



# Acute and sustained increase in endothelial biomarkers in COVID-19

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## ABSTRACT

Endothelial injury is related to poor outcomes in respiratory infections yet little is known in relation to COVID-19. Performing a longitudinal analysis (on emergency department admission and post-hospitalisation follow-up), we evaluated endothelial damage via surrogate systemic endothelial biomarkers, that is, proadrenomedullin (proADM) and proendothelin, in patients with COVID-19. Higher proADM and/or proendothelin levels at baseline were associated with the most severe episodes and intensive care unit admission when compared with ward-admitted individuals and outpatients. Elevated levels of proADM or proendothelin at day 1 were associated with in-hospital mortality. High levels maintained after discharge were associated with reduced diffusing capacity.

## INTRODUCTION

Direct endothelial viral damage and/or perivascular inflammation have been described as important pathogenic mechanisms in altered microcirculation and organ damage in patients with COVID-19. Postmortem studies have revealed the presence of endothelial injury, disrupted endothelial cell membrane and angiogenesis in patients who died from COVID-19.<sup>1</sup> Moreover, several studies have uncovered endothelial damage using complex techniques such as the detection of circulating endothelial cells or transendothelial electrical resistance.<sup>2,3</sup> Endothelial biomarkers can vary, including the likes of proadrenomedullin (proADM) and proendothelin, two endothelium-derived markers mirroring endothelial dysfunction.<sup>4-6</sup> These biomarkers have been shown to hold prognostic power in community-acquired pneumonia (CAP) and sepsis.<sup>7</sup> To our knowledge, though, no studies have evaluated endothelial injury via the use of soluble biomarkers at diagnosis and follow-up. Therefore, using various time points, we aimed to study endothelial biomarkers (proADM and proendothelin) in patients experiencing the acute phase of COVID-19 and their association with mortality.

## METHODS

We conducted a longitudinal study in both patients with COVID-19, with a confirmed infection by reverse transcription PCR, and a control group at La Fe University and Polytechnic Hospital in Valencia, Spain. Patients visited emergency department (ED) between 8 March and 4 June 2020. We included all patients with either initial ward admission, initial

intensive care unit (ICU) admission (within the first 24 hours) or on an outpatient basis. The control cohort included volunteers without symptoms of COVID-19 infection who presented with a negative SARS-CoV-2 antibody serology. Additionally, we obtained biomarkers at ED (T1) and during a post-hospitalisation follow-up visit (T2). Peripheral venous blood was drawn from patients and control cohort individuals to be stored in EDTA tubes. EDTA tubes were centrifugated (2500 rpm) for 10 min within the first 8 hours of extraction to obtain plasma and later aliquoted for storage at  $-80^{\circ}\text{C}$  until analysis of proADM and proendothelin. Further details of methods are detailed in the online supplemental file. The main outcome for the initial analysis of biomarkers (T1) was in-hospital mortality.

## RESULTS

In this study, we enrolled 400 patients. Of these, 210 (23 outpatients; 179 with initial ward admission and 8 with initial ICU admission) were included in the analysis of biomarkers at day 1 (online supplemental figure 1). Additionally, in 97 of 210 patients, biomarker data were obtained at both day 1 and during follow-up visit (online supplemental table 1 depicts and compares characteristics of those included in and excluded from the study). Baseline characteristics are described in table 1. In brief, the cohort had a median age of 64 years, with 67.6% having at least one comorbidity. Of this group, 7.6% required mechanical ventilation, and 27 deaths occurred. The median length of hospitalisation was 12 days.

Levels of proADM and proendothelin at T1 were higher in patients than in the control group (figure 1A,B). Detailed biomarker levels of patients and control group individuals are shown in online supplemental table 2. After we excluded patients with probable, previous endothelial damage (arterial hypertension, history of smoking, diabetes, dyslipidaemia or chronic heart disease), similar findings were found for ProADM, although not for proendothelin (online supplemental figure 2A,B). ProADM and proendothelin levels differed significantly among patients, being at their lowest in outpatients and at their highest in ICU-admitted patients (figure 1C,D). A correlation analysis was performed between proADM and proendothelin and other recognised severity markers in COVID-19. A positive correlation was found with C reactive protein (CRP), D-dimer (DD), lactate dehydrogenase (LDH); conversely, a negative correlation was

