Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Clinica Chimica Acta 523 (2021) 185–190

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Endothelial dysfunction and Mid-Regional proAdrenomedullin: What role in SARS-CoV-2 infected Patients?

Martina Zaninotto [a,*], Monica Maria Mion [a], Lucio Marchioro [a], Andrea Padoan [a], Mario Plebani [a,b]

[a] Department of Laboratory Medicine, University-Hospital, Padua, Italy
[b] Department of Medicine-DIMED, University of Padua, Italy

ARTICLE INFO

Keywords:
Endothelial damage
Endothelitis
MR-proADM
SARS-CoV-2
Prognosis

ABSTRACT

Background: Endothelial dysfunction, a major complication of SARS-CoV-2 infectionplaying a key-role in multi-organ damage, carries high risk of mortality.
Aim: To investigate the potential role of Mid-Regional pro-Adrenomedullin (MR-proADM) in detecting endothelial damage with a view to stratifying the risk of adverse events (length of stay, death, admission in Intensive Care Unit) and/or disease resolution.
Materials and Methods: In 135 consecutive patients with SARS-CoV-2 infection, MR-proADM was measured in EDTA-K2 plasma samples using B.R.A.H.M.S. KRYPTOR® COMPACT Plus method (Thermo Fisher Scientific, Hennigsdorf, Germany)
Results: Patients were subdivided into three groups based on their MR-proADM value (nmol/L): 1 (n = 20, MR-proADM ≤ 0.55); 2 (n = 82, 0.55 < MR-proADM ≤ 1.50); 3 (n = 33, MR-proADM > 1.50). The higher the MR-proADM value, the greater the patients' age, the more frequent the occurrence of pneumonia, the requiring of more aggressive treatment, the longer the hospitalization and the more frequent a fatal event. Significant differences were found between the three groups for MR-proADM, White-blood cell count, Neutrophil count, D-dimer, C-reactive Protein, Procalcitonin and hs-Troponin I. At logistic regression,it was found that MR-proADM and Log_{10}D-dimer were the most significant predictors of adverse events.
Conclusion: The findings made in the present study highlight the relevance of MR-proADM values in providing clinically useful information, particularly for stratifying COVID-19 patients according to the risk of a more severe form of disease and to the development of adverse events.

1. Introduction

The vascular endothelium, an active paracrine, endocrine, and autocrine system, plays a fundamental role in both the regulation of vascular tone and the maintenance of vascular homoeostasis [1,2].

Endothelial dysfunction is a main determinant of microvascular dysfunction since it shifts the vascular equilibrium towards a greater vasoconstriction with subsequent organ ischemia, inflammation with associated tissue oedema, and a pro-coagulant state [3].

SARS-CoV-2 infects the host by means of the Angiotensin Converting Enzyme2 (ACE2) receptor, which is expressed in several organs, including the lung, heart, kidney, and intestine as well as the endothelial cells [4]. Moreover, findings reported in the literature [1], have demonstrated the presence of viral elements within endothelial cells and an accumulation of inflammatory cells, thus suggesting that SARS-CoV-2 infection facilitates the induction of endotheliitis in several organs as a direct consequence of the viral involvement and of the host inflammatory response.

The clinical status for subjects with SARS-CoV-2 infection ranges from lack of symptoms to severe pneumonia, with a mortality rate ranging from 4 to 13%, mainly in cases of acute respiratory distress syndrome (ARDS) [5–7], being the disease severity evaluated mainly on the basis of clinical and radiological findings [8]. Moreover, as occurs in several different diseases, also in SARS-CoV-2 patients the use of biomarkers may help clinicians to evaluate disease severity and stratify the risk of an adverse outcome [9,10].

Several papers in the literature [11–15] have evidenced the relevance of the endothelium dysfunction in the development and severity

* Corresponding author at: Biol. Sci Department of Laboratory Medicine, University-Hospital, Padua, Italy.
E-mail address: martina.zaninotto@aopd.veneto.it (M. Zaninotto).

https://doi.org/10.1016/j.cca.2021.09.016
Received 12 July 2021; Received in revised form 17 September 2021; Accepted 17 September 2021
Available online 20 September 2021

of COVID-19 disease focusing on the clinical usefulness of the biomarkers measurements in assessing endothelial damage in this specific disease, as well as in several other conditions in which endothelial cells are activated. On the other hand, a small number of biomarkers and a few commercially available methods have been validated to evaluate endothelial function in clinical practice.

Adrenomedullin (ADM),first discovered by Kitamura et al. in 1993 [16], is a vasodilator peptide with antimitogenic and natriuretic properties that participates in blood pressure control. The main function of this peptide is to trigger vasodilatation in both vascular resistance and capacitance vessels resulting in a blood flow increase. ADM further reduces vasoconstriction by inhibiting the renin-angiotensin-aldosterone system and maintains endothelial integrity by reducing vascular permeability [17]. A disruption of the ADM system results in vascular leakage, which is the first step in the inflammation and the coagulation cascade activation [18,19].

