



**Original Article**

**Elevated P-Selectin in Severe Covid-19: Considerations for Therapeutic Options**

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**Competing interests:** The authors declare no conflict of Interest.

**Abstract. Background:** Coronavirus disease 2019 (COVID-19) is mainly a respiratory tract disease and acute respiratory failure with diffuse microvascular pulmonary thrombosis are critical aspects of the morbidity and mortality of this new syndrome.

**Purpose:** The aim of our study was to investigate, in severe COVID-19 hospitalized patients, the P-selectin plasma concentration as a biomarker of endothelial dysfunction and platelet activation.

**Methods:** 46 patients with severe or critical SARS-CoV-2 infection were included in the study. Age-matched patients then were divided in those requiring admission to the intensive care unit (ICU, ICU cases) vs those not requiring ICU hospitalization (non-ICU cases). Blood samples of severe COVID-19 patients were collected at the time of hospital admission. The quantification of soluble P-selectin was performed by ELI, assay.

**Results:** Our study showed a higher P-selectin plasma concentration in patients with Covid-19, regardless of ICU admission, compared to the normal reference values and compared to ten contextually sampled healthy donors (HD); (COVID-19): median 65.2 (IQRs: 45.1-81.1) vs. HD: 40.3 (IQRs: 24.3-48.7), p=0023. Moreover, results showed a significant reduction of P-selectin after platelets removal in HD, in contrast, both ICU and non-ICU COVID-19 patients showed similar high levels of P-selectin with and without platelets.

**Conclusion:** Elevation of P-selectin suggests a central role of platelet endothelium interaction as part of the multifaced pathogenic mechanism of COVID-19 leading to the local activation of hemostatic system forming pulmonary thrombi. Further work is necessary to determine the therapeutic role of antiplatelets agents or of the anti P-selectin antibody Crizanlizumab.

**Keywords:** P-selectin; Covid-19; Endothelium; Platelets.

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**Introduction.** Despite a worldwide spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection approaching, in January 2021, one hundred million cases and two million deaths, this disease's pathophysiology remains inadequately defined and largely understood.

COVID-19 is mainly a respiratory tract disease, and acute respiratory failure and diffuse microvascular pulmonary thrombosis are critical aspects of the morbidity and mortality of the coronavirus disease 2019 (Covid-19).<sup>1</sup> However, both autopsy findings and clinical observations have described vascular damages

and thrombotic complications in a wide range of organs.

Available published data suggest that from one-third to one-half of patients hospitalized with COVID-19 have hemostatic laboratory parameters suggestive of a pro-thrombotic state leading to a coagulopathy. These patients also manifest a hyperinflammatory state characterized by elevated inflammatory markers, strongly associated with severe pneumonia and a high mortality rate.<sup>3</sup>

SARS-CoV-2 enters human cells by binding to the angiotensin-converting-enzyme 2 (ACE2) receptor, expressed on respiratory epithelial cells and other cell types, including endothelial cells.<sup>2</sup>

Direct infection of endothelial cells, as well as the inflammatory environment, might result in an endothelial activation that drives the expression of P-selectin and tissue factor (TF), thus promoting platelet recruitment and aggregation.<sup>4</sup> Subsequent accumulation of mononuclear cells provides a platform for the initiation of plasma coagulation by triggering prothrombin's cleavage to thrombin and fibrin formation.<sup>5</sup>