**Table 1** Baseline characteristics

Characteristic	Patients with biomarkers at day 1 (n=210)	Patients with biomarkers at day 1 and follow-up visit (n=97)	Control (n=50)
Age in years, median (IQR)	64 (51–76)	55 (47–67)	43 (40–49)
Female sex, no (%)	99 (47.1)	43 (44.3)	33 (66)
Current or former smokers, no (%)	52 (24.8)	27 (27.8)	5 (10)
Coexisting conditions, no (%)			
Any	142 (67.6)	52 (55.6)	7 (14)
Hypertension	92 (43.8)	27 (27.8)	3 (6)
Diabetes	48 (22.9)	15 (15.5)	0 (0)
Dyslipidaemia	64 (30.5)	27 (27.8)	4 (8)
Chronic heart disease	26 (12.4)	8 (8.2)	1 (2)
Chronic renal disease	26 (12.4)	1 (1)	1 (2)
Chronic liver disease	6 (2.9)	4 (4.1)	1 (2)
Neurological disease	25 (11.9)	3 (3.1)	0 (0)
Chronic respiratory disease	23 (11)	12 (12.4)	1 (2)
Days since onset of symptoms, median (IQR)	7 (5–10)	7 (6–10)	NA
SpO <sub>2</sub> /FiO <sub>2</sub> at admission (IQR)	452.4 (429–461.9)	452.4 (438.1–457.1)	NA
Radiological data at admission			
Bilateral infiltrates, no (%)	117 (55.7)	61 (62.9)	NA
Severity			
PSI score, median (IQR)	68 (48–92)	55 (44–72)	NA
SOFA score, median (IQR)	1 (0–2)	1 (0–1)	NA
Respiratory support			
HFNC/CPAP/NIV, no (%)	22 (10.6)	14 (14.4)	NA
MV, no (%)	16 (7.6)	9 (9.3)	NA
Median length of MV, days, median (IQR)	14 (11–17)	12 (10–16)	NA
Treatment			
Remdesivir, no (%)	1 (0.5)	0 (0)	NA
Steroids, no (%)	74 (35.2)	28 (28.9)	NA
Hydroxychloroquine/chloroquine, no (%)	178 (84.8)	95 (97.9)	NA
Lopinavir/ritonavir, no (%)	39 (18.6)	28 (28.9)	NA
Tocilizumab, no (%)	35 (16.7)	18 (18.6)	NA
Statins (chronic treatment), no (%)	51 (24.3)	21 (21.6)	4 (8)
Aspirin (chronic treatment), no (%)	20 (9.5)	4 (4.1)	0 (0)
Outcomes			
Length of hospitalisation, days, median (IQR)	12 (9–20)	12 (10–16)	NA
In-hospital mortality, no (%)	27 (12.9)	NA	NA

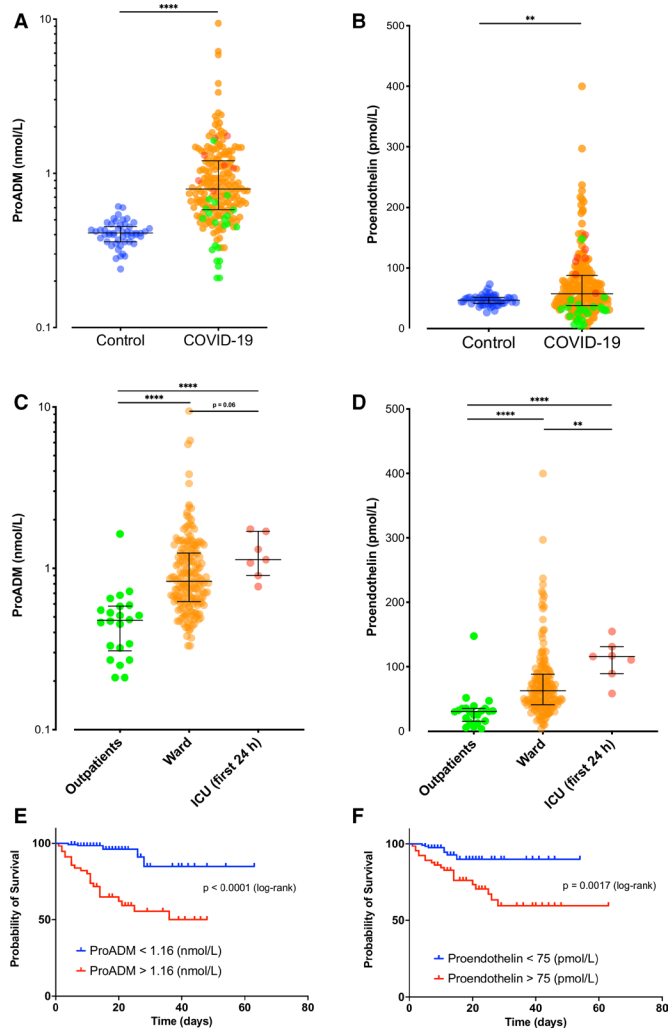
HFNC, high-flow nasal cannula; MV, mechanical ventilation; NA, not applicable; NIV, non-invasive ventilation; PSI, Pneumonia Severity Index; SOFA, Sequential Organ Failure Assessment; SpO<sub>2</sub>/FiO<sub>2</sub>, peripheral blood oxygen saturation/fraction of inspired oxygen.

observed with lymphocyte count (online supplemental figure 3).