The values for mid-regional pro-adrenomedullin (MR-proADM), which is derived from proADM, directly reflect the effects of its less stable and less easily detectable precursor. MR-proADM has therefore recently been introduced into clinical practice as a prognostic marker in patients with bacterial infection [20]. A significant relation between MR-proADM values and bacterial pneumonia severity has been highlighted [21].

Despite most ofthe studies evaluating the role of MR-proADM in bacterial infections leading to sepsis, scant evidence is available on patients with viral infections, including Covid-19 [22–25].The aim of our study was therefore to investigate in Covid-19 infected patients, the potential role of MR-proADM circulating concentrations in detecting endothelial damage and providing clinically relevant information for stratifying the patients according to the risk of adverse events or probability of disease resolution.

2. Materials and methods

Between November 12thand 24th2020, 135 consecutive hospitalized patients with microbiology proven COVID-19 infection were enrolled for the study. The CT scans performed at the time of admission, revealed abnormal results in 85% of patients being the ground-glass opacity (55.4%) and bilateral patchy shadowing (50.8%) the most common patterns. The biochemical parameters [Glucose, Creatinine, Lactate Dehydrogenase (LDH), Albumin, Ferritin] were measured in samples collected in lithium heparin tubes (Becton Dickinson) during the hospitalization period. All measurements have been carried out on Cobas 8000 system (Roche Diagnostics, GmbH, Mannheim, Germany) with the exception ofProcalcitonin (PCT) (Liaison Brahms PCT II gen, Diasorin SpA, Saluggia, Italy),C-reactive protein (CRP) (Dimension Vista, Siemens Healthcare Diagnostics Inc, Tarrytown USA), cardiac troponin I (hs-cTnI, Architect 2000, Abbot Diagnostics) and D-dimer (Sclavo reagents, Sysmex CS-5100), while hematological data were obtained using Sysmex XE 2100 (Sysmex, Kobe, Japan).

MR-proADM measurement was performed in plasma EDTA-K2 using the TRACE technology (Time-Resolved Amplified Cryptate Emission, B.R.A.H.M.S. KRYPTOR® COMPACT Plus, Thermo Fisher Scientific, Hennigsdorf, Germany). During the study, the method's analytical performance was monitored by determining the Internal Quality Control materials (B.R.A.H.M.S. MR-proADM KRYPTOR QC) that provided an intra-assay imprecision (CV%) ranging from $\leq$ 3.1% (0.50 $<$ MR-proADM $<$ 2.00 nmol/L)to $\leq$ 1.2% (2.00 $<$ MR-proADM $<$ 6.00 nmol/L). The measurement was carried out in all samples in the same analytical run in order to avoid any bias between different calibrations. On the basis of MR-proADM URL and risk stratification cut-off proposed by the manufacturer (URL = 0.55 nmol/L, risk stratification = 1.50 nmol/L respectively), the patients studied were subdivided into 3 groups. In particular, Group 1, n = 20, MR-proADM $\leq$ 0.55 nmol/L; Group 2, n = 82, MR-proADM $>$ 0.55 nmol/L $\leq$ 1.50 nmol/L; Group 3, n = 33, MR-proADM $>$ 1.50 nmol/L.

The study protocol (number 23307) was approved by the Ethics Committee of the University-Hospital of Padua and was conducted according to the principles of the Declaration of Helsinki. Need for the informed consent was waived as regard to the study design (retrospective).

2.1. Statistical analysis

Statistical analyses were made using Stata v13.1 (StataCorp, Lakeway Drive, TX, USA) and Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA). For descriptive statistics, the median and interquartile range (IQR) were used to summarize results as appropriate. Kruskal-Wallis equality-of-populations rank test was employed to define differences across groups of subjects, with or without Bonferroni's criteria for adjusting *p*-values for multiple testing.

Chi-square test and Fisher's exact test were used for comparing proportions across groups. Logistic regressions were employed to estimate the association between the studied outcomes and the predictor variables with or without adjusting estimators for confounding variables, including age and gender.

The Mann-Whitney *U* test was used to compare the values of the MR-proADM in the two clinical outcomes.

The backward-selection stepwise logistic regression model, with variables inclusion at *p*equal 0.1, was used to derive multivariate models. Non-parametric receiver operating characteristics curve analyses were used to estimate the biomarker's performance in predicting outcomes. Areas under two or more ROC curves were compared using the DeLong criteria.

