Hemostatic alterations in COVID-19

by Flora Peyvandi, Andrea Artoni, Cristina Novembrino, Stefano Aliberti, Mauro Panigada, Marco Boscarino, Roberta Gualtierotti, Federica Rossi, Roberta Palla, Ida Martinelli, Giacomo Grasselli, Francesco Blasi, and Armando Tripodi

Haematologica 2020 [Epub ahead of print]


Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
Hemostatic alterations in COVID-19

Flora Peyvandi,¹,² Andrea Artoni,¹ Cristina Novembrino,¹ Stefano Aliberti,²,³ Mauro Panigada,⁴ Marco Boscarino,¹ Roberta Gualtierotti,¹,² Federica Rossi,¹ Roberta Palla,² Ida Martinelli,¹ Giacomo Grasselli,²,⁴ Francesco Blasi,²,³ and Armando Tripodi¹

¹Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy
²Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milan, Italy
³Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, UOC Pneumologia, Milan, Italy
⁴Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, UOC Anestesia e Terapia Intensiva Adulti, Milan, Italy

Running title: COVID-19 acquired coagulopathy

Text word count: 1483

Corresponding author
Armando Tripodi
Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center,
via Pace 9,
20122 Milan, Italy
Tel +39 02 55035414
Fax +39 02 54100125
E-mail: armando.tripodi@unimi.it
Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),\textsuperscript{1,2} that, first identified in China, has spread globally. A coagulopathy is common, particularly in patients admitted to intensive care units (ICU).\textsuperscript{3} Although controversial,\textsuperscript{4-6} high rates of venous thromboembolism (VTE) are also reported.\textsuperscript{7} The International Society on Thrombosis and Haemostasis (ISTH) released a statement suggesting prophylactic low molecular weight heparin (LMWH).\textsuperscript{8} However, the optimal strategy for prophylaxis remains controversial,\textsuperscript{9} owing to limited knowledge on how COVID-19 affects hemostasis.

At the beginning of the pandemic, COVID-19 patients were reported to present with abnormalities mimicking the coagulopathies like disseminated intravascular coagulation (DIC) or sepsis induced coagulopathy (SIC).\textsuperscript{10} However, a more recent study in a small group of patients severe enough to be admitted to the ICU failed to confirm DIC, because patients presented with marked increase of D-dimer but without hypofibrinogenemia or thrombocytopenia, i.e., the hallmarks of DIC with consumption coagulopathy.\textsuperscript{3} With this controversial background, we report the results obtained using an array of hemostasis measurements in COVID-19 patients, admitted first to the emergency room and then to different wards characterized by delivery of different levels of intensity-care depending on disease severity.

After the viral diagnosis, 62 patients, depending on their severity, were consecutively admitted to three wards, characterized by low-intensity care (n=21), when hypoxia could be handled by ventilation support with high-flow nasal cannulas; intermediate sub-intensive care (n=21), when hypoxia prompted the use of continuous positive airway pressure, or high-intensity care (n=20) when hypoxia warranted intubation and mechanical ventilation in ICU. In this context, we designed the project COHERENT (COVID-19: HEmostasis, immune Response, ENdothelial perTurbation and complement), aimed to investigate the mechanism of thrombosis in COVID-19 patients. The project received approval by Comitato Etico Area2, Milano (no.360_2020). Patients started prophylaxis with low-dose LMWH on admission and dosages were then adjusted by attending physicians after patient transfer to the hospital wards. LMWH dosages were as follows: low-intensity, enoxaparin 70U/Kg o.d.; intermediate-intensity, 70U/kg b.i.d.; high-intensity, 100U/kg o.d. Venous blood was collected, not earlier than 72h after the beginning of LMWH prophylaxis and before the administration of the daily dose in vacuum-tubes containing 1/10 volumes of trisodium citrate 0.109 M. Specimens were centrifuged for 20 minutes at 3000g.
Prothrombin and activated partial thromboplastin time (PT, APTT) were performed using Recombiplastin-2G and Synthasil APTT (Werfen, Orangeburg, NY) with results expressed as clotting time ratios (patient-to-normal). Factor VIII (FVIII) and FII were measured by the one-stage assay based on APTT and FVIII-deficient plasma and PT-based assay and FII-deficient plasma, respectively (Werfen). von Willebrand factor antigen (VWF:Ag) and ristocetin cofactor activity (VWF:RCo) were measured by commercial kits (Werfen). Fibrinogen was measured according to Clauss. D-dimer and free protein S (PS) antigen were measured by latex-based assays (Werfen). Antithrombin and protein C (PC) activity were measured by chromogenic assays (Werfen). Platelet counts and markers of inflammation and acute-phase reactions (C-reactive-protein and ferritin) were obtained from the patients’ records.

