COVID-19: Elevated von Willbrand Factor at Hospital Admission Predicts Clinical Outcomes

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Abstract
Background: Recent studies reported higher thromboembolic complications in COVID-19 with associated mortality. The virus SARS-CoV-2 utilizes ACE2 receptors expressed in endothelial cells. Von Willebrand factor (VWF), released from the vascular endothelium following an injury results in platelet adhesion and aggregation.

Objectives: To study the role of VWF antigen level and other factors associated with thrombogenesis in COVID-19 patients and to correlate with their clinical outcome.

Methodology: A prospective study where COVID-19 patients underwent VWF antigen level, fibrinogen, platelet count, activated partial thromboplastin time, international normalized ratio, Protein C, Protein S, Antithrombin III and D-dimer at the time of hospitalization.

Results: 30 (85.7%) out of 35 COVID-19 patients had elevated VWF antigen level during hospitalization. Twenty-one (60%) patients developed complications significantly elevated VWF antigen level (P= 0.037). There was a positive correlation between VWF antigen level and number of complications in COVID-19 patients. Eleven patients (31.54%) who developed Acute Respiratory Distress Syndrome (ARDS) had a statistically higher VWF antigen level compared to patients with no ARDS (mean 235.1 vs 182.1%; P= 0.024). Patients who died with significantly elevated VWF antigen level (mean 289.9 vs 187%; P= 0.002), lower platelet count (mean 159 vs 275×10⁹/L; P= 0.01), and elevated D-dimer (mean 4253.5 vs 278.5ng/ml; P= 0.001) in comparison to survivors. In addition, there was a positive correlation between VWF antigen level with ferritin (r= 0.628), CRP (r= 0.611) and IL-6 (r= 0.782).

Conclusion: Our study shows that patients with COVID-19 have elevated VWF antigen level correlating with complications and poor outcome.

Background
COVID-19 pandemic caused by SARS-CoV-2 is increasing rapidly in several countries across the world. As of 14th December 2020, 72,655,939 cases have been reported, with a case fatality rate of 2.23%. Despite stringent measures such as social distancing, wearing masks, and imposing lockdown in controlling the disease, the second wave of COVID-19 has emerged in many countries across the world, thus showing no decline in the daily infection.¹ Mortality of COVID-19 is higher in patients, with advanced age, diabetes mellitus, hypertension, and obesity.²

The understanding of COVID-19 has changed significantly since the beginning of the pandemic. Initial studies from China demonstrated COVID-19 as predominantly a respiratory viral illness with fever and flu-like symptoms. The virus utilizes ACE2 receptors in type 2 alveolar cells causing significant lung damage leading to Acute respiratory distress syndrome (ARDS).³ Subsequent studies revealed COVID-19 involvement in other systems such as vascular endothelium, gastrointestinal system, liver, and heart due to the abundance of ACE2 receptors in these tissues.⁴ Higher endothelial expression of ACE2 receptors in these organs attracts binding of SARS-CoV-2 spiked protein.⁵ This causes widespread disruption and damage to the endothelial lining. Endothelial disruption, the release of proinflammatory cytokines further damages the endothelium leading to edema and increased leakiness resulting in Acute Respiratory Distress Syndrome (ARDS).

Up to 25% of COVID-19 patients have shown to develop thromboembolic complications, particularly in those with severe disease.⁶ Atypical presentation of COVID-19 with Myocardial Infarction and stroke have been described.⁷ Currently thromboembolic complications are the most common cause of morbidity and mortality among COVID-19 patients. Post-mortem studies revealed alveolar edema, interstitial lymphocytic inflammation, and importantly, a higher proportion of COVID-19 patients were found to have extensive fibrin thrombi with activated megakaryocytes involving smaller blood vessels.⁸ Distinctly, these patients were found to have severe endothelial damage, loss of endothelial cell tight junctions, widespread microthrombi in both pulmonary arterioles and alveolar capillaries. COVID-19 patients developed significant endothelial damage in comparison to influenza