2.2. Role of funding source

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

3. Results

The main patient demographic characteristics, clinical outcomes and laboratory findings during the hospitalization are summarized in Table 1 (A and B respectively)**:** in particular, the biochemical data reported (Table 1B) have been obtained at the enrollment for the MR-proADM measurement**.** Single specimen collection for MR-proADM measurement was carried out for each patient during study period (median time elapesd from the hospital admission to MR-proADM measurement,7-days). After their discharge from the Emergency Department (ED), most of the patients (88.2%), were admitted to Infectious Disease or Internal Medicine wards. Depending on the severity of the disease, and its evolution, 65 patients (48.1%) were moved to the Intensive Care Unit (ICU) or to the Respiratory Pathophysiology ward; 16 patients (11.8%) were directly transferred from the ED to the ICU. These clinical paths were considered Intermediate Outcome in our study. Fourteen of the 135 patients (10.4%) died, the fatality being identified as Outcome.

As reported in Table 2, the patients presenting higher MR-proADM values (MR-proADM Group 1 $<$ Group 2 $<$ Group 3), were elderly (51 *vs*. 68 *vs* 75 years), developing more frequently pneumonia (75% *vs* 96% *vs* 94%), requiring more aggressive treatment (ICU admission: 40% *vs* 38% *vs* 79%), undergoing a longer period of hospitalization (13 *vs* 16 *vs* 37 days) and suffering a fatal event (0 *vs* 1 *vs* 13 subjects) (Table 2A).

On evaluating additional habit and clinical characteristics that may be relevant risk factors for a severe course of COVID-19 disease (such as smoking habit, diabetes, hypertension, dyslipidemia or metabolic syndrome),no significant difference was observed between the three patient groups (Table 2A) whereas in group 3 patients, a higher, statistically significant prevalence of stroke and vasculopathy, acute and chronic kidney failure was observed (p = 0.002 and p = 0.001, respectively).

A statistically significant difference was found between groups for concentrations of the biomarkers evaluated in the study, in particular:

Table 1
Demographic and clinical characteristics (1A), and laboratory findings (1B) of the study patients n = 135 (IQR, Interquartile Range; y, years).

Demographic and Clinical Characteristics (Table 1A)		
Gender Males, n (%); Females, n (%)	100 (74); 35 (26)	
Age Median, IQR (years)	67, 58–77	
Enrollment Period	From 12th to 24th November 2020	
MR-proADM, nmol/L	0.93, 0.64–1.46	
Time from symptoms onset to MR-proADM measurement Median, IQR (days)	12, 7–21	
Time from hospital presentation-admission to MR-proADM measurementMedian, IQR (days)	7, 2–15	
Hospital stayMedian, IQR (days)	17, 10–30	
Clinical outcomesdischarged n (%); deceased n (%)	121 (89.6); 14 (10.4)	
Laboratory Findings (Table 1B)		
Biomarker, measuring unit (Reference Interval)	**Number of Patients (%)**	**Median (IQR)**
White blood-cell count, 10^9/L(4.4–11)	135 (1 0 0)	8.6, 6.5–11.7
Lymphocyte count, 10^9/L(1.1–4.8)	132 (97.8)	1.16, 0.83–1.87
Monocyte count, 10^9/L(0.20–0.96)	132 (97.8)	0.63, 0.40–0.91
Neutrophil count, 10^9/L(1.8–7.8)	132 (97.8)	6.3, 4.36–9.70
Platelet count, 10^9/L(150–450)	135 (1 0 0)	243, 197–309
Hemoglobin, g/L(females: 123–153; males: 140–175)	135 (1 0 0)	130, 118–139
C-reactive Protein, mg/L(0–6)	134 (99.3)	39, 9–97
Procalcitonin, μg/L(0.0–0.5)	115 (85.2)	0.16, 0.05–0.39
Ferritin, μg/L(females: 11–328; males: 31–409)	108 (80.0)	757, 409–1387
D-dimer, μg/L(0–59 y: 0–250; 60–69 y: 0–300; 70–79 y: 0–350; >79 y: 0–400)	130 (96.3)	296, 176–718
High-sensitivity Troponin I, ng/L(females: 0–16; males: 0–34)	108 (80.0)	10.2, 4.1–20.0
Lactate dehydrogenase, U/L (females: 135–214; males: 135–225)	122 (90.4)	297, 239–381
Glucose, mmol/L(3.7–5.6)	131 (97.0)	6.5, 5.2–8.4
Creatinine, μmol/L(females: 45–84; males: 59–104)	135 (1 0 0)	79, 67–106
Albumin, g/L(→60 y: 35–52; 60–90: 32–46)	105 (77.8)	29, 25–33

MR-proADM ($p < 0.0001$), White-blood cell count ($p < 0.001$ for 1 *vs* 3 and 2 *vs* 3), Neutrophil count ($p < 0.001$ for 1 *vs* 3 and 2 *vs* 3), D-dimer ($p < 0.001$ for 1 *vs* 3 and 2 *vs* 3), C-reactive Protein ($p < 0.0001$ for 1 *vs* 3 and 2 *vs* 3), Procalcitonin ($p < 0.0001$ for 1 *vs* 3 and 2 *vs* 3), and hs-cTnI ($p < 0.0001$ for 1 *vs* 3 and 2 *vs* 3) (Table 2B).

