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The vasoactive peptide MR-pro-adrenomedullin in COVID-19 patients: an observational study

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Abstract

Objectives: Midregional pro-adrenomedullin (MR-proADM) is a vasoactive peptide with key roles in reducing vascular hyperpermeability and thereby improving endothelial stability during infection. While MR-proADM is useful for risk stratification in patients with sepsis, clinical data about prediction accuracy in patients with severe acute respiratory syndrome coronavirus 2 disease (COVID-19) is currently missing.

Methods: We included consecutively adult patients hospitalized for confirmed COVID-19 at a tertiary care center in Switzerland between February and April 2020. We investigated the association of MR-proADM levels with in-hospital mortality in logistic regression and discrimination analyses.

Results: Of 89 included COVID-19 patients, 19% (n=17) died while in the hospital. Median admission MR-proADM levels (nmol/L) were increased almost 1.5-fold increased in non-survivors compared to survivors (1.3 [interquartile range IQR 1.1–2.3]) vs. 0.8 [IQR 0.7–1.1]) and showed good discrimination (area under the curve 0.78). An increase of 1 nmol/L of admission MR-proADM was independently associated with a more than fivefold increase in in-hospital mortality (adjusted odds ratio of 5.5, 95% confidence interval 1.4–21.4, p=0.015). An admission MR-proADM threshold of 0.93 nmol/L showed the best prognostic accuracy for in-hospital mortality with a sensitivity of 93%, a specificity of 60% and a negative predictive value of 97%. Kinetics of follow-up MR-proADM provided further prognostic information for in-hospital treatment.

Conclusions: Increased levels of MR-proADM on admission and during hospital stay were independently associated with in-hospital mortality and may allow a better risk stratification, and particularly rule-out of fatal outcome, in COVID-19 patients.

Keywords: biomarker; COVID-19; in-hospital mortality; prognostic markers; SARS-CoV-2.

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus, is currently affecting millions of people worldwide. Coronavirus disease 2019 (COVID-19) is caused by SARS-CoV-2, was declared a global pandemic on March 11th, 2020 by the World Health Organization (WHO). COVID-19 is highly contagious and key mechanisms that may have a role in the pathophysiology of multi-organ injury secondary to infection with SARS-CoV-2 include direct viral toxicity, endothelial cell damage and thrombo-inflammation, dysregulation of the immune response, and possibly a dysregulation of the renin–angiotensin–aldosterone system [1, 2]. The increased incidence of cardiovascular and thromboembolic complications [3, 4], the immune cell deactivation [5, 6], and sepsis-like multiple organ failure [7, 8] suggest the involvement of multiple pathways. There is preliminary evidence that

most of the patients (around 85%) show only mild symptoms of upper respiratory tract infection, but 10% of patients progress to severe lower respiratory manifestations requiring hospital admittance, and 5% require intensive care unit treatment due to severe disease progression, primarily characterized by pulmonary and multi-organ failure [9].

Besides protective and preventive measures to reduce and minimize large-scale outbreaks, a timely assessment of the individual risk profile of a patient presenting to the emergency department may help to improve early decisions about triaging, site-of-care and initiation of COVID-19 targeted treatments. Having early objective measures of a patient's risk for in-hospital mortality is thus important to best allocate hospital resources. Herein, prognostic biomarkers may help to estimate a patient's individual risk and provide objective and measurable results within short time after a patient's hospital admission. Such markers may also improve the understanding of the pathophysiology behind COVID-19 infection and its adverse outcomes.

Whilst different cytokines and blood markers have been compared in patients with different severities of COVID-19 and biochemical biomarker alterations in COVID-19 have been analyzed [10], no study till date has investigated the potential role of adrenomedullin (ADM) during the host response to a SARS-CoV-2-infection [11]. ADM and its mid-regional prohormone fragment midregional pro-adrenomedullin (MR-proADM) has been linked to endothelial dysfunction and the risk for organ dysfunction in patients with sepsis and infection of the lung as it is directly related to the status of the endothelium [12–15]. Previous studies corroborated that MR-proADM serves as an accurate marker for risk assessment in patients with pneumonia, sepsis and critical illness and thereby improving clinical scores such as the sequential organ failure assessment score (SOFA-Score) [16–18]. Therefore, MR-proADM is mostly used to improve the identification of organ dysfunction and disease progression to sepsis or septic shock [19]. MR-proADM has also been shown to be a good predictor for short- and long-term mortality in patients with lower respiratory tract infections and sepsis [20–22]. Previously, a MR-proADM cut-off of 0.87 nmol/L has been proposed in a large multinational study to best classify patients with assumed infection into a low or high risk group concerning mortality [23]. Saeed et al. [23] showed in two cohorts that a MR-proADM concentration of 0.87 nmol/L is a valuable cut-off to be able to increase outpatient treatment by at least 15%, with decreased hospital readmission rates and no increase in mortality. The aim of this study was to investigate the association and prognostic accuracy of initial and follow-up MR-proADM levels with in-hospital mortality in patients with confirmed SARS-CoV-2 infection.

Materials and methods

Study design and setting

This prospective observational study included all consecutively hospitalized adult patients (≥18 years) due to a confirmed SARS-CoV-2 infection at the Cantonal Hospital Aarau, a tertiary care medical center in Switzerland, between February 26th, 2020 and April 30th, 2020. The study was approved by the local Ethical Committee (EKZN, 2020-01306) and performed in conformance with the Declaration of Helsinki ethical guidelines.

