High levels of mid-regional proadrenomedullin in ARDS COVID-19 patients: the experience of a single, Italian Center

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Abstract. – **OBJECTIVE:** This study evaluated the ability of mid-regional proadrenomedullin (MR-proADM) to identify disease severity in Coronavirus disease 2019 (COVID-19) patients in comparison to conventional inflammatory biomarkers and clinical scores.

PATIENTS AND METHODS: In an observational trial, COVID-19 acute respiratory distress syndrome (ARDS) patients were enrolled. MR-proADM, C-reactive protein (CRP), procalcitonin (PCT) and lactic acid (LA) were measured in all patients at admission (T0), at 24 hours (T1) and in the third (T3) and fifth day (T5) of hospitalization. The aims of this study were to determine the role of MR-proADM to detect patients with high risk of mortality and compare the prognostic value of MR-proADM with commonly used clinical scores (Sequential Organ Failure Assessment score – SOFA score, Acute Physiologic Assessment and Chronic Health Evaluation II score – APACHE II score, and Simplified Acute Physiological score II – SAPS II score).

RESULTS: Twenty-one COVID-19 ARDS patients admitted to the Intermediate Care Unit (IMCU) were enrolled. The median MR-proADM values were 2.28, 2.41, 1.96 and 1.89 nmol/L at T0, T1, T3 and T5, respectively. The 30-day all-cause mortality rate was 52.4%. Mean MR-proADM T0 value was significantly higher in non-survivors compared with survivors (3.5 *vs.* 1.1 nmol/L, $p < 0.05$). No significant differences were found for the other inflammatory biomarkers. In terms of the area under the receiver-operating characteristic curve (AUC), MR-proADM showed a similar discriminatory power compared with APACHE II, SOFA and SAPS II score (0.81, 0.91, 0.70 and 0.78, respectively). The optimal MR-proADM cut-point cut-off point was 1.07 nmol/L, which corresponds to a sensitivity of 91% and a specificity of 71%.

CONCLUSIONS: MR-proADM, in addition to the clinical scores, could be useful to predict outcome in COVID-19 ARDS patients.

Key Words:
 COVID-19, ARDS, MR-proADM.

Introduction

New Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is associated with a broad spectrum of clinical respiratory syndromes, ranging from mild upper airway symptoms to progressive life-threatening viral pneumonia[1-3]. Clinically, the cardinal features in COVID-19 patients are acute-onset dyspnea, tachypnea, and progressive hypoxemia. Mechanical ventilation is often required to maintain oxygenation and ventilation in these patients[4,5]. Radiographically, peripheral lung ground-glass opacities on computed tomographic imaging of the chest fulfill the Berlin criteria for ARDS[6]. Histologically, the hallmark of the early phase of ARDS is diffuse alveolar damage with edema, hemorrhage, and intra-alveolar fibrin deposition, as described by Katzenstein et al[7]. Although diffuse alveolar damage is a nonspecific finding, since it may have noninfectious or infectious

causes, the distinctive feature of COVID-19 is the acute endothelial dysfunction associated with the disease, which increases vascular permeability, promotes activation of the coagulation cascade and tissue edema and compromises the perfusion of vital organs[8-10].

One of the most challenging tasks clinicians face when assessing patients with COVID-19 is to stratify them. The initial risk stratification has important clinical implications, because identifying or more promptly treating patients in high-risk groups is crucial to decrease the mortality rate.

In these months, many studies[11,12] have tried to identify clinical risk factors and laboratory biomarkers that can help with the prediction of outcomes in COVID-19 patients. In particular, advanced age (>60), male sex, smoking, obesity, comorbidities (particularly hypertension), severe lymphopenia and high levels of lactate dehydrogenase (LDH) are believed to be risk factors for severe disease and poor outcomes, after Emergency Department (ED) admission.

MR-proADM is a peptide secreted from various organs and tissues and it regulates vascular tone and endothelial permeability[13-15]; in the last years, several studies have reported increased MR-proADM levels in septic shock, lower respiratory tract infections, heart failure, lung transplantation and thoracic surgery[16-20]. Moreover, MR-proADM seems to be an early marker of severity in septic patients, providing a more accurate mortality risk stratification compared to other biomarkers and clinical scores[21,22].

To date, the MR-proADM circulating levels in COVID-19 patients and its potential role in the clinical management of these patients, were not clearly investigated.

The primary aim of the present study was to describe the variation of MR-proADM plasma concentration during COVID-19 infection and to evaluate its prognostic value in IMCU patients with a diagnosis of COVID-19 ARDS. The secondary aim was to analyze the added value of MR-proADM as a risk stratification tool in comparison with other biomarkers and clinical severity scores.