At Univariate Analysis (Table 3) of demographic parameters and laboratory findings in relation to Outcome and Intermediate Outcome, it was found that most of the variables considered, in particular White blood cells and Neutrophil counts, *Log*$_{10}$D-dimer, C-reactive Protein, Lactate Dehydrogenase (LDH), Albumin, Neutrophil-to-Lymphocyte *ratio* (NLR) and MR-proADM, were significantly associated with both Outcome and Intermediate Outcome. In particular, *Log*$_{10}$d-Dimer and MR-proADM shows the highest statistically significant Odds Ratio (OR) for both Outcome and Intermediate Outcome.

At logistic regression, performed to estimate the association between the established outcomes (Outcome and Intermediate Outcome) and the predictor variables, with or without adjusting estimators for confounding variables (Age and Gender), it was found that the most significant predictors are MR-proADM (OR: 2.43 and 2.68) and *Log*$_{10}$ D-dimer (OR: 7.42 and 13.31) values, in addition to NLR (OR: 1.12 and 1.27)and LAD (OR: 1.01 and 1.01) levels. At Multivariate Model analysis, conducted with the inclusion of the parameters found to be significant at Univariate Analysis (MR-proADM, Age, NLR, LDH, Glucose, *Log*$_{10}$ d-Dimer, Gender, WBC and PCR), the combination of MR-proADM + NLR + Age, (Model 1) exhibits an Area Under Curve (AUC) for Outcome equal to 0.924 (95 %CI 0.867–0.981) while the Model 2 combining LDH + Glucose + *Log*$_{10}$D-dimer values, yielded an AUC of 0.853 (95 %CI 0.787–0.920) for Intermediate Outcome (Fig. 1a and 1b)

4. Discussion

The aim of our retrospective study was to ascertain whether circulating concentrations of Mid-Regional pro-Adrenomedullin (MR-proADM), a peptide derived from pro-adrenomedullin (ADM hormone precursor), might provide clinically relevant information on the pathophysiological mechanism and subsequent organ dysfunction induced by COVID-19. Indeed, in addition to its vasodilator properties (1), ADM exerts various protective physiological effects on the cardiovascular, respiratory, renal, immune, and neuroendocrine systems (25). Therefore, the increased expression and activity of ADM might reflect a response to organ damage and dysfunction: the measurement of circulating concentrations may provide additional clinical informations on the microcirculatory impairment and functional disorders of all inner organs induced by SARS-CoV-2 [26,27].

The population studied (Table 1), comparable for clinical and demographic characteristics to SARS-CoV-2 patients evaluated in our previous studies [28,29], seems however to suffer from fatal clinical events (death) in a significantly higher percentage (10.4 % *vs* 8.0% and 6.2%)., The median MR-proADM value, was higher than the URL for healthy subjects (0.93, 0.64–1.46 *vs*. 0.55 nmol/L) and, in33 out of 135 patients (24%), higher than the risk-stratification cut-off (1.55 mmol/L). MR-proADM seems to be closely associated with disease severity, and with the occurrence of major events a weak relationship having been demonstrated with clinical or demographics characteristics (Table 2).

The obtained results suggest that MR-proADM measurement is of additional clinical value in stratifying risk and establishing the prognosis of COVID-19 patients. In fact, on considering the two clinical outcomes defined in our study, the MR-proADM values observed in patients who died (Outcome) are significantly higher than those in the Intermediate Outcome group (2.42, 2.08–3.33 nmol/L *vs* 1.29, 0.91–2.20 nmol/L; $p = 0.0008$), with a greater prevalence of pneumonia ($p = 0.008$), longer hospitalization period ($p < 0.001$), greater number of subjects moved to the ICU and/or Respiratory Pathophysiology ward ($p < 0.001$), and higher mortality ($p < 0.001$) (Table 2).

Findings at univariate analysis (Table 3) confirm the clinical and pathophysiological relevance of the measurement of the biomarker, which provides the highest Odds Ratio (OR) for both Outcome (OR 2.48, 95 %CI 1.56–3.95) and Intermediate Outcome (OR 2.36, 95% CI 1.43–3.91), in addition to *Log*$_{10}$ d-dimer (OR 4.89, 95 %CI 1.81–13.25; OR 12.33, 95 %CI4.01–37.96 for Outcome and Intermediate Outcome respectively). Finally, the accuracy of MR-proADM in predicting Outcome (AUC 0.900, 95 %CI 0.827–0.974)and Intermediate Outcome (AUC 0.757, 95 %CI 0.662–0.851) increases slightly on adding, in the multivariate analysis, NLR and Age (Model 1-Outcome: AUC 0.916, 95 %CI 0.853–0.979; Intermediate Outcome: AUC 0.783, 0.698–0.867). Noteworthy seems to be the data provided by *Log*$_{10}$ D-dimer values: from the pathophysiological viewpoint, both biomarkers (MR-proADM and D-dimer) provide complementary information suggesting a possible prothrombotic condition in these patients.