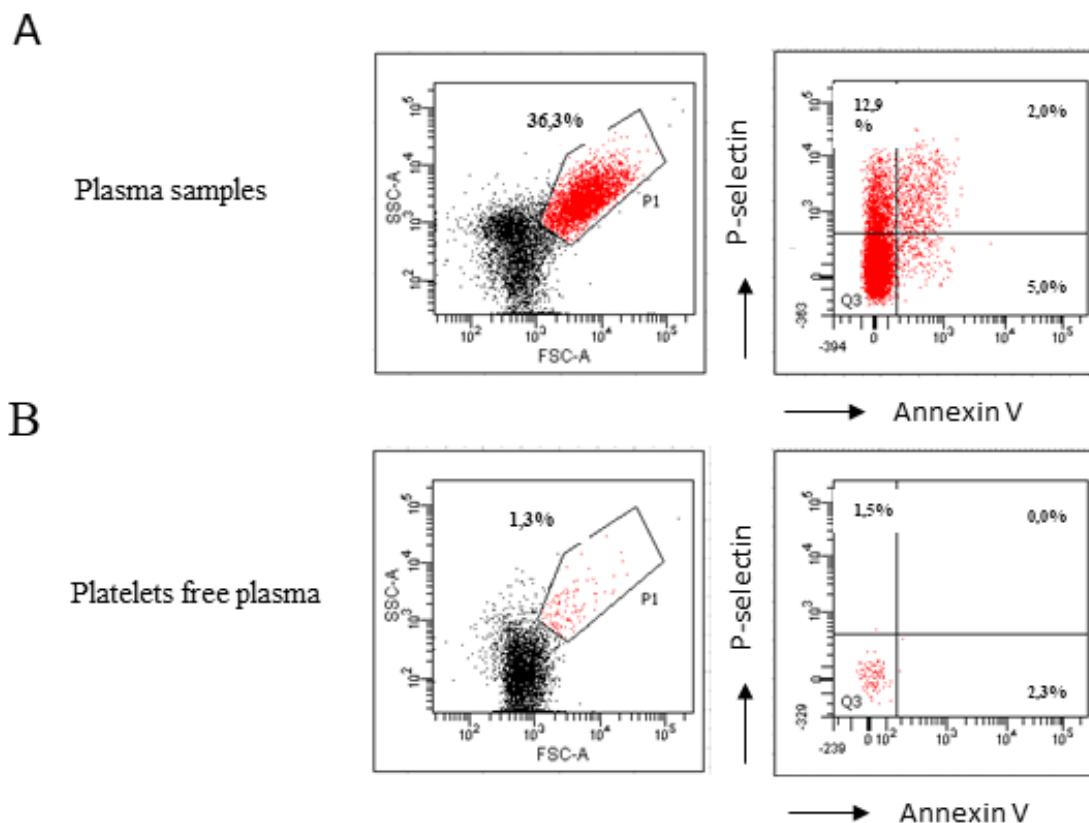
The molecular interaction between P-selectin expressed in platelets and endothelial cells rapidly triggers TF exposure on monocytes,<sup>6</sup> and this may represent a mechanism by which platelets and mononuclear cells contribute to disproportionate intravascular micro-thrombosis in SARS-CoV-2.

The aim of our study was to investigate, in COVID-

19 hospitalized patients compared to healthy adult human controls, the ex-vivo P-selectin plasma concentration as a biomarker of endothelial dysfunction and platelet activation. The association between this parameter at the time of hospital admission and the severity and the outcomes of the disease with subsequent admittance into the intensive care unit (ICU) was finally assessed.

**Study Population.** A group of 46 patients with confirmed SARS-CoV-2 infection, admitted to our Institute between March and April 2020, was included in the study. All enrolled patients had severe illness (respiratory rate >30, SpO<sub>2</sub> <93% on room air at sea level, PaO<sub>2</sub>/FiO<sub>2</sub> <300, or lung infiltrates >50%), or critical illness (association of acute respiratory distress syndrome (ARDS), septic shock, cardiac dysfunction, cytokine storm and/or exacerbation of underlying comorbidities). Age-matched patients were then divided into those requiring admission to the intensive care unit (ICU, ICU cases) vs. those non requiring ICU hospitalization (non-ICU cases). A significant effort was made to exclude from the study population those with prior administration of anti-platelet agents or anticoagulant drugs.

A group of ten age-matched healthy donors (HD) were enrolled in the study as controls. Characteristics of enrolled patients are described in **Figure 1**.



**Figure 1.** The expression of P-selectin and Annexin V on platelet surface was evaluated in plasma samples by flow cytometry (A). The removal of platelets/vesicles in EV-free plasma samples was confirmed by flow cytometry (B).

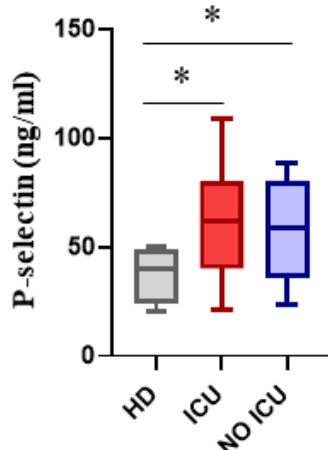
**Material and Methods.** Blood samples of severe COVID-19 patients were collected at the time of hospital admission. Heparin peripheral blood was centrifuged at 1200 rpm for 10 minutes at room temperature to obtain plasma samples containing extracellular vesicles and platelets (Plasma). After that, 500 ul of plasma samples were further centrifuged at 5000 rpm for 5 minutes at room temperature to eliminate platelets and extracellular vesicles (EV-free plasma). To verify the removal of platelets/vesicles in EV-free plasma, we performed a flow cytometry analysis. Specifically, plasma and EV-free plasma were stained with P-selectin and Annex V for 15 minutes at room temperature and then acquired to a FACS Canto II cytometer (**Figure 2**). The quantification of soluble P-selectin was performed by ELISA assay (R&D system; average value in heparin plasma: mean 39 ng/ml (range: 25-53).