Patients and Methods

Study Design

The BIOMKS-COVID19 is an observational, single-center study in COVID-19 ARDS patients admitted to the IMCU of ED in the Santi Antonio e Biagio e Cesare Arrigo Hospital of Alessandria, between March 2020 and April 2020. This institution is a tertiary-care public hospital of the Community of Alessandria (Northern Italy), with a reference population of 430,000 people that attended about 60,000 visits to the Emergency Room last year. The trial protocol was approved by the Local Scientific Ethics Committee; the trial was performed in accordance with the principles of the Declaration of Helsinki.

Patients

Twenty-one patients diagnosed with ARDS related to COVID-19 and admitted to the IMCU were recruited. SARS-CoV-2 infection was confirmed by positive detection of viral RNA in nasopharyngeal secretions using a specific Polymerase Chain Reaction test. COVID-19 ARDS was diagnosed on the basis of the Berlin 2012 ARDS diagnostic criteria: acute hypoxemic respiratory failure, presentation within one week of worsening respiratory symptoms; bilateral airspace disease on chest x-ray, computed tomography or ultrasound that is not fully explained by effusions, lobar or lung collapse or nodules; and cardiac failure is not the primary cause of acute hypoxemic respiratory failure[6].

Exclusion criteria were age less than 18 years, pregnancy and malignancies.

MR-proADM Analysis

A sample of blood from an EDTA-containing tube was centrifugated at 3500 rpm for eight minutes, and then, a plasma aliquot was immediately frozen and stored at -20°C. The MR-proADM measures were determined by the B.R.A.H.M.S. KRYPTOR® compact PLUS (Thermo Fisher Scientific, Hennigsdorf, Germany) automated method using the TRACE (Time-Resolved Amplified Cryptate Emission) technique. The detection limit of the assay was 0.05 nmol/L, while intra-and inter-assay coefficients of variation were under 4% and 11%, respectively.

Trial Procedures

The data of demographics, comorbidities, and treatments were recorded. SOFA score, APACHE II score and SAPS II score were calculated at admission.

MR-proADM, CRP, PCT and LA were measured in all patients at admission (T0), after 24 hours (T1) and in the third and fifth day of hospitalization (T3 and T5, respectively). Blood sam-

ples drawn upon arrival for full blood panel, renal function, LDH, ferritin, D-dimer and myocardial enzymes were also collected.

Outcomes

The primary aim of the study was to describe the variations of MR-proADM plasma concentration during acute phase of SARS-CoV2 infection and its correlation with 30-day all-cause mortality. Secondary aim was to analyze the added value of MR-proADM as a risk stratification tool in comparison with other biomarkers and clinical severity scores.

Statistical Analysis

Statistical analysis was done with Stata version 16. Descriptive statistics for the continuous variables are presented as mean (min-max), while groups are represented by frequencies and percentages. Whenever, continuous variables are compared between groups the Wilcoxon-Mann-Whitney test is used. To measure the classification capability of biomarkers, we used the time-dependent ROC curves[23] considering death within 30 days after ED admission as outcome. As a classification measure, we used the AUC.

Results

Characteristics of the Patients

Twenty-one patients (15 males and 6 females) affected by COVID-19 ARDS were included in this study. The characteristics of patients are summarized in Table I. The median age of the patients was 70.9 years (range 54-85). Four patients (19%) had no relevant medical history. Seventeen patients (80.9%) had one or more co-morbidities, including hypertension (14 patients, 66.6%), cardiovascular diseases (7 patients, 33.3%), chronic obstructive pulmonary disease (3 patients, 14.3%) and diabetes mellitus (7 patients, 33.3%). Eight patients (38.1%) suffered from chronic kidney disease (CKD): 4 patients were in CKD stage I, 1 patient was in CKD stage II, 3 patients were in CKD stage III according to K/DOQI guidelines[24]. No patient was on chronic kidney replacement therapy.

The mean APACHE II score was 13.61 (min-max 3-27), the mean SOFA score was 3.52 (min-max 1-9) and the mean SAPS II score was 33.86 (min-max 19-67). Commonly tested blood values were reported in Table II.

Five patients (23.8%) were transferred to the Intensive Care Unit (ICU); 6 patients (28.6%) were moved to other less intensive care wards; 5 patients (23.8%) were discharged at home from IMCU.

A total of 11 patients died within 30 days from ED admission (52.4%), among which 5 patients died in the IMCU (23.8%), 6 patients died in the ICU (28.6%) (Figure 1).

MR-proADM Values and Association Between Biomarkers and Clinical Scores With Mortality

The mean values of MR-proADM at T0, T1, T3 and T5 were 2.28, 2.41, 1.96 and 1.89 nmol/l, respectively (Figure 2).

