Received: 14 June 2024 | Revised: 9 August 2024 | Accepted: 12 August 2024

https://doi.org/10.1016/j.rpth.2024.102551

RESEARCH LETTER

Fibrinolytic profile depends on disease severity in pediatric patients with cirrhosis: illustration by 2 different plasma-based fibrinolysis assays

1 | INTRODUCTION

The fibrinolytic state in cirrhotic patients is still a debated subject. Historically, cirrhotic patients were thought to be in a hyperfibrinolytic state that was thought to contribute to bleeding risk [1]. More recently, this hyperfibrinolytic state and its association with bleeding risk have been put into question. Patients with cirrhosis show simultaneous changes in profibrinolytic and antifibrinolytic mechanisms. Typically, a decrease in plasminogen, α2-antiplasmin, and thrombin activatable fibrinolysis inhibitor and an increase in tissue plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1) are seen [2]. Depending on the type and severity of liver disease, hypofibrinolysis, normal fibrinolysis, and hyperfibrinolysis have been described [3]. Few reports on fibrinolysis are available in pediatric patients with cirrhosis. Similar to adult patients with cirrhosis, hypofibrinolysis, normal fibrinolysis, and hyperfibrinolysis have been described [4]. Which assays can best be used to evaluate fibrinolysis remains uncertain, and cutoffs for various available assays are not always firmly established. Since the process of fibrinolysis in clotting blood or plasma is slow, activators are added in most assays to estimate fibrinolytic potential more reliably and rapidly. Whereas fibrinolytic assays in whole blood may better represent physiology, plasma-based assays have been much more extensively used to estimate fibrinolytic potential in cirrhosis. One of the plasma-based assays used has been extensively clinically validated in the context of thrombosis in the general population and may therefore have clinical relevance in patients with cirrhosis as well [5].

In this study, we analyzed fibrinolytic status in pediatric patients with cirrhosis just before and 3 months after liver transplantation with 2 different plasma-based fibrinolysis assays, an in-house assay, and a commercially available test called the Lysis Timer (Nodia). This is, to our knowledge, the first report of Lysis Timer results in a pediatric population.

2 | METHODS

2.1 | Study population

Pediatric patients with cirrhosis, listed for liver transplantation at Cliniques Universitaires Saint-Luc, Brussels, Belgium, were enrolled prospectively. Informed consent was obtained from the parents or legal representative. A blood draw was performed just before and 3 months after liver transplantation in patients. In addition, an age-matched control group was included comprising healthy controls requiring minor surgery. The study was approved by the local ethical committee (2020/12MAR/157) based on the Declaration of Helsinki.

2.2 | Blood collection

Peripheral blood was collected in citrate tubes (Monovette plastic 3.0 mL, 3.2% citrate, 106 mM [Sarstedt]; 9:1 blood-to-citrate ratio) after drawing a discard tube. Citrate plasma was prepared by double centrifugation at 2500*g* for 10 minutes. Samples were stored at −80 °C until analysis.

2.3 | Fibrinolysis assays

Clot lysis time (CLT) was performed as previously described [6]. In short, a mixture of tissue factor (Dade Innovin, Siemens Healthineers, 1:1000 final dilution), $CaCl_2$, phospholipids, and tPA (56 ng/mL) was added to plasma. During clot formation and clot lysis, turbidimetry at 405 nm was performed at 37 °C. The time in minutes between the midpoint of coagulation and midpoint of fibrinolysis (CLT) was registered. Lysis Timer was also executed as described before [7]. Patient plasma was mixed with silica and tPA (160 ng/mL). Coagulation was then triggered with human α-thrombin (4 National Institute of Health/mL) and $CaCl_2$.

