Journal of Thrombosis and Haemostasis, **17**: 110–112 DOI: 10.1111/jth.14328

COMMENTARY

Crosslinked clots formed independently of factor XIII and without fibrinogen-to-fibrin conversion – is this a liver-specific phenomenon?

T. LISMAN

Surgical Research Laboratory and Section of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

To cite this article: Lisman T. Crosslinked clots formed independently of factor XIII and without fibrinogen-to-fibrin conversion – is this a liver-specific phenomenon?. *J Thromb Haemost* 2019; **17**: 110–2.

See also Poole LG, Pant A, Baker KS, Kopec AK, Cline-Fedewa HM, Iismaa SE, Flick MJ, Luyendyk JP. Chronic liver injury drives non-traditional intrahepatic fibrin(ogen) cross-linking via tissue transglutaminase. This issue, pp 113–25.

Both basic and applied studies dealing with aspects of hemostasis in patients with liver diseases have spurred significant controversy over the last two decades [1]. Clinically, we have learnt that patients with liver diseases do not necessarily have a hemostatic defect resulting in a bleeding tendency. Instead, these patients appear to be in hemostatic balance, owing to declines in both prohemostatic and antihemostatic pathways, and may experience both bleeding and thrombotic complications when this reset hemostatic balance is offset [2]. These insights into the hemostatic status of these patients have led to the realization that correction of hemostasis before interventional procedures is frequently not required, and can even do harm. Major procedures, notably liver transplantation, can be performed in patients with profound 'coagulopathy' (defined as thrombocytopenia and/or prolongations in the prothrombin time) without the requirement for any prohemostatic interventions [3]. Not all proceduralists, however, accept the change in dogma, and require correction of hemostasis prior to minor invasive procedures with an established low bleeding risk. Despite overwhelming clinical and laboratory evidence arguing against correction of the prothrombin time prior to invasive procedures in patients with chronic liver disease [4], some guidelines specifically state that correction should be performed [5]. In organized attempts to convince proceduralists and professional organizations, my colleagues and I have frequently heard 'Are you telling me that everything I have been taught in medical school was wrong?' Obviously, this is not the case, as tests such as the prothrombin time were never designed to predict (procedural) bleeding, but, unfortunately, they have been used as such. Acceptance of changes in dogma is sometimes slow.

In this issue of the *Journal of Thrombosis and Haemostasis*, Poole *et al*. present data that made me wonder whether everything I have been taught in the 20 years in which I have studied thrombosis and hemostasis is wrong [6]. In a mouse model of chronic liver injury, it was demonstrated that crosslinked fibrin(ogen) is deposited in a process that does not require fibrinogen-to-fibrin conversion or factor XIII. Rather, these 'non-traditional' clots can be formed from fibrinogen that is crosslinked by tissue transglutaminase (TG2), which is a multifunctional and widely expressed protein that has been implicated in diabetes and celiac disease, and as a player in inflammation, apoptosis, and matrix remodeling [7]. Although TG2 is known to crosslink fibrin(ogen) *in vitro* [8], this study is the first to document TG2-dependent fibrin(ogen) crosslinking *in vivo*.

Interestingly, intrahepatic deposition of non-traditional fibrin(ogen) had no effect on the severity of disease in this model, and this challenges a second dogma in the field of hemostasis and liver diseases. This study provides the first evidence that the beneficial effects of anticoagulants in delaying the progression of liver disease in animals and humans probably result from inhibition of thrombin rather than inhibition of fibrin formation, and therefore argues against the theory of 'parenchymal extinction', i.e. microthrombosis caused by intrahepatic clot formation, as the driver of progression of chronic liver disease [9,10].

The two seminal findings from this study – crosslinked clots can be formed without thrombin-mediated

Correspondence: Ton Lisman, University Medical Center Groningen, Department of Surgery, BA33, Hanzeplein 1, 9713 GZ Groningen, the Netherlands
Tel.: +31 50 361 9028
E-mail: j.a.lisman@umcg.nl

Received: 25 October 2018
Manuscript handled by: P. H. Reitsma
Final decision: P. H. Reitsma, 26 October 2018

fibrinogen-to-fibrin conversion, without the requirement for activated FXIII (FXIIIa) activity, and intrahepatic fibrin(ogen) deposition does not alter the progression of chronic liver disease, give rise to a wide array of questions.

