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Endothelium-associated biomarkers mid-regional proadrenomedullin and C-terminal proendothelin-1 have good ability to predict 28-day mortality in critically ill patients with SARS-CoV-2 pneumonia: A prospective cohort study

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ABSTRACT

Purpose: We assessed the ability of mid-regional proadrenomedullin (MR-proADM) and C-terminal proendothelin-1 (CT-proET-1) to predict 28-day mortality in critically ill patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia.
Methods: Biomarkers were collected during the first seven days in this prospective observational cohort study. We investigated the relationship between biomarkers and mortality in a multivariable Cox regression model adjusted for age and SOFA score.
Results: In 105 critically ill patients with confirmed SARS-CoV-2 pneumonia 28-day mortality was 28.6%. MR-proADM and CT-proET-1 were significantly higher in 28-day non-survivors at baseline and over time. ROC curves revealed high accuracy to identify non-survivors for baseline MR-proADM and CT-proET-1, AUC 0.84, (95% CI 0.76–0.92), $p < 0.001$ and 0.79, (95% CI 0.69–0.89), $p < 0.001$, respectively. The AUC for prediction of 28-day mortality for MR-proADM and CT-proET-1 remained high over time. MR-proADM ≥1.57 nmol/L and CT-proET-1 ≥ 111 pmol/L at baseline were significant predictors for 28-day mortality (HR 6.80, 95% CI 3.12–14.84, $p < 0.001$ and HR 3.72, 95% CI 1.71–8.08, p 0.01).
Conclusion: Baseline and serial MR-proADM and CT-proET-1 had good ability to predict 28-day mortality in critically ill patients with SARS-CoV-2 pneumonia.
Trial registration: NEDERLANDS TRIAL REGISTER, NL8460.

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List of abbreviations

COVID-19	corona virus disease
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
ICU	intensive care unit
ARDS	acute respiratory distress syndrome
ADM	adrenomedullin
ET-1	Endothelin-1
MR-proADM	mid-regional proadrenomedullin
CAP	community-acquired pneumonia
CT-proET-1	C-terminal proendothelin-1
ETZ	Elisabeth -Tweesteden Ziekenhuis
METC	Medisch Ethische Toetsingscommissie
RT-PCR	real-time reverse transcriptase-polymerase chain reaction
WHO	World Health Organization
PEEP	positive end expiratory pressure
CPAP	continuous positive airway pressure
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology Statement
EDTA	Ethylene Diamine Tetra Acetic acid
CV	coefficient of variation
CLSI EP17A	Clinical & Laboratory Standards Institute Evaluation Protocol 17A
IQR	interquartile range
ROC	curve receiver operating characteristics curve
SOFA	Sequential Organ failure Assessment
APACHE IV	Acute Physiological and Chronic Health Evaluation IV
CURB-65	Confusion blood Urea nitrogen Respiratory rate Blood pressure age 65 or older
LOS	length of stay
cTnT	cardiac troponin T
PCT	procalcitonin
AUC	area under the curve
CI	confidence interval
PPV	positive predictive values
NPV	negative predictive value
LR+	positive likelihood ratio
LR-	negative likelihood ratio
HR	hazard ratio
CRP	C-reactive protein
ED	emergency department
SAPS II	Simplified Acute Physiology Score II
ACE2	angiotensin converting enzyme 2
TNF-alpha	tumor necrosis factor alpha

1. Introduction

Corona virus disease 2019 (COVID-19), the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1,2], has turned out to be an enormous challenge to intensive care units (ICU) worldwide [3-5]. A substantial part of the patients deteriorated quickly and needed to be admitted to the ICU with signs and symptoms consistent with acute respiratory failure and/or acute respiratory distress syndrome (ARDS). Many of those patients had prolonged treatment periods at the ICU and large variations in mortality (26–61.5%) were reported [3-5]. Up to 25% of the patients with COVID-19 developed prothrombotic complications, like deep venous thrombosis or pulmonary embolisms [6].

As virus-induced endothelial dysfunction and damage, endotheliitis, has been proposed as one of the potential mechanisms of COVID-19 [7,8], there may be a role for endothelium related adrenomedullin (ADM) and Endothelin-1 (ET-1). The midregion and C-terminal part of these prohormones are more stable [9,10], and therefore measuring the midregion or C-terminal prohormone is more feasible for clinical purposes. Mid-regional proadrenomedullin (MR-proADM) is the midregion of the prohormone of ADM [9]. ADM is a peptide generated by endothelial and vascular smooth muscle cells, with anti-inflammatory effects on vascular endothelial cells, protecting the microcirculation against endothelial permeability in sepsis [11,12]. In lower respiratory tract infections MR-proADM levels were rapidly induced and baseline MR-proADM measurements proved to be a good predictor of short and long-term survival in community-acquired pneumonia (CAP) patients admitted to the emergency room or ICU [13,14]. C-terminal proendothelin-1 (CT-proET-1) is the C-terminal part of the prohormone of ET-1 [10]. ET-1 is a strong vasoconstrictor peptide and pro-inflammatory cytokine that is released from activated endothelial cells [15]. Elevated concentrations of CT-pro-ET-1 were found in patients with CAP and sepsis [14,16,17].

In the present study we aimed to investigate the prognostic value of MR-proADM and CT-proET-1 at baseline to predict 28-day mortality in critically ill patients with confirmed SARS-CoV-2 pneumonia. Secondary aim was testing of these two biomarkers over time in the ICU.