Among biomarkers tested, only the median concentration of MR-proADM and LDH was significantly higher in non-survivor patients than in survivor patients (MR-proADM T0: 2.3 *vs.* 1.1 nmol/l, $p = 0.006$; LDH T0: 1045.0 *vs.* 705.5 U/l, $p = 0.024$). No differences were found about CRP (12.2 *vs.* 11.8 mg/dl, $p = 0.622$), PCT (0.4 *vs.* 0.2 ng/ml, $p = 0.188$), LA (1.7 *vs.* 1.1 mmol/l, $p = 0.168$). The mean APACHE II, SOFA and SAPS II score was also significantly higher in non-survivors than in survivors (14.0 *vs.* 9.7, $p < 0.001$; 4.0 *vs.* 2.6, $p = 0.004$; 36.0 *vs.* 27.8, $p = 0.015$, respectively) (Table III). As shown in Figure 3, 30-day survival was worse in patients with MR-proADM values above the median.

When comparing the ability of the scores and MR-proADM as classifiers for overall mortality, the AUC was 0.91, 0.70 and 0.78 for APACHE

Table I. Baseline characteristics of the study population.

|	
Sex	
Female	6 (28.6%)
Male	15 (71.4%)
Smoking history	
Active smoker	1 (4.8%)
Former smoker	3 (14.3%)
Never smoked	17 (80.9%)
Comorbidities	
Hypertension	14 (66.6%)
Cardiovascular diseases	7 (33.3%)
Chronic obstructive pulmonary disease	3 (14.3%)
Diabetes mellitus	7 (33.3%)
Chronic kidney disease	8 (38.1%)
No comorbidities	4 (19.0%)

Data are presented as absolute numbers with percentages in brackets.

Table II. Baseline patient characteristics, inflammatory biomarkers and blood chemistry values.

Variable	Mean	SD	Min	Max
Age	70.9	10.9	54	85
SAPSII	33.8	11.9	19	67
APACHE II	13.6	6.8	3	27
SOFA	3.5	2.3	0	9
CPR T0 (mg/dl)	11.7	6.5	1.9	27.1
MR-proAdm T0	2.3	2.7	0.1	9.7
PCT T0 (ng/ml)	0.8	0.1	0.02	7.0
LA T0 (mmol/l)	1.6	1.7	0.2	8.7
PaO_2/FiO_2	227.9	61.0	98	298
WBC (×1000/mcl)	10.8	8.7	3.3	38.4
Lymphocytes (×1000/mcl)	1.2	1.9	360	9.7
PTL (×1000/mcl)	301.8	180.5	80	685
Creatinine (mg/dl)	1.2	0.9	0.4	4.2
LDH (U/l)	825	288.2	458	1533
D-dimer (mcg/ml)	2.5	2.1	0.3	7.5
Ferritin (ng/ml)	1617.5	1415.2	78	5.284
Troponin (ng/l)	132.1	207.1	5	726

SAPS II: Simplified Acute Physiological Score II; APACHE II: Acute Physiological and Chronic Health Evaluation II score; SOFA: Sequential Organ Failure Assessment score; CPR T0: C-reactive protein at admission; MR-proADM T0: Mid-regional proadrenomedullin at admission; PCT T0: procalcitonin at admission; LA T0: lactic acid at admission; PaO2/FiO2: Arterial oxygen partial pressure/Fractional inspired oxygen with a minimum positive end expiratory pressure of 5 cmH20; WBC: white blood cell count; PTL: platelet count; LDH: lactate dehydrogenase; SD: standard deviation; Min: minimum; Max: maximum.

II, SOFA and SAPS II score, respectively, and 0.81 for MR-proADM (Figure 4). The optimal MR-proADM cutpoint cut-off point was 1.07 nmol/L, which corresponds to a sensitivity of 91% and to a specificity of 71%. The AUC for PCR, PCT and LA was 0.57, 0.54 and 0.56, respectively.
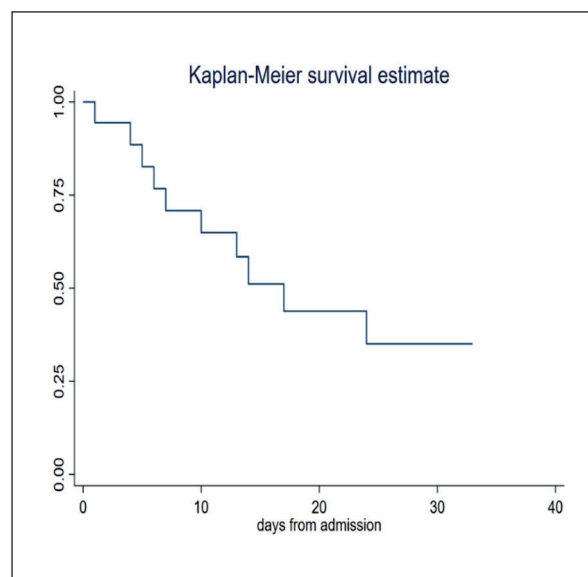
Figure 1. Overall survival Kaplan-Meier analyses of over-all survival in the study population.