Our study has some limitations, such as the punctual value of MR-proADM, the performance of a single centre study, the limited period of patients enrollment, the lack of clinical severity score, Moreover, we did not study the kinetic release of the endothelial biomarker, that has been evaluated in other papers [30,31] showing that the constantly higher values observed over time in non-surviving patients [29] represent the more relevant prognostic information provided by the biomarker measurement. In our study, the single MR-proADM value obtained during the patients hospitalization seem to provide a comparable and clinically relevant prognostic information.

In conclusion, it has been widely recognized that a crucial role is played by endothelial dysfunction during SARS-CoV-2 infection, the

Table 2
Demographic and Clinical Characteristics, Habits (2A) and Laboratory Findings (2B) of the three Groups.

2 A Demographic, Clinical Characteristics and Habits -

Patients Group	Patients number (%)	Gender M, n,(%) F, n (%)	Age Median, IQR (*years*)	Pneumonia n, (%)	ICU stay n, (%)	Hospital stay Median, IQR (*days*)	Death n, (%)	Smoke n, (%)	Diabetes n, (%)	Hypertension n, (%)	Dyslipidemia and Metabolic Syndrome n, (%)	Stroke and Vasculopathy n, (%)	Acute and Chronic Kidney Failure n, (%)
1	20 (15)	17 (85) 3 (15)	51, 42–60	15 (75)	8 (40)	13, 7–33	0 (0)	0 (0)	4 (20)	4 (20)	2 (10)	0 (0)	1 (5)
2	82 (61)	57 (70) 25 (30)	68, 58–76	79 (96)	31 (38)	16, 10–22	1 (1.2)	7 (8.5)	25 (30.5)	45 (54.9)	17 (20.7)	7 (8.5)	3 (3.7)
3	33 (24)	26 (79) 4 (21) §, $p = 0.367$	75, 67–80 $\chi^2 = 32.9$, $p < 0.001$ (all pairwise comparison)	31 (94) §, $p = 0.008$	26 (79) §, $p < 0.001$	37, 24–45 $\chi^2 = 25.06$, $p < 0.001$ (1 *vs* 3 and 2 *vs* 3)	13 (39.4) §, $p < 0.001$	6 (18.2) §, $p = 0.292$	6 (18.2) §, $p = 0.418$	24 (72.7) §, $p = 0.234$	4 (12.1) §, $p = 0.567$	5 (15.2) §, $p = 0.002$	9 (27.3) §, $p = 0.001$

2B. Laboratory Findings - Median, IQR - (reference range)

Patients' Group	MR-proADM, nmol/L	White blood-cell count, 10^9/L, (4.4–11)	Neutrophils count, 10^9/L, (1.8–7.8)	D-dimer, μg/L (0–59 y: 0–250; 60–69 y: 0–300; 70–79 y: 0–350; >79 y: 0–400)	CRP mg/L (0–6)	PCT μg/L (0.0–0.5)	Ferritin, μg/L (females: 11–328; males: 31–409)	hs-cTnI, ng/L	Creatinine, μmol/L
1	0.50, 0.37–0.52	7.13, 4.08–10.50	3.92, 2.39–7.90	150, 150–269	8.7, 2.9–29.2	0.04, 0.04–0.14	706, 219–1465	3.5, 2.0–7.9	69, 56–81
2	0.87, 0.69–1.13	8.10, 6.46–11.47	5.80, 4.56–8.26	249, 174–513	25.0, 6.4–88.0	0.11, 0.04–0.27	608, 418–1320	8.5, 4.3–13.5	76, 66–88
3	2.38, 1.88–3.25 $\chi^2 = 100.0$, p<0.0001	11.43, 8.88–20.01 $\chi^2 = 16.47$ p<0.0001 (1 *vs*. 3 and 2 *vs*. 3)	10.29, 7.55–19.11 $\chi^2 = 28.4$ p<0.0001 (1 *vs*. 3 and 2 *vs*. 3)	880, 425–2358 $\chi^2 = 31.1$ p<0.0001 (1 *vs*. 3 and 2 *vs*. 3)	100.0 53.0–126.7 $\chi^2 = 30.9$ p<0.0001 (1 *vs*. 3 and 2 *vs*. 3)	0.53, 0.26–1.72 $\chi^2 = 34.7$ p<0.0001 (1 *vs*. 3 and 2 *vs*. 3)	1118, 506–1840 χ^2 =2.73, p=0.254	21.0, 15.0–86.2 χ^2 = 40.6p<0.0001 (1 *vs*. 3 and 2 *vs*. 3)	126, 100–175 $\chi^2 = 31.1$ p<0.0001 (1 *vs*. 3 and 2 *vs*. 3)

Group 1 = MR-proADM ≤ 0.55 nmol/L.
Group 2 = 0.55 nmol/L < MR-proADM ≤ 1.50 nmol/L.
Group 3 = MR-proADM > 1.50 nmol/L.
IQR = Interquartile Range; M = male; F = female; § = Fisher's exact test; χ^2 = chi square test.