2. Material and methods

2.1. Study design and selection criteria

In a single centre prospective observational cohort study, we enrolled patients with confirmed SARS-CoV-2 pneumonia, admitted to the ICU of the Elisabeth-Tweesteden (ETZ) Hospital (Tilburg, the Netherlands) from March 11 until May 27, 2020. The study protocol was approved by the METC Brabant (Medisch Ethische Toetsingscommissie Brabant) (Tilburg, the Netherlands) (NW 2020–86). Informed consent was achieved from participating patients. Inclusion criteria were adults ≥18 years of age, admitted to the ICU with pneumonia and SARS-CoV-2 infection confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay of nasopharyngeal or bronchial swabs. Patients who did not meet the inclusion criteria or without informed consent were excluded. SARS-CoV-2 pneumonia was defined according to the interim guidance of World Health Organization (WHO) for clinical management of COVID-19 [18]. Both severe and critical type diseases defined by the WHO interim guidance were included. Severe disease; severe pneumonia was designated when the patients had clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) plus one of the following symptoms or physiological signs: respiratory rate > 30 breaths/min, severe respiratory distress or $SpO_2 < 90\%$ on room air. Chest imaging (radiograph, CT scan or lung ultrasound) may assist in diagnosis and identify or exclude pulmonary complications [18]. Critical disease; ARDS was designated when the symptoms of pneumonia lasted less than one week or when there were new or worsening symptoms, chest imaging showed bilateral ground glass lobar opacities, lobar or lung collapse, or nodules and respiratory failure could not be solely explained by cardiac failure or fluid overload. Additionally, signs of oxygenation impairment ($PaO_2/FiO_2 \leq 300$ mmHg with positive end expiratory pressure (PEEP) ≥ 5 cmH_2O or continuous positive airway pressure (CPAP) ≥ 5 cmH_2O) needed to be present [18]. All patients received selective decontamination of the digestive tract. Prophylactic antibiotics were given during the first four days to all patients as part of this decontamination strategy. STROBE (Strengthening the Reporting of Observational Studies in Epidemiology Statement) guidelines for reporting observational studies were followed [19]. Only cases with an index test comprising MR-proADM and CT-pro-ET-1 at baseline were included. Primary outcome measure was the prediction of 28-day mortality by baseline biomarker. Testing of the changes of biomarkers in COVID-19 patients over time in the ICU was the secondary aim of the study.

2.2. Procedures

Clinical data, microbiological and laboratory results were collected on a daily basis in patients enrolled in the study. These data were obtained from the accepting hospitals if patients had an early transfer to another ICU. Additional blood samples were collected into EDTA-tubes on a daily basis for seven days, or until discharge or death. Plasma was separated by centrifugation and stored in aliquots at −80 °C. MR-proADM and CT-pro-ET-1 levels were measured using an automated immunofluorescent sandwich assay on a Kryptor Compact Plus analyzer (BRAHMS AG, Henningsdorf, Germany) at the central diagnostic laboratory in Maastricht, the Netherlands. The Kryptor measures the signal

that is emitted from an immunocomplex by time-resolved amplified cryptate emission. MR-proADM and CT-proET-1 assays have a limit of detection of 0.05 nmol/L and 2.94 pmol/L. The functional sensitivity (lowest value with an interassay coefficient of variation (CV) < 20% as described by the manufacturer) of 0.25 nmol/L (MR-proADM) and 9.78 pmol/L (CT-proET-1), respectively. Imprecision of the assays were verified according to the Clinical & Laboratory Standards Institute Evaluation Protocol 17-A (CLSI EP17-A), using a low and high sample, measured for five days in triplicate. Intra and Inter CV values were all ≤10% for MR-proADM and CT-proET-1.

2.3. Statistical analysis

All non-normally distributed data (Kolmogorov-Smirnov test $p < 0.05$) were expressed as median (with interquartile range, IQR) or as number of patients (percentage) where appropriate. Patient characteristics and outcomes were compared using a Mann-Whitney *U* -test for skewed distributed continuous variables and a chi-square test was used to analyse categorical variables. To analyse the time course of MR-proADM and CT-proET-1 profiles in the different patient groups a linear mixed-models analysis for repeated measures was used, including time and 28-day survival as independent factors. Testing for interaction was performed. The association between mortality and each biomarker or severity score at admission was assessed using area under the receiver operating characteristics (ROC) curves. Optimal cut-off points were calculated for each biomarker and severity score. Continuous variables were transformed to dichotomous variables (below or equal and above the cut-off point) and then included in the Cox regression model to study the effects on outcome. Considering the total number of deaths within 28 days in our study ($n = 30$) and to avoid overfitting in the model, MR-proADM and CT-proET-1 were tested in a separate multivariable model with two other variables: age and Sequential Organ failure Assessment (SOFA) score. The cumulative survival was analysed by applying the Kaplan-Meier curves and differences in mortality were compared with the Log Rank test. All tests were two-sided and a *p*-value <0.05 was considered statistically significant. All data were analysed using a statistical software package (SPSS Inc., version 24, Chicago, IL, USA).

