



Baseline endothelial-related biomarkers in hemodialysis patients and risk of developing severe SARS-Cov-2 infection

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Coronavirus disease 2019 (COVID-19) represents a major challenge for health systems worldwide, due to its pandemic spread and its high mortality rate. By mid-January 2021, COVID-19 had caused more than 2 million deaths worldwide. Brazil is one of the countries with the highest number of infected patients (more than 8 million) and deaths (~ 210,000) [1]. Direct viral infection of the endothelial cell and diffuse endothelial inflammation were demonstrated in a series of patients with COVID-19, with subsequent organ ischemia and a procoagulant state [2].

Here, we describe preliminary findings from a cohort of maintenance hemodialysis patients with previous baseline measurement of endothelium-related biomarkers. This cohort was first created to evaluate the association of endothelial derangement with cognitive derangement [3]. Among the endothelium-related biomarkers, we have chosen four that comprise different endothelial functions/structures: intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion protein-1 (VCAM-1), which is related to endothelial cell activation; angiotensin-2 (AGPT2), an endothelial growth factor that promotes polymorphonuclear cell infiltration and induces endothelial cell apoptosis; and syndecan-1,

a newly explored marker of endothelial glycocalyx derangement, that has been reported as increased in hemodialysis patients [4].

SARS-Cov-2 infection is associated with several vascular complications, including pulmonary thrombosis, contributing to its severity and mortality [5]. Endothelial dysfunction can also lead to vasoconstriction with subsequent ischemia, inflammation with tissue edema, and a procoagulant state [2]. Our main objective was to evaluate whether these biomarkers predict the risk of developing severe COVID-19 in maintenance hemodialysis patients.

We gathered data on all our adult patients on maintenance hemodialysis. By the end of August 2020, sera from patients without a previous COVID-19 diagnosis were tested using the Mybiosource SARS-CoV-2 IgG assay. In September 2019, these patients had blood samples collected as part of a previous study evaluating cognition and endothelial alterations [3]. At that moment, endothelium-related biomarkers were measured. The present study was approved by the institutional review board of the Ethical Committee of Universidade de Fortaleza and the original cohort by Universidade Federal do Ceará.

Furthermore, the following variables were gathered: age, gender, arterial hypertension, diabetes mellitus, cardiovascular disease (coronary artery disease and/or peripheral vascular disease), dialysis vintage, body mass index (BMI) > 30 kg/m², hemoglobin, serum albumin and the endothelium-related biomarkers VCAM-1, ICAM-1, AGPT-2, and syndecan-1. The outcome was severe COVID-19, defined as SARS-Cov-2-related hospital admission based on the attending physician's judgment, or mortality due to COVID-19.

The study cohort consisted of 122 patients. Of these, 29 (23.8%) had symptomatic COVID-19 diagnosed by RT-PCR, 15/29 (51.7%) required hospital admission and 6/29

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(21.4%) died. Further 22/122 (18.0%) patients had asymptomatic infection diagnosed only retrospectively by antibody testing by ELISA. These 51 patients (29 symptomatic and 22 asymptomatic COVID infection) were selected and included in the final analysis. The majority of patients were males ($n = 32/51$, 62.7%) and the mean age was 53.5 ± 17.1 years. The median dialysis vintage was 84 [12–144] months. The prevalence of arterial hypertension was 68.6% and diabetes mellitus was 49.0%. Only four patients had $BMI > 30 \text{ kg/m}^2$.

Patients with severe COVID-19 infection were older, had longer dialysis vintage and had more cardiovascular disease. No difference was observed regarding gender or systemic arterial hypertension diagnosis. Although no difference was observed at the baseline levels of ICAM, VCAM-1 and AGPT-2 levels, syndecan-1 levels were higher in patients with severe COVID-19 than in those with asymptomatic/non-severe COVID-19 see Table 1. Only syndecan-1 was correlated with clinical variables—age ($r_s = 0.378$, $p = 0.001$) and dialysis vintage ($r_s = 0.422$, $p < 0.001$).