Table 3
Univariate analysis.

	Outcome			Intermediate Outcome		
Variable	OR	95% C.I.	p	OR	95% C.I.	p
Gender	5.08	0.64–40.35	0.124	1.44	0.66–3.15	0.359
Age	1.07	1.01–1.13	0.012	1.01	0.98–1.03	0.531
White blood- cell count	1.09	1.02–1.19	0.012	1.20	1.09–1.32	0.000
Limphocyte count	0.42	0.16–1.12	0.084	0.83	0.58–1.21	0.336
Neutrophil count	1.18	1.07–1.29	0.000	1.38	1.21–1.59	0.000
Monocyte count	0.84	0.28–2.54	0.763	1.15	0.79–1.68	0.463
Hemoglobin	0.98	0.95–1.01	0.135	0.98	0.96–1.00	0.073
Platelet count	0.99	0.99–1.00	0.227	1.00	0.99–1.00	0.616
Log$_{10}$ D-dimer	4.89	1.81–13.25	0.002	12.33	4.01–37.96	0.000
High-sensitivity Troponin I	1.01	1.00–1.02	0.011	1.01	0.99–1.02	0.128
Glucose	0.99	0.84–1.18	0.994	1.16	1.03–1.31	0.016
Procalcitonin	1.03	0.97–1.10	0.290	0.99	0.93–1.05	0.767
C-reactive Protein	1.01	1.00–1.02	0.001	1.01	1.01–1.02	0.000
Creatinine	1.00	0.99–1.00	0.293	1.00	0.99–1.00	0.190
Lactate dehydrogenase	1.00	1.00–1.01	0.007	1.01	1.00–1.01	0.000
Albumin	0.85	0.74–0.98	0.021	0.77	0.69–0.87	0.000
Ferritin	1.00	1.00–1.00	0.023	1.00	0.99–1.00	0.054
MR-proADM	2.48	1.56–3.95	0.000	2.36	1.43–3.91	0.001
NLR	1.13	1.06–1.20	0.000	1.26	1.13–1.40	0.000

n.e. = not estimable.
NLR = neutrophil-to-lymphocyte ratio.

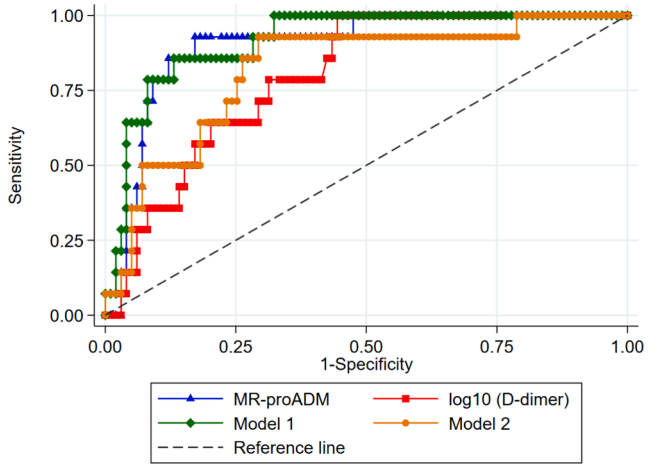

Fig. 1a. Receiver operating characteristics (ROC) analyes of MP-proADM,log_{10} D-dimer and of biomarkers combination models (Model 1 = MR-proADM + NLR + Age; Model 2 = LAD + Glucose + *Log*$_{10}$D-dimer) in prediction of the Outcome. Area under the curve analyses showed that AUC (95 %CI) were 0.900 (0.827–0.974) for MR-proADM; 0.797 (0.699–0.894) for log_{10} D-dimer; 0.916 (0.853–0.979) for Model-1 and 0.820 (0.705–0.936) for Model 2 respectively.