Baseline characteristics of this cohort was recently published elsewhere [24]. In brief, COVID-19 was defined by a positive real-time reverse transcription polymerase chain reaction (RT-PCR) taken from nasopharyngeal swabs or lower respiratory tract specimens, according to the WHO guidance [25]. In addition, most patients showed typical clinical symptoms (e.g., respiratory symptoms with or without fever, and/or pulmonary infiltrates and/or anosmia/dysgeusia). All data of interest were assessed as part of the clinical routine during the hospitalization.

Data collection

Clinical information, including socio-demographics and comorbidities, home medications and COVID-19-specific in-patient medication were assessed until hospital discharge or death and extracted from the electronic health records. Experimental antiviral treatment was recorded if given and included Hydroxychloroquine (alone or in combination with Azithromycin) and sometimes Tocilizumab. For all patients the age-adjusted Charlson comorbidity index [26] and the Clinical Frailty Score (up to nine points) [27] were calculated as part of routine clinical care. Comorbidities were also assessed through chart review and based on International Statistical Classification of Diseases and Related Health Problems codes (ICD10). Subsequently, patient outcomes including in-hospital mortality, admission to the intensive care unit (ICU), length of hospital stay (LOS), and length of ICU stay were collected by chart review. Laboratory test results were available according to clinical routine. MR-proADM was batch-tested later and therefore, not available to the treating team during the index hospitalization.

Endpoint with study objective

The primary endpoint was all cause in-hospital mortality. The main objective of this study was to investigate the ability of MR-proADM to predict in-hospital mortality in confirmed COVID-19 patients, in order to classify patients at high or low risk for in-hospital mortality.

Measurements of MR-proADM

After hospital admission plasma and serum samples were collected using BD Vacutainer® heparin and SST tubes. Routine left-over

samples were stored at −80 °C until analysis. Results from routine laboratory tests were recorded. MR-proADM was assessed in batch using a commercially available automated fluorescent sandwich immunoassay (KRYPTOR™, B.R.A.H.M.S Thermo Fisher Scientific, Germany), as described in detail elsewhere [28, 29]. Briefly, the immunoassay employs two polyclonal antibodies to the amino acids 45–92 of pre-pro-adrenomedullin, the MR-proADM and has a limit of detection (LOD) of 0.05 nmol/L [29]. The functional assay sensitivity, defined as the MR-proADM concentration with an inter-assay coefficient of variation of <20%, was 0.25 nmol/L. Values for the analytes followed a Gaussian distribution in healthy individuals without significant differences between males and females [29]. The laboratory technicians who measured MR-proADM were blinded to the characteristics of the patients and the characteristics of the study.

Different time points during hospitalization were analyzed, depending on the available data:

- T_0 (initial blood draw upon hospital admission).
- T_1 (day 3/day 4).
- T_2 (day 5/day 6).
- T_3 (day 7/day 8).

Statistical analyses

Discrete variables are expressed as frequency (percentage) and continuous variables as medians with interquartile ranges (IQR) or mean with standard deviation. Multivariable logistic regression model was used to examine the association of MR-proADM levels with the primary endpoint. As predefined, regression models were adjusted for gender and age-adjusted Charlson comorbidity Index. Odds ratios (OR) and corresponding 95% confidence intervals (CIs) were reported as a measure of association and C-statistics (area under the operating receiver curve (ROC-AUC) as a measure of discrimination. We also validated the prognostic value of different pre-defined MR-proADM cut-offs based on previous studies in other populations, namely 0.75 nmol/L [13, 30], 0.87 nmol/L [23], 1.5 nmol/L [13, 30] as well as 2.5 nmol/L [31] and analyzed cut-offs representing median of the investigated time points. Survival analyses and estimation of adjusted Cox-proportional hazard ratios were used to compare in-hospital mortality according to different MR-proADM-cutoffs. Additionally, we assessed sensitivity, specificity, positive and negative predictive values of different MR-proADM cut-offs to predict in-hospital mortality. Groups were compared with Wilcoxon rank sum test. A two-sided p-value of <0.05 was considered significant. Statistical analysis was performed using Stata 15.1 (StataCorp, College Station, TX, USA).

Results

A total of 103 patients were hospitalized with confirmed COVID-19, these included direct admissions (n=74) and 29 transfers from other hospitals (three cases from France, one case from the Canton Ticino, 25 cases from regional hospitals not accepting COVID-19 admissions or in whom treatment at a tertiary care hospital was indicated). Four patients had to be excluded from the analysis due to declined general informed consent, in nine cases aliquots for biomarker analysis were not available. One patient was still hospitalized at the time of the analysis and therefore excluded from the data set. Figure 1 provides an overview of the study flow.

Baseline characteristics

Baseline characteristics in the overall cohort and stratified data according to the primary endpoint are summarized in Table 1. Median age was 67 years (IQR 58–74) and 35% (n=31) were female. A total of 22% (n=20) of patients were taking angiotensin-converting enzyme inhibitors before hospitalization, whilst 19% (n=17) had angiotensin II receptor blockers and few patients were taking corticosteroids or other immunosuppressive treatments. Patients had a high burden of comorbidities with a median age-adjusted Charlson comorbidity index of three points and a median frailty score of three points. The most common comorbidities included hypertension (58%, n=52), chronic kidney disease (27%, n=24), and obesity (29%, n=26). Overall, 49% of patients received experimental treatment (mostly Hydroxychloroquine, rarely Ritonavir-boosted Lopinavir). A total of 26% (n=23) of patients developed severe COVID-19 progression characterized by need for ICU treatment, of whom 18 received mechanical ventilation.

