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JTH COMMENTARY

Laboratory testing of factor XI inhibitors: methodological challenges remain

Michael Hardy[1,2,3] | Henri Thonon[1,2,4] | Thomas Lecompte[5,6] | François Mullier[1,2]

[1]Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Louvain-la-Neuve, Belgium

[2]Hematology Laboratory, Namur Thrombosis and Hemostasis Center, Namur Research Institute for Life Sciences, CHU UCL Namur, Yvoir, Belgium

[3]Anesthesiology Department, CHU UCL Namur, Yvoir, Belgium

[4]Emergency Department, CHU UCL Namur, Yvoir, Belgium

[5]Pharmacy Department, University of Namur, Namur, Belgium

[6]Vascular Medicine Department, University Hospital of Nancy, University of Lorraine, Nancy, France

Correspondence
François Mullier, Department of Laboratory Medicine, CHU UCL Namur, Avenue Docteur Gaston Thérasse 1, 5530 Yvoir, Belgium.
Email: francois.mullier@chuuclnamur.uclouvain.be

In this issue of *Journal of Thrombosis and Haemostasis*, Yin et al. [1] investigated the impact of depleting factor (F)XII or FXI, as well as pharmacological inhibition of activated FXI (FXIa) by milvexian, on coagulation initiated on a surface, ie, a catheter segment. They observed that only trace amounts of FXIa were required for coagulation to proceed on surfaces. They also showed that at concentrations mirroring plasma concentrations achieved in clinical trials, milvexian was less effective than dabigatran in mitigating catheter-initiated coagulation. The use of dabigatran as a control in this context seems questionable, and one must be cautious when studying dabigatran with the thrombin generation assay, even in spiking experiments [2]. Heparins may be a more appropriate control in this context, given that clinically, this is the gold standard for prevention of catheter-induced thrombosis. The meticulous work reported by the authors raises further issues about the clinical positioning of FXI-directed anticoagulants and underscores the methodological uncertainties that complicate their assessment.

FXI-directed anticoagulants were developed with the aim of providing effective thromboprophylaxis while limiting the risk of bleeding inherent to anticoagulant drugs. Although clinical data demonstrating this premise are still awaited, their ability to prevent thrombosis triggered by artificial surfaces has scarcely been explored in humans, apart from limited data in patients with end-stage renal disease [3]. Yin et al.'s [1] study demonstrated that at concentrations currently achieved in clinical trials, milvexian was less effective than dabigatran in mitigating catheter-associated coagulation, corroborating our recently published findings [4]. The authors elegantly demonstrated that almost complete depletion of FXI was necessary to blunt catheter-initiated coagulation in a platelet-poor plasma medium, with residual FXI activity as low as 2% still permitting coagulation. Such depletion studies are of paramount importance to dissect the involvement of the actors, but they heavily depend on the quality of the depletion and the added-back protein, either isolated or with normal plasma (and then the issue arises of its quality, including preanalytical activation). An inaugural study of milvexian identified that a concentration of 3.6 μM, ie, the peak concentration achieved when taking 100 mg twice a day, reduced the coagulant activity of FXI by only 90%, an insufficient level to suppress coagulation on a surface [5]. Whether higher milvexian concentrations can safely achieve more complete FXI inhibition, and thus fully prevent surface-triggered coagulation, remains to be determined and warrants further investigation. Interestingly, Yin et al.'s [1] findings suggest that targeting FXII alone may be suboptimal because FXI can be activated independently of FXII, probably because FXI can also self-activate on a surface [6]. Furthermore, even trace amounts of thrombin produced downstream of FXIIa or FXIa can drive robust positive feedback activation of FXI [7]. Taken together, these findings indicate that extensive preclinical work remains necessary before considering the use of FXI or FXII inhibitors in clinical trials in the context of foreign surfaces.