1 Are these 'non-traditional' fibrinogen clots also formed in humans? As TG2-crosslinked fibrinogen has unique structural characteristics (i.e. specific α–γ hybrid crosslinks) [8], examination of fibrin(ogen) structures in, for example, biopsies of human diseased liver, or in human vascular clots (see below), should be possible. In fact, it was already stated in an article published in 1991 that 'Thus, whenever a tissue transglutaminase might escape from cells into the plasma environment where it could directly interact with fibrinogen … diagnostic analysis should include a search for this internally crosslinked monomeric form of fibrinogen' [11], but, to my knowledge, this proposal was never followed up.
2 Are these clots truly 'clots' or are they, for example, thin 'sheets' of crosslinked fibrinogen that are attached to collagen, as suggested by the authors? The shape and localization of intrahepatic fibrin(ogen) have not been extensively explored, and more in-depth (electron) microscopy studies are urgently needed to provide a better understanding of the nature of these fibrin(ogen) structures that have only been identified with immunohistochemical labeling of liver sections [12].
3 What initiates intrahepatic fibrinogen deposition? If intrahepatic activation of coagulation is not required as such, what signals are required for fibrinogen deposition and TG2-mediated crosslinking? Deposition of collagen, which is the hallmark of hepatic fibrosis, could be the key driver.
4 Is non-traditional fibrin(ogen) deposition the hallmark of all types of liver injury? Fibrin(ogen) has been found in many experimental settings of chronic or acute liver injury. However, the mechanisms by which fibrin(ogen) is deposited may be different. For example, in acetaminophen-induced acute liver injury, in which no deposition of collagen occurs, there is massive intrahepatic crosslinked fibrin deposition, which appears not to affect injury, but does facilitate repair [13]. In a model of cholestatic acute liver injury, however, fibrin(ogen) deposits occur and appear to accelerate injury [14]. Is this because the types of clot differ between these models? Also, in a model of fatty liver disease, crosslinked fibrin(ogen) deposits have been identified in the liver and adipose tissue [15], and again it would be of interest to identify the make-up of these deposits.
5 Is parenchymal extinction, i.e. the progression of liver injury by intrahepatic microthrombi, not a relevant pathological mechanism at all, or does this depend on the nature, extent and phase of liver injury? The observation that fibrinogen knockout is protective against acute biliary injury induced by the hepatotoxicant α-naphthylisothiocyanate suggests that parenchymal extinction may be relevant in some forms of liver injury [14]. A role of fibrin in many other models has been suggested, but evidence has always been circumstantial, and it has been suggested that fibrin(ogen) deposition primarily acts as an innocent bystander [12]. Perhaps a systematic screen of various clinically relevant liver injury models in the fibrinogen knockout or fibrinogen AEK mouse would be of interest.
6 Are these non-traditional clots formed elsewhere? If collagen is the initiator of non-traditional fibrin(ogen) deposition, these clots may be formed in fibrotic lesions in other organs. Models of cardiac [16], pulmonary [17] and renal fibrosis [18], for example, are known to be accompanied by fibrin(ogen) deposition, and it would be of interest to assess the requirement for fibrinogen cleavage by thrombin, and crosslinking by FXIIIa, in these models. Could non-traditional clots be formed intravascularly? Portal vein thrombosis is a poorly understood, but common, complication of chronic liver disease that is frequently resistant to anticoagulant therapy [19]. It is tempting to speculate that (some) portal vein thrombi are non-traditional clots, which will be, by definition, unresponsive to anticoagulants. Although TG2 is thought to be absent from plasma under normal conditions, it is present in red blood cells, and TG2 could contribute to fibrin(ogen) crosslinking in localized or systemic thrombotic diseases. A contribution of TG2 to clot crosslinking, particularly under hemolytic conditions, was suggested in the late 1980s [20], but this concept has never been extensively validated. Thrombotic conditions accompanied by hemolysis, including sickle cell disease, disseminated intravascular coagulation, thrombotic microangiopathies such as thrombotic thrombocytopenic purpura, hemolytic–uremic syndrome, and HELLP syndrome, are all difficult-to-treat conditions characterized by deposition of fibrin within organs. It is not inconceivable that some of these fibrin clots are, in fact, the non-traditional fibrinogen deposits identified by Poole *et al.*