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mediated by heparin. Thrombosis is an orchestrated phenomenon involving multiple pro-thrombotic, anti-thrombotic, and fibrinolytic factors. Tissue or endothelial injury causes a homeostatic imbalance of these factors resulting in accelerated coagulation, thrombogenesis, and related complications. Von Willebrand factor (VWF) is a large multimeric glycoprotein, expressed in vascular endothelial cells and platelets. VWF gene is located in the locus of short arm of chromosome 12, and measures about 178 kilobase. Under physiological conditions, VWF is produced as a monomer, and undergoes dimerization in the endoplasmic reticulum. Subsequent multimerization occurs in the golgi complex and is stored in Weibel-Palade bodies (WPB) in endothelial cells and in megakaryocytes it gets stored in alpha granules and later gets partitioned into platelet or released into circulation. Each monomeric sub-unit weighs about 225kDa (kiloDaltons) and the multimers may range from 500 to 20,000kDa. Endothelial injury results in the activation and release of stored VWF multimers, leading to platelet adhesion and aggregation on the damaged surface. Protein C, Protein S, and Antithrombin III (AT III) are endogenous anticoagulants involved in the regulation of coagulation. Protein C is activated by Protein S in the presence of thrombomodulin, which in turn inhibits factors V and VIII in the coagulation cascade. AT-III is a plasma glycoprotein responsible for thrombin inhibition in the blood coagulation cascade and its biological activity is mediated by heparin.

Aim
To evaluate the serum levels of VWF antigen and other factors associated with thrombogenesis in COVID-19 patients and to correlate with clinical outcomes.

Methodology

Patients
A prospective observational study of thrombogenic factors on consecutive COVID-19 patients admitted to our institute between 1st July 2020 to 12th July 2020 was carried out following informed consent at the time of hospitalization. All patients above 16 years of age with COVID-19 were included in the study. Patients who were already on antithrombotic agents or anticoagulants, pregnant women and patients unwilling to participate were excluded. All hospitalized COVID-19 patients underwent investigations as per the unit protocol and inflammatory markers such as C-reactive protein (CRP), ferritin, Interleukin-6 (IL-6), and lactate dehydrogenase (LDH). Radiological investigations were carried out upon clinical indications to assess COVID-19 disease severity. Following parameters were tested at the time of hospital admission: VWF antigen level, fibrinogen level, platelet count, Activated partial thromboplastin time (APTT), International normalized ratio, Protein C, Protein S, AT III, and D-dimer. COVID-19 patients received corticosteroids, anticoagulants, antibiotics, antiviral medications, and vitamin supplements as per our institution protocol.

Materials
About 3.0ml blood was collected in a Greiner bio-one citrated (3.2% disodium citrate) vacutainer at the time of hospital admission. Laboratory analysis was performed on the citrated plasma after separation based on the following methods VWF antigen level using latex enhanced immunoassay, fibrinogen level using clot-based assay, D-Dimer using high sensitive latex enhanced immunoassay. Similarly, Protein C and AT III using automated chromogenic assay, Protein S activity was carried out using a clot-based assay with fully automated Haemostasis analyzer ACL TOP-750 (Instrumentation Laboratory-a Werfen company, USA). The normal reference range of VWF antigen level expressed in percentage (%) was calculated according to the patient’s blood group. For blood group O the reference range was 41.1% to 125.9% and for non-O blood group 61.3% to 157.8%. Normal range were set as following, fibrinogen 220-496 mg/dL, D-Dimer 0-250 ng/mL, Protein C 70-140 %, Protein S 63.5-149 %, AT III 83-128 %. Instrumentation Laboratory Normal Control was processed with each batch of the run to monitor the method performance. The obtained control result agreed with the certified values. The performance of these tests was correlated with COVID-19 severity and clinical outcomes. Clinical complications were defined as patients requiring ICU stay, worsening lung infiltrates accounting for ARDS, prolonged hospital stay, multi-organ dysfunction or death.

Ethics Approval
Institutional Ethics Committee approval obtained

Statistical analysis
Data entries was analyzed using Statistical Package for the Social Sciences (SPSS Statistics v21.0) software. Descriptive statistics were used to summarize the basic features and the visualization of the data. Mean, standard variation, frequency and percentage of variables were calculated.

Student t-test was utilized to compare the means in factors associated with thrombosis with respect to patients COVID-19 disease severity, complications and clinical outcome. Correlation coefficient (r) and scatter plots were used to quantify the association between the thrombogenic factors, and inflammatory markers.

Univariate regression analysis was used to infer the relationships between the VWF antigen level (X or Dependant variable) and the independent variables (Y) such as duration of hospital stay, ferritin, CRP, and IL-6. P-value of <0.05 was considered as statistically significant.

Results

35 COVID-19 patients underwent tests for thrombosis at the time of hospitalization, with a median age of 50 (IQR:44.5-55.7) years and male-female ratio of 1.69:1. Fever (n= 18; 51.4%) and dry cough (n= 11; 31.4%) were the most common presenting symptoms. Twenty-three (65.7%) patients had underlying co-morbidities such as diabetes mellitus (n= 14; 40%) and hypertension (n= 11; 31.4%).