The DIC score was calculated using ISTH criteria. In patients with sepsis, SIC score is more sensitive than the DIC score to detect an associated coagulopathy, thus we also calculated this score that is based on platelet count, PT-INR and the Sequential Organ Failure Assessment (SOFA) score that includes data on respiratory, cardiovascular, hepatic and renal dysfunction, but also on the presence of hemostasis alterations such as thrombocytopenia and PT-INR.

Patients characteristics did not differ in the 3 groups. No differences for well-known risk factors and comorbidities (age, BMI, hypertension, diabetes) between the groups according to the intensity of care were observed. In the entire cohort we recorded three deaths and 25 thrombotic events (40%) in 25 patients, i.e., 16 deep-vein thrombosis, 8 pulmonary embolism, 1 visceral venous thrombosis.

Median (min-max) values of the hemostasis measurements in COVID-19 patients are in Table 1. The PT-ratio was slightly increased in patients at high and intermediate care intensity compared with those at low-intensity. The APTT-ratio was slightly less than unity in all patients irrespective of care intensity. Median platelet counts for patients at intermediate or high care intensity were higher than those at low-intensity; the lowest observed platelet count (i.e., 80x10^9/L) being higher than 50x10^9/L, usually considered the threshold for DIC. Fibrinogen for patients admitted to the three care intensity wards were higher than the upper limit of normal range, with a gradient of increase across the care intensities and with values in patients at high-intensity care as high as 1035mg/dL. The lowest observed fibrinogen level (i.e., 150mg/dL) was higher than 100mg/dL that is incorporated in the DIC score as threshold value to assign points. A similar trend of positive association with the level of care intensity was observed for D-dimer; median
values ranging from 870 (low-intensity) to 1,347 or to 2,217ng/mL (intermediate- or high-intensity care) (Table 1). The median (min-max) DIC score for the whole patient cohort was 2 (0-4), with only one patient scoring 4. SIC scores were similar in the three groups, all being below the cut-off of 4. Median FVIII, already high (208U/dL) in low-intensity patients, increased steadily in those at intermediate (223U/dL) or high-intensity (302U/dL). Median antithrombin varied from 87U/dL (low-intensity) to 100U/dL (high-intensity). PC was increased in patients at low-intensity (i.e.,126U/dL) or high-intensity of care (i.e.,143U/dL). PS free antigen was lower than 100U/dL, with small variations according to the intensity of care (Table 1, Fig 1). Median VWF:Ag was high in patients at low-intensity (i.e.,262U/dL) and increased further at intermediate (i.e.,371U/dL) and high-intensity (i.e.,466U/dL). VWF:RCo values paralleled those of VWF:Ag, albeit at a lower level, so that the VWF:RCo/VWF:Ag ratio ranged between 0.85 (low), 0.86 (intermediate) and 0.81 (high) care intensity (Table 1, Fig 2). The median FVIII/VWF:Ag ratio ranged between 0.81 (low), 0.61 (intermediate) and 0.65 (high) care intensity. Median ferritin was extremely high, i.e., 380, 705 and 788µg/L in patients at low, intermediate, and high-intensity care. C-reactive protein was 1.00, 3.32 and 5.05mg/dL in patients at low, intermediate and high-intensity care (Table 1).