Discussion

On February 2020 Italy, especially the northern regions, was hit by an epidemic of SARS-CoV-2 that spread from China between December 2019 and January 2020[25,26]. SARS-CoV-2 is an RNA virus whose tropism for the respiratory system causes a pneumonia identified through fever, dyspnea and acute respiratory symptoms and named COVID-19. This disease can lead to ARDS, whose prevalence among COVID-19
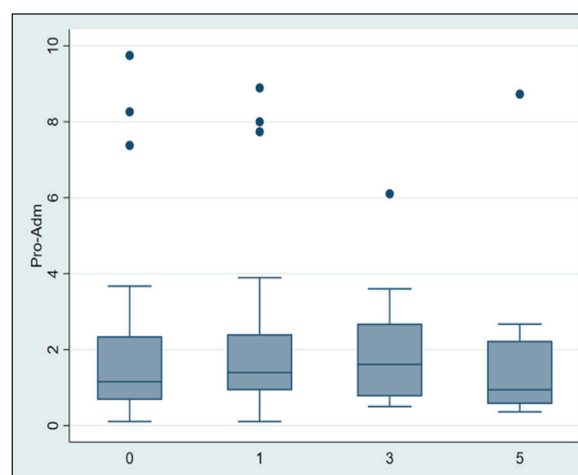
Figure 2. MR-ProADM values at T0, T1, T3, T5 (nmol/l).

Table III. Patients characteristics at baseline with regards to 30-day survival.

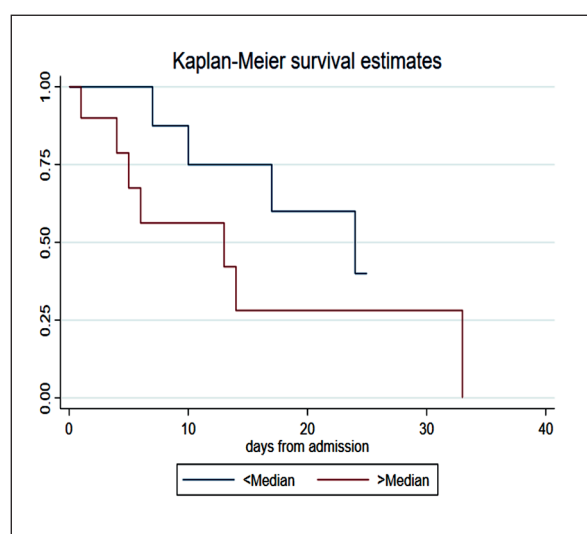|	Survivors (N = 10)		Non-survivors (N = 11)		
	Mean	**Median**	**Mean**	**Median**	**p**
Age (years)	67.6	68.0	76.0	73.9	0.244
SAPS II	27.8	28.0	36.0	40.0	0.015
APACHE II	9.7	10.0	14.0	18.0	< 0.001
SOFA	2.6	2.0	4.0	4.8	0.004
CRP T0 (mg/dl)	11.8	9.0	12.2	12.0	0.622
MR-proAdm T0	1.1	0.8	2.3	3.5	0.006
PCT T0 (ng/ml)	0.2	0.1	0.4	1.5	0.188
LA T0 (mmol/l)	1.1	1.0	1.7	2.1	0.168
WBC (×1000/mcl)	7.5	7.0	10.2	13.8	0.438
Lymphocytes (×1000/mcl)	995	930	1.9	1.6	0.888
PTL (×1000/mcl)	351	306	545	262	0.139
Creatinine (mg/dl)	0.9	0.9	1.3	1.5	0.105
LDH (U/l)	705.5	684.0	1045.0	956.4	0.024
D-dimer (mcg/ml)	2.4	1.9	4.6	2.6	0.643
Ferritin (ng/ml)	1430.9	1280.8	2635.0	1973.9	0.425
Troponin (ng/l)	47.6	31.0	143.0	180.8	0.567

