating ACE-2 levels can be expected in COVID-19 patients [35]. A recent report by Turk *et al.* (European Review for Medical and Pharmacological Sciences 2020; 24: 8606-8620) supports this idea and proposes that in the initial phase of COVID-19 ACE-2 gene expression takes place.[36] Interestingly, however, there are still controversies in the literature regarding serum ACE-2 levels and adverse outcomes in COVID-19 patients in which results show elevated, normal or even lowered circulating ACE-2 levels compared with healthy controls [8,37-41]. These controversies could be related to the ACE-2 gene polymorphism that result in differences in disease susceptibility and disease severity [42]. Based on these contradictory data and the still-limited evidence of RAAS peptide alterations in the setting of SARS-CoV-2 infection, we found low serum ACE-2 levels in patients with proven COVID-19. We think that SARS-CoV-2-induced decrease in ACE-2 expression could result in feedback control leading to increased production of Angiotensin-2, which could intensify the severity of COVID-19.

This study found low serum ACE-2 levels in COVID-19 patients and showed the predictive value of ACE-2 in different prognostic models. In a recent study by Mortaz *et al.* [8], baseline circulating ACE-2 levels were found to be lower in severe and moderate COVID-19 patients compared to healthy controls. Moreover, the authors demonstrated that treatment significantly increased ACE-2 levels in patients with moderate and severe disease. In a recent study by Osman *et al.* [43], the expression of ACE-2 messengers ribonucleic acid (mRNA), ACE-2 cell-surface protein, and the plasma concentrations of soluble ACE-2 were investigated in 44 patients (30 prolonged viral shedders and 14 short viral shedders) compared to 15 healthy volunteers. Soluble ACE-2 plasma concentrations were found to be decreased in prolonged viral shedders compared to healthy volunteers, while the concentration of soluble ACE-2 returned to normal in the short viral shedders after treatment. Although our finding of low levels of circulating ACE-2 upon admission to the ED and its correlation with adverse outcomes is of great importance, this study could not examine circulating ACE-2 levels seen over time, especially following appropriate treatments. This raises the question of the clinical relevance of low ACE-2 levels at the baseline in patients who probably would have poor outcomes

and a possible restoration of "normal" levels over time during recovery.

Clinical studies investigating plasma renin levels as a marker of organ perfusion in distinct disease states have been widely recognized [44-46]. In this context, an increase in plasma renin levels and a lack of perfusion in critical tissues in septic patients were recently demonstrated by Gleeson *et al*. [46]. The authors suggested that plasma renin levels were a significant predictor of ICU mortality, and the measurements were not interfered with by diurnal variation, medications or renal replacement therapies. Given that the involvement of RAAS in COVID-19 is well known, the role of plasma renin activity in COVID-19 was first investigated by Melegari *et al.* [47] during the first wave of the disease in April 2020. Plasma renin levels were measured in the first 24 hours, in the following 72 hours and after one week in eight COVID-19 patients admitted to the ICU without a previous hospitalization. Although the sample size of the study did not allow clinical clear associations to be made, the mean plasma renin levels of not survivors steadily increased during the follow-up on the patients. In another study by Kutz *et al.* [12], the serum levels of RAAS peptides in hospitalized COVID-19 patients compared to those of patients suffering from SARS-CoV-2-negative respiratory infections were assessed. Plasma renin activity was found to be lower in the COVID-19 patients, and no difference in ACE and ACE-2 plasma activity between the groups was observed.

This study also explored the importance of circulating ACE-2 and renin levels in identifying different levels of lung involvement risk within the NEWS strata. According to both crude and adjusted models, we found that COVID-19 patients with a NEWS ≥ 2 have increased lung involvement risk and that inclusion of circulating ACE-2 and renin levels increased the accuracy of identifying those patients who have increased risk for COVID-19-associated lung involvement. Patients with a NEWS score ≥ 2 and renin ≤ 92.5 had 113.4 times (95% CI 13.6–928.7) higher risk of lung involvement compared to patients who had a NEWS < 2 and renin > 92.5. Similarly, patients with a NEWS score ≥ 2 and ACE-2 ≤ 3 had a > 74-fold increased risk of lung involvement compared to patients with a NEWS score < 2 and ACE-2 > 3. However, to the best of our knowledge, this is the first study to assess the effectiveness of these blood markers combined with NEWS for the prediction of lung involvement in COVID-19 patients. Therefore, we think that our data significantly reflect the importance of adding NEWS to other laboratory variables to improve the decision-making process regarding healthcare management in COVID-19 patients.

Although our study has several strengths, the authors are aware that the present study has some limitations. First, while our study included a moderate-sized cohort, the results may not be generalizable to all COVID-19 patient populations. Second, it would have been noteworthy if we simultaneously measured other RAAS components, including angiotensin-1, angiotensin-2 and angiotensin-(1–7) at baseline. Third, this study only assessed the circulating RAAS components at one time point (on admission to the ED), and evaluations at serial time points may shed further insight on how the RAAS fluctuates over time in COVID-19. Fourth, although the number of ICU admissions and deaths in our study population was relatively low, we believe that the robustness of our multivariate analyses and consistency across models support the reliability of our findings. Finally, the present study was underpowered to investigate the association between other possible confounding covariates that may alter the RAAS in patients with COVID-19, such as variables associated with cytokine storms (e.g., IL-6 and IL-8), body mass index and corticosteroid use. For instance, ACE and ACE-2 upregulation have been demonstrated by the use of dexamethasone, which is a standard treatment option in patients with severe COVID-19 [48].

Conclusions

In conclusion, serum ACE-2 and renin levels can prognosticate lung involvement in COVID-19 and have good correlation and agreement with NEWS. In this regard, the measurement of circulating RAAS components in COVID-19 patients will provide further understanding of the RAAS in adverse outcomes associated with COVID-19.

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Ethics approval

The study was approved by the institutional review board of the COMU Medical Center (Approval No: 2011-KAEK-27/2021-E.2100041801). It was carried out in accordance with the guidelines of the Helsinki Declaration and written informed consent was obtained from all study participants.

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Conflict of interests
No conflict of interests is declared.

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