The effect of prostacyclin infusion on markers of endothelial activation and damage in mechanically ventilated patients with SARS-CoV-2 infection

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ABSTRACT

Background: In a pilot study, we found a significant reduction in mean daily sequential organ failure assessment score in mechanically ventilated patients with COVID-19 who received prostacyclin, compared to placebo. We here investigate the effect on biomarkers of endothelial activation and damage.
Methods: Post-hoc study of a randomized controlled trial in adult patients with confirmed SARS-CoV-2 infection, mechanically ventilated, with soluble thrombomodulin (sTM) plasma levels >4 ng/mL. Patients received prostacyclin infusion (1 ng/kg/min) or placebo. Blood samples were collected at baseline and 24 h.
Results: Eighty patients were randomized (41 prostacyclin, 39 placebo). The median changes in syndecan-1 plasma levels at 24 h were −3.95 (IQR: −21.1 to 2.71) ng/mL in the prostacyclin group vs. 3.06 (IQR: −8.73 to 20.5) ng/mL in the placebo group (difference of the medians: -7.01 [95% CI: −22.3 to −0.231] ng/mL, corresponding to −3% [95% CI: −11% to 0%], $p = 0.04$). Changes in plasma levels of sTM, PECAM-1, p-selectin, and CD40L did not differ significantly between groups.
Conclusions: Prostacyclin infusion, compared to placebo, resulted in a measurable decrease in endothelial glycocalyx shedding (syndecan-1) at 24 h, suggesting a protective effect on the endothelium, which may be related to the observed reduction in organ failure.

1. Introduction

As of December 15th, 2021, more than 270 million people have been infected with SARS-CoV-2 and more than 5.3 million have died with COVID-19 [1]. Mortality rates of 30–40% have been reported among infected patients admitted to intensive care units (ICU), highest in patients requiring mechanical ventilation [2,3]. Endothelial damage and microvascular thrombosis seem to be intimately involved in the pathophysiology of COVID-19-associated acute respiratory distress syndrome [4-6], and it has been proposed that COVID-19 is essentially a disease of the endothelium [7,8]. Prostacyclin is a hormone produced and secreted by endothelial cells, which, in addition to acting as a vasodilator and platelet inhibitor [9], maintains and protects the endothelium and the endothelial glycocalyx layer through several known mechanisms [10-15].

We hypothesized that intravenous prostacyclin infusion might attenuate the endotheliopathy seen in COVID-19 and thereby improve clinical outcomes. To investigate this, we conducted a randomized controlled trial of prostacyclin infusion in mechanically ventilated patients with COVID-19 and severe endotheliopathy. We found that 72 h infusion of 1 ng/kg/min prostacyclin significantly reduced organ failure as measured by mean daily sequential organ failure assessment (SOFA) score during follow-up [16] and we speculate that this effect may be attributed to the endothelioprotective effect of the intervention.

The aim of the present study was, therefore, to investigate whether infusion of prostacyclin had measurable effects on markers of endothelial activation, endothelial damage, and platelet-endothelial interaction, when compared to placebo 24 h after initiation of the intervention.

2. Methods

The study design and main results have previously been published in detail [16,17]. This is a post-hoc study of a Danish multicenter, randomized (1:1, active:placebo), blinded, parallel-grouped exploratory trial of low-dose continuous infusion of prostacyclin vs. placebo for 72 h in mechanically ventilated patients with COVID-19 and severe endotheliopathy (Danish National Committee on Health Research Ethics journal no. H-20026049; EudraCT no. 2020–001296-33; NCT no. 04420741). The study was conducted in accordance with Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. Patients, 18 years or older, with confirmed SARS-CoV-2 infection who were invasively mechanically ventilated and had a level of circulating soluble thrombomodulin (sTM) above 4 ng/mL measured using a lateral flow immunoassay (BioPorto Diagnostics A/S) were included in the study. Patients were randomly assigned to the synthetic prostacyclin analog iloprost (Ilomedin, Bayer Pharma AG) at a dose of 1 ng/kg/min or placebo (equal volume saline) which was administered as a continuous intravenous infusion for 72 h. Other aspects of patient care were provided according to the standard of care at each site, including systemic dexamethasone.

2.1. Blood samples

Blood samples were obtained at baseline and 24 h and were collected in tubes anticoagulated with ethylenediaminetetraacetic acid (EDTA) or citrate. Tubes were centrifuged twice at 1800 g for 10 min at 5 °C to obtain cell-free plasma aliquots which were frozen at −80 °C and stored in a dedicated research biobank.