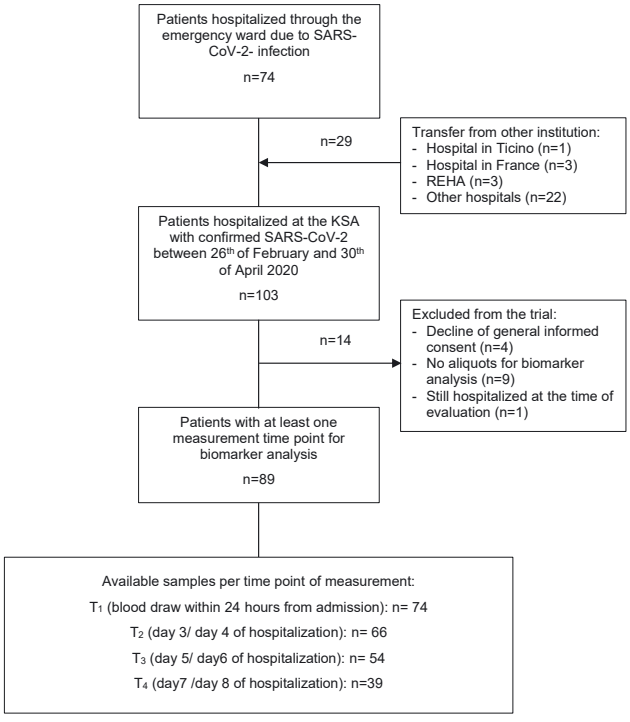

Figure 1: Flow chart of the study.
KSA, cantonal hospital Aarau; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Overall, patients hospitalized due to COVID-19 had a median length of hospital stay (LOS) of nine days (IQR 5.0–18.0). 19% (n=17) of the hospitalized patients died during hospitalization.

A majority of patients presented with a severe clinical course, particularly regarding the respiratory system with an increased respiratory rate and evidence of compromised oxygenation. C-reactive protein was elevated with a

Table 1: Demographic data, comorbidities, in-hospital treatment and in-hospital endpoints in the study population.

	Overall n=89	Survivors n=72	Non-survivors n=17	p-Value
Socio-demographics				
Age, years median (IQR)	67.0 (58.0–74.0)	63.0 (55.5–74.0)	74.0 (69.0–80.0)	**<0.01**
Female gender, n (%)	31 (35)	30 (42)	1 (6)	**<0.01**
Nationality, n (%)				
France	3 (4)	3 (4)	0 (0)	0.80
Italy	6 (7)	5 (7)	1 (6)	
Switzerland	56 (63)	46 (64)	10 (59)	
Turkey	4 (4)	4 (6)	0 (0)	
Others	20 (22)	14 (19)	6 (35)	
Pre-existing risk-factors and medication				
Active smoker, n (%)	6 (9)	5 (9)	1 (8)	0.95
Corticosteroid use, n (%)	2 (2)	1 (1)	1 (6)	0.26
Immunosuppressant, n (%)	4 (4)	2 (3)	2 (12)	0.11
Angiotensin converting enzyme-inhibitor, n (%)	20 (22)	14 (19)	6 (35)	0.16
Angiotensin II receptor blockers, n (%)	17 (19)	13 (18)	4 (24)	0.61
Pre-admission history				
Symptom onset before admission (days), median (IQR)	8.0 (5.0–10.0)	8.0 (4.0–11.0)	7.0 (5.0–8.0)	0.47
Transfer from another hospital, n (%)	27 (30)	21 (29)	6 (35%)	0.62
Comorbidities				
Age-adjusted Charlson comorbidity index, median (IQR)	3.0 (2.0–6.0)	3.0 (2.0–6.0)	5.0 (3.0–9.0)	**<0.01**
Clinical frailty score, median (IQR)	3.0 (2.0–4.0)	3.0 (2.0, 4.0)	3.0 (3.0–4.0)	0.27
Active malignancy/cancer, n (%)	9 (10)	5 (7)	4 (24)	**0.04**
Hypertension, n (%)	52 (58)	39 (54)	13 (76)	0.09
Coronary artery disease, n (%)	23 (26)	16 (22)	7 (41)	0.11
Congestive heart failure, n (%)	3 (3)	3 (4)	0 (0)	0.39
Asthma, n (%)	14 (16)	11 (15)	3 (18)	0.81
COPD, n (%)	7 (8)	4 (6)	3 (18)	0.10
Obstructive sleep apnea, n (%)	12 (13)	9 (13)	3 (18)	0.58
Solid organ transplant recipient, n (%)	1 (1)	1 (1)	0 (0)	0.63
Rheumatic disease, n (%)	2 (2)	1 (1)	1 (6)	0.26
Chronic kidney disease, n (%)	24 (27)	16 (22)	8 (47)	**0.04**
Obesity (BMI > 30 kg/m^2), n (%)	26 (29)	22 (31)	4 (24)	0.57
Diabetes mellitus, n (%)	21 (24)	17 (24)	4 (24)	0.99
In-hospital treatment				
Treatment specification, n (%)				
Hydroxychloroquine	36 (40)	27 (38)	9 (53)	**0.04**
Hydroxychloroquine + azithromycin	3 (3)	3 (4)	0 (0)	
Hydroxychloroquine + tocilizumab	1 (1)	1 (1)	0 (0)	
Lopinavir/ritonavir	2 (2)	2 (3)	0 (0)	
Tocilizumab	2 (2)	0 (0)	2 (12)	
Symptomatic treatment only	45 (51)	39 (54)	6 (35)	
Antibiotic treatment, n (%)	38 (43)	25 (35)	13 (76)	**<0.01**
ICU care, n (%)	23 (26)	16 (22)	7 (41)	0.11
Need for mechanical ventilation, n (%)	18 (78)	12 (75)	6 (86)	0.09
Overall length of hospital stay (days), median (IQR)	9.0 (5.0–18.0)	9.0 (5.0–12.5)	15.0 (5.0–24.0)	0.08

BMI, body-mass-index; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; IQR, interquartile range. p-Values in bold: statistically significant.

median level of 88.5 mg/L (IQR 38.5, 149.0) while procalcitonin (PCT) levels were low (median of 0.11 µg/L [0.05, 0.34]).

