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LETTERS TO THE EDITOR

Validation of viscoelastic coagulation tests during cardiopulmonary bypass: comment

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We read with interest the publication by Ortmann *et al*. regarding the validation of viscoelastic coagulation tests during cardiopulmonary bypass (CPB) [1]. This study is a valuable addition to the existing body of evidence on this topic, highlighting the need for accurate coagulation testing to guide hemostatic therapy in cardiac surgery. The study found that although thrombelastography (TEG) and thromboelastometry (ROTEM) fibrin-based test results taken towards the end of CPB are clinically comparable to those after heparin reversal, this was not the case for Clauss assay results [1]. In fact, fibrinogen concentration measurements taken during CPB were lower by a mean of 1.2 g L^{-1}. The authors proposed that this difference was due to heparin present in blood samples taken during CPB, as Gertler *et al*. have shown that the Clauss assay is affected by heparin concentrations > 2 IU mL^{-1} [2]. It would be worth exploring this hypothesis further by confirming the heparin sensitivity of the Clauss reagents used. The package insert for the reagent brand used by Ortmann *et al*. (HemosIL Fibrinogen-C XL, Instrumentation Laboratory, Milan, Italy) states it is unaffected by heparin up to 1 U mL^{-1}, indicating that the presence of heparin may have affected the Clauss assay results. A recent study carried out by Erdoes *et al*. concluded there was no significant difference between on-CPB and post-CPB results for Clauss fibrinogen [3]. This study used reagent 9 (Table 1), which is reported to be unaffected by heparin levels < 2 IU mL^{-1}. Similar results have been observed by Sato *et al*. using one of the STA reagents (numbers 10–12, Table 1); these are also reportedly unaffected by heparin levels < 2 IU mL^{-1} [4]. In a study of aortic surgery, Solomon *et al*. performed the Clauss assay using Dade Thrombin Reagent (number 3, Table 1), which has a reported heparin sensitivity threshold of 0.6 IU mL^{-1}; on-CPB values were similar to those after heparin neutralization [5,6]. A multicentric comparison of Clauss fibrinogen values measured in sets of cardiac surgery plasma samples that were distributed to six laboratories has also been performed [7]. Eight different combinations of coagulometers, Clauss reagents and calibrators were used in the study, seven of which provided on-CPB results that were comparable with those after heparin neutralization [7]. A significant difference between the two time-points was apparent with reagent 9 but not with reagent 3; this result is surprising considering the higher heparin sensitivity threshold with reagent 9 vs. reagent 3 (< 2 IU mL^{-1} vs. < 0.6 IU mL^{-1}, respectively; Table 1). The mean anti-factor Xa activity was 2.8 U mL^{-1} and the mean anti-factor IIa activity was 2.1 U mL^{-1}, with maximum values of 4.6 U mL^{-1} and 2.7 U mL^{-1}, respectively [8]. The mismatch between heparin sensitivities reported in package inserts and those observed in practice demonstrates a need for real-world validation studies. Should one decide to perform the Clauss fibrinogen assay while the patient is still on CPB (because of the long turnaround time) [6,9], it seems important not only to select a brand with low reported heparin sensitivity, but also to confirm the sensitivity level by in-house validation.

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Table 1 Heparin sensitivity of different brands of Clauss assays