SAPS II: Simplified Acute Physiological Score II; APACHE II: Acute Physiological and Chronic Health Evaluation II score; SOFA: Sequential Organ Failure Assessment score; CPR T0: C-reactive protein at admission; MR-proADM T0: Mid-regional proadrenomedullin at admission; PCT T0: procalcitonin at admission; LA T0: lactic acid at admission; WBC: white blood cell count; PTL: platelet count; LDH: lactate dehydrogenase.
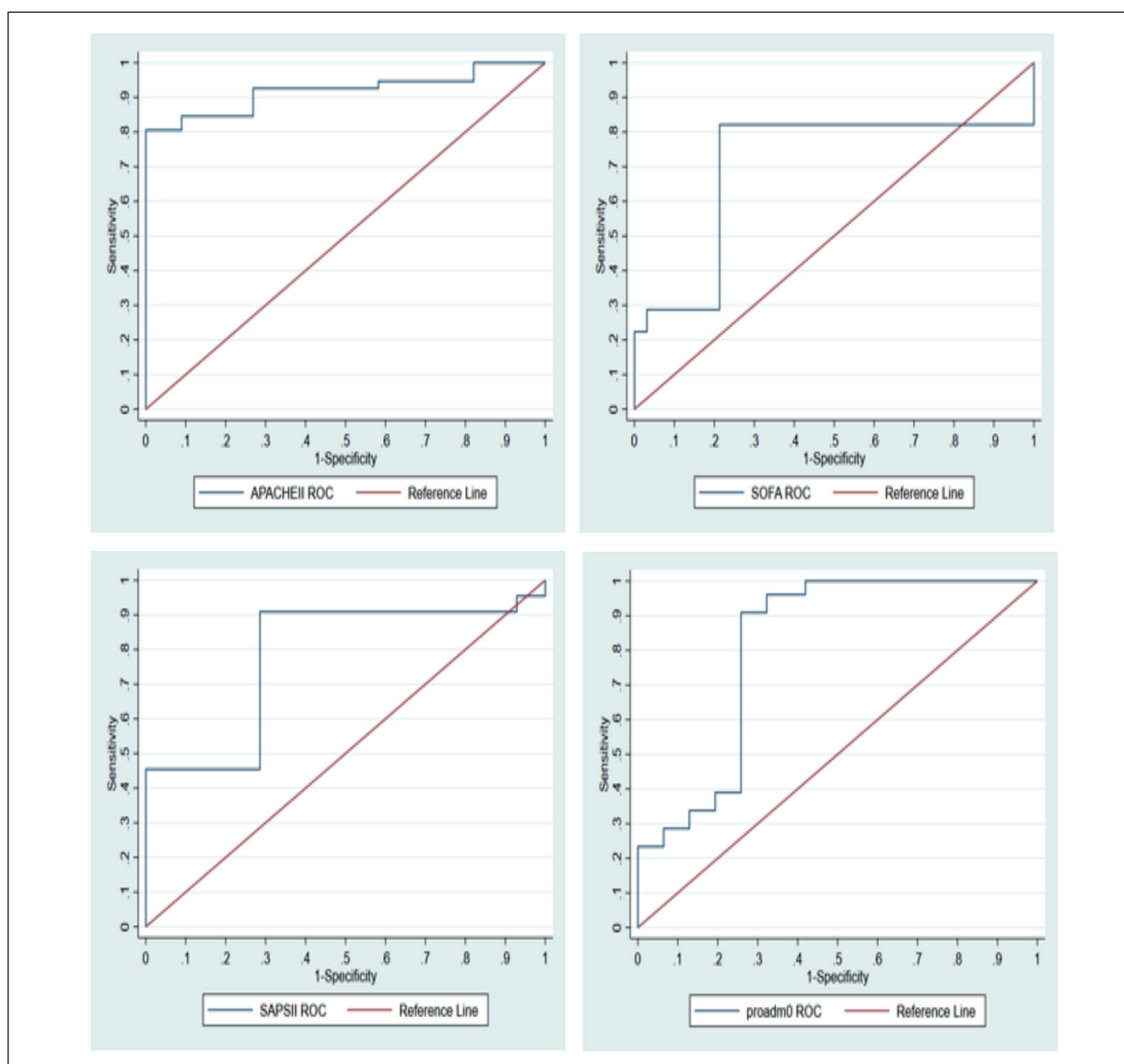
patients has been reported to be up to 17%. This severe acute respiratory syndrome has a dramatic impact on the health organization and health of the population due to the high need for mechanical ventilation, frequent hospitalization in ICU and significant increase in mortality[27-29].

More recently, emerging data[30,31] have focused the attention on the role of endothelial dysfunction during SARS-CoV-2 infection. The vascular endothelium is involved in barrier function, vasomotor tone control and immune response regulation. Histopathological studies[32-34] have evidenced a diffuse endothelial damage due to direct viral invasion or secondary to increased levels of pro-inflammatory cytokines, such as soluble interleukin 2 receptor (IL-2R), interleukin-6 (IL-6) and tumor necrosis factor alfa (TNF-alfa). Endothelial dysfunction induced by SARS-CoV-2 results in a pro-thrombotic state with microthrombi formation and pulmonary microvessels occlusion resulting in ventilation-perfusion mismatch and clinical phenotype of refractory ARDS, multisystem organ failure and death[35,36].

Figure 3. Survival Kaplan-Meier analyses stratified according to median MR-proADM T0 value.