Association of MR-proADM levels and in-hospital mortality

Median admission MR-proADM levels were almost 1.5-fold higher in non-survivors compared to survivors (1.3 nmol/L [IQR 1.1–2.3] vs. 0.8 nmol/L [IQR 0.7–1.1]). A 1 nmol/L increase in admission MR-proADM levels was independently associated with a more than five times higher risk of in-hospital mortality OR of 5.5 (95% CI 1.4–21.4, p=0.015 [Table 2]). MR-proADM assessed on admission showed a good discrimination performance for in-hospital mortality (AUC of 0.78).

Furthermore, prognostic accuracy of MR-proADM to predict in-hospital mortality was analyzed for different cut-offs (Table 3). Based on the Youden Index, we found an optimal cut-off at 0.93 nmol/L which was close to the median with a sensitivity of 92.9% (95% CI 66.1–99.8%) and a specificity of 60% (95% CI 46.5–72.4%). Furthermore, the cut-off at 0.93 nmol/L revealed the highest negative predictive value of 97.3% (95% CI 85.5–99.9%). Results were similar for other previously proposed cut-offs (i.e., at 0.75 and 0.87 nmol/L). The use of higher cut-offs such as 1.5 and 2.5 nmol/L showed a lower sensitivity (42.9 and 21.4%, respectively) but higher specificity and positive predictive values.

Performing time to event analyses, we found a higher survival rate among patients with an admission MR-proADM level below 0.75 nmol/L as compared with those having a MR-proADM level between 0.75 and 1.5 nmol/L (HR 3.1 [95% CI 0.3–24.5]) or >1.5 nmol/L (HR 4.8 [95% CI 1.4–16.6]). Also in patients with an admission MR-proADM level below 0.87 nmol/L and below 0.93 nmol/L a higher survival rate was found compared with those with MR-proADM levels ≥0.87 nmol/L (HR 9.9 [95% CI 1.2–82.0]) and ≥0.93 nmol/L (HR 11.7 [95% CI 1.4–97.7]) (Figure 2).

Kinetics of serial measurements of MR-proADM

The kinetics of MR-proADM levels during the follow-up period stratified by survival status is shown in Figure 3. MR-proADM values in non-survivors were significantly higher compared to survivors at every measured time point. Importantly, in survivors, MR-proADM remained low during the whole follow-up period, whereas in non-survivors MR-proADM showed a step-wise increase after baseline. MD-MR-proADM assessed during follow-up showed also good discrimination for in-hospital mortality. The best value was observed around day 5/6 of hospitalization (time point 3) with an AUC of 0.92.

Discussion

This prospective study involving patients with confirmed COVID-19 in the early phase of the pandemic aimed to investigate the prognostic accuracy of initial and follow-up MR-proADM levels for predicting in-hospital mortality. We found that levels of admission MR-proADM, a marker that reflects permeability and endothelial damage, increased 1.5-fold in patients with a fatal outcome and were thus strongly associated with in-hospital mortality, also in statistical models adjusted for age, gender and comorbid-burden. Further, when looking at the kinetics of MR-proADM, we also found a further increase in its level in non-survivors while survivors had lower levels that remained low during follow-up. These results suggest that MR-proADM may be helpful in the early risk stratification (on hospital admission) and in monitoring of patients with COVID-19. Results indicated that the high prognostic value of MR-proADM particularly allows safe rule-out of in-hospital mortality in patients with low MR-proADM levels, which could potentially improve patient triage in the ED, and early discharge of patients on the medical ward.

Early identification of COVID-19 patients at high risk for disease aggravation is crucial in order to predict severe disease progression, triaging and reduce COVID-19 associated mortality. Yet, prediction rules for patient's individual risk profile as well as the ability to predict outcomes are still lacking. Whilst several pro-inflammatory cytokines and prognostic markers have already been investigated in patients with COVID-19, no study has examined the prognostic value of MR-proADM for the identification of COVID-19 patients at high risk for mortality. However, MR-proADM holds great promise as a biomarker in COVID-19 as it plays a key role in reducing vascular permeability and promotes endothelial stability and integrity following severe infection, and thus may help in identifying patients at risk of COVID-19 induced endotheliitis. Indeed, a recent study investigating gene up-regulation in patients with systemic capillary leak syndrome, characterized by plasma leakage into peripheral tissue and transient episodes of hypotensive

Table 2: Univariable and multivariable logistic regression analyses for different MR-proADM cut-offs at different time points.