3. Results

3.1. Descriptive characteristics of the patients

A cohort of 133 critically ill patients with a suspected SARS-CoV-2 pneumonia was identified during the study period. In 105 patients, SARS-CoV-2 was confirmed by RT-PCR (in 88 nasopharyngeal swabs and 17 tracheal aspirations), informed consent was achieved and MR-proADM and CT-pro-ET-1 levels were measured at admittance and subsequent days. The patient flow diagram shows the flow of patients along with the primary endpoint of 28-day survival (Fig. 1). Fifty-five (52.4%) patients were transferred to another ICU due to national government policy in order to distribute COVID-19 patients over the country. The median ICU-time before transfer to another ICU was three days (IQR 2–5).

Demographics and clinical characteristics of the 105 included patients are shown in Table 1. Twenty-three (22%) of the 105 included patients had severe pneumonia and 82 (78%) fulfilled the Berlin criteria for ARDS [20], with severe ARDS in 19 (18%) patients. There was a low number of coinfections with bacteria, fungi or other viruses (table S1, appendix p 2). *Enterococcus spp* ($n = 5$) and *S. pneumonia* ($n = 3$) were most frequently found as bacterial coinfections and *A fumigatus* was found in deep respiratory tract secretions in seven patients.

The 28-day all-cause mortality was 28.6%. Patients were divided in survivors and non-survivors with regards to survival up to 28 days. Both groups were comparable except for older age, higher SOFA score, Acute Physiological and Chronic Health Evaluation IV (APACHE IV) and Confusion blood Urea nitrogen Respiratory rate Blood pressure age 65 or older (CURB-65) score in non-survivors. Duration of invasive

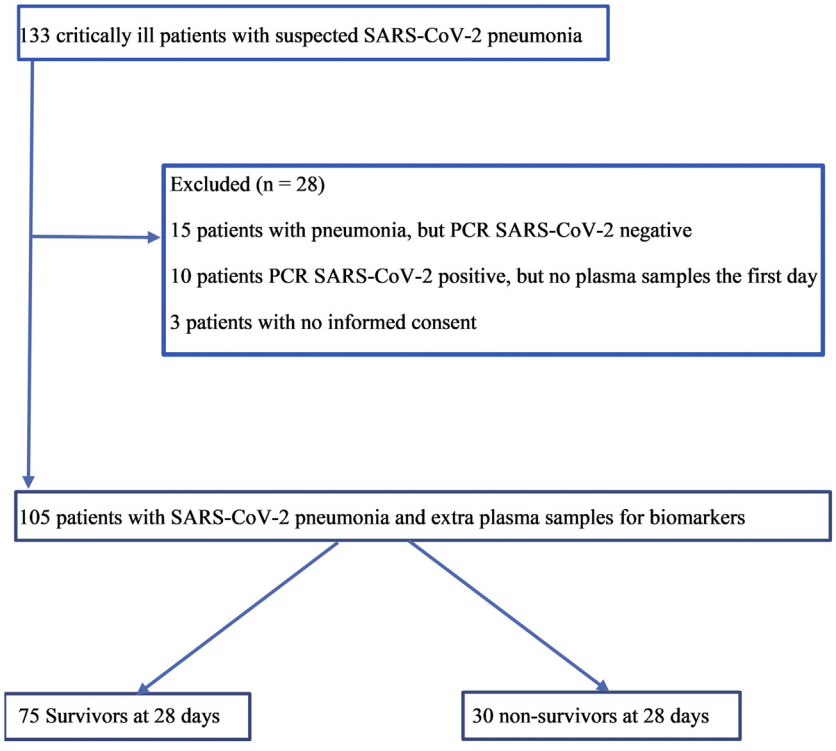

Fig. 1. Legends: Patient flow diagram.

Table 1
Characteristics of patients with SARS-CoV-2 pneumonia with regards to survival up to 28 days.