Thirty (85.7%) COVID-19 patients had elevated VWF antigen level (Blood group-O:41.1-125.9%; Blood group A, B and AB:61.3-157.8%) at the time of hospital admission (overall mean:198.1); whereas 4 (13.3%), 3 (8.5%), 0 (0%), 0 (0%), 3 (8.5%), 1 (2.9%) ,5 (14.3%) and 14 (40%) patients had abnormal levels of fibrinogen, platelets,
Table 1: Comparison of factor levels between COVID-19 patients with complications and without complications

<table>
<thead>
<tr>
<th></th>
<th>Complications (n= 21; 60%)</th>
<th>No complications (n= 14; 40%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pro-coagulant factors</strong></td>
<td></td>
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</tr>
<tr>
<td>VWF (O group: 41.1-125.9 %; non-O group: 61.3-157.8 %)</td>
<td>216.1 71.6 183.58-248.88</td>
<td>172.7 46.7 145.76-199.73</td>
<td>0.037</td>
</tr>
<tr>
<td>Fibrinogen (238-496 mg/dL)</td>
<td>385.5 129.4 323.18-447.97</td>
<td>301.6 96.3 268.86-366.39</td>
<td>0.054</td>
</tr>
<tr>
<td>Platelet (150-450 x10^9/L)</td>
<td>250.7 95.2 206.17-295.33</td>
<td>277.4 87.7 209.98-344.90</td>
<td>0.471</td>
</tr>
<tr>
<td>APTT (25-35 secs)</td>
<td>33.0 14.1 26.06-39.96</td>
<td>33.3 6.0 26.01-34.71</td>
<td>0.495</td>
</tr>
<tr>
<td>INR (0.90-1.15)</td>
<td>1.01 0.12 0.96-1.07</td>
<td>1.02 0.14 0.93-1.13</td>
<td>0.807</td>
</tr>
<tr>
<td><strong>Anti-coagulant factors</strong></td>
<td></td>
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<tr>
<td>Protein C (70-140 %)</td>
<td>94.8 20.9 85.29-104.33</td>
<td>97.1 28.4 80.69-113.59</td>
<td>0.795</td>
</tr>
<tr>
<td>Protein S (63.5-149 %)</td>
<td>99.8 28.9 86.73-113.06</td>
<td>103.5 23.5 89.97-117.17</td>
<td>0.683</td>
</tr>
<tr>
<td>Antithrombin III (83-128 %)</td>
<td>98.3 14.1 91.92-104.75</td>
<td>97.8 97.8 82.09-113.63</td>
<td>0.953</td>
</tr>
<tr>
<td><strong>Pro-fibrinolytic factors</strong></td>
<td></td>
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<tr>
<td>D-dimer (0-250 ng/mL)</td>
<td>894.6 2268.8 -359.83-2149.03</td>
<td>248.4 212.4</td>
<td>0.297</td>
</tr>
</tbody>
</table>

APTT, INR, Protein C, Protein S, AT III and D-dimer respectively at the time of admission.

Complications vs no complications

Twenty-one (60%) COVID-19 patients developed complications during the study period. Patients who developed complications had significantly elevated VWF antigen level compared to those with no complications (mean 216.1 vs 172.7 %; P= 0.037). Other parameters showed no statistical difference; fibrinogen level (mean 385.5 vs 301.6 mg/dL; P= 0.054), platelet count (mean 250.7 vs 277.4 x10^9/L; P=0.471), APTT (mean 33.0 vs 33 secs; P=0.495), INR (mean 1.01 vs 1.02; P= 0.807), Protein C (mean 94.8 vs 97.1 %; P= 0.795), Protein S (mean 99.8 vs 103.5 %; P= 0.683), AT III (mean 98.3 vs 97.8 %; P= 0.953) and D-dimer (mean 894.6 vs 248.4 ng/mL; P= 0.297) as illustrated in Table 1.

Out of 21 patients who had complications, 10 (47.6%), 3 (14.2%), 4 (19%), 4 (19%) had one, two, three, and four complications respectively. Detailed analysis is presented in the supplementary table.

On further analysis, there was a positive correlation between VWF antigen level and the number of complications in COVID-19 patients (Figure 1).