	Survivors n=72	Non-survivors n=17	p-Value	AUC	Univariable OR (95% CI), p-Value	Multivariable[a] OR (95% CI), p-Value
MR-proADM time point 0 (within 24 h from admission), n=74						
MR-proADM overall (nmol/L), median (IQR)	0.8 (0.7–1.1)	1.3 (1.1–2.3)	**<0.01**	0.78	3.2 (1.3–8.1), **p=0.012**	5.5 (1.4–21.4), **p=0.015**
MR-proADM cut-offs (nmol/L), n (%)						
<0.75	20 (33%)	1 (7%)	**0.02**		Reference	Reference
>0.75	32 (53%)	7 (50%)			4.4 (0.50–38.3), p=0.182	3.6 (0.4–33.9), p=0.265
≥1.5	8 (13%)	6 (43%)			15.0 (1.6–145.2), **p=0.019**	14.4 (1.0–202.3), **p=0.048**
MR-proADM 0.87-cut-off (nmol/L), n (%)						
<0.87	33 (55%)	1 (7%)	**<0.01**		Reference	Reference
≥0.87	27 (45%)	13 (93%)			15.9 (1.9–129.3), **p=0.010**	11.8 (1.2–112.4), **p=0.032**
MR-proADM median-cutoff (nmol/L), n (%)						
<0.93	36 (60%)	1 (7%)	**<0.01**		Reference	Reference
≥0.93	24 (40%)	13 (93%)			19.5 (2.4–159.0), **p=0.006**	14.4 (1.2–139.8), **p=0.021**
MR-proADM time point 1 (day 3/day 4 of hospitalization), n=66						
MR-proADM overall (nmol/L), median (IQR)	1.0 (0.8–1.5)	2.5 (1.4–4.0)	**<0.01**	0.84	2.8 (1.4–5.6), **p=0.003**	2.8 (1.3–6.0), **p=0.006**
MR-proADM cut-offs (nmol/L), n (%)						
<0.75	11 (21%)	0 (0%)	**<0.01**		NA	NA
>0.75	28 (54%)	4 (29%)			NA	NA
≥1.5	13 (25%)	10 (71%)			NA	NA
MR-proADM[§] 0.87-cut-off (nmol/L), n (%)						
<0.87	21 (40%)	0 (0%)	**<0.01**		NA	NA
≥0.87	31 (60%)	14 (100%)			NA	NA
MR-proADM median-cut-off (nmol/L), n (%)						
<1.1	32 (62%)	2 (14%)	**<0.01**		Reference	Reference
≥1.1	20 (38%)	12 (86%)			9.6 (1.9–47.4), **p=0.006**	7.5 (1.4–41.3), **p=0.021**
MR-proADM time point 2 (day 5/day 6 of hospitalization), n=54						
MR-proADM overall (nmol/L), median (IQR)	0.9 (0.6–1.4)	3.8 (2.6–8.3)	**<0.01**	0.92	2.0 (1.2–3.4), **p=0.006**	1.9 (1.2–3.3), **p=0.012**
MR-proADM cut-offs (nmol/L), n (%)						
<0.75	16 (36%)	0 (0%)	**<0.01**		NA	NA
>0.75	19 (43%)	1 (10%)			NA	NA
≥1.5	9 (20%)	9 (90%)			NA	NA
MR-proADM 0.87-cut-off (nmol/L), n (%)						
<0.87	21 (48%)	0 (0%)			NA	NA
≥0.87	23 (52%)	10 (100%)	**<0.01**		NA	NA
MR-proADM median-cut-off (nmol/L), n (%)						
<1.1	28 (64%)	0 (0%)	**<0.01**		NA	NA
≥1.1	16 (36%)	10 (100%)			NA	NA
MR-proADM time point 3 (day 7/day 8 of hospitalization), n=39						
MR-proADM overall (nmol/L), median (IQR)	1.3 (0.8–1.8)	2.5 (1.6–9.2)	**0.01**	0.82	1.3 (1.0–1.7), p=0.055	1.3 (1.0–1.7), p=0.087
MR-proADM cut-offs (nmol/L), n (%)						
<0.75	8 (24%)	0 (0%)	0.09		NA	NA
>0.75	13 (39%)	1 (17%)			NA	NA
≥1.5	12 (36%)	5 (83%)			NA	NA
MR-proADM 0.87-cut-off (nmol/L), n (%)						
<0.87	10 (30%)	0 (0%)			NA	NA
≥0.87	23 (70%)	6 (100%)	0.1		NA	NA
MR-proADM median-cut-off (nmol/L), n (%)						
<1.3	19 (58%)	1 (17%)	0.07		Reference	Reference
≥1.3	14 (42%)	5 (83%)			6.8 (0.7–64.7), p=0.096	4.7 (0.5–48.7), p=0.194

AUC, area under the curve; CI, confidence interval; IQR, interquartile range; MR-proADM, midregional pro-adrenomedullin; OR, odds ratio.
[a]Adjusted for, gender and age-adjusted Charlson comorbidity index. p-Values in bold: statistically significant.

Table 3: Prognostic accuracy of different MR-proADM cut-offs at baseline.

MR-proADM cut-off values	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive predictive value, % (95% CI)	Negative predictive value, % (95% CI)
0.75 nmol/L	92.9 (66.1–99.8)	33.3 (22.7–46.7)	24.5 (13.8–38.3)	95.2 (76.2–99.9)
0.87 nmol/L	92.9 (66.1–99.8)	55.0 (41.6–67.9)	32.5 (18.6–49.1)	97.1 (84.7–99.9)
0.93 nmol/L	92.9 (66.1–99.8)	60.0 (46.5–72.4)	35.1 (20.2–52.5)	97.3 (85.8–99.9)
1.5 nmol/L	42.9 (17.7–71.1)	86.7 (75.4–94.1)	42.9 (17.7–71.1)	86.7 (75.4–94.1)
2.5 nmol/L	21.4 (4.7–50.8)	98.3 (91.1–100.0)	75.0 (19.4–99.4)	84.3 (73.6–91.9)

CI, confidence interval; MR-proADM, midregional pro-adrenomedullin.

shock and edema, found that MR-proADM was not only one of the most up-regulated genes, but that subsequent application to endothelial cells resulted in a protective effect on vascular barrier function [19]. Our study evaluated the prognostic performance of initial and follow-up measurements of MR-proADM in confirmed COVID-19 patients treated in a Swiss tertiary care hospital. Our results suggest that MR-proADM levels together with clinical evaluation and further laboratory findings may help to early identify and classify patients presenting to the emergency department into low and high risk for adverse outcome. Particularly, by applying a cut-off of 0.93 nmol/L revealed a high negative predictive value, allowing safe exclusion of low-risk patients.