	Total ($N = 105$)	Survivors ($N = 75$)	Non-survivors ($N = 30$)	p value
Age (years) (median, IQR)	68 (59–74)	65 (58–73)	72 (67–76)	0.01
Male gender (N, %)	80 (76.2%)	56 (74.7%)	24 (80%)	0.56
BMI (kg/m^2) (median, IQR)	28.4 (25.8–32.7)	28.3 (25.8–32.3)	29.2 (25.5–33.3)	0.65
Pre-existing comorbidities (N, %)				
Obesity (BMI ≥ 30 kg/m^2)	42 (40%)	29 (38.7%)	13 (43.3%)	0.66
Hypertension	29 (27.9%)	20 (27%)	9 (30%)	0.76
Congestive heart failure	17 (16.2%)	11 (14·7.7%)	6 (20%)	0.50
COPD	16 (15.2%)	12 (16%)	4 (13.3%)	0.73
Diabetes mellitus	24 (22.9%)	17 (22.7%)	7 (23.3%)	0.94
Cerebrovascular disease	7 (6.7%)	3 (4%)	4 (13.3%)	0.08
Malignancy	15 (14.3%)	10 (13.3%)	5 (16.7%)	0.66
Chronic renal disease	5 (4.8%)	2 (2.7%)	3 (10%)	0.11
Auto-immune disorder	8 (7.6%)	7 (9.3%)	1 (3.3%)	0.30
Initial symptoms (N, %)				
Fever (temp >38.0 °C)	79 (75.2%)	54 (72%)	25 (83.3%)	0.22
Cough	98 (93.3%)	71 (94.7%)	27 (90%)	0.39
Sputum	24 (22.9%)	14 (18.7%)	10 (33.3%)	0.11
Dyspnea	86 (81.9%)	61 (81.3%)	25 (83.3%)	0.81
Nausea or vomiting	15 (14.3%)	12 (16%)	3 (10%)	0.43
Diarrhoea	19 (18.1%)	16 (21.3%)	3 (10%)	0.17
Myalgia	15 (14.3%)	12 (16%)	4 (10%)	0.43
Time course of illness – days				
Time from illness onset to ICU admission (days) (median, IQR)	8 (7–11)	8 (7–11)	7 (5–12)	0.06
Time from RT-PCR diagnosis to ICU admission (days) (median, IQR)	0 (−2.5–1)	-1 (−3–0)	0 (0–1)	0.01
Severity of illness at baseline				
Sepsis-3, sepsis (N, %)	102 (97.1%)	72 (96%)	30 (100%)	0.27
Sepsis-3, septic shock (N, %)	11 (10.5%)	6 (8%)	5 (16.7%)	0.19
SOFA (points) (median, IQR)	6 (3–7)	5 (3–6)	7 (4–7)	0.01
APACHE IV (points) (median, IQR)	47 (40–59)	43 (35–53)	53 (45–71)	<0.001
CURB-65 (points) (median, IQR)	2 (1–2)	1 (0–2)	2 (2–3)	<0.001
PaO_2/FiO_2 ratio, mmHg	163 (119–194)	167 (116–199)	162 (132–177)	0.51
Therapy during ICU (N, %)				
HFNO (only)	7 (6.7%)	5 (6.7%)	2 (6.7%)	1.00
IMV	98 (93.3%)	70 (93.3%)	28 (93.3%)	1.00
Prone position ventilation	58 (55.2%)	41 (54.7%)	17 (56.7%)	0.85
Vasopressor	87 (82.9%)	60 (80%)	27 (90%)	0.22
CRRT	9 (8.6%)	4 (5.3%)	5 (16.7%)	0.06
Anti-COVID-19 treatment				
- Chloroquine only	79 (75.2%)	59 (78.7%)	20 (66.7%)	0.20
- Chloroquine + Lopinavir/Ritonavir	26 (24.8%)	16 (21.3%)	10 (33.3%)	0.20
- Methylprednisolone	6 (5.7%)	3 (4%)	3 (10%)	0.23
- IL-1RA	2 (1.9%)	1 (1.3%)	1 (3.3%)	0.50
Outcome (median, IQR)				
Duration IMV (days)	14 (9–26)	19 (10−30)	10 (3–17)	<0.001
ICU LOS (days)	17 (10−32)	24 (12–35)	11 (4–18)	<0.001
Hospital LOS (days)	23 (12–37)	30 (18–44)	12 (6–18)	<0.001
Biomarkers at baseline (median, IQR)				
WBC, 10E9/L	8.2 (6.1–11.2)	7.9 (6.0–11.3)	8.8 (6.5–11.2)	0.51
Neutrophil, 10E9/L	5.9 (4.0–8.9)	5.9 (3.8–8.9)	6.8 (4.2–9.2)	0.45
Lymphocyte, 10E9/L	0^0.7(0.5–0.9)	0.8 (0.5–1.0)	0.7 (0.4–0.9)	0.22
Platelets, 10E9/L	227 (180–287)	228 (1749–278)	223 (178–305)	0.94
D-dimer (ng/mL)	1381 (797–4080)	1279 (751–3405)	2223 (1173–11,838)	0.06
cTnT (ng/mL)	0.02 (0.01–0.03)	0.01 (0.01–0.02)	0.03 (0.02–0.07)	<0.001
CRP (mg/L)	141 (90–207)	141 (91–196)	146 (89–240)	0.60
PCT (ng/mL)	0.5 (0.2–1.1)	0.4 (0.2–0.9)	0.9 (0.3–2.4)	0.04
Ferritin (mcg/L)	1320 (720–2317)	1115 (583–1917)	1449 (1047–3604)	0.06
Lactate (mmol/L)	1.2 (0.9–1.6)	1.1 (0.8–1.5)	1.6 (1.1–1.9)	0.01
MR-proADM (nmol/L)	1.16 (0.85–1.71)	1.01 (0.80–1.28)	1.88 (1.35–2.64)	<0.001
CT-proET-1 (pmol/L)	93.5 (72.1–122.9)	84.2 (66.7–101.1)	132.1 (100.7–156.2)	<0.001

Legends: All continuous data are presented as median (interquartile range) and categorical data as number (percentage). BMI: body mass index, COPD: Chronic obstructive pulmonary disease, SOFA: Sequential organ failure assessment, APACHE IV: Acute physiology and chronic health evaluation IV, CURB-65: Confusion blood Urea nitrogen Respiratory rate Blood pressure age 65 or older, HFNO: high flow nasal oxygen, IMV: Invasive mechanical ventilation, CRRT: continuous renal replacement therapy, IL-1RA: Recombinant interleukin-1 receptor antagonist, LOS: Length of stay, WBC: White blood cells, cTnT: cardiac Troponin T, CRP: C-reactive protein, PCT: Procalcitonin, MR-proADM: Mid-regional proadrenomedullin, CT-proET-1: C-terminal pro-endothelin-1.

mechanical ventilation, Length of stay (LOS) at the ICU and hospital were, due to early death, significant lower in 28-day non-survivors.

