Increased Von Willebrand factor platelet-binding capacity is related to poor prognosis in COVID-19 patients.

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Abstract:
None

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Increased Von Willebrand factor platelet-binding capacity is related to poor prognosis in COVID-19 patients.

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Coronavirus-associated disease-2019 (COVID-19) is still a global health emergency. Its causative agent, the novel coronavirus SARS-CoV-2, has infected more than 300 million people and caused
more than 5 million deaths worldwide.

SARS-CoV-2 infection increases the risk of thrombotic complications and patients with pre-existing cardiovascular disease (CVD) comorbidities have a higher risk of experiencing severe symptoms and death. The pro-thrombotic state observed in severe patients is not just an epiphenomenon that occurs in patients experiencing a cytokine storm and virus-driven endothelial damage, but also contributes to the rapid worsening of the disease. In the pulmonary microcirculation, microthrombi (1, 2) impair pulmonary gas exchange and aggravate the acute respiratory distress syndrome (ARDS) (3). Systemically, platelet-mediated thrombotic microangiopathy and consumptive coagulopathy may lead to multiple organ failure (4).

Nevertheless, the mechanisms responsible for the formation of thrombi in COVID-19 patients have yet to be fully elucidated. One of the key features of COVID-19 is endothelial injury (5) that results in a dramatic increase of the circulating levels of von Willebrand factor (VWF)(6). The imbalance between VWF and ADAMTS13 increases the hypercoagulable state promoted by COVID-19 disease (7) and it is associated with disease severity (8, 9) and short-term mortality (4). In a previous study, our research network provided evidence that critically ill COVID-19 patients experience increased VWF-induced platelet agglutination (10). To assess if the ability of platelets to bind VWF is different between severe and mild COVID-19 patients we performed a prospective study in which we evaluated VWF antigen levels (VWF:Ag; ASSERACHROME VWF:AG by Stago), VWF ristocetin cofactor activity (VWF:RCo; BCS XP instrument by Siemens), and ristocetin-induced platelet agglutination (RIPA) in COVID-19 patients admitted at the Umberto I Hospital from April to December 2020 (n=49) in relation to disease severity and to the short-term mortality. The RIPA assay was performed by adding scalar doses of ristocetin (0.75, 1, 1.25, 1.5 and 2 mg/ml) to platelet-rich-plasma. If the response to 0.75 mg/ml ristocetin was higher than 40%, ristocetin was also tested at a lower dose (0.5 mg/ml). Sars-COV-2-positive patients (age >18 y, at least one positive swab for COVID-19) were stratified based on their required respiratory support at time of blood sampling, among severe (on assisted or controlled mechanical ventilation and
PaO\textsubscript{2}/FiO\textsubscript{2} <200; n=35) and mild (on nasal cannula or face mask; n=14). Severe patients were further stratified based on short-term mortality (n=21 of 35), defined as mortality occurring less than 20 days after presentation to the hospital. For comparison we evaluated the same parameters in a group of healthy Sars-COV-2-negative volunteers (HV, n=20). The clinical characteristics of patients and HV upon enrollment are shown in the table. The study was approved by the Ethics Committee of the Umberto I Hospital of Rome.

In agreement with previous reports (8), both VWF:Ag and VWF:RCo were significantly higher in severe compared to mild patients (Figure 1A), and, both patient populations displayed significantly higher levels compared to the normal reference values (50-160 IU/dl for VWF:Ag; 41-130 IU/dl for VWF:RCo). However, in the RIPA assay, mild patients required the same amount of ristocetin as healthy volunteers to achieve 20% or 40% platelet agglutination, while severe patients needed significantly less ristocetin to achieve the same level of agglutination (Figure 1B). This data suggested that platelets of severe COVID-19 patients bind more easily to plasmatic VWF, and that this does not depend solely on the VWF concentration. In addition, no correlation was found between ristocetin sensitivity and VWF concentration (data not shown). Interestingly, the minimal ristocetin concentration capable of inducing 20% or 40% platelet agglutination for patients with survival less than 20 days was significantly reduced compared to patients with survival greater than 20 days (p=0.0268 and 0.0005 respectively), to mild COVID-19 patients (p=0.0117 and p=0.0010 respectively) and healthy volunteers (p=0.0004 and p<0.0001 respectively) (Figure 1C). No differences were found between patients with survival greater 20 days compared to both mild patients and HV. Thus, platelets from patients experiencing short-term mortality appear to bind more readily to VWF compared to platelets from patients with survival greater 20 days that have comparable amounts of plasmatic VWF.

VWF binds to platelets via the glycoprotein (GP)Ib-IX receptor. Spontaneous or aberrant adhesion to VWF has been associated with thrombo-inflammation (11). Thus, we speculate that platelet agglutination is facilitated by differences in VWF-GPIb interactions. However we detect no
differences in the levels of VWF:Ag and VWF:RCo among the two subpopulations studied (data not shown). Moreover, previous studies show that, even though ADAMTS13 level and activity is reduced in COVID-19, circulating levels of ultra large VWF multimers are not increased in severe patients (12).

A potential explanation could be that the increased platelet agglutination is due to other components present in the blood of severe patients. A recent study demonstrates that COVID-19 severity correlates with the presence of high plasmatic concentrations of anti-SARS-CoV-2 spike IgG with aberrant glycosylation (hypofucosylated) (13, 14). Moreover a new study by Bye and colleagues (11) went on to show that immune complexes formed of hypofucosylated anti-SARS-CoV-2 IgG and the spike protein increase thrombus formation on VWF in vitro by triggering FcgRIIA signaling. There are indeed reports suggesting that inside-out signaling can upregulate ristocetin-dependent platelet binding to VWF (15).

