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Response to “Studies on hemostasis in COVID-19 deserve careful reporting of the laboratory methods, their significance and their limitation”: Don’t throw the baby out with the bathwater

Dear Editor,

We appreciate the opportunity to respond to the letter from Dr Hardy and colleagues.1 The authors stress the large variability observed between different anti-Xa kits available on the market, they contest our results of thrombin generation assay (TGA) in patients with COVID-19 receiving standard- or high-dose prophylaxis and question the relevance of thromboelastography as a global hemostasis assay. We would like to respond to the methodological concerns raised by the authors.

The key concern they raised is the choice of the anti-Xa kit. We used HemosIL Liquid anti-Xa kit (Werfen, Le Pré-Saint-Gervais, France) containing dextran sulfate but no exogenous antithrombin. It is indeed true that different anti-Xa methods can give different results.2 The anti-Xa methods that are the least influenced by plasma proteins, ie, with dextrans and appropriate dilution, approach the plasma concentration best. In a single plasma sample there is only one heparin concentration, determined essentially by the number of high-affinity pentasaccharide sequences per unit volume. The effect of that concentration differs significantly in normal and even more in patients’ plasmas. An anti-Xa test that is sensitive to binding of heparin by acute phase plasma proteins is sensitive to the same plasma variables as the in vivo thrombin generation process and thus shadows part of the heparin resistance in patients with COVID-19 infection. It therefore is a conceptual mistake that some tests “overestimate” anti-Xa values, but we must admit that inappropriate tests may underestimate them. As an example, in the last survey of the External Quality Control for Assays and Tests with Focus on Thrombosis and Haemostasis (ECAT survey 2019-M4), among 142 participant centers, 55 hospital laboratories using Stago Liquid anti-Xa kit without dextran, also used by Hardy et al in their study, underestimated the effect of heparin (0.10 IU/mL with a CV 35.8%) in a plasma sample loaded with unfractionated heparin (UFH) 0.30 IU/mL, compared to HemosIL Liquid anti-Xa kit with dextran used in our study or Hyphen Biomed Biophen LRT with dextran used by Hardy et al (0.29 IU/mL; CV 8.9% and 0.33 IU/mL; 10.2%, respectively). In the same survey, no difference was observed between kits when low molecular weight heparin (LMWH) was present in the plasma sample (LMWH 0.65 IU/mL). Observation reported here by Hardy et al was earlier reported by previous ECAT surveys, which pointed out that Stago Liquid anti-Xa kit underestimates the effect of UFH compared to other kits.

Hardy et al report their own results obtained in COVID-19 patients with two anti-Xa reagents containing (or not) dextran sulfate with different types of heparin and they show, as the ECAT survey did, no significant difference is observed between two methods for plasmas from patients treated with enoxaparin. In our study, the large majority (91%) of patients received prophylaxis with enoxaparin and according to the findings reported by Hardy et al our results should not be influenced by the presence of dextran contained in the
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... anti-Xa kit. Of note, we did not observe any statistically significant difference between thrombin generation capacity of our 71 patients treated with LMWH prophylaxis (endogenous thrombin potential [ETP] 1739 ± 513; peak 343 ± 111) and 7 others who received UFH prophylaxis (ETP 1492 ± 798 [P > .05]; peak 254 ± 155 [P > .05]).

The second concern raised by the authors was about thrombin generation experiments. Lack of the preanalytical conditions was mentioned. We thank the authors for this pertinent comment and agree that preanalytical variables may have crucial impact on the results of TGA. We used preanalytical conditions recommended by the International Society on Thrombosis and Haemostasis (ISTH), essentially those of the seminal article by Hemker et al, le blood was drawn by direct venipuncture into a tube containing 106mM sodium citrate, using 21-gauge needles and light tourniquet. Platelet poor plasma was prepared within 2 hours of collection with double centrifugation (2500 × g 15 minutes at room temperature) and platelet poor plasma was collected very carefully, taking care not to disturb the buffy coat and then was frozen at −80°C immediately.

The authors also had a concern on the choice of thrombin generation reagent used. We agree with the authors that to investigate the buffy coat and then was frozen at −80°C immediately.

The third concern of the authors refers to “heparin resistance” in COVID-19. We admit that our use of the term “heparin resistance” may have caused confusion. Heparin resistance is defined as the need for more than 35,000 units/24 hours to achieve a therapeutic activated prothrombin time (APTT). This doesn’t apply to heparin prophylaxis. We wanted to point out the insufficiency of the recommended doses of heparin prophylaxis to downregulate coagulation activation in patients with COVID-19. Recent ISTH guidelines do not recommend treatment dose heparin for primary prevention in patients with COVID-19. Standard- or intermediate-dose LMWH or UFH, as administered in our study, are recommended for primary prophylaxis. We admit that heparin resistance is not only induced by antithrombin deficiency but can also be the effect of other causes, such as excessive neutralization by plasma proteins. It has been recently reported that spike protein of SARS-Cov2 can bind heparin and heparin sulfate with high affinity, which might suggest additional, not-well-defined mechanisms of interference of the virus with the anticoagulation process. However, because the common denominator of all anticoagulant therapy is lowering of the amount of active thrombin available, by lowering prothrombin (anti-vitamin K), by inhibiting prothrombin conversion (anti-vitamin K, anti-Xa activity of heparins, or direct oral anticoagulants [DOACs]), by draining thrombin (anti-IIa activity) or by inhibiting thrombin (anti-IIa DOACs), it can be safely assumed that all these measures diminish the TG curve. This has been extensively demonstrated for heparins. We therefore were surprised to observe no decrease of TG in patients with COVID-19 receiving appropriate heparin prophylaxis and feel that it is an observation worth communicating.

We almost might agree with the last sentence of this paragraph; “Altogether, to our opinion, the observation of normal TG profiles despite heparin administration rather reflects the low heparin levels measured with regards to the hyperinflammatory state observed in most COVID-19 patient,” except for the fact that heparin levels are confused with heparin effect, due to the abovementioned conceptual error.

The last concern of the authors was about the concept of viscoelastometric tests and the validity of the test after addition of exogenous tissue plasminogen activator (t-PA). The debate on the performance of different global hemostasis assays in different clinical settings is still open and needs more information from clinical studies. However, a large number of such studies already demonstrated that rotational thromboelastometry (ROTEM) can detect fibrin clot formation abnormalities, eg, factor XIII (FXIII) deficiency and abnormal fibrinolysis, eg, hyperfibrinolysis in trauma patients. The test is validated and widely used in hospital laboratories. We added exogenous t-PA to improve the sensitivity of the test to hypofibrinolysis and reported the intra- and inter-assay precision of the modified assay in our hands. The same approach was used by other groups in other clinical situations. This week our data were corroborated when Blasi et al, using TGA with tissue factor 5pM and ROTEM with exogenous t-PA, showed that in COVID-19 patients thrombin generation was preserved despite heparin prophylaxis and that fibrinolytic capacity in these patients was significantly decreased.

We believe that the two global hemostasis assays, TGA and ROTEM, bring different and complementary information and that both add to our understanding of COVID-19-associated coagulopathy, essential for further improvements in clinical care. We hope and expect that further studies will unveil more details of this coagulopathy. In the meantime, we would suggest not to throw the baby with the bathwater and accept that our study has demonstrated that patients with COVID-19 present high thrombin generation and a hypofibrinolytic state.

Conflicts of Interest
The authors decline no competing interests.

Author Contributions
Y. Dargaud and C. Nougier drafted the manuscript. All authors reviewed the manuscript for critical content and approved the final version.

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