3.2. Association between baseline MR-proADM, CT-proET-1 and 28-day mortality

Non-survivors at 28 days had significant higher concentrations MR-proADM and CT-proET-1 at baseline than survivors (Table 1). Baseline cardiac Troponin T (cTnT), procalcitonin (PCT) and lactate concentrations were also significant higher in non-survivors. There were no significant differences in other biomarkers at baseline between 28-day survivors and non-survivors (Table 1). ROC curves revealed that baseline MR-proADM and CT-proET-1 had high accuracy to identify non-survivors, area under the curve (AUC) 0.84, (95% confidence interval (CI) 0.76–0.92), $p < 0.001$ and 0.79, (95% CI 0.69–0.89), $p < 0.001$, respectively (Table 2)(Fig. 2). CURB-65 and cTnT had comparable high

Table 2
Prediction of 28-day mortality by clinical score and biomarker at baseline.

	AUC (95% CI)	*p* value	cut-off	sens	spec	PPV	NPV	LR+	LR-
SOFA	0.66 (0.54–0.78)	0.01	7	76%	57%	81%	49%	1.77	0.42
APACHE IV	0.73 (0.63–0.83)	<0.001	52	71%	53%	79%	43%	1.51	0.55
CURB-65	0.78 (0.68–0.87)	<0.001	3	88%	47%	80%	61%	1.57	0.26
D-dimer	0.63 (0.50–0.75)	0.06	NA	NA	NA	NA	NA	NA	NA
cTnT	0.76 (0.66–0.86)	<0.001	0.025	77%	70%	87%	55%	2.57	0.33
CRP	0.53 (0.41–0.66)	0.60	NA	NA	NA	NA	NA	NA	NA
PCT	0.63 (0.51–0.75)	0.04	1.0	77%	50%	80%	45%	1.54	0.46
Ferritin	0.64 (0.50–0.78)	0.06	NA	NA	NA	NA	NA	NA	NA
Lactate	0.68 (0.57–0.79)	0.01	1.5	73%	57%	81%	46%	1.70	0.47
MR-proADM	0.84 (0.76–0.92)	<0.001	1.57	88%	67%	87%	69%	2.67	0.18
CT-proET-1	0.79 (0.69–0.89)	<0.001	111	83%	63%	85%	59%	2.24	0.27

Legends: AUC: area under the curve, sens: sensitivity, spec: specificity, PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio, SOFA: Sequential organ failure assessment, APACHE IV: Acute physiology and chronic health evaluation IV, CURB-65: Confusion blood Urea nitrogen Respiratory rate Blood pressure age 65 or older, cTnT: cardiac Troponin T, PCT: Procalcitonin, MR-proADM: Mid-regional proadrenomedullin, CT-proET-1: C-terminal pro-endothelin-1.

AUCs in ROC analysis and SOFA, APACHE IV and other biomarkers had lower AUCs (Table 2). When MR-proADM or CT-proET-1 at baseline were combined with SOFA, APACHE IV or CURB-65, the combination of MR-proADM and CURB-65 yielded the highest AUC (0.86. 95% CI 0.78–0.94, $p < 0{\cdot}001$) (table S2, appendix p 3) (fig. S1, appendix p 6). The combination of baseline MR-proADM and CT-proET-1 did not yield a higher AUC (table S2, appendix p 3). Optimal cut-off points were calculated for each biomarker and sensitivity, specificity, positive and negative predictive values (PPV, NPV) and positive and negative likelihood ratios (LR+, LR-) for each severity score and biomarker were calculated. MR-proADM, CT-proET-1 and cTnT yielded the highest LR+ (Table 2). Continuous variables were transformed to dichotomous variables and included in the univariable Cox regression analysis. Univariable Cox regression analysis demonstrated that, MR-proADM ≥1.57 nmol/L, CT-proET-1 ≥ 111 pmol/L and cTnT ≥0.025 ng/mL had the strongest association with increased risk of 28-day mortality compared to patients with values below the cut-off point (table S3, appendix p 4). MR-proADM and CT-proET-1 were separately included in a multivariable Cox regression model with age and SOFA as co-predictors (Table 3). MR-proADM ≥1.57 nmol/L and CT-proET-1 ≥ 111 pmol/L were significant predictors for 28-day mortality with high hazard ratios (HR 6.80, 95% CI 3.12–14.84, $p < 0.001$ and HR 3.72, 95% CI 1.71–8.08, p 0.01). Patients with baseline MR-proADM and CT-proET-1 equal or above their cut-off point had increased risk of 28-day mortality in Kaplan-Meier analysis compared with the values below the cut-off points (fig. S2 a-b, appendix p 7–8).

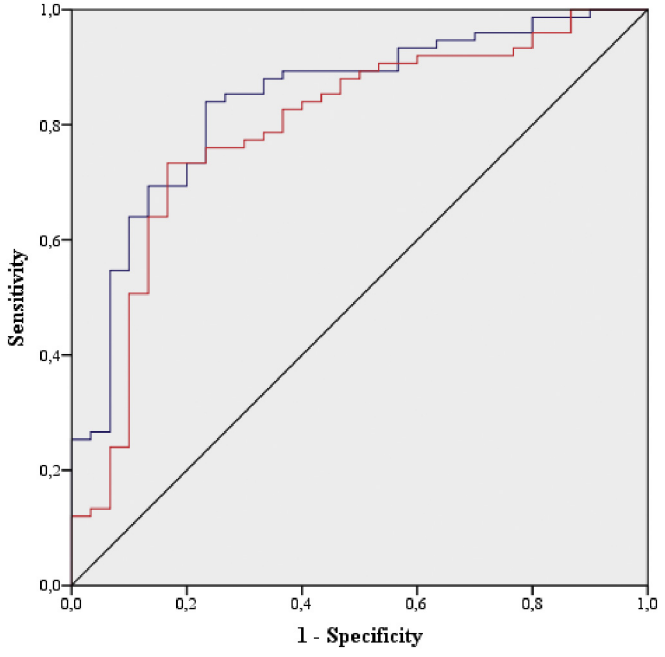

Fig. 2. Legends: ROC curve for biomarker at baseline in predicting 28-day mortality. Blue line = MR-proADM (AUC 0.84, 95% CI 0.76–0.92, $p < 0.001$), red line = CT-pro-ET-1 (AUC 0.79, 95% CI 0.69–0.89, $p < 0.001$), black line = reference line.