2.2. Enzyme-linked immunosorbent assay (ELISA)

Soluble markers of glycocalyx degradation (syndecan-1), thrombomodulin cleavage (soluble thrombomodulin; sTM), tight-junction integrity (platelet endothelial cell adhesion molecule-1; PECAM-1), endothelial cell activation (platelet-selectin; p-selectin), and platelet-endothelial interaction (CD40 ligand; CD40L) were measured by commercially available immunoassays according to the manufacturer's recommendations: syndecan-1 (sCD138; Nordic Biosite, Copenhagen, Denmark), sTM (Nordic Biosite, Copenhagen, Denmark), PECAM-1 (CD31; R&D Systems, Abingdon, UK), p-selectin (Nordic Biosite, Copenhagen, Denmark), and CD40L (Nordic Biosite, Copenhagen, Denmark).

2.3. Statistical analyses

Statistical analyses were conducted in RStudio. All analyses were performed in the intention-to-treat population defined as all randomized patients. Clinical outcomes were compared as described in the main publication of the trial [16]. Missing biomarker values (6% in both groups) were considered missing at random and, therefore, imputed by random forest imputation [18]. Absolute biomarker levels at 24 h and relative change in biomarker levels from baseline to 24 h were compared between groups using Wilcoxon rank sum test and effects quantified by differences in medians. Confidence intervals of the estimated location difference were based on continuity-corrected normal approximations [19].

3. Results

Between June 2020 and January 2021, 138 patients were screened and 80 randomized to receive prostacyclin ($n = 41$) or placebo ($n = 39$). The intervention was discontinued in four patients in the prostacyclin group (three were transferred to a non-participating hospital and one withdrew consent) and one patient in the placebo group (withdrew consent).

3.1. Baseline characteristics

Baseline clinical characteristics and endothelial biomarker levels are presented in Table 1. Mean age was 67 years (IQR: 58–74) and 66% were male. Baseline characteristics appeared similar between groups.

3.2. Clinical outcomes

Clinical outcomes are presented in detail in the main publication of the trial [16]. The median number of days alive and free of mechanical ventilation at 28 days, the primary endpoint of the trial, were 16 vs. 5 days in the prostacyclin and placebo groups, respectively (difference of the medians: 11 [95% confidence interval, CI: −5 to 21] days, $p = 0.07$). The mean daily SOFA score during ICU stay was 5.75 vs. 6.67 in the prostacyclin and placebo groups, respectively (adjusted difference of the means: 1.1 [95% CI: 0.28 to 1.96], $p = 0.01$), and 28-day mortality was 22% vs. 44% in the prostacyclin and placebo groups, respectively (risk ratio: 0.50 [95% CI: 0.24 to 0.96], $p = 0.06$).

Table 1
Patient characteristics.

	Prostacyclin group, n (%) or median (IQR)	Placebo group, n (%) or median (IQR)
Baseline characteristics		
Age, years	68 (60; 73)	66 (57; 75)
Male sex	30 (73%)	23 (59%)
Chronic cardiovascular disease	26 (63%)	23 (59%)
Chronic respiratory disease	6 (15%)	4 (10%)
Metastatic cancer	1 (2%)	0 (0%)
Hematological cancer	1 (2%)	3 (8%)
End-stage renal disease	1 (2%)	1 (3%)
Admitted from		
Emergency room	9 (22%)	11 (28%)
Ward	28 (68%)	27 (69%)
Operating room/post-operative care	1 (2%)	0 (0%)
Other intensive care unit	3 (7%)	1 (3%)
Baseline clinical observations		
Lowest SBP within 24 h, mmHg	85 (77; 93)	82 (74; 89)
Vasopressor use within 24 h	37 (90%)	34 (87%)
RRT within 24 h	1 (2%)	2 (5%)
Acute surgery within 24 h	1 (2%)	0 (0%)
Baseline endothelial biomarker levels		
Syndecan-1, ng/mL	213 (200; 227)	205 (129; 219)
sTM, ng/mL	11.4 (9.21; 20.4)	18.5 (10.1; 21.2)
PECAM-1, ng/mL	14.6 (11.8; 17.2)	13.9 (10.6; 15.4)
P-selectin, ng/mL	2.21 (2.02; 2.57)	2.22 (1.93; 2.64)
CD40L, ng/mL	0.201 (0.119; 0.421)	0.234 (0.154; 0.452)

IQR: interquartile range; MV: mechanical ventilation; RRT: renal replacement therapy; SBP: systolic blood pressure; SOFA: sequential organ failure assessment; VT: vasopressor therapy

3.3. Endothelial biomarkers

Absolute and relative endothelial biomarker levels at 24 h are presented in Table 2.