Adrenomedullin (ADM) is a 52-aminoacid peptide that was first isolated in 1993 from extracts of a human pheochromocytoma. The ADM belongs to the calcitonin gene peptide superfamily which also includes calcitonin, PCT, the calcitonin gene-related peptide (CGRP) and amylin[37]. ADM synthesis is widely distributed in many mammalian tissues, including kidney, lung, blood vessels, heart, adipose tissue and endothelial cells; it has been found moreover in all epithelial surfaces separating the internal environment of the external and all bodily secretions, suggesting the possibility that ADM has a function of protection against external

Figure 4. Receiver operating characteristic (ROC) curves of APACHE II, SOFA, SAPS II and MR-proADM.

pathogens. Its fundamental biological effects include potent vasodilator, positive inotropic, diuretic, natriuretic and bronchodilator, inhibitor of insulin secretion, aldosterone and adrenocorticotropic hormone (ACTH) inhibitor[38-40]. ADM high levels were described not only in septic patients[41], but also in other diseases characterized by endothelium damage, such as hypertension, heart, respiratory and renal failure and cirrhosis[42]. However, the measurement of ADM is technically challenging because it has a short half-life and not available outside the research setting. For this reason, commercial immunoassays of more stable analytes were tested.

MR-proADM is the more stable mid-regional fragment of the ADM precursor and it is directly reflective of the level of the rapidly degraded active ADM peptide. Clinically, MR-proADM is commonly used due to its better technical viability than that of ADM[43].

Several studies on ED patients or ICU patients found that MR-proADM has been associated with poor prognosis in sepsis and it seems to be a superior biomarker compared with others (such as CRP, PCT and soluble triggering receptor expressed on myeloid cells-1) for prognostic purposes in sepsis[44,45]. Moreover, its assessment could accurately identify disease progression in initially non-severe

patients, safely increase outpatient treatment with no subsequent mortalities and identify high risk patients requiring early antibiotic treatment or ICU admission[46,47]. Mearelli et al[48] tested two prognostic models composed of clinical variables routinely measured at ED admission and biomarkers, such as MR-proADM, resulting to be more robust than SOFA and quick SOFA score alone in predicting mortality and in ruling out the high risk of death among in undifferentiated patients with suspected sepsis at ED admission and in those, among them, definitively diagnosed with sepsis at the end of clinical work-up.

On the other hand, MR-proADM has been extensively investigated for its prognostic value in community-acquired pneumonia (CAP) and a recent meta-analysis demonstrated that MR-proADM is predictive of increased complications and higher mortality rates in patients suffering from CAP. In particular, MR-proADM displayed moderate diagnostic accuracy for predicting complications in CAP, with an AUC of 0.74 (95% CI: 0.70-0.78) and an elevated MR-proADM level was associated with increased risk of death from CAP (RR 6.16, 95 % CI 4.71-8.06)[49].

Although in these studies the sepsis and CAP were general due to a bacterial infection, similar hypotheses can also be formulated for viral infections such as SARS-CoV-2.

Our observational study enrolled ARDS COVID-19 patients admitted to the IMCU of our Hospital. Elevated circulating MR-proADM levels were found, suggesting that virus-induced endothelial dysfunction and damage could play a crucial role in pathophysiological mechanisms of organ failure. Moreover, early MR-proaADM levels showed the strongest association with 30-days mortality compared to all other clinically used biomarkers. Finally, MR-proADM, as a stand-alone biomarker to predict outcome, exhibited an AUC higher than all the other biomarkers and comparable to clinical scores. We also identified a cut-off of 1.073 nmol/L, which corresponds to better sensitivity and specificity (91% and 71%, respectively).

The study does have limitations. First, stratification of patients by age group, comorbidity and other variables was not possible given our small sample size. Second, we don't know whether MR-proADM is only a biomarker of infection or it plays an active role in the pathogenesis of endothelial damage and dysfunction due to SARS-CoV-2. Finally, the study was conducted on COVID-19 ARDS patients requiring hospi-

talization in the IMCU; we don't know whether MR-ProADM could be useful in less severe COVID-19 patients.

Conclusions

Our study evaluated MR-proADM circulating levels in patients with COVID-19-related ARDS. MR-proADM provides a more accurate disease severity and mortality risk stratification compared to clinically established biomarkers. MR-proADM may be helpful in early identification of high-risk patients who may be eligible for an early ICU admission. Interventional studies to confirm these hypotheses are essential and should be viewed as mandatory before incorporation MR-proADM into routine clinical use.

Conflict of Interest
The Authors declare that they have no conflict of interests.

References

1) Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W, China novel coronavirus investigating and research team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020; 382: 727-733.

2) Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, Xing X, Xiang N, Wu Y, Li C, Chen Q, Li D, Liu T, Zhao J, Liu M, Tu W, Chen C, Jin L, Yang R, Wang Q, Zhou S, Wang R, Liu H, Luo Y, Liu Y, Shao G, Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam TTY, Wu JT, Gao GF, Cowling BJ, Yang B, Leung GM, Feng Z. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020; 382: 1199-1207.