3.3. Dynamic changes in MR-proADM and CT-proET-1 during ICU stay

Time dependent analysis of MR-proADM, CT-proET-1, PCT, and C-reactive protein (CRP) were performed. MR-proADM levels were significantly different between non-survivors and survivors, with higher levels MR-proADM in non-survivors (p 0.01). MR-proADM increased faster over time in non-survivors, as demonstrated by a significant time*28-day survival interaction term, (p 0.01) (Fig. 3a). CT-proET-1 levels were significantly different between non-survivors and survivors, with higher levels in non-survivors ($p < 0.001$). These differences in CT-proET-1 levels between non-survivors and survivors were constant over time, with a non-significant time*28-day survival interaction term (p 0.43) (Fig. 3b). Moreover, the AUC for prediction of 28-day non-survivors remained high over days for MR-proADM and CT-proET-1 (table S4, appendix p 5). There were no significant differences in PCT

Table 3
Multivariable Cox regression models for the prediction of 28-day mortality with baseline biomarker values.

	Patients (N)	Events (N)	Multivariable analysis	
			HR (95% CI)	*P* value
Model 1				
Age	105	30	1.07 (1.01–1.13)	0.03
SOFA day 1	105	30		
< 7			1.0 (Reference)	
≥ 7			2.36 (1.13–4.92)	0.02
MR-proADM day 1	105	30		
< 1.57 nmol/L			1.0 (Reference)	
≥ 1.57 nmol/L			6.80 (3.12–14.84)	<0.001
Model 2				
Age	105	30	1.05 (0.99–1.11)	0.07
SOFA day 1	105	30		
< 7			1.0 (Reference)	
≥ 7			2.41 (1.16–5.01)	0.02
CT-proET-1 day 1	105	30		
< 111 pmol/L			1.0 (Reference)	
≥ 111 pmol/L			3.72 (1.71–8.08)	0.01

Legends: HR = hazard ratio, CI = confidence interval, SOFA: Sequential organ failure assessment, MR-proADM: Mid-regional proadrenomedullin, CT-proET-1: C-terminal pro-endothelin-1.