Deceased patients had higher levels of proADM and proendothelin at T1 when compared with survivors (online supplemental table 3). ProADM and proendothelin display a better Somers'  $D_{xy}$  (0.69 and 0.55, respectively) for in-hospital mortality when compared with DD (0.51), CRP (0.49), LDH (0.36), as well as for survival when compared with lymphocyte count (0.36). ProADM and proendothelin levels were independently associated with an increased risk of mortality after multivariable adjustment (online supplemental table 4). A cut-off point was established at 1.16 nmol/L and 75 nmol/L for proADM and proendothelin using the Youden Index. Additionally, a Kaplan-Meier analysis (figure 1E,F) showed an association between a

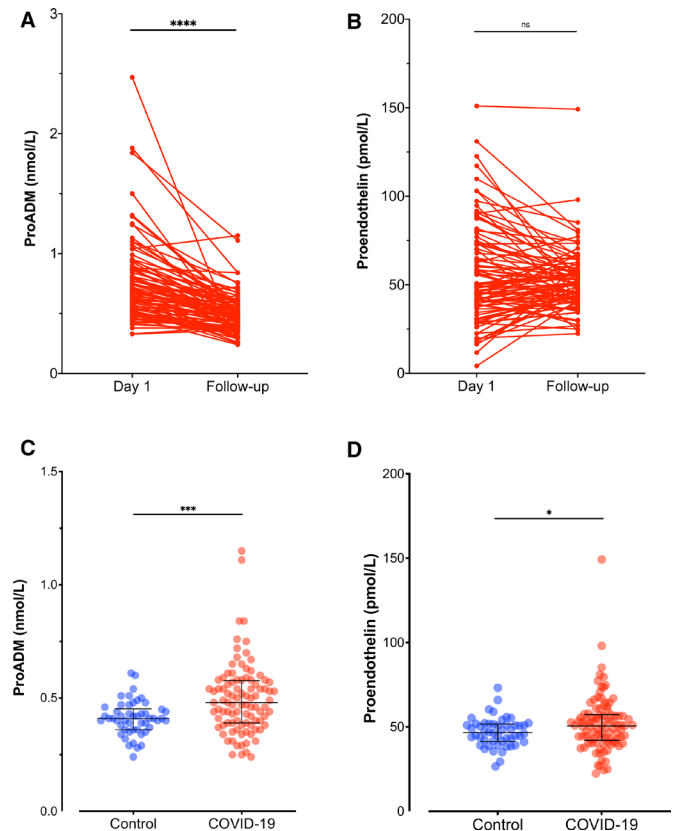
shorter time to death and levels of proADM >1.16 nmol/L and proendothelin >75 nmol/L in patients requiring hospitalisation. The Cox analysis only demonstrated an association between mortality and levels of proADM >1.16 nmol/L (online supplemental table 4).

The median time to post-hospitalisation follow-up visits was 65 (55–70) days. ProADM levels, although not those of proendothelin, were lower at follow-up than on day 1 (figure 2A,B). Furthermore, both proADM and proendothelin levels at follow-up remained higher than those in the control group (figure 2C,D and online supplemental table 2). ProADM and proendothelin at T2 presented with a significantly positive correlation with CRP, troponin T, pro b-type natriuretic peptide



**Figure 1** Proadrenomedullin (proADM) and proendothelin levels at day 1 ( $n=210$ ). Green dots: outpatients; orange dots: ward-admitted; red dots: ICU-admitted (within the first 24 hours). (A) Higher proADM levels in patients with COVID-19 than in those belonging to the control group (point estimate  $-0.39$  (95% CI  $-0.49$  to  $-0.29$ ;  $p<0.0001$ )). (B) Higher proendothelin levels in patients with COVID-19 than in those belonging to the control group (point estimate  $-11.4$  (95% CI  $-20.28$  to  $-3.17$ ;  $p<0.01$ )). (C) Increasing proADM levels were found among outpatients, patients with initial ward admission and patients with initial ICU admission. Outpatients versus ward, point estimate  $0.39$  (95% CI  $0.26$  to  $0.56$ ;  $p<0.0001$ ). Outpatients versus ICU, point estimate  $0.69$  (95% CI  $0.45$  to  $1.01$ ;  $p<0.001$ ). Ward versus ICU, point estimate  $-0.30$  (95% CI  $-0.60$  to  $0.03$ ;  $p=0.068$ ). (D) Increasing proendothelin levels were found among outpatients, patients with initial ward admission and patients with initial ICU admission. Outpatients versus ward, point estimate  $33.65$  (95% CI  $21.58$  to  $45.25$ ;  $p<0.0001$ ). Outpatients versus ICU, point estimate  $84.79$  (95% CI  $58.99$  to  $104.01$ ;  $p<0.0001$ ). Ward versus ICU, point estimate  $-48.05$  (95% CI  $-72.75$  to  $-18.17$ ;  $p<0.001$ ). (E) Survival analysis by Kaplan-Meier analysis (log-rank test) according to proADM levels. (F) Survival analysis by Kaplan-Meier analysis (log-rank test) according to proendothelin levels. \*\*\*\* $p<0.0001$ ; \*\*\* $p<0.001$ ; \*\* $p<0.01$ ; \* $p<0.05$ . ICU, intensive care unit.