COVID-19: ICU vs non-ICU Patients

Eight (22.8%) patients required ICU support. Table 2 illustrates the levels of thrombotic factors between ICU and non-ICU patients. COVID-19 patients requiring ICU had a significantly higher levels of VWF antigen level (mean 252.9 vs 182.7 %; P= 0.006), fibrinogen (mean 432 vs 326.7 mg/dL; P= 0.037) and D-dimer (mean 2075.7 vs 253.7 ng/mL; P= 0.043). Platelet count (mean 246.2 vs 263.9 x10^9/L; P= 0.672), INR (mean 1.09 vs 0.99; P= 0.04), APTT (mean 38.7 vs 29.9 secs; P= 0.361), Protein C (mean 92.7 vs 96.6 %; P= 0.572), Protein S (mean 96.6 vs 102.7 %; P= 0.488) and AT III (mean 96.7 vs 98.3 %; P= 0.804) were not different between the groups. The Odds ratio of COVID-19 patients requiring ICU support with VWF antigen level >200% requiring ICU support was 11.08 (95%CI: 1.24-99.15; P= 0.006).

Prolonged Hospital Stay

Twenty-one patients (60%) had ≥7 days of hospital stay. Patients with prolonged hospital stay had significantly increased VWF antigen level (mean 216.2 vs 172.7 %; P= 0.037). There was a significant logarithmic correlation between VWF antigen level and the duration of hospitalization in COVID-19 (Figure 2). However, there was no statistical significance noted among the other parameters.

ARDS vs no ARDS

Eleven patients (31.54%) developed ARDS. Patients with ARDS had statistically higher VWF antigen level compared to patients with no ARDS (mean 235.1 vs 182.1 %; P= 0.024).

Dead vs Alive

Four (11.4%) COVID-19 patients died during the study period. Patients who died had higher VWF antigen (mean 289.9 vs 187 %; P= 0.002), lower...
platelet count (mean 159.3 vs 275 ×109/L; P< 0.01) and elevated D-dimer (mean 4265.3 vs 278.5 ng/mL; P< 0.001) in comparison to alive patients. (supplementary table)

Correlation between thrombogenic factors and inflammatory markers

COVID-19 patients with elevated VWF antigen level had higher inflammatory markers, such as ferritin (mean 459.2 [95% CI:250-668] vs 63.3 mg/dL [95%CI:26-152.8]; P< 0.001), LDH (mean 287 [95%CI: 229-346] vs 185.8 U/L [95%CI:163-209]; P< 0.002), CRP (mean 46.8 [95%CI:21-72] vs 1.2 mg/dL [95%CI:0.27-2.2]; P< 0.001) and IL-6 (mean 49.3 [95%CI 27.7-70.9] vs 3.5 pg/mL [95%CI 2.3-4.7]; P< 0.00) compared to patients with normal VWF antigen level. In addition, the was a positive correlation between VWF antigen level with ferritin (r= 0.628), CRP (r= 0.611) and IL-6 (r= 0.782) levels. (Table 3)

Discussion

COVID-19 is associated with a higher incidence of thromboembolic complications, particularly in those with severe and progressive disease. Autopsy studies revealed widespread endothelial damage with extensive microvascular thrombosis involving pulmonary as well as systemic vasculature. Our study demonstrates that 85.7% of COVID-19 patients admitted to the hospital have elevated VWF antigen level. A similar study published earlier showed elevated VWF antigen in 94% of COVID-19 patients.16 Interestingly, the cut off values of VWF antigen level varies depending on the blood group. Blood group O patients tend to have a higher cut off level than the non-blood group O. We stratified and adjusted VWF antigen level accordingly for better results.

Our data shows that COVID-19 patients requiring ICU care had significantly higher VWF antigen level in comparison to non-ICU patients (mean 252.9 vs 182.7 9.9); P< 0.006). In addition, we found a clear association between elevated VWF antigen level and COVID-19 patients with complications. The levels were much higher in patients with ARDS (mean 235.1 vs 182.1 %; P< 0.024), prolonged hospital stay (mean 216.9 vs 172.7 %; P= 0.037), and in particular, in those who died (mean 289.9 vs 187 %; P= 0.002) compared to those with no ARDS, with shorter hospital stay (<7 days) and survived. The mortality rate in our COVID-19 patients with elevated VWF antigen level was 11.4%. Our mortality was slightly lower compared to a study by Ladikou et al, where the mortality rate was 16.7%, involving 24 patients.17 However, a larger study by Helms et al, in COVID-19 patients with ARDS showed a mortality of 8.7 %, and a much higher median VWF antigen level compared to our study (455% vs 216.3%).18 These features may be an indication of accelerated prothrombotic state in patients with severe COVID-19. Further analysis showed a positive correlation between VWF antigen level and the number of complications. We do not know whether elevated VWF antigen level is a consequence or cause of complications. We evaluated the VWF antigen level at the time of hospital admission when the patient did not have clinical complications. This indicates that elevated VWF antigen level may occur prior to the onset of complications. Interestingly, in comparison with other studies, the median VWF antigen levels were low in our study. Patients with mild COVID-19 in our series had a VWF antigen level of 197.7 whereas, in a study by Cugno et al, the median level was 263%. Similarly, in patients with severe COVID-19, the levels were 216.6% in our series as compared to 455% from the west. It is unclear whether these differences explain the lower mortality in our series of COVID-19 patients.19