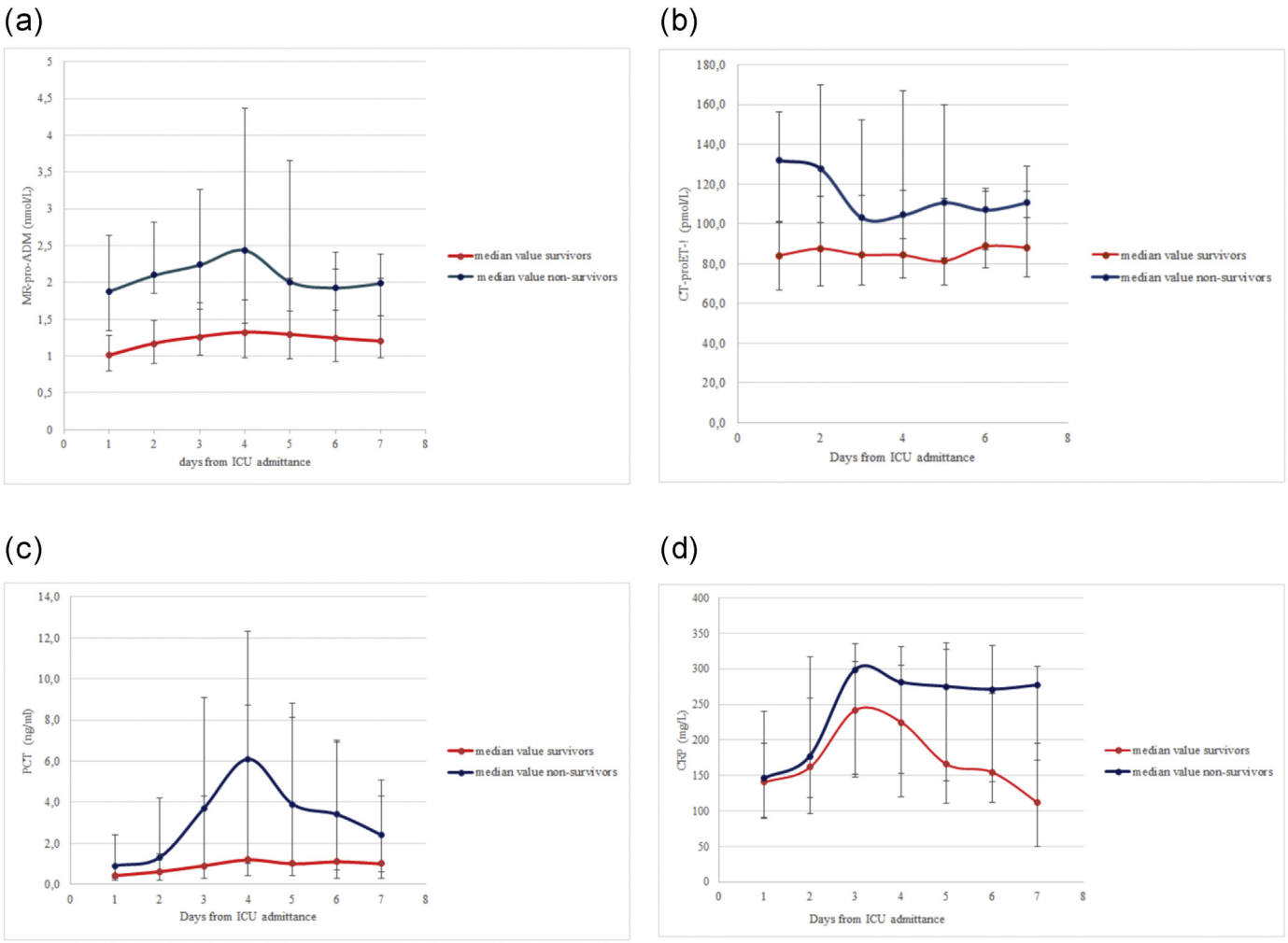

Fig. 3. a. Legends: Temporal changes in MR-proADM. Median values with IQR. Time dependent analysis of MR-proADM was significant different between non-survivors and survivors (p 0.01) and significant over time (time*group interaction term, p 0.01).
b. Legends: Temporal changes in CT-proET-1. Median values with IQR. Time dependent analysis of CT-proET-1 was significant different between survivors and non-survivors ($p < 0.001$), but constant over time (time*group interaction term p 0.43).
c. Legends: Temporal changes in PCT. Median values with IQR. Time dependent analysis of PCT was non-significant different between survivors and non-survivors (p 0.18) and non-significant over time (p 0.46).
d. Legends: Temporal changes in CRP. Median values with IQR. Time dependent analysis of CRP was non-significant different between survivors and non-survivors (p 0.64), but significant over time (time*group interaction term, p 0.02).

and CRP levels between both groups at any of the timepoints (p 0.18 and p 0.64, respectively). Only CRP differed over time (time*28-day survival interaction term p 0.02). (Fig. 3c and d).

4. Discussion

The primary aim of the study was to investigate the prognostic value of baseline MR-proADM and CT-proET-1 to predict 28-day mortality in a well-described cohort critically ill patients with SARS-CoV-2 pneumonia. Testing of the changes of the two biomarkers over time was the secondary aim. We reported two main findings. First, baseline MR-proADM and CT-proET-1 had high accuracy to identify 28-day non-survivors in ROC curves and were significant predictors for 28-day mortality with high hazard ratios in a multivariable Cox regression model with age and SOFA score. A higher mortality characterized patients presenting with MR-proADM and CT-proET-1 equal or exceeding their cut-off points (1.57 nmol/L and 111 pmol/L, respectively). The prognostic accuracy of baseline MR-proADM and CT-proET-1 to identify 28-day non-survivors was higher compared with most commonly measured laboratory parameters and APACHE IV and SOFA severity scores. Secondly, these significant higher levels of MR-proADM and CT-proET-1 in non-survivors persisted over time. Whereby MR-proADM increased faster in non-survivors. The differences in CT-proET-1 levels between non-survivors and survivors were constant over time. Moreover, the AUC for the prediction of 28-day mortality remained high for both MR-proADM and CT-proET-1 over time. Finding a biomarker able to identify patients with worst outcome in the ICU emerged as a priority during the COVID-19 pandemic. Both MR-proADM and CT-proET-1 appeared to be biomarkers with strong prognostic values. It might be argued that patients with highest values would benefit most from anti-inflammatory therapies such as steroids and interleukin-6 receptor antagonists.

Laboratory abnormalities are frequently reported in COVID-19 patients. Lymphocytopenia, increased values of CRP and D-dimer were most frequent predictive of adverse outcome [21]. We could not report similar results for these biomarkers in our study. Our findings of good ability of MR-proADM to predict 28-day mortality are in line with several studies. MR-proADM was frequently studied in patients with non-COVID-19 respiratory infections [13,14,22]. However, most of these patients were not admitted to the ICU [13,14,22]. Baseline MR-proADM was studied in an observational cohort study of 728 CAP patients

admitted to the emergency department (ED) [14]. MR-proADM had the best performance for prediction of 28-day mortality (HR 3·67 and AUC 0·85). In another observational cohort study of 1175 ED patients MR-proADM was able to identify patients requiring rapid administration of antibiotics or triage to the ICU [22]. Baseline MR-proADM performed well in predicting 28-day mortality in comparison with APACHE II and Simplified Acute Physiology Score II (SAPSII) when all patients admitted to the ICU were included [23]. The value of MR-proADM as prognostic marker in adult patients hospitalized with SARS-CoV-2 infection was studied in several observational studies [24-29]. Increased levels of MR-proADM were independently associated with mortality [24-29]. However, not all patients were admitted to the ICU [24,25,28,29]. CT-proET-1 was studied in non-COVID-19 patients admitted to the ED with CAP [14,16]. CT-proET-1 correlated with disease severity of CAP and was an independent predictor of mortality in both studies with CAP patients [14,16]. CT-proET-1 was studied in in critically ill patients with sepsis admitted to the ICU [17]. CT-proET-1 levels >74 pmol/L at ICU admission independently predicted ICU death [17]. Our search did not reveal studies with CT-proET-1 as marker in patients with SARS-CoV-2 infection.