and DD levels at T2 (online supplemental figure 4). Patients with a sustained elevation of endothelium-derived markers (28 of 97) more frequently presented with altered diffusing lung capacity for carbon monoxide ( $D_{LCO}$ ) ( $<80\%$  predicted) (online supplemental table 5).



**Figure 2** Longitudinal assessment of proadrenomedullin (proADM) and proendothelin levels ( $n=97$ ). (A) ProADM levels at day 1 and post-hospitalisation follow-up, as well as their differences per mixed linear regression (adjusted for the time between obtaining the two samples). Point estimate  $-0.26$  (95% CI  $-0.33$  to  $-0.20$ ;  $p<0.001$ ). (B) Proendothelin levels at day 1 and follow-up. No differences were found according to the mixed linear regression (adjusted for the time between collection of both samples). Point estimate  $-3.88$  (95% CI  $-8.34$  to  $0.57$ ;  $p=0.09$ ). (C) Higher proADM levels at follow-up in patients with COVID-19 than in those belonging to the control group (point estimate  $-0.07$  (95% CI  $-0.011$  to  $-0.03$ ;  $p<0.001$ )). (D) Higher proendothelin levels at follow-up in patients with COVID-19 than in those belonging to the control group (point estimate  $-3.62$  (95% CI  $-7.17$  to  $-0.05$ ;  $p<0.05$ )). \*\*\*\* $p<0.0001$ ; \*\*\* $p<0.001$ ; \*\* $p<0.01$ ; \* $p<0.05$ ; ns, non-significant.

## DISCUSSION

The main findings of the present study were: (1) COVID-19 provokes an increase in systemic proADM and proendothelin levels, which can be related to initial severity; (2) initial higher levels of proADM or proendothelin are associated with poor outcomes in the short term; (3) a proportion of patients exhibited a sustained increase in endothelium-derived markers, which was also associated with altered  $D_{LCO}$ .

We found significantly higher proADM and proendothelin levels in the most severe patients (ICU-admitted) when compared with outpatients and those admitted to the ward. Moreover, survival significantly decreased in those with elevated levels of proADM or proendothelin. These markers were good predictors of mortality. Endothelial damage is an important signature of sepsis, CAP and other severe infections. It can cause increased vascular permeability, oedema and ischaemia, favouring local and systemic organ damage.<sup>8,9</sup>

A sustained rise in biomarkers at day 65 of follow-up was

found in 29% of patients. In those with a persistent increase in endothelium-derived markers, we observed more functional respiratory impairment, such as altered  $D_{LCO}$ . This could support prior claims where endothelial damage has been suggested as a precursor to post-COVID-19 pulmonary fibrosis.<sup>10</sup>

Protecting the endothelium is a potential strategy worth considering in COVID-19. Some observational studies have already demonstrated the beneficial effects of endothelium stabilisers.<sup>11–13</sup> In line with such considerations, we believe that analysing endothelial biomarkers may provide useful information in future trial designs. Long-term consequences of sustained endothelial damage perhaps due to a combination of direct effect of SARS-CoV-2 on a potentially prior endothelial injury have yet to be elucidated; few studies have aimed to investigate this topic during the post-acute phase of COVID-19.<sup>14</sup>

The study has several limitations. This is a single-centre study, and further analyses are needed. Prior endothelial damage and baseline  $D_{LCO}$  of patients were unknown. In any case, only 12 of 97 patients had chronic respiratory disease that might have led to low  $D_{LCO}$  values. No objective metric for radiological findings was used at baseline. Irrespective of the possibly pre-existing endothelial dysfunction in patients with COVID-19, biomarker measurements are capable of detecting endothelial dysfunction during and after COVID-19, and can be related to mortality and poorer  $D_{LCO}$  evolution. Also, there was a significant loss during follow-up visits, so follow-up results should be interpreted with caution. Lastly, our study does not allow us to ascertain if biomarker evolution occurred as a consequence of either the disease's course or treatment.

In conclusion, COVID-19 provokes an increase in proADM and proendothelin to perhaps mirror endothelial injury, which could persist after discharge. The intensity of endothelial injury at COVID-19 diagnosis is related to initial severity and prognosis. A significant proportion of patients exhibited a sustained increase in endothelium-derived markers, which was related to impaired  $D_{LCO}$ .

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