Multivariate regression and mediation analyses [6] were performed to assess the hypothesized associations between clinical demographic characteristics and selected endothelial-related biomarkers with severe COVID-19. Age (OR 1.07 95% CI 1.01–1.13, per each year) and dialysis vintage (OR 1.10 95% CI 1.02–1.21, per each 10 month) remained significantly associated with severe COVID-19. However, after including baseline syndecan-1 levels in the model, neither variable was associated (age with OR 0.98

95% CI 0.87–1.10 and dialysis vintage with OR 1.08 95% CI 0.92–1.23) with severe COVID-19, and only syndecan-1 was independently associated with this outcome—OR 1.34 95% CI 1.05–1.72, per each 10 ng/mL.

Aiming to further explore the association between syndecan-1 and COVID-19 severity, we performed a mediation analysis. In the unadjusted analysis, the putative impact of age and dialysis vintage on COVID-19 severity seemed to be mediated by syndecan-1 levels. The direct effect was no longer significant on either age or dialysis vintage. After adjustment for confounders, the results were very similar—see Table 2.

In the present study, we performed a secondary analysis of a cohort of maintenance hemodialysis patients using endothelium-related biomarkers measured within 6-months before the SARS-Cov-2 pandemic. The presence of this cohort gave us the opportunity to evaluate whether the baseline endothelium status evaluated through several biomarkers would be associated with severe COVID-19. The main finding of our study is that baseline levels of syndecan-1 measured in hemodialysis patients before SARS-Cov-2 infection is associated with severe COVID-19. Moreover, our data suggest that endothelium glycocalyx derangement can be an important mediator of the increased risk of severe COVID-19 infection associated with age and dialysis vintage in maintenance HD patients.

Our cohort has a prevalence of COVID-19 infection of 41.8% up to September 2020. Our data are similar to those

Table 1 Baseline demographics and clinical characteristics stratified by COVID-19 severity

	All patients (n = 122)	COVID-19 patients (n = 51)	Non-severe COVID-19 (n = 36)	Severe COVID-19 (n = 15)	p
Age (years), mean \pm SD	51.6 \pm 16.8	53.5 \pm 17.1	49.8 \pm 15.3	62.5 \pm 18.2	0.014
Male, n (%)	74 (60.7)	32 (66.7)	22 (61.1)	10 (66.7)	0.708
Hypertension, n (%)	88 (72.1)	35 (68.6)	23 (63.9)	12 (80.0)	0.259
Diabetes Mellitus, n (%)	56 (45.9)	25 (49.0)	18 (50.0)	07 (46.7)	0.828
Cardiovascular disease, n (%)	16 (13.1)	10 (19.6)	4 (11.1)	6 (40.0)	0.018
Severe Obesity (BMI > 30 kg/m ²), n (%)	6 (4.9)	4 (7.8)	2 (5.6)	2 (13.3)	0.571
Dialysis vintage (months), median(IQR)	62 (11–240)	84 (12–144)	60 (8–120)	196 (36–244)	0.005
Hemoglobin (g/dL), mean \pm SD	11.8 \pm 1.6	11.8 \pm 1.7	11.8 \pm 1.8	12.0 \pm 1.4	0.738
Albumin (g/dL), mean \pm SD	4.1 \pm 0.4	4.1 \pm 0.3	4.1 \pm 0.3	4.2 \pm 0.4	0.236
ICAM-1 (ng/mL), median (IQR)	184 (142–242)	190 (159–241)	185 (151–248)	207 (173–237)	0.725
VCAM-1 (ng/mL), median (IQR)	1,230 (1,125–1510)	1,229 (1,124–1,480)	1,205 (1,129–1,479)	1,366 (1,025–1,527)	0.885
AGPT2 (pg/mL), median (IQR)	1.34 (0.72–2.10)	1.48 (0.76–2.23)	1.56 (0.67–2.31)	1.20 (0.76–1.94)	0.522
Syndecan-1 (ng/mL), median (IQR)	162 (117–276)	188 (116–310)	141 (106–215)	345 (306–381)	<0.001

Table 2 Multiple mediation analyses for severe COVID-19 according to age and dialysis vintage and with syndecan-1 as Mediator