	Assay reagents	Manufacturer	Heparin levels at which results are unaffected*
1.	Fibrinogen kit	BioMed Diagnostics, White City, OR, USA	< 0.4 IU mL^{-1}
2.	FIBROQUANT System Pack	The Tulip Group, Goa, India	< 0.4 IU mL^{-1}
3.	Dade Thrombin Reagent	Siemens Healthcare Diagnostics (formerly Dade Behring), Marburg, Germany	< 0.4 U mL^{-1} (LMWH) < 0.6 U mL^{-1} (UFH)
4.	Fibrinogen Assay Kit	Helena Laboratories, Beaumont, TX, USA	< 0.6 USP mL^{-1}
5.	MDA Fibriquik	Tcoag (formerly bioMérieux), Bray, Ireland	< 0.6 U mL^{-1}
6.	HemosIL Fibrinogen-C XL	Instrumentation Laboratory, Milan, Italy	< 1 U mL^{-1}
7.	Fibrinogen (Clauss method)	InterMedical, Grassobbio, Italy	< 1 U mL^{-1}
8.	HemosIL Q.F.A. Thrombin (Bovine) Kit	Instrumentation Laboratory, Milan, Italy	< 2 U mL^{-1}
9.	Multifibren U	Siemens Healthcare Diagnostics, Marburg, Germany	< 2 U mL^{-1}
10.	STA Fib 2	Diagnostica Stago S.A.S., Asnières sur Seine, France	< 2 IU mL^{-1}
11.	STA Fib 5	Diagnostica Stago S.A.S., Asnières sur Seine, France	< 2 IU mL^{-1} (LMWH and UFH)
12.	STA Liquid Fib	Diagnostica Stago S.A.S., Asnières sur Seine, France	< 2 IU mL^{-1} (LMWH and UFH)
13.	Fibrinogen Reagent Kit	Technoclone GmbH, Vienna, Austria	< 2 IU mL^{-1} (UFH)
14.	TriniCLOT Fibrinogen Kit	Tcoag, Bray, Ireland	< 3 USP U mL^{-1}
15.	TEClot	TECO, Niederbayern, Germany	< 5 U mL^{-1}
16.	Biopool Fibrinogen Assay Kit	Trinity Biotech, Bray, Ireland	< 5 U mL^{-1}
17.	Fibrotek FIB Fibrinogen Assay Kit	r^2 Diagnostics Inc., South Bend, IN, USA	Therapeutic levels do not significantly interfere with test results
18.	Pacific Hemostasis Fibrinogen Assay Set	Thermo Fisher Scientific, Waltham, MA, USA	Therapeutic levels do not significantly interfere with test results
19.	Fibrinogen Clauss	BioSystems S.A., Barcelona, Spain	Unspecified/no information provided
20.	DiaFibrinogen	DiaMed, Morat, Switzerland	Unspecified/no information provided
21.	Dia-FIB	Diagon Ltd, Budapest, Hungary	Unspecified/no information provided
22.	Sclavo Fibrinogen Kit	Sclavo Diagnostics, Sovicille, Italy	Unspecified/no information provided

LMWH, low-molecular-weight heparin; UFH, unfractionated heparin
*Information obtained from package inserts for each assay

Another point to consider is that the Clauss assay, as for any other coagulation factor activity measurement, is not based on a physico-chemical technique and therefore cannot measure the absolute fibrinogen concentration in a sample [10,11]. Instead, it is a chronometric assay based on estimations using a reference standard [12], and there is unavoidable variability in locally prepared and calibrated reference standards [13]. Using the World Health Organization (WHO) Standard [14] could improve the inter-laboratory comparability; however, differences between coagulometers and reagents would still make standardization challenging [7]. Consequently, caution is advised when establishing algorithm cut-offs or guidelines for fibrinogen supplementation based on Clauss fibrinogen measurement without in-house validation.

Alternatives to the Clauss assay could be considered, including the Reptilase® Time test based on thrombin-like snake-venom that is unaffected by heparin [15], or the fibrin-based assays on ROTEM and TEG, which are designed to counteract heparin, for example by adding polybrene to the reagents, or by using heparinase-coated cups (information taken from assay package inserts). The fibrin-based viscoelastic tests provide a rapid assessment of hemostasis at the point-of-care [16]. Both FIBTEM and Functional Fibrinogen (FF) assays showed clinically comparable results on-CPB and after heparin reversal in a number of investigations, including the present work [1,3,8]. Gertler *et al*. confirmed *in vitro* that FIBTEM MCF based on the r ex-tem reagent was not affected by heparin levels $\leq$ 4 IU mL^{-1} [2]; the FIBTEM package insert states that spiking up to 5 IU mL^{-1} of unfractionated heparin to whole blood did not show a significant influence on FIBTEM. However, some reagents are sensitive to heparin, such as FF performed in non-heparinase cups, FF in heparinase cups when the samples have high heparin concentration (equivalent to 400 IU kg^{-1} bw) [17] or fib-tem S that does not contain a heparin inhibitor (package insert states that spiking approximately 0.2 IU mL^{-1} unfractionated heparin to whole blood has been shown to influence FIBTEM S results).

Although the selection of a Clauss reagent with low heparin sensitivity may enable an accurate assessment of fibrinogen under heparinization, the measurements could still become futile if the fibrinogen level in a patient changes quickly (e.g. due to dynamic bleeding and/or volume resuscitation combined with a long turnaround time of the Clauss measurement). Choosing Clauss and viscoelastic reagents with low heparin sensitivity to assess the fibrinogen concentration and fibrin-based clot strength, respectively, in combination with a fast turn-

around time [18,19], may be key to optimizing fibrinogen and fibrin assessment on CPB.

Addendum

All authors read the paper by Ortmann *et al*. This letter to the editor was drafted by C. Solomon, with review and editing by K. Fickenscher, L. Ormonde and M. Ranucci.

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Disclosure of Conflict of Interests

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