**Results.** Our study showed a higher P-selectin plasma concentration in patients with Covid-19, regardless of ICU admission, compared to the normal reference values and compared to contextually sample healthy donors; (COVID-19): median 65.2 (IQRs: 45.1-81.1) vs. HD: 40.3 (IQRs: 24.3-48.7),  $p=0.0023$ ). Moreover, results showed a significant reduction of P-selectin after platelet removal in HD, suggesting that most of this molecule was trapped in the platelets. In contrast, both ICU and non-ICU COVID-19 patients showed similar P-selectin levels with and without platelets, suggesting that Covid-19 induced a release of these molecules from activated platelets/cells (Figure 1C). A similar platelet count has been observed in the two groups ranging within the standard value (150-400/mm<sup>3</sup>). More significantly lower lymphocyte count was observed in ICU patients, confirming an association between lymphocytopenia and disease severity.<sup>6,7</sup>

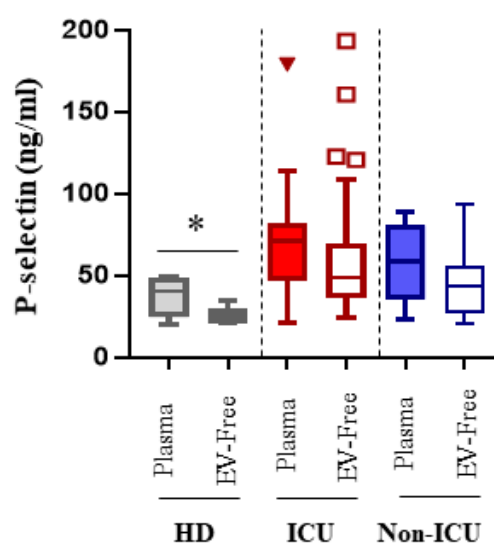
A

	Age (years)	Gender	Platelets	Lymphocytes
	Median (IQRs)	M/F	(n <sup>o</sup> /mmc)	(n <sup>o</sup> /mmc)
			Median (IQRs)	Median (IQRs)
ICU	67 (59-74)	17/10	248 (183-368)	0,79 (0,6-0,9)
non-ICU	67 (50-80)	17/2	210 (168-326)	1,18 (0,95-1,56)

B



C



**Figure 2.** Clinical features of enrolled COVID-19 patients (A). Soluble P-selectin was quantified in plasma samples (B-C) and in extracellular-free plasma samples (EV-free, C) from healthy donors (HD, n=10), ICU (n=27) and in non-ICU (n=19) COVID-19 patients by ELISA assay. Data were compared by Mann-Whitney test. \*  $p<0.05$  was considered significant.

**Discussion.** Our results suggest a central role of platelet endothelium interaction as part of the multifaced pathogenic mechanism of COVID-19, leading to the local activation of the hemostatic system forming pulmonary thrombi. More, these interactions amplify the leukocyte recruitment, increasing chemokine expression on the endothelial surface with extensive adhesion, activation, and leukocyte trafficking across the endothelial wall.<sup>8</sup>

It will be interesting to examine whether therapies inhibiting platelet-endothelium interaction or inhibiting platelet function might improve microvascular perfusion, reduce thrombo-inflammation, and finally reduce COVID-19 morbidity and mortality.

In this perspective, we suggest studying, in the early phases of COVID-19 disease, the role of anti-platelet agents, acetylsalicylic acid, GPIIb, GPIIIa antagonists, and P2Y<sub>12</sub> antagonists, not only in de novo therapy initiation but also in patients previously in prophylaxis or in treatment for cardiovascular disorders. The suggested mechanism to study is not only the direct P-selectin/platelet interaction but also the neutrophil extracellular trap (NET) production as described in sepsis and transfusion-related acute lung injury (TRALI).<sup>9,10,11</sup> Further, Crizanlizumab-tmca, a selectin blocker humanized IgG2 kappa monoclonal antibody that binds to P-selectin, and approved to reduce the frequency of vaso-occlusive crises (VOCs) in adult and pediatric patients, might be evaluated in severe cases not responding or in combination to anti-platelet therapy.<sup>12,13</sup>

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