The prognostic relevance of MR-proADM was already analyzed and proved in several prior studies investigating patients with community-acquired pneumonia [32–34], chronic obstructive pulmonary disease (COPD) [35, 36], and cardiovascular diseases [37, 38]. Our analysis is in line with this research and further expands the field to COVID-19, mirroring a severe disease course as well as a particular pathophysiology involving different organ systems as demonstrated in recent studies [2]. Interestingly, we also demonstrated that a previously proposed cut-off level of 0.87 nmol/L showed high sensitivity and negative likelihood ratio to rule out in-hospital mortality in COVID-19 patients [23]. Similar to our analysis, a previous study showed that MR-proADM had a high prognostic accuracy with an AUC of 0.81 for the prediction of ICU mortality in patients with sepsis [28]. Zhoue et al. [6] suggested that already existing risk scores like CRB-65 (confusion-respiratory rate – blood pressure-age ≥65 years) and quick sepsis related organ failure assessment (qSOFA) may be helpful to identity COVID-19 affected patients with a poor prognosis, but the rate of false-positive patients was limiting the study's conclusion. Consequences would be a higher than needed demand of limited resources. In this respect, other studies, not referring to COVID-19, confirmed that MR-proADM is more accurate compared to risk scores alone and that the addition of MR-proADM might improve the accuracy of these risk scores when used in combination [16, 31]. Further, MR-proADM can be easily performed by a biomarker assay in contrast to scores that are often complex to calculate.

We found an optimal cut-off at 0.93 nmol/L in our analyzed cohort that can be recommended for the assessment of disease severity, disease progression, risk for in-hospital mortality and also for decisions regarding patient disposition. This cut-off is close to already defined and validated MR-proADM cut-offs at 0.75 [13, 30] and 0.87 nmol/L [23]. Higher cut-offs between 1.5 and 2.5 nmol/L showed too low sensitivity. Thus, an initial MR-proADM value below 0.93 nmol/L assessed within the first 24 h after hospital admission should be interpreted as low-risk concerning in-hospital mortality and might predict a mild course of COVID-19. Whether this proposed MR-proADM cut-off will improve hospital resource allocation and might help to facilitate site-of-care decisions at an early stage of disease, needs further prospective investigation. However, this would be essential in regions where healthcare systems reach their maximum capacity during peaks of the COVID-19 pandemic.

Limitations

This study has a number of limitations: Firstly, the number of analyzed cases was small due to the single center design of this study. Secondly, clinical data was limited and not all evaluated laboratory parameters and characteristics were available for all patients, resulting in few missing data. Thirdly, due to incomplete data, smoking and body mass index, which represent two important confounders of MR-proADM levels were not addressed in the adjusted regression models. Despite these, our findings suggests that MR-proADM can potentially assist in identifying the most severe cases and clinical decision makings in COVID-19.