Several studies reported that COVID-19 patients have an acquired coagulopathy with an increased risk of VTE in critically ill patients.4-7 However, the frequency varies greatly and there is still unsettled strategy for prophylaxis.12 Therefore, besides the need of well-designed randomized clinical trials, we deemed crucial to better understand mechanistically thrombosis, with the ultimate goal to implement more targeted approaches to management. With this background, we investigated coagulation in infected patients hospitalized on the basis of their clinical severity in three different intensity-care wards by employing an array of measurements centralized in the same laboratory, with special emphasis on those used to diagnose DIC and SIC, the pro- and anticoagulant factors and those indicating endothelial perturbation. Our results did not confirm DIC, because high D-dimer was the only compatible result, whereas other parameters indicating consumption coagulopathy, as low fibrinogen and platelet counts, were normal or often increased. Furthermore, none of the patients had a DIC score of 5 or more (the threshold indicating a high likelihood of DIC according to the ISTH criteria).11 The vast majority of patients had a score of 2 or less and only one had a score of 4, driven by remarkably high levels of D-dimer (i.e.,38,847ng/mL). Similarly, SIC scores were similar in the three groups and all below the cut-off value of 4, so that these patients differed from those with sepsis. FVIII,
one of the most potent procoagulants, was strikingly increased with a gradient from low to high intensity-care, suggesting a state of hypercoagulability roughly proportional to disease severity. VWFAg was even higher than FVIII, causing the reduction of the FVIII/VWF:Ag ratio proportionally to the degree of disease severity and thus suggesting that endothelial cell perturbation concurs with hypercoagulability to explain mechanistically the clinical manifestations of VTE associated with COVID-19. These views are supported by the findings of Goshua et al\textsuperscript{13} who recently showed that VWF and D-dimer were significantly higher in ICU versus non-ICU patients.

Overall, the above findings are consistent with a complex crosstalk between inflammation, hemostasis and endothelial cells that, once activated during inflammation, acquire a prothrombotic phenotype that in turn contributes to the procoagulant imbalance. These findings are mechanistically plausible with the increased VTE risk in COVID-19 patients, with a possible added contribution from fibrinolysis derangement not explored in this study. The clinical picture of hospitalized COVID-19 patients in Milan differed not only from DIC\textsuperscript{3} but also from other disorders characterized by hypercoagulability and endothelial perturbation, triggered by systemic inflammation, such as the hemophagocytic lymphohistiocytosis/macrophage activation syndrome\textsuperscript{14} and bacterial sepsis.\textsuperscript{15} The reasons for such differences may rest on evaluation of patients at different disease stages and/or the early start of LMWH prophylaxis, even though the striking hypercoagulability was present notwithstanding the implementation of prophylaxis.

In conclusion, this study in COVID-19 patients characterizes an acquired coagulopathy associated with hyperacute inflammation, hypercoagulability and endothelial perturbation broadly proportional to the clinical severity of the infection and to the levels of intensity of care needed by the patients.

Acknowledgments

This work was partially supported by the Italian Ministry of Health - Bando Ricerca Corrente and partially financed by Italian fiscal contribution "5x1000" 2017 devolved to Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico. The authors would like to thank Prof Pier Mannuccio Mannucci for his critical revision of the manuscript.

Authorship contribution

FP, AA and AT conceived the study. AA supervised blood samples and data collection. SA, MP, RG, IM, GG and FB managed patients. CN, FR and RP made testing. MB made
statistical analysis. FP and AT wrote the manuscript. All the authors reviewed data and revised the manuscript.

**Conflict-of-interest statements**
FP reports personal fees from Roche, Sanofi, Sobi, Spark, and Takeda, outside the submitted work; CN reports personal fee from Instrumentation Laboratory, Roche, Bayer, Novonordisk, Sobi, outside the submitted work; SA reports grants and personal fees from Bayer Healthcare, Aradigm Corporation, Grifols, Chiesi, and INSMED, and personal fees from Astra Zeneca, Basilea, Zambon, Novartis, Raptor, Actavis UK Ltd, and Horizon, outside the submitted work; RG reports personal fees from Biomarin and Takeda, outside the submitted work; IM reports personal fees from Bayer, Daiichi-Sankyo, Pfizer, Werfen, Grifols, Italfarmaco, outside the submitted work; RP reports personal fees from Novonordisk, outside the submitted work; GG reports personal fees from Biotest, Draeger Medical, Getinge Thermofisher and Fisher&Paykel, outside the submitted work. FB reports grants and personal fees from Astrazeneca, Chiesi, GSK, Insmed, and Pfizer, grants from Bayer, personal fees from Guidotti, Grifols, Menarini, Mundipharma, Novartis, and Zambon, outside the submitted work; AT reports speaker’s fees from Werfen, Stago, Sobi. The other authors (AA, MP, MB, FR) have nothing to disclose.
REFERENCES


Table 1. Median (min-max) values of the hemostasis measurements in COVID-19 patients.