3) Wu JT, Leung K, Leung GM. Nowcasting and forecasting the potential domestic and international sprehead of t2019-nCoV outbreak originating in Wuhan, China: a modelling study. Lancet 2020; 395: 689-697.

4) Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020; 323: 1239-1242.

5) Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen

PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020; 382: 1708-1720.

6) ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS. Acute Respiratory Distress Syndrome: The Berlin Definition. JAMA 2012; 307: 2526-2533.

7) Katzenstein AL, Bloor CM, Leibow AA. Diffuse alveolar damage--the role of oxygen, shock, and related factors. A review. Am J Pathol 1976; 85: 209-228.

8) Ware LB, Matthay MA, Parsons PE, Thompson BT, Januzzi JL, Eisner MD; National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome Clinical Trials Network. Pathogenetic and prognostic significance of altered coagulation and fibrinolysis in acute lung injury/acute respiratory distress syndrome. Crit Care Med 2007; 35: 1821-1828.

9) Becker RC. COVID-19 update: Covid-19-associated coagulopathy. J Thromb Thrombolysis 2020; 15: 1-14.

10) Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzankov A, Li WW, Li VW, Mentzer SJ, Jonigk D. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. N Engl J Med 2020; 383: 120-128.

11) Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395: 497-506.

12) Mattiuzzi C, Lippi G. Which lessons shall we learn from the 2019 novel coronavirus outbreak? Ann Transl Med 2020; 8: 48.

13) Hinson JP, Kapas S, Smith DM. Adrenomedullin, a multifunctional regulatory peptide. Endocr Rev 2000; 21: 138-167.

14) Tanenao E. A review of the biological properties and clinical implications of adrenomedullin and pro adrenomedullin N-terminal peptide (PAMP), hypotensive and vasodilating peptides. Peptides 2001; 22: 1693-1711.

15) Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo E, Eto T. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 1993; 192: 553-560.

16) Suberviola B, Castellanos-Ortega A, Llorca J, Ortiz F, Iglesias D, Prieto B. Prognostic value of proadrenomedullin in severe sepsis and septic shock patients with community acquired pneumonia. Swiss Med Wkly 2012; 142: 13542.

17) Alcoba G, Manzano S, Lacroix L, Galleto-Lacour A, Gervaix A. Proadrenomedullin and copeptin in pediatric pneumonia: a prospective diagnostic accuracy study. BMC Infectious Diseases 2015; 15: 347.

18) Valenzuela-Sanchez F, Valenzuela-Mendez B, Rodriguez-Guitierrez J, Estella-Garcia A, Gonzalez-Garcia MA. New role of biomarkers: mid-regional pro-adrenomedullin, the biomarker of organ failure. Ann Transl Med 2016; 4: 329.

19) Pepe MS, Feng Z, Huang Y, Longton G, Prentice R, Thompson IM, Zheng Y. Integrating the predictiveness of a marker with its performance as a classifier. Am J Epidemiol 2008; 167: 362-368.

20) Jougasaki M, Burnett JC Jr. Adrenomedullin: potential in physiology and pathophysiology. Life Sci 2000; 66: 855-872.

21) Christ-Crain M, Morgenthaler NG, Struck J, Harbarth S, Bergmann A, Muller B. Mid-regional pro-adrenomedullin as a prognostic marker in sepsis: an observational study. Crit Care 2005; 9: 816-824.

22) Travaglino F, De Berardinis B, Magrini L, Bongiovanni C, Candelli M, Silveri NG, Legramante J, Galante A, Salerno G, Cardelli P, Di Somma S. Utility of Procalcitonin (PCT) and Mid regional pro-Adrenomedullin (MR-proADM) in risk stratification of critically ill febrile patients in Emergency Department (ED). A comparison with APACHE II score. BMC Infect Dis 2012; 12: 184.

23) Heagerty PJ, Lumley T, Pepe, MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. Biometrics 2004; 56: 337-344.

24) National Kidney FoundationK/DOKI. Clinical practice guidelines for chronic kidney disease: evaluation classification and stratification. Am J Kidney Dis 2002; 39.

25) Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun 2020; 109: 102433.

26) Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579: 270-273.

27) Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Resp Med 2020; 8: 420-422.

28) Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. JAMA 2020; 323: 1061-1069.

29) Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020; 382: 1708-1720.

30) Marchetti M. COVID-19-driven endothelial damage: complement, HIF-1, and ABL2 are potential pathways of damage and targets for cure. Ann Hematol 2020; 99: 1701-1707.

31) Pons S, Fodil S, Azoulay E, Zafrani L. The vascular endothelium: the cornerstone of organ dysfunction in severe SARS-CoV-2 infection. Crit Care 2020; 24: 353.

32) Su H, Yang M, Wan C, Yi LX, Tang F, Zhu HY, Yi F, Yang HC, Fogo AB, Nie X, Zhang C. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. Kidney Int 2020; 98: 219-227.

33) Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, Zhang X, Zhang M, Wu S, Song J, Chen T, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest 2020; 130: 2620-2629.

34) Wang F, Hou H, Luo Y, Tang G, Wu S, Huang M, Liu W, Zhu Y, Lin Q, Mao L, Fang M, Zhang H, Sun Z. The laboratory tests and host immunity of COVID-19 patients with different severity of illness. JCI Insight 2020; 5: 137799.

35) Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, Kaptein FHJ, van Paassen J, Stals MAM, Huisman MV, Endeman H. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res 2020; 191: 145-147.

36) Zhang MD, Xiao M, Zhang S, Xia P, Caio W, Jiang W. Coagulopathy and antiphospholipid antibodies in patients with Covid-19. N Engl J Med 2020; 382: 38.

37) Kangawa K, Kitamura K, Minamino N, Matsuo H. Adrenomedullin: a new modulator of vascular tone. J Card Fail 1996; 2: 135-140.

38) Jougasaki M, Burnett JC Jr. Adrenomedullin: potential in physiology and pathophysiology. Life Sci 2000; 66: 855-872.

39) Nagaya N, Mori H, Murakami S, Kangawa K, Kitamura S. Adrenomedullin: angiogenesis and gene therapy. Am J Physiol Regul Integr Comp Physiol 2005; 288: 1432-1437.

40) Tsuruda T, Kato J, Kuwasako K, Kitamura K. Adrenomedullin: continuing to explore cardioprotection. Peptides 2019; 111: 47-54.

41) Nishio K, Akai Y, Murao Y, Doi N, Ueda S, Tabuse H, Miyamoto S, Dohi K, Minamino N, Shoji H, Kitamura K, Kangawa K, Matsuo H. Increased plasma concentrations of adrenomedullin correlate with relaxation of vascular tone in patients with septic shock. Crit Care Med 1997; 25: 953-957.

42) Eto T, Kitamura K, Kato J. Biological and clinical roles of adrenomedullin in circulation control and cardiovascular diseases. Clin Exp Pharmacol Physiol 1999; 26: 371-380.

43) Struck J, Tao C, Morgenthaler NG, Bergmann A. Identification of an Adrenomedullin precursor fragment in plasma of sepsis patients. Peptides 2004; 25: 1369-1372.

44) Schuetz P, Christ-Crain M, Morgenthaler NG, Struck J, Bergmann A, Müller B. Circulating precursor levels of endothelin-1 and adrenomedullin, two endothelium-derived, counteracting substances, in sepsis. Endothelium 2007; 14: 345–351.

45) Christ-Crain M, Morgenthaler NG, Struck J, Harbarth S, Bergmann A, Müller B. Mid-regional pro-adrenomedullin as a prognostic marker in sepsis: an observational study. Crit Care 2005; 9: 816–824.

46) Saeed K, Wilson DC, Bloos F, Schuetz P, van der Does Y, Melander O, Hausfater P, Legramante JM, Claessens YE, Amin D, Rosenqvist M, White G, Mueller B, Limper M, Callejo CC, Brandi A, Macchi MA, Cortes N, Kutz A, Patka P, Yañez MC, Bernardini S, Beau N, Dryden M, van Gorp ECM, Minieri M, Chan L, Rood PPM, Del Castillo JG. The early identification of disease progression in patients with suspected infection presenting to the emergency department: a multi-centre derivation and validation study. Crit Care 2019; 23: 40.

47) Gonzalez Del Castillo J, Wilson DC, Clemente-Callejo C, Román F, Bardés-Robles I, Jiménez I, Orviz E, Dastis-Arias M, Espinosa B, Tornero-Romero F, Giol-Amich J, González V, Llopis-Roca F; INFURG-SEMES. Biomarkers and clinical scores to identify patient populations at risk of delayed antibiotic administration or intensive care admission. Crit Care 2019; 23: 335.

48) Mearelli F, Barbati G, Casarsa C, Giansante C, Breglia A, Spica A, Moras C, Olivieri G, Occhipinti AA, De Nardo M, Spagnol F, Fiotti N, Di Girolamo FG, Ruscio M, Castello LM, Colonetti E, Marino R, Ronco C, Zanetti M, Lupia E, Muiesan ML, Di Somma S, Avanzi GC, Biolo G. The integration of qSOFA with clinical variables and serum biomarkers improves the prognostic value of qSOFA alone in patients with suspected or confirmed sepsis at ED admission. J Clin Med 2020; 9: 1205.

49) Liu D, Xie L, Zhao H, Liu X, Cao J. Prognostic value of mid-regional pro-adrenomedullin (MR-proADM) in patients with community-acquired pneumonia: a systematic review and meta-analysis. BMC Infect Dis 2016; 16: 232.