Based on this evidence, we hypothesize that the increased ability of platelets to agglutinate that we observe in severe patients with short-term mortality is due to the presence of antibodies with aberrant glycosylation in the plasma of these patients. In our cohort we did not detect any difference in the levels of anti-spike IgG based on the short-term mortality (data not shown). Future studies in vivo are needed to confirm this hypothesis.

In other virus infections, IgG antibodies contribute to the worsening of the disease (16) and greater adhesion of platelets with VWF has been reported (17). Collectively these data suggest that COVID-19 thrombotic complications are not solely dependent on Sars-COV-2 but are the result of the aberrant antibody response that amplifies the virus-induced endothelial injury and the VWF-induced platelet adhesion. As other viruses such as Adenovirus, Dengue and Epstein-Barr virus can induce platelet activation and thrombosis (18) we could envisage that pathogens other than SARS-CoV2, could impair the haemostatic balance with a similar mechanism.

A limitation of our work is that we did not measure collagen binding or ADAMTS13 activity as COVID-19 patients had a reduced activity of ADAMTS 13 (8).
In conclusion, we report that platelet adhesion to VWF, observed using ristocetin-induced platelet agglutination, correlates with the mortality of patients and can be a useful tool to identify patients with high risk of clinical deterioration. We also provide indirect supporting evidence that a plasmatic component, possibly immune-complexes containing hypofucosylated IgG, could synergize with VWF to recruit platelets into microthrombi that precipitate the condition of severe COVID-19 patients. The growing understanding of SARS-CoV-2 infection pathogenesis and how it contributes to critical illness and its complications, may help to improve risk stratification, and develop targeted therapies to reduce the acute and long-term consequences of the disease.

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Author contribution: L.S. designed research, analyzed data, and wrote the paper. F.R. designed research, analyzed data, selected patients and collected clinical and epidemiological data. M.C. performed research and analyzed data. E.S. performed research and analyzed data. A.C. performed research and analyzed data. R.M. performed research and analyzed data. M.V. performed research and analyzed data. G.C. analyzed data, selected patients and collected clinical and epidemiological data. G.D. analyzed data, selected patients and collected clinical and epidemiological data. S.C. performed research and analyzed data. O.G. performed research and analyzed data. E.F.M selected patients and collected clinical and epidemiological data. V.Z. selected patients and collected clinical and epidemiological data. F.P. designed research, analyzed data, selected patients and collected clinical and epidemiological data. F.M.P. designed research, performed research, analyzed data, and wrote the paper.

References


Table 1: Characteristics of COVID-19 Patients (Severe and Mild) and Healthy Volunteers (HV)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>COVID-19 Severe n=35</th>
<th>COVID-19 Mild n=14</th>
<th>HV n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-yr</td>
<td>67.9 ±11.2</td>
<td>66.6±19.9</td>
<td>36.3 ± 9</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>19/16</td>
<td>11/11</td>
<td>9/11</td>
</tr>
<tr>
<td>Characteristics</td>
<td>Mortality &lt; 20 d</td>
<td>Survival &gt; 20 d</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td><strong>Demographic characteristics</strong></td>
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</tr>
<tr>
<td># 21</td>
<td>#14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-yr</td>
<td>68.2 ± 11.7</td>
<td>63.5 ± 11.2</td>
<td></td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>9/12</td>
<td>6/8</td>
<td></td>
</tr>
<tr>
<td>SAPS II</td>
<td>39.9 ± 11.9</td>
<td>45.3 ± 13.8</td>
<td></td>
</tr>
<tr>
<td><strong>Medical history</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>76.1 %</td>
<td>71.1 %</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>33.3 %</td>
<td>27.3 %</td>
<td></td>
</tr>
<tr>
<td>Smoke</td>
<td>23.8 %</td>
<td>9.1 %</td>
<td></td>
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<tr>
<td>Obesity</td>
<td>19.1 %</td>
<td>18.1</td>
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<tr>
<td><strong>Symptoms at disease onset</strong></td>
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</tr>
<tr>
<td>Fever</td>
<td>100 %</td>
<td>100 %</td>
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<tr>
<td>Dyspnea</td>
<td>52.4 %</td>
<td>45.4 %</td>
<td></td>
</tr>
</tbody>
</table>

Table 1b Characteristics of severe COVID-19 Patients divided for mortality and survival
**Cough** 66.7% 62.5%

**Figure Legend**

Figure 1

A: Box Plot of the plasma concentration of vonWillebrand factor antigen (VWF:Ag) and vonWillebrand Ristocetin Cofactor (VWF:RCo) evaluated in severe and mild COVID-19 patients. Normal references value were 50–160 IU/dl for VWF:Ag and 41-130 IU/ dl for VWF:RCo.

B: Dot blot of ristocetin (0.75-2 mg/ml) induced platelet agglutination in severe (N=35) and mild (N = 22) COVID-19 patients versus Healthy Volunteers (HV, N=20). The results are reported as the ristocetin concentrations that exceed 20% and 40% of agglutination. Statistical data were evaluated by Wilcoxon-Mann-Whitney rank sum test for upaired data.

C: Dot blot of ristocetin (0.75-2 mg/ml) induced platelet agglutination in the subpopulation of severe COVID-19 patients between who survival greater than 20 days (> 20 days; N=14) and those who died within 20 days from enrollment (< 20 days; N=21). The results are reported as the ristocetin concentrations that exceed 20% and 40% of agglutination. Statistical data were evaluated by Wilcoxon-Mann-Whitney rank sum test for upaired data.