The median absolute plasma levels at 24 h of sTM were 14.6 vs. 18.0 ng/mL in the prostacyclin and placebo groups, respectively (difference of the medians: −3.35 [95% CI: −5.14 to −0.0700] ng/mL, $p = 0.04$). Absolute plasma levels of syndecan-1, PECAM-1, p-selectin, and CD40L did not differ significantly between groups.

The median relative plasma levels at 24 h of syndecan-1 were 3.95 ng/mL decrease vs. 3.06 ng/mL increase in the prostacyclin and placebo groups, respectively (difference of the medians: −7.01 [95% CI: −22.3 to −0.231] ng/mL, p = 0.04). Relative plasma levels of sTM, PECAM-1, p-selectin, and CD40L did not differ significantly between groups.

4. Discussion

In this post-hoc analysis we found that low-dose prostacyclin, in mechanically ventilated patients with COVID-19 and severe endotheliopathy, resulted in a subtle but significant decrease in syndecan-1 levels at 24 h, compared to placebo. Furthermore, sTM levels at 24 h were significantly lower in the prostacyclin group, compared to placebo, but this difference was not significant after adjusting for baseline levels.

Few months after the outbreak of the COVID-19 pandemic, it was reported in autopsy series that tissues from deceased patients were characterized by severe endothelial damage and microvascular thrombi [4-6]. It was later proposed that critical COVID-19 is basically an endothelial disease which results in systemic inflammation, platelet activation, hypercoagulability, microvascular thrombosis, and organ failure [7,8].

Prostacyclin is synthesized and released from healthy endothelial cells and exerts paracrine effects on the endothelium, vascular smooth muscle, and platelets, resulting in dose dependent vasodilation and platelet inhibition [9]. In addition, synthetic prostacyclin analogues have been reported to stimulate reendothelialization through regulation of integrin expression [10], prevent capillary leakage through up-regulation of vascular endothelial cadherin (VE-cadherin) [11], and protect against ischemia/reperfusion injury through stimulation of heme oxygenase-1 expression [12]. Prostacyclin also attenuates the inflammatory hit on the endothelium through inhibition of nuclear factor-κB (NF-κB) and tumor necrosis factor α (TNF-α) activation [13,14], inhibition of interleukin 2 (IL-2) and IL-6 production, stimulation of IL-10 production, and inhibition of T-cell priming capacity and macrophage inflammatory protein-3 beta (MIP-3β) induced migration of dendritic cells [15]. We have previously reported that continuous infusion of prostacyclin at a dose of 1 ng/kg/min is safe and does not induce hypotension or bleeding complications in patients undergoing percutaneous coronary intervention [20] or pancreaticoduodenectomy [21], or in patients with septic shock [22].

Shedding of the endothelial glycocalyx, as measured by syndecan-1, has been reported to correlate with disease severity in patients with COVID-19 [23]. The glycocalyx exerts pivotal functions to maintain the endothelium in a healthy state, including acting as a physical barrier and thereby inhibiting direct cell-cell interaction with the vascular cells [24]. In addition, the glycocalyx acts as a mechano-sensor governing endothelial cell organization to meet the specific local requirements to maintain cell integrity [24]. It has previously been reported that prostacyclin increases messenger ribonucleic acid (mRNA) levels for heparan sulfate proteoglycan, an important constituent of the glycocalyx matrix, and it could be speculated that this would also occur in the prostacyclin treated patients in the present study [25]. We found a significant decrease in plasma levels of syndecan-1 at 24 h in patients who received prostacyclin, compared to controls, suggesting that prostacyclin infusion may have limited glycocalyx shedding. The subtlety of the changes may reflect that the full effect of the intervention is not yet seen at 24 h. Given the directionality of the changes in the two groups, it can also be speculated that the results may be subject to regression to the mean.