Exposure variable	Crude OR (95% CI)*	Adjusted OR (95% CI)*—model 1-
Age, per each year		
Total effect of older age	1.05 (1.01–1.10)	1.07 (1.01–1.13)
ACME of higher syndecan-1	1.05 (1.01–2.96)	1.06 (1.02–4.11)
ADE of older age	1.00 (0.94–1.07)	1.01 (0.93–1.10)
Dialysis Vintage, per each 10 months		
Total effect of higher dialysis vintage	1.12 (1.04–1.21)	1.10 (1.02–1.21)
ACME of higher syndecan-1	1.10 (1.03–4.89)	1.08 (1.03–6.67)
ADE of higher dialysis vintage	1.12 (0.99–1.25)	0.98 (0.87–1.10)

ACME average causal mediation effect—a variable that explains how much of the putative effect of the variable on COVID-19 severity is explained by the possible effect of the mediator, *ADE* average direct effect—a variable that explains how much of the putative effect of the variable on COVID-19 severity is still explained by the variable after considering the effect of any given mediator, *OR* odds ratio. Model 1: adjusted for age, gender, hypertension, diabetes mellitus, cardiovascular disease, dialysis vintage, obesity, hemoglobin and serum albumin

*All estimates and Quasi-Bayesian CIs generated after 5000 simulations

of other studies from countries with a high COVID-19 incidence. Clarke et al. [7] reported a seroprevalence of 36% in hemodialysis patients from England. In our cohort, we could not detect any difference regarding an association of baseline ICAM-1 or AGPT-2, biomarkers already known to be related to endothelial cell activation and endothelial growth factor, with severe COVID-19. However, there was a strong and independent association between syndecan-1 and COVID-19 severity, suggesting that previous endothelial glycocalyx damage is a risk factor in hemodialysis patients.

It has been suggested that the syndecan-1 response is biphasic, where syndecan-1 plays a role in initial injury, but with chronic development of the underlying damage, cellular syndecan-1 expression is lost again resulting in an inverse association of syndecan-1 and disease parameters. Also, lower extracellular volume was associated with higher plasma syndecan-1 levels in hemodialysis patients and better endothelial remodeling [8], although our data could not confirm this association. Taken together, it is possible that repeated glycocalyx injuries and remodeling in hemodialysis patients, instead of chronic endothelial damage, may predispose to more severe COVID-19 infection.

In this cohort, in addition to the association between syndecan-1 and severe COVID-19, we were able to investigate the hypothesis that syndecan-1 could mediate the negative effects of age and dialysis vintage on COVID-19 severity, with complete mediation being suggested in our model. Because of the sample size limitation and the use of a parsimonious model to avoid overfitting, caution is necessary before concluding that all effects of age and dialysis vintage can be statistically mediated by a single biomarker. However, our data strongly indicate that baseline endothelium glycocalyx derangement can be an important factor for COVID-19 severity, at least in the maintenance HD population.

Our study has several limitations. First, we measured baseline endothelium-related biomarkers six months before the first COVID-19 case was detected in our cohort and we were unable to measure the syndecan-1 levels at the time of infection; however, the stability of such biomarkers can be determined by a high correlation between our samples collected in 2016 and those collected in 2019. Second, although we performed the ELISA test to detect COVID-19 antibodies in all participants, some lack of knowledge persists about the actual sensitivity of serological tests to diagnose previously asymptomatic infections in patients infected by COVID-19. In a recent study [7] carried out in hemodialysis patients, of 79 PCR-positive patients, only 2 were seronegative. It has also been suggested that up to 40% of asymptomatic individuals that were initially seropositive became seronegative in the early convalescent phase. However, another population-based study demonstrated that over 90% of quantitative polymerase-chain-reaction-positive individuals tested positive at antibody assays and remained seropositive for 4 months after the diagnosis, with no decrease in antibody levels [9]. Finally, our criteria for defining severe COVID-19 were based on need for hospital admission, which depends on the assistant physician's criteria.

In conclusion, when analyzing several endothelium-related biomarkers prior to the SARS-Cov-2 infection, syndecan-1 was independently associated with severe COVID-19. Moreover, it seems to mediate the negative effects of age and dialysis vintage on COVID-19 severity in a cohort of maintenance HD patients.

Declarations

Conflict of interest The authors have no conflict of interest.

Ethical statement This study was approved by the local Ethics Committee and was performed according to the Declaration of Helsinki. Participants received an explanatory statement and gave their written informed consent to participate in the study.

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