Falsely Low Fibrinogen Levels in COVID-19 Patients on Direct Thrombin Inhibitors

To the Editor

We read with great interest Ranucci et al's[1] review on the "trials and tribulations" of fibrinogen level determination that was recently published in *Anesthesia & Analgesia*. This topic has become even more important with the rise of the coronavirus pandemic since severe hyperfibrinogenemia is a characteristic finding in patients critically ill with coronavirus disease 2019 (COVID-19) respiratory failure.[2] We wanted to take this opportunity to bring another limitation of the Clauss method of fibrinogen determination to readers' attention, as it can have important implications for care of these patients.

Given the hypercoagulability seen in COVID-19 patients, anticoagulation with low-molecular-weight heparin has been recommended by the International Society of Thrombosis and Hemostasis.[3] However, since antithrombin levels can be significantly lower than normal in this patient population,[2] heparin's effectiveness may be limited in some cases. Our institution has been selectively using direct thrombin inhibitors (DTIs) to overcome this problem. Unbeknownst to clinicians, this affected our laboratory's method for measuring fibrinogen levels, causing them to be vastly underestimated. An example of the magnitude in underestimation is provided in the Figure for illustrative purposes.

The Clauss method of fibrinogen measurement is similar to a thrombin time. Platelet poor plasma is exposed to a reagent containing supraphysiologic concentrations of thrombin and clot formation is sensed by mechanical or photo-optical means. The time to clot detection is compared against reference plasma to generate a corresponding fibrinogen level. The concentration of thrombin in the Clauss reagent varies by manufacturer and what instrumentation is being used. When present in the patient sample, DTIs inhibit the thrombin in the Clauss reagent, prolonging the time to clot formation, and thus underestimating the fibrinogen concentration. Reagents with lower thrombin concentrations are more susceptible to DTI inference. This problem has been reported on several different commercial platforms with samples containing both bivalirudin and argatroban.[4,5]

The Clauss assay in our particular laboratory uses a reagent with the highest commercially available thrombin concentration—100 NIH units (UNIH)/mL (QFA Thrombin, Instrumentation Laboratories, Bedford, MA). Despite this high level, "inhibitors" can still interfere with fibrinogen assessment. This can be assessed by performing a dilution procedure. This involves taking the patient plasma sample and performing a 1:1 dilution with HemosIL Factor Diluent (Instrumentation Laboratories), a nonactive buffer solution. This reduces the effect of the DTI. The Figure provides an example of how large a difference this can make on the measurement of fibrinogen levels.

Viscoelastic testing represents an alternative to the Clauss method for following fibrinogen levels in the setting of DTIs. The platelet–fibrinogen interactions assessed by maximum amplitude on thromboelastography (TEG) (Haemonetics, Boston, MA) or maximum clot formation on rotational thromboelastometry (ROTEM) (Instrumentation Laboratories) are relatively unaffected by the presence of DTIs.[6] In the example provided in the Figure, a ROTEM was obtained following the reported severe drop in fibrinogen on day 4 and resulted in the following notable parameters: EXTEM clotting time of 477 seconds (normal range 43–82 seconds) and FIBTEM maximum clot firmness of 39 mm (normal range 7–24 mm). The clotting time was appropriately prolonged, indicating thrombin inhibition by the argatroban, while the increased maximum clot firmness was still able to reflect the hyperfibrinogenemia that was present. This discordance between the ROTEM findings and the reported fibrinogen level by the Clauss method prompted the initial investigations into diluting the DTI samples.

The hypercoagulability caused by COVID-19 is still not well understood. Fibrinogen levels are an important piece of the puzzle, not only from a research aspect but for patient care. However, it is important for both scientists and clinicians to understand that their measurement is not always entirely straightforward.

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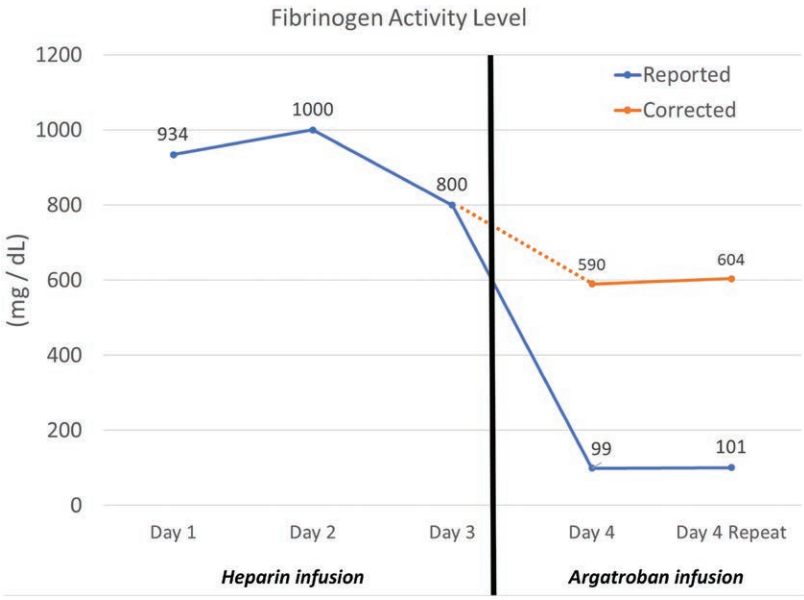

Figure. The graph demonstrates an example of the magnitude of which an argatroban infusion can have upon the measurement of fibrinogen levels determined by the Clauss assay. The patient's initially reported levels (blue line) on the day argatroban was started (which were repeated for confirmation) were almost an order of magnitude lower from previous measurements taken while on a heparin infusion. These samples were diluted as described in the text to decrease the effects of the argatroban and rerun, producing a "corrected" fibrinogen level (orange line).

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