Thrombomodulin (TM) is an essential constituent of the protein C anticoagulant system where it exerts its effect by binding to thrombin, thereby decreasing levels of circulating thrombin, and by inactivating factors Va and VIIIa by potentiating the generation of activated protein C [26]. TM also has a protective effect on endothelial cell vasculature by depressing inflammatory injuries [27]. Furthermore, the lectin-like domain of TM has anti-inflammatory and cytoprotective effects as it attenuates mitogen-activated protein kinase (MAPK) pathways and NF-κB translocation and interferes with neutrophil adhesion to endothelial cells, partly by suppressing intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1) [28]. Cleavage of TM from the luminal part of the endothelial cell, thus, induces a prothrombotic phenotype, which is a hallmark of COVID-19, and it could be speculated that prostacyclin mitigates these adverse effects [4,5]. Interestingly, complement activation is boosted during the progression of COVID-19 and loss of TM from the luminal part of the endothelial cell membrane may contribute to this pathology as the lectin-like domain has inhibitory effect on complement activation [29]. Indeed, increased levels of circulating sTM is associated with mortality in patients with severe infections [30], including COVID-19 [8]. For this reason, sTM levels were used as a screening tool to identify patients with severe endotheliopathy in the present study. We found that patients who received prostacyclin, compared to controls, had significantly lower absolute levels of circulating sTM at 24 h, suggesting that prostacyclin infusion may have limited protein C system impairment. However, the difference was not significant after adjusting for baseline levels.

We found no measurable difference between study groups regarding p-selectin, which is in alignment with the findings of a recent

Table 2
Endothelial biomarker levels at 24 h.

	Prostacyclin group, median (IQR)	Placebo group, median (IQR)	Difference of the medians (95% CI)	P-value
Absolute plasma levels at 24 h				
Syndecan-1, ng/mL	205 (187; 225)	203 (153; 224)	1.43 (−6.96; 15.4)	0.79
sTM, ng/mL	14.6 (9.93; 18.0)	18.0 (13.0; 21.1)	−3.35 (−5.14; −0.0700)	0.04
PECAM-1, ng/mL	14.4 (12.1; 17.1)	14.2 (11.0; 15.7)	0.235 (−0.693; 2.45)	0.25
P-selectin, ng/mL	2.21 (1.73; 2.61)	2.12 (1.98; 2.60)	0.0890 (−0.295; 0.195)	0.89
CD40L, ng/mL	0.216 (0.104; 0.387)	0.197 (0.120; 0.339)	0.0185 (0.0740; 0.103)	0.84
Relative plasma levels at 24 h				
Syndecan-1, ng/mL	−3.95 (−21.1; 2.71)	3.06 (−8.73; 20.5)	−7.01 (−22.3; −0.231)	0.04
sTM, ng/mL	−0.148 (−1.06; 1.51)	0.935 (−1.40; 1.90)	−1.08 (−1.68; 0.795)	0.53
PECAM-1, ng/mL	−0.258 (−1.38; 1.34)	0.589 (−0.344; 1.32)	−0.847 (−1.54; 0.203)	0.14
P-selectin, ng/mL	−0.0400 (−0.359; 0.166)	−0.0510 (−0.203; 0.180)	0.0110 (−0.236; 0.127)	0.64
CD40L, ng/mL	−0.0330 (−0.0990; 0.0340)	−0.0365 (−0.0647; 0.0160)	0.00352 (−0.0510; 0.0430)	0.83

CD40L: cluster of differentiation 40 ligand; CI: confidence interval; IQR: interquartile range; PECAM-1: platelet endothelial cell adhesion molecule-1; p-selectin: platelet-selectin; sTM: soluble thrombomodulin.

meta-analysis [31]. Similarly, no significant differences between study groups concerning PECAM-1 and CD40L were observed at 24 h.

This study has important limitations including a small sample size which precludes firm conclusions. Blood samples were obtained only at baseline and 24 h after start of the intervention, which limits the certitude of our interpretations regarding both magnitude and directionality of the effect of the intervention. Also, all patients were enrolled in ICUs in the Capital Region of Denmark, which may reduce the generalizability of the results. Furthermore, a potential effect of co-enrollment of the patients also in other interventional clinical trials cannot be excluded.

5. Conclusions

In mechanically ventilated patients with COVID-19 and severe endotheliopathy, we found that low-dose prostacyclin infusion, compared to placebo, resulted in a measurable decrease in endothelial glycocalyx shedding (syndecan-1) at 24 h, suggesting a protective effect on the endothelium, which may be related to the observed reduction in organ failure.

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Ethics approval and consent to participate

Danish National Committee on Health Research Ethics journal no. H-20026049. The study was conducted in accordance with Good Clinical Practice guidelines and the principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

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

Author contributions

Conceptualization: PJ, PS, AP, MB, JS, NC, KK. Formal analysis: MV, PJ, TL. Writing – original draft: MV, PJ. Writing – review and editing: PS, MB, NC, KK, JS, AP.

Declaration of Competing Interest

PJ is co-inventor on a patent concerning the diagnostic biomarker soluble thrombomodulin and the use of low-dose prostacyclin as a therapeutic in acute critical illness. All other authors declare no competing interests.

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