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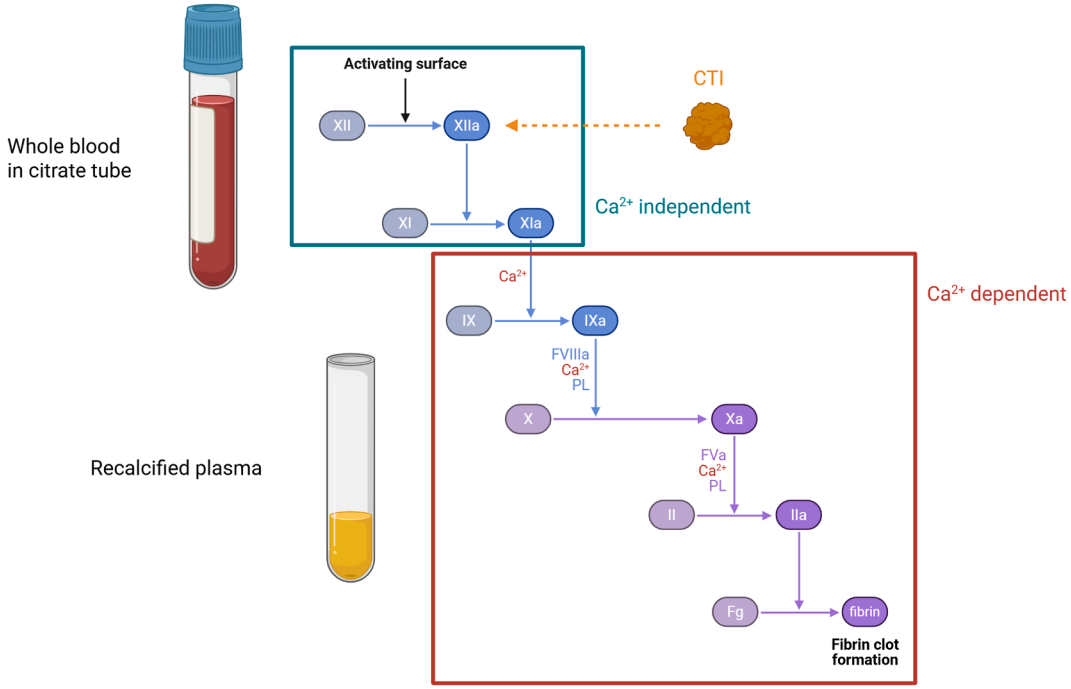

FIGURE Contact activation of factor (F)XII and FXI readily occurs on foreign surfaces under the low-calcium (Ca^{2+}) conditions of citrated blood or plasma inside collection tubes. Activated FXI (FXIa) is generated during this preanalytical phase. Corn trypsin inhibitor (CTI) largely inhibits this artifactual activation only if it is added to the collection tube before venipuncture; adding CTI to plasma after centrifugation is less effective. Fg, fibrinogen; FVa, activated factor V; FVIIIa, activated factor VIII; XIIa, activated factor XII; PL, procoagulant phospholipid vesicles.

Nevertheless, the optimal conditions for studying the effect of FXI-directed anticoagulants *in vitro* or *ex vivo* have yet to be firmly established, while the appropriate assay and conditions for clinical monitoring are still to be defined [8]. Identifying the bleeding phenotype of patients with inherited FXI deficiency with a laboratory approach was reported to require the presence of platelets, inhibition of preanalytical activation of the contact pathway, and the use of a low concentration of tissue factor [9–11]. In line with this, the authors reported that the effect of milvexian was greater when studied in platelet-rich plasma compared with platelet-poor plasma. When activated, platelets release polyphosphates from dense granules, which form on their surface nanoparticles, promoting FXI activation [12–14]. Platelets were also reported to exhibit low intrinsic FXI activity (<1% of plasma pool), the nature of which is debated, but which could locally amplify thrombin generation and compensate for certain forms of FXI deficiency [15]. Platelet counts are best left unadjusted to prevent artifacts introduced by diluting the specimen with platelet-poor plasma, provided that the native count already lies within an assay-tolerant window (ie, 50-400 $\times$ 10^9/L in the thrombin generation assay used by the authors) [16]. However, the significance of these mechanisms in the context of pharmacological FXI inhibition remains to be fully elucidated.

Activation of the contact pathway during the preanalytical phase, occurring as soon as blood collection and further during processing, yields FXIa within the sample (Figure). FXII can be activated on the tube walls, even under low-calcium conditions (ie, in a citrated medium); FXIIa, thereafter, activates FXI [17]. However, the activation of FIX and downstream factors requires calcium. Consequently, an inhibitor acting upstream of FXIa can exert its full inhibitory potential *in vitro* or *ex vivo* only if this preanalytical activation is prevented. This objective can only be achieved by ensuring the presence of a contact phase inhibitor at the time of blood collection—most often corn trypsin inhibitor (targeting FXIIa) added to the collection tube, or the FXII or FXI inhibitor drug studied, if present at a "sufficient" concentration. Indeed, the addition of corn trypsin inhibitor to plasma does not guarantee complete suppression of contact activation [18]. For instance, this point is critical when evaluating abelacimab, a dual inhibitor of FXI and FXIa *in vitro*, as we recently reported [4].

Notwithstanding the aforementioned limitations, Yin et al.'s [1] study deepens the uncertainty surrounding the clinical potential of FXI-directed anticoagulants, illustrating that the proportion of FXIa inhibition currently achieved in clinical trials may be insufficient to prevent surface-initiated coagulation, as also suggested in other settings [19]. It also emphasizes the urgent need to determine the optimal conditions for the *in vitro* and *ex vivo* testing of these drugs, particularly concerning the presence of platelets and the control of preanalytical contact activation, to enable their unbiased evaluation.

AUTHOR CONTRIBUTIONS

M.H., H.T., T.L., and F.M. wrote the original draft and reviewed and edited the manuscript.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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