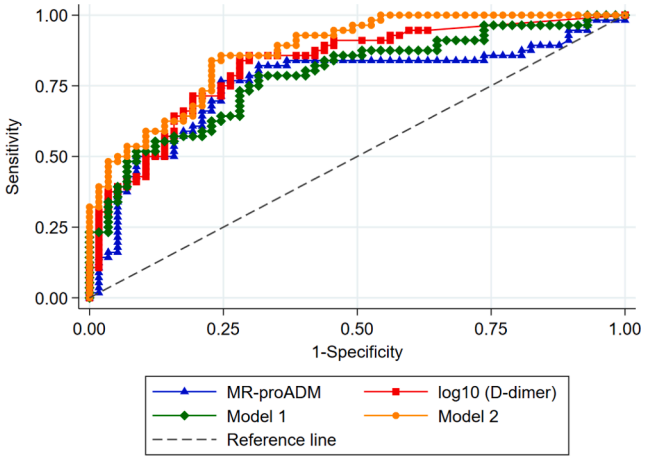

Fig. 1b. .Receiver operating characteristics (ROC) analyes of MP-proADM,log_{10} D-dimer and of biomarkers combination models (Model 1 = MR-proADM + NLR + Age; Model 2 = LDH + Glucose + *Log*$_{10}$D-dimer) in prediction of the Intermediate Outcome. Area under the curve analyses showed that AUC (95 % CI) were 0.757 (0.662–0.851) for MR-proADM; 0.822, (0.744–0.899) for log_{10} D-dimer; 0.783 (0.698–0.867) for Model-1 and 0.869 (0.806–0.932) for Model-2 respectively.

endothelium being a direct target of the virus, as well as the main actor in orchestrating a pro-inflammatory and pro-coagulant state in COVID-19 patients (12,15,26).

The aim of the present study was to evaluate in patients with COVID-19 disease, the behavior of MR-proADM in the maintenance of endothelial integrity by reducing vascular permeability. The results obtained confirm that the concentrations of this biomarker provide clinically useful information in patients with COVID-19, being particularly effective in the identification of the patients at risk of more severe disease, as well as of fatal event. As reported in other clinical situations [32], high MR-pro ADM values suggests a more severe degree of endothelium dysfunction that defines the general risk of the patients. Therefore, the measurement of the biomarker concentrations during hospitalization, and after disease remission, may provide further biochemical insight on the status of endothelial function, severity of damage, and/or confirmation of recovery.

Declaration of Competing Interest

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

References

[1] Z. Varga, A.J. Flammer, P. Steiger, M. Haberecker, R. Andermatt, A.S. Zinkernagel, M.R. Mehra, R.A. Schuepbach, F. Ruschitzka, H. Moch, Endothelial cell infection and endotheliitis in COVID-19, Lancet 395 (10234) (2020) 1417–1418.
[2] A.J.Flammer, T.Anderson, D.S.Celermajer, et al. The assessment of endothelial function: from research into clinical practice. Circulation(2012)126: 753-67.
[3] P.O. Bonetti, L.O. Lerman, A. Lerman, Endothelial dysfunction-A marker of atherosclerotic risk, Arterioscl. Throm. Vas. 23 (2) (2003) 168–175.
[4] C.M. Ferrario, J. Jessup, M.C. Chappell, D.B. Averill, K.B. Brosnihan, E.A. Tallant, D.I. Diz, P.E. Gallagher, Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2, Circulation 111 (20) (2005) 2605–2610.