<table>
<thead>
<tr>
<th></th>
<th>Intensity of care</th>
<th>Tests for DIC diagnosis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (min-max)</td>
<td>Intermediate (min-max)</td>
<td>High (min-max)</td>
</tr>
<tr>
<td>PT ratio</td>
<td>1.02 (0.85-1.33)</td>
<td>1.12 (0.95-1.44)</td>
<td>1.06 (0.96-1.133)</td>
</tr>
<tr>
<td>APTT ratio</td>
<td>0.93 (0.79-1.22)</td>
<td>0.91 (0.78-1.10)</td>
<td>0.95 (0.78-1.15)</td>
</tr>
<tr>
<td>Platelet count, n x 10^9/L</td>
<td>275 (138-480)</td>
<td>362 (120-556)</td>
<td>366 (80-584)</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>344 (150-861)</td>
<td>471 (285-830)</td>
<td>531 (224-1035)</td>
</tr>
<tr>
<td>D Dimer, ng/mL</td>
<td>870 (203-38,847)</td>
<td>1347 (525-6,910)</td>
<td>2,217 (564-6,410)</td>
</tr>
<tr>
<td>Pro- and anticoagulant factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor II, U/dL</td>
<td>116 (65-140)</td>
<td>94 (76-128)</td>
<td>104 (75-143)</td>
</tr>
<tr>
<td>Factor VIII, U/dL</td>
<td>208 (121-347)</td>
<td>223 (109-423)</td>
<td>302 (178-374)</td>
</tr>
<tr>
<td>Antithrombin, U/dL</td>
<td>87 (61-133)</td>
<td>94 (63-135)</td>
<td>100 (71-143)</td>
</tr>
<tr>
<td>Protein C, U/dL</td>
<td>120 (60-234)</td>
<td>126 (72-210)</td>
<td>143 (85-232)</td>
</tr>
<tr>
<td>Protein S free antigen, U/dL</td>
<td>75 (38-98)</td>
<td>72 (26-95)</td>
<td>84 (56-110)</td>
</tr>
<tr>
<td>Endothelial-derived factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VWF:Ag, U/dL</td>
<td>262 (90-577)</td>
<td>371 (132-769)</td>
<td>466 (231-746)</td>
</tr>
<tr>
<td>VWF:RCo, U/dL</td>
<td>210 (88-447)</td>
<td>303 (129-539)</td>
<td>383 (195-528)</td>
</tr>
<tr>
<td>VWF:RCo /Ag ratio</td>
<td>0.85 (0.65-1.02)</td>
<td>0.86 (0.62-0.98)</td>
<td>0.81 (0.69-1.01)</td>
</tr>
<tr>
<td>FVIII/VWF:Ag Ratio</td>
<td>0.81 (0.40-2.05)</td>
<td>0.61 (0.32-1.00)</td>
<td>0.65 (0.40-0.97)</td>
</tr>
<tr>
<td>Inflammation markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin, µg/L</td>
<td>380 (32-1,587)</td>
<td>705 (124-4,081)</td>
<td>788 (212-5,064)</td>
</tr>
<tr>
<td>C-reactive protein, mg/dL</td>
<td>1.00 (0.07-11.71)</td>
<td>3.32 (0.19-18.3)</td>
<td>5.05 (0.6-25.5)</td>
</tr>
</tbody>
</table>
LEGENDS TO FIGURES

Fig 1. Box plots of results for factor VIII, antithrombin, protein C and protein S for patients at low, intermediate and high intensity of care.

Fig 2. Box plots of results for von Willebrand factor antigen (VWF:Ag), von Willebrand factor ristocetin-cofactor (VWF:RCO), VWF:RCO/Ag ratio and FVIII/VWF:Ag ratio for patients at low, intermediate and high intensity of care.
Fig. 2