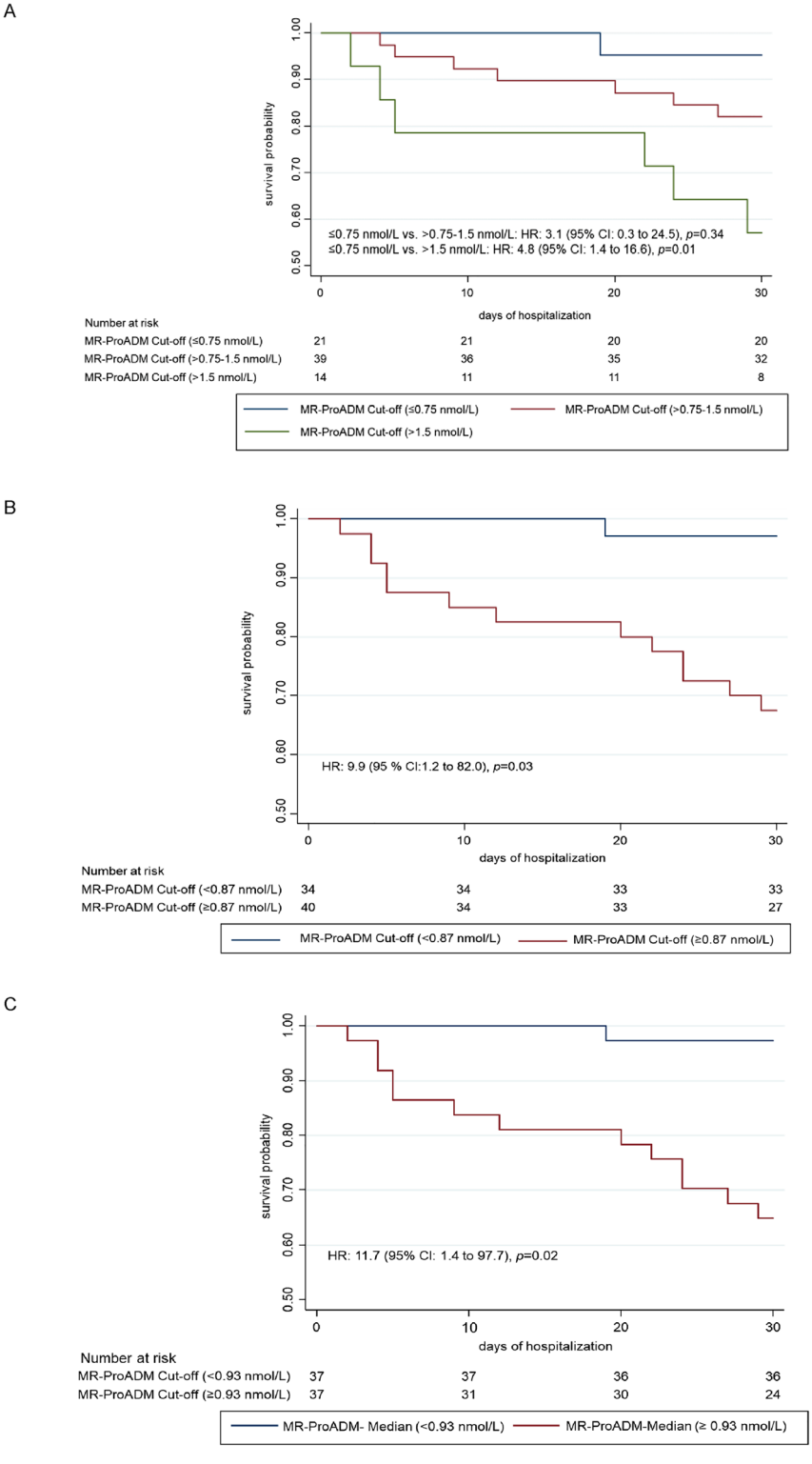

Figure 2: Survival according to different MR-proADM cut-offs and median within 24 h from admission.
(A) ≤0.75 nmol/L, >0.75–1.5 nmol/L, >1.5 nmol/L; (B) <0.87 nmol/L, ≥0.87 nmol/L; (C) Median: <0.93 nmol/L, ≥0.93 nmol/L. MR-proADM, mid-regional pro-adrenomedullin.

Conclusions

To our knowledge this is the first study evaluating the prognostic accuracy of MR-proADM in patients with COVID-19 that confirms the high prognostic value of MR-proADM and allows good discrimination, particularly to rule-out in-hospital mortality in patients at low-risk. Thus, when using this marker together with clinical and

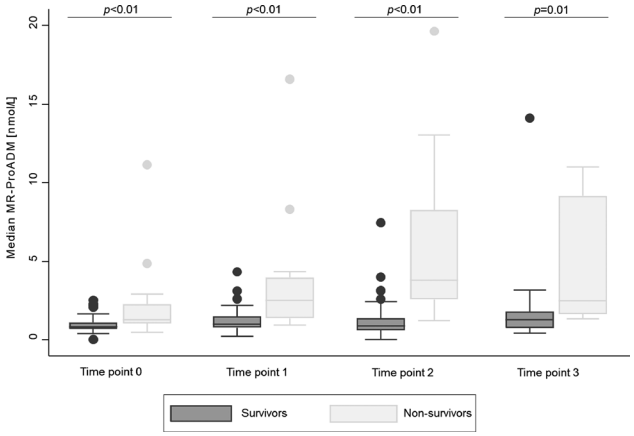

Figure 3: Median MR-proADM values at the different measurement time points for survivors and non-survivors.
MR-proADM, midregional pro-adrenomedullin.

other laboratory findings, MR-proADM may improve early risk stratification, a major obstacle in pandemics.

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Author contributions: CG, AK, and PS had the idea, wrote the protocol and initiated the study. CG managed the trial and collected data. LB and AHL managed the laboratory investigations. CG, DK and SH performed the statistical analyses and CG, DK and PS drafted the manuscript. AK, SH, AC, LB, AHL, KS and BM, amended and commented on the manuscript. All authors approved the final version. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: PS and BM received research support paid to the Institution from Thermofisher, bioMerieux, Roche Diagnostics, Nestle Health Science and Abbott Nutrition. PS received sponsoring for biomarker-measurement costs (reagents) from BRAHMS. KS reports grands from Thermofisher and Pfizer. All other authors reported no conflicts of interest.
Informed consent: Informed consent was obtained from all individuals included in this study.
Ethical approval: The study was approved by the local Ethical Committee (EKZN, 2020-01306) and performed in conformance with the Declaration of Helsinki ethical guidelines.

References

1. Li R, Pei S, Chen B, Song Y, Zhang T, Yang W, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). Science 2020;368: 489–93.
2. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. Nat Med 2020;26:1017–32.
3. Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Biondi-Zoccai G, et al. Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 pandemic. J Am Coll Cardiol 2020;75:2352–71.
4. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, et al. COVID-19 and cardiovascular disease. Circulation 2020;141: 1648–55.
5. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal Transduct Target Ther 2020;5:33.
6. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395: 1054–62.
7. Zaim S, Chong JH, Sankaranarayanan V, Harky A. COVID-19 and multiorgan response. Curr Probl Cardiol 2020;45:100618.
8. Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, et al. SARS-CoV-2 and viral sepsis: observations and hypotheses. Lancet 2020;395: 1517–20.
9. 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. J Am Med Assoc 2020.
10. Ciaccio M, Agnello L. Biochemical biomarkers alterations in coronavirus disease 2019 (COVID-19). Diagnosis (Berl) 2020;7: 365–72.
11. Wilson DC, Schefold JC, Baldira J, Spinetti T, Saeed K, Elke G. Adrenomedullin in COVID-19 induced endotheliitis. Crit Care 2020;24:411.
12. Valenzuela-Sanchez F, Valenzuela-Mendez B, Rodriguez-Gutierrez JF, 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.
13. Kutz A, Hausfater P, Amin D, Amin A, Canavaggio P, Sauvin G, et al. The TRIAGE-ProADM score for an early risk stratification of medical patients in the emergency department – development based on a multi-national, prospective, observational study. PLoS One 2016;11:e0168076.
14. Renaud B, Schuetz P, Claessens YE, Labarere J, Albrich W, Mueller B. Proadrenomedullin improves Risk of Early Admission to ICU score for predicting early severe community-acquired pneumonia. Chest 2012;142:1447–54.