[5] N. Potere, E. Valeriani, M. Candeloro, M. Tana, E. Porreca, A. Abbate, S. Spoto, A. W.S. Rutjes, M. Di Nisio, Acute complications and mortality in hospitalized patients with coronavirus disease 2019: a systematic review and meta-analysis, Crit. Care 24 (1) (2020), https://doi.org/10.1186/s13054-020-03022-1.
[6] B. Gallo Marin, G. Aghagoli , K. Lavine et al. Predictors of COVID severity: a literature review. Rev Med Virol (2021) January; doi 10.1002/rvm.2146.
[7] E. Azoulay, L. Zafrani, A. Mirouse, E. Lengliné, M. Darmon, S. Chevret, Clinical phenotypes of critically ill COVID-19 patients, Intensive Care Med 46 (8) (2020) 1651–1652.
[8] World Health Organization. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected: Interim guidance. 2020. WHO reference number: WHO/2019-nCoV/clinical/2020.4 2020.
[9] G. Lippi, E.J. Favaloro, D-dimer is associated with severity of coronavirus disease 2019: a pooled analysis, Thromb. Haemost. 120 (05) (2020) 876–878.
[10] G. Lippi, M. Plebani, B.M. Henry, Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis, Clin. Chim. Acta. 506 (2020) 145–148.
[11] https://www.aboutpharma.com/blog/2020/10/06/un-test-del-sangue-per-misurare-la-gravità-di-covid-19/ (last access: 30 June 2021).
[12] F. Vieceli Dalla Sega, F. Fortini, S. Spadaro, et al. Time course of endothelial dysfunction markers and mortality in COVID-19 patients: A pilot study. Clin. Transl. Med. (2021) Mar; 11(3): e283.
[13] K. Inoue, T. Kodama, H. Daida, Pentraxin 3: A novel biomarker for Inflammatory cardiovascular disease, Int. J. Vasc. Med. 2012 (2012) 1–6.
[14] https://www.sciencedirect.com/topics/neuroscience/pentraxin-3 (last access: 30 June 2021).
[15] M.P. Nägele, B. Haubner, F.C. Tanner, F. Ruschitzka, A.J. Flammer, Endothelial dysfunction in COVID-19: current findings and therapeutic implications, Atherosclerosis 314 (2020) 58–62.
[16] K. Kitamura, K. Kangawa, M. Kawamoto, Y. Ichiki, S. Nakamura, H. Matsuo, T. Eto, Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma, Biochem. Biophys. Res. Commun. 192 (2) (1993) 553–560.
[17] A.A. Voors, D. Kremer, C. Geven, J.M. ter Maaten, J. Struck, A. Bergmann, P. Pickkers, M. Metra, A. Mebazaa, H.-D. Düngen, J. Butler, Adrenomedullin in heart failure: pathophysiology and therapeutic application, Eur. J. Heart Fail. 21 (2) (2019) 163–171.
[18] D.C. Wilson, J.C. Schefold, J. Baldirà, T. Spinetti, K. Saeed, G. Elke, Adrenomedullin in COVID-19 induced endotheliitis, Crit. Care. 24 (1) (2020), https://doi.org/10.1186/s13054-020-03151-7.
[19] C. Ince, P.R. Mayeux, T. Nguyen, et al. The endothelium in sepsis. Shock(2016) 45 (3):259-270.
[20] N.G. Morgenthaler, J. Struck, C. Alonso, et al. Measurement of mid-regional pro-adrenomedullin in plasma with an immuno-luminometric assay. Clin. Chem.(2005) 51(10):1823-1829.
[21] S. Spoto, J.M. Legramante, M. Minieri, M. Fogolari, A. Terrinoni, E. Valeriani, C. Sebastiano, S. Bernardini, M. Ciccozzi, P.S. Angeletti, How biomarkers can improve pneumonia diagnosis and prognosis: procalcitonin and mid-regional pro-adrenomedullin, Biomark. Med. 14 (7) (2020) 549–562.
[22] S. Angeletti, S. Spoto, M. Fogolari, M. Cortigiani, M. Fioravanti, L. De Florio, B. Curcio, D. Cavalieri, S. Costantino, G. Dicuonzo, Diagnostic and prognostic role of procalcitonin (PCT) and MR-proadrenomedullin (MR-proADM) in bacterial infections, APMIS 123 (9) (2015) 740–748.
[23] F. Valenzuela Sanchez, B. Valenzuela Mendez, J.F. Rodríguez Gutierrez, R. Bohollo de Austria, J. Rubio Quiñones, L. Puget Martínez, I. Valiente Alemán, A. Estella García, Initial levels of MR-proadrenomedullin: a predictor of severity in patients with influenza a virus pneumonia, Intensive CareMed. Exp. 3 (S1) (2015), https://doi.org/10.1186/2197-425X-3-S1-A832.
[24] M. Michels, K. Djamiatun, S.M. Faradz, et al., High plasma mid-regional pro-Adrenomedullin levels in children with severe dengue virus infection, J. Clin. Virol. 50 (1) (2011) 8–12.
[25] G. Elke, F. Bloos, D.C. Wilson, et al., The use of mid-regional pro-Adrenomedullin to identify disease severity and treatment response to sepsis-a secondary analysis of a large randomized controlled trial, Crit. Care 22 (1) (2018) 79.
[26] S. Pons, S. Fodil, E. Azoulay, L. Zafrani, The vascular endothelium: the cornerstone of organ dysfunction in severe SARS-CoV-2 infection, Crit. Care 24 (1) (2020), https://doi.org/10.1186/s13054-020-03062-7.
[27] Endothelial dysfunction in Covid-19: a position paper of the ESC Working Group for Atherosclerosis and vascular Biology, and the Council of Basic Cardiovascular Science. https://doi.org/ 10.1093/cvr/cvaa230.
[28] M. Zaninotto, M.M. Mion, C. Cosma, D. Rinaldi, M. Plebani, Presepsin in risk stratification of SARS-CoV-2 patients, Clin. Chim. Acta 507 (2020) 161–163.
[29] M. Zaninotto, M.M. Mion, A. Padoan, L. Babuin, M. Plebani, Cardiac Troponin I in SARS-CoV-2-patients: The additional prognostic value of serial monitoring, Clin. Chim. Acta 511 (2020) 75–80.
[30] G. Montrucchio, G. Sales, F. Rumbolo, et al. Effectiveness of mid-regional pro-adrenomedullin (MR- proADM) as prognostic marker in COVID-19 critically ill patients: An observational prospective study. https://doi.org/10.371/journal.pone.0246771.
[31] I. Benedetti, D. Spinelli, T. Callegari, et al., High levels of mid-regional proadrenomedullin in ARDS COVID-19 patients: the experience of a single, Italian Center, Eu. Re. Med. Pharmacol. Sc. 25 (2021) 1743–1751.
[32] S. Masson, P. Caironi, E. Spanuth, et al, Presepsin /soluble CD14 subtype) and procalcitonin levels for mortality prediction in sepsis: data from the Albumin Italian Outcome Sepsis trial. Crical Care (2014) 18: R6. https://ccforum.com/content/18/1/R6 (last access: 30 June 2021).