Evidence of direct viral infection of the endothelial cell and diffuse endothelial inflammation was found in post-mortem analysis of COVID-19 patients [8]. These findings suggested that SARS-CoV-2 infection facilitated the induction of endothelial dysfunction and damage, endotheliitis [8]. ADM is a peptide hormone, produced by endothelial and vascular smooth muscle cells due to pro-inflammatory cytokines, bacterial toxins, hypoxia or volume overload. ADM binds to receptors in especially cardiovascular and pulmonary tissues and has anti-inflammatory effects on vascular endothelial cells, stabilizing the endothelial barrier function and protects the microcirculation against permeability in sepsis [11,12,30]. Besides its action on the endothelium ADM has important effects on the vascular system, ADM reduces vasoconstriction through inhibition of the renin-angiotensin-aldosterone system [11,30]. ET-1 is released from activated endothelial cells. It is a strong vasoconstrictor peptide and pro-inflammatory cytokine [15]. Besides blood vessels, ET-1 receptors are also found in other tissues, with the highest levels in the lungs [15]. ET-1 release is stimulated by bacterial toxins and inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha) or interleukin-6 [17]. Both MR-proADM and CT-proET-1 are the more stable midregion and C-terminal part of the prohormones that correlate with the release of the active peptides [9,10]. Renal failure could be another reason for elevated levels MR-proADM and CT-proET-1, most probably due to inappropriate renal clearance [31]. A small portion of the patients had chronic renal disease as comorbidity, and only two patients were on renal replacement therapy when biomarkers were collected. MR-proADM and CT-proET-1 levels could decrease faster during renal replacement therapy [31].

Some limitations of our study need to be addressed. First, we did a prospective observational study in a cohort critically ill patients with SARS-CoV-2 pneumonia from March 11 until May 27, 2020, which was the first period of the COVID-19 pandemic in the Netherlands. The treatment of COVID-19 has changed during the last year. Treatment with Chloroquine and Lopinavir/Ritonavir is obsolete and none of these patients were treated with dexamethasone during the first 10 days of hospitalization or interleukin-6 receptor antagonists. There may be differences in 28-day mortality due to changes in COVID-19 treatment. We must rely on older data of clinical practice leading to potential observational bias. Second, plasma samples could not be collected for seven days in all patients due to early ICU discharge, transfer to another ICU or early death. Incomplete longitudinal biomarker data might result in withdrawal bias. Clinical and microbiological data were obtained from the accepting hospitals if patients had an early transfer to another ICU. We may have lost some data by this method of collecting. Third, asymmetry in numbers survivors and non-survivors could influence the statistical significance between both groups. Fourth, although we have lost only a small number (28/133) of the total eligible patients during the study period by selecting only patients with SARS-CoV-2 infection confirmed by RT-PCR, with index tests at the first day and informed consent we may have introduced selection bias. Both observational, withdrawal and selection bias may have led to potential underestimation of the prognostic performance of MR-proADM and CT-proET-1.

A biomarker, as single baseline value or serial measured, will always oversimplify the interpretation of important clinical and other laboratory variables and therefore, MR-proADM and CT-proET-1 are meant, rather than to supersede, to complement clinician's judgement.

A strong feature of our analysis is that it is a real-life study performed on a mixed ICU in the Netherlands with patients with SARS-CoV-2 pneumonia, reflecting routine clinical ICU practice during the first period of the COVID-19 pandemic.

5. Conclusions

Baseline MR-proADM and CT-proET-1 had good ability to predict 28-day mortality in critically ill patients with SARS-CoV-2 pneumonia. Moreover, MR-proADM and CT-proET-1 appeared to be biomarkers with persistent strong prognostic value in the following days. MR-proADM and CT-proET-1 may help clinicians to identify patients at higher risk of adverse outcome and improve the decisions about ICU treatment.

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BRAHMS provided free MR-proADM and CT-proET-1 laboratory kits for this study. BRAHMS had no involvement in data collection, data analysis, data interpretation, trial design or patient recruitment, or the decision submit the paper for publication.

Ethics approval and consent to participate

The study protocol was approved by the METC Brabant (Tilburg, the Netherlands) (METC number NW2020–86).

Consent for publication

Not applicable.

Authors' contributions

Jos AH van Oers: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Writing original draft, Review & Editing. Yvette Kluiters: Resources, Review & Editing. Judith AP Bons: Resources, Review & Editing. Mariska de Jongh: Formal analysis, Methodology, Software, Validation, Review & Editing. Sjaak Pouwels: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Review & Editing. Dharmanand Ramnarain:Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Review & Editing. Dylan W de Lange: Supervision, Review & Editing. Harm-Jan de Grooth: Formal analysis, Methodology, Software, Validation, Review & Editing. Armand RJ Girbes: Supervision, Review & Editing.

Data sharing

Data collected for the study, including deidentified participant data and related documents, including the protocol, and informed consent form, will be made available to researchers after publication of the manuscript upon reasonable request via application to the corresponding author.

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Not applicable.

Declaration of Competing Interest

The authors declare that they did not have any financial support.

The manufacturer provided free MR-proADM and CT-proET-1 laboratory kits for this study.

The authors declare that they have no conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcrc.2021.07.017.

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