Thus, it is of interest to establish whether these 'non-traditional' clots are specific for liver diseases or clinical conditions in which intrahepatic fibrin deposition occurs, or whether they represent a more widespread phenomenon, perhaps even contributing to intravascular thrombosis. Next, it will be of interest to ascertain whether there are situations in which these non-traditional clots do harm. In the current study, the non-traditional clots appeared to be true innocent bystanders, as progression of liver injury was identical between wild-type and TG2 knockout mice. However, the authors' conclusion that 'these studies provide strong experimental evidence that the mechanism connecting coagulation activity to liver fibrosis occurs via a pathway independent of hepatic fibrin(ogen) deposition in the chronically damaged liver' may be premature, as the role of these clots may be context-dependent [12]. Also, it is possible that intrahepatic clots do not

drive progression of disease, but do affect complications of chronic liver disease, notably portal hypertension. A recent study demonstrated relief of portal hypertension by rivaroxaban in carbon tetrachloride-treated rats [21]. This effect was proposed to be multifactorial, with reduced intrahepatic microthrombosis as one of the contributing mechanisms. Even if intrahepatic thrombosis proves not to significantly contribute to the progression of liver diseases, exploration of a functional role of non-traditional fibrinogen clots in other settings, notably intravascular thrombosis, remains of interest.

Should there be situations in which TG2-crosslinked fibrinogen has a functional role, an interesting question will be whether TG2 is a viable target for pharmacological interventions. TG2 has multiple functions, not only as a transglutaminase, but also as a G-protein for several seven transmembrane receptors and as a coreceptor for β_1 and β_3 integrins, and has been implicated in a wide variety of diseases [7]. Nevertheless, small-molecule inhibitors of TG2 have been developed, and one of these is undergoing early clinical studies, specifically for patients with celiac disease (EudraCT Number: 2017-002241-30). Whether the inhibition of alternative functions of TG2 disqualifies TG2 inhibitors as agents to prevent intravascular or extravascular 'non-traditional' fibrin(ogen) deposition would be an important area of investigation.

In summary, the study by Poole *et al*. has provided solid evidence against a role of intrahepatic fibrin deposition in the progression of disease, at least in the chronic carbon tetrachloride model. During the study of the role of fibrin deposition in driving liver injury, the authors have detailed a thrombin-independent and FXIII-independent way in which fibrinogen can be deposited and crosslinked, at least within the liver. Whether this observation has wider implications remains to be investigated, but it is certainly an attractive hypothesis.

Disclosure of Conflict of Interests

The author states that he has no conflict of interest.

References

1 Lisman T, Porte RJ. Pathogenesis, prevention, and management of bleeding and thrombosis in patients with liver diseases. *Res Pract Thromb Haemost* 2017; **1**: 150–61.

2 Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease: evidence and clinical consequences. *Blood* 2010; **116**: 878–85.

3 Massicotte L, Thibeault L, Roy A. Classical notions of coagulation revisited in relation with blood losses, transfusion rate for 700 consecutive liver transplantations. *Semin Thromb Hemost* 2015; **41**: 538–46.

4 Lisman T, Porte RJ. Value of preoperative hemostasis testing in patients with liver disease for perioperative hemostatic management. *Anesthesiology* 2017; **126**: 338–44.

5 Patel IJ, Davidson JC, Nikolic B, Salazar GM, Schwartzberg MS, Walker TG, Saad WA; Standards of Practice Committee, with Cardiovascular and Interventional Radiological Society of Europe (CIRSE) Endorsement. Consensus guidelines for periprocedural management of coagulation status and hemostasis risk in percutaneous image-guided interventions. *J Vasc Interv Radiol* 2012; **23**: 727–36.

6 Poole LG, Pant A, Baker KS, Kopec AK, Cline-Fedewa HM, Lismaa SE, Flick MJ, Luyendyk JP. Chronic liver injury drives non-traditional intrahepatic fibrin(ogen) cross-linking via tissue transglutaminase. *J Thromb Haemost* 2018; **17**: 113–25.