15. Labarere J, Schuetz P, Renaud B, Claessens YE, Albrich W, Mueller B. Validation of a clinical prediction model for early admission to the intensive care unit of patients with pneumonia. Acad Emerg Med 2012;19:993–1003.
16. Schuetz P, Wolbers M, Christ-Crain M, Thomann R, Falconnier C, Widmer I, et al. Prohormones for prediction of adverse medical outcome in community-acquired pneumonia and lower respiratory tract infections. Crit Care 2010;14:R106.
17. Bellia C, Agnello L, Lo Sasso B, Bivona G, Raineri MS, Giarratano A, et al. Mid-regional pro-adrenomedullin predicts poor outcome in non-selected patients admitted to an intensive care unit. Clin Chem Lab Med 2019;57:549–55.
18. Agnello L, Bivona G, Parisi E, Lucido GD, Iacona A, Ciaccio AM, et al. Presepsin and midregional proadrenomedullin in pediatric oncologic patients with febrile neutropenia. Lab Med 2020;51: 585–91.
19. Elke G, Bloos F, Wilson DC, Brunkhorst FM, Briegel J, Reinhart K, et al. The use of mid-regional proadrenomedullin to identify disease severity and treatment response to sepsis – a secondary analysis of a large randomised controlled trial. Crit Care 2018;22:79.
20. Huang DT, Angus DC, Kellum JA, Pugh NA, Weissfeld LA, Struck J, et al. Midregional proadrenomedullin as a prognostic tool in community-acquired pneumonia. Chest 2009;136:823–31.
21. Christ-Crain M, Morgenthaler NG, Stolz D, Muller C, Bingisser R, Harbarth S, et al. Pro-adrenomedullin to predict severity and outcome in community-acquired pneumonia [ISRCTN04176397]. Crit Care 2006;10:R96.
22. Kruger S, Ewig S, Giersdorf S, Hartmann O, Suttorp N, Welte T, et al. Cardiovascular and inflammatory biomarkers to predict short- and long-term survival in community-acquired pneumonia: results from the German Competence Network, CAPNETZ. Am J Respir Crit Care Med 2010;182:1426–34.
23. Saeed K, Wilson DC, Bloos F, Schuetz P, van der Does Y, Melander O, et al. 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.
24. Gregoriano C, Koch D, Haubitz S, Conen A, Fux CA, Mueller B, et al. Characteristics, predictors and outcomes among 99 patients hospitalised with COVID-19 in a tertiary care centre in Switzerland: an observational analysis. Swiss Med Wkly 2020; 150:w20316.
25. WHO. Clinical management of severe acute respiratory infection when novel coronovirus (nCov) infection is suspected; interim guidance. Geneva: WHO; 2020.
26. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol 1994;47: 1245–51.
27. Juma S, Taabazuing MM, Montero-Odasso M. Clinical frailty scale in an acute medicine unit: a simple tool that predicts length of stay. Can Geriatr J 2016;19:34–9.
28. 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: R816–24.
29. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. Clin Chem 2005;51:1823–9.
30. Albrich WC, Dusemund F, Ruegger K, Christ-Crain M, Zimmerli W, Bregenzer T, et al. Enhancement of CURB65 score with proadrenomedullin (CURB65-A) for outcome prediction in lower respiratory tract infections: derivation of a clinical algorithm. BMC Infect Dis 2011;11:112.
31. Legramante JM, Mastropasqua M, Susi B, Porzio O, Mazza M, Miranda Agrippino G, et al. Prognostic performance of MR-pro-adrenomedullin in patients with community acquired pneumonia in the Emergency Department compared to clinical severity scores PSI and CURB. PLoS One 2017;12:e0187702.
32. Cavallazzi R, El-Kersh K, Abu-Atherah E, Singh S, Loke YK, Wiemken T, et al. Midregional proadrenomedullin for prognosis in community-acquired pneumonia: a systematic review. Respir Med 2014;108:1569–80.
33. Gordo-Remartinez S, Calderon-Moreno M, Fernandez-Herranz J, Castuera-Gil A, Gallego-Alonso-Colmenares M, Puertas-Lopez C, et al. Usefulness of midregional proadrenomedullin to predict poor outcome in patients with community acquired pneumonia. PLoS One 2015;10:e0125212.
34. Espana PP, Capelastegui A, Mar C, Bilbao A, Quintana JM, Diez R, et al. Performance of pro-adrenomedullin for identifying adverse outcomes in community-acquired pneumonia. J Infect 2015;70: 457–66.
35. Grolimund E, Kutz A, Marlowe RJ, Vogeli A, Alan M, Christ-Crain M, et al. Long-term prognosis in COPD exacerbation: role of biomarkers, clinical variables and exacerbation type. COPD 2015; 12:295–305.
36. Schuetz P, Marlowe RJ, Mueller B. The prognostic blood biomarker proadrenomedullin for outcome prediction in patients with chronic obstructive pulmonary disease (COPD): a qualitative clinical review. Clin Chem Lab Med 2015;53:521–39.
37. Shah KS, Marston NA, Mueller C, Neath SX, Christenson RH, McCord J, et al. Midregional proadrenomedullin predicts mortality and major adverse cardiac events in patients presenting with chest pain: results from the CHOPIN trial. Acad Emerg Med 2015;22:554–63.
38. Yuyun MF, Narayan HK, Ng LL. Prognostic significance of adrenomedullin in patients with heart failure and with myocardial infarction. Am J Cardiol 2015;115:986–91.