Elevated inflammatory markers such as ferritin, CRP, and IL-6 were associated with severity in COVID-19. Our data showed a clear correlation between VWF antigen level and inflammatory markers. In addition, there was a positive correlation between the VWF antigen level and the severity of inflammation.

Non-survivors in our series had higher VWF antigen level, lower platelets, and elevated D-dimer. This probably represents the onset of DIC as a terminal event leading to death. In concurrence, both ours and the study by Berger et al, showed higher mortality in patients with elevated D-dimer levels compared to patients with normal levels (20% vs 2% in our study vs 29.9% vs 10.8%).20

Overall, our study shows elevated VWF antigen level in patients with COVID-19 which correlates with disease severity, complications, and mortality. Elevated VWF antigen probably an indication of underlying endothelial injury could be due to caused directly by SARS-CoV-2 virus or secondary to systemic inflammation or maybe an immune dysfunction leading to endothelitis.

Our study is limited by smaller patient numbers, based predominantly on VWF antigen level and other routine clinical parameters. We do not know the baseline VWF antigen level in the general population. However, our study throws open to a number of questions that need further research.

In conclusion, our study shows that COVID-19 have elevated VWF antigen level correlating with complications and poor outcome. Larger studies

Table 2: Comparison of thrombotic factors in COVID-19 patients requiring ICU and non-ICU

<table>
<thead>
<tr>
<th></th>
<th>ICU (n= 8; 22.8%)</th>
<th>Non-ICU (n= 27; 77.2%)</th>
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<tbody>
<tr>
<td><strong>Pro-coagulant factors</strong></td>
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<td>VWF (O group:41.1-125.9%; non-O group:61.3-157.8%)</td>
<td>252.9 ± 106.6</td>
<td>163.7± 342.1</td>
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<tr>
<td>Fibrinogen (238-496 mg/dL)</td>
<td>432.0</td>
<td>159.2</td>
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<tr>
<td>Platelet (150-450 ×10^9/L)</td>
<td>246.2</td>
<td>100.4</td>
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<td>APTT (25-35 secs)</td>
<td>38.7</td>
<td>23.36</td>
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<tr>
<td>INR (0.9-1.15)</td>
<td>1.09</td>
<td>0.07</td>
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<td>Protein C (70-140%)</td>
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<td>Antithrombin III (83-128%)</td>
<td>96.7</td>
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<td><strong>Pro-fibrinolytic factors</strong></td>
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<tr>
<td>D-Dimer (0-250 ng/mL)</td>
<td>2075.7</td>
<td>4492.0</td>
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### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>95% CI</th>
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<th>95% CI</th>
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<td>342.1± 197.7</td>
<td>0.006</td>
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<tr>
<td>Fibrinogen (238-496 mg/dL)</td>
<td>432.0</td>
<td>159.2</td>
<td>298.9-565.1</td>
<td>263.9</td>
<td>91.1</td>
</tr>
<tr>
<td>Platelet (150-450 ×10^9/L)</td>
<td>246.2</td>
<td>100.4</td>
<td>162.3-330.2</td>
<td>96.6</td>
<td>18.6</td>
</tr>
<tr>
<td>APTT (25-35 secs)</td>
<td>38.7</td>
<td>23.36</td>
<td>17.1-60.4</td>
<td>29.9</td>
<td>4.56</td>
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<tr>
<td>INR (0.9-1.15)</td>
<td>1.09</td>
<td>0.07</td>
<td>1.01-1.16</td>
<td>0.99</td>
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</table>
are required to ascertain the utility of VWF antigen level in patients with COVID-19, particularly with the commencement of the second wave in several countries.

This study was approved by the hospital’s internal ethical committee.

**Declarations**

**Ethics Approval**

Institutional Ethics Committee approval obtained

**References**