7 Odii BO, Coussons P. Biological functionalities of transglutaminase 2 and the possibility of its compensation by other members of the transglutaminase family. *ScientificWorldJournal* 2014; **2014**: 714561.

8 Murthy SN, Lorand L. Cross-linked A alpha.gamma chain hybrids serve as unique markers for fibrinogen polymerized by tissue transglutaminase. *Proc Natl Acad Sci USA* 1990; **87**: 9679–82.

9 Wanless IR, Wong F, Blendis LM, Greig P, Heathcote EJ, Levy G. Hepatic and portal vein thrombosis in cirrhosis: possible role in development of parenchymal extinction and portal hypertension. *Hepatology* 1995; **21**: 1238–47.

10 Anstee QM, Wright M, Goldin R, Thursz MR. Parenchymal extinction: coagulation and hepatic fibrogenesis. *Clin Liver Dis* 2009; **13**: 117–26.

11 Murthy SN, Wilson J, Guy SL, Lorand L. Intramolecular crosslinking of monomeric fibrinogen by tissue transglutaminase. *Proc Natl Acad Sci USA* 1991; **88**: 10601–4.

12 Kopec AK, Luyendyk JP. Role of fibrin(ogen) in progression of liver disease: guilt by association? *Semin Thromb Hemost* 2016; **42**: 397–407.

13 Kopec AK, Joshi N, Cline-Fedewa H, Wojcicki AV, Ray JL, Sullivan BP, Froehlich JE, Johnson BF, Flick MJ, Luyendyk JP. Fibrin(ogen) drives repair after acetaminophen-induced liver injury via leukocyte alphaMbeta2 integrin-dependent upregulation of Mmp12. *J Hepatol* 2017; **66**: 787–97.

14 Luyendyk JP, Mackman N, Sullivan BP. Role of fibrinogen and protease-activated receptors in acute xenobiotic-induced cholestatic liver injury. *Toxicol Sci* 2011; **119**: 233–43.

15 Kopec AK, Abrahams SR, Thornton S, Palumbo JS, Mullins ES, Divanovic S, Weiler H, Owens AP 3rd, Mackman N, Goss A, van Ryn J, Luyendyk JP, Flick MJ. Thrombin promotes diet-induced obesity through fibrin-driven inflammation. *J Clin Invest* 2017; **127**: 3152–66.

16 Schnitt SJ, Stillman IE, Owings DV, Kishimoto C, Dvorak HF, Abelmann WH. Myocardial fibrin deposition in experimental viral myocarditis that progresses to dilated cardiomyopathy. *Circ Res* 1993; **72**: 914–20.

17 Shea BS, Probst CK, Brazee PL, Rotile NJ, Blasi F, Weinreb PH, Black KE, Sosnovik DE, Van Cott EM, Violette SM, Caravan P, Tager AM. Uncoupling of the profibrotic and hemostatic effects of thrombin in lung fibrosis. *JCI Insight* 2017; **2**: e86608.

18 Craciun FL, Ajay AK, Hoffmann D, Saikumar J, Fabian SL, Bijol V, Humphreys BD, Vaidya VS. Pharmacological and genetic depletion of fibrinogen protects from kidney fibrosis. *Am J Physiol Renal Physiol* 2014; **307**: F471–84.

19 Loffredo L, Pastori D, Farcomeni A, Violi F. Effects of anticoagulants in patients with cirrhosis and portal vein thrombosis: a systematic review and meta-analysis. *Gastroenterology* 2017; **153**: 480–7.

20 Lorand L, Dailey JE, Turner PM. Fibronectin as a carrier for the transglutaminase from human erythrocytes. *Proc Natl Acad Sci USA* 1988; **85**: 1057–9.

21 Vilaseca M, Garcia-Caldero H, Lafoz E, Garcia-Irigoyen O, Avila MA, Reverter JC, Bosch J, Hernandez-Gea V, Gracia-Sancho J, Garcia-Pagan JC. The anticoagulant rivaroxaban lowers portal hypertension in cirrhotic rats mainly by deactivating hepatic stellate cells. *Hepatology* 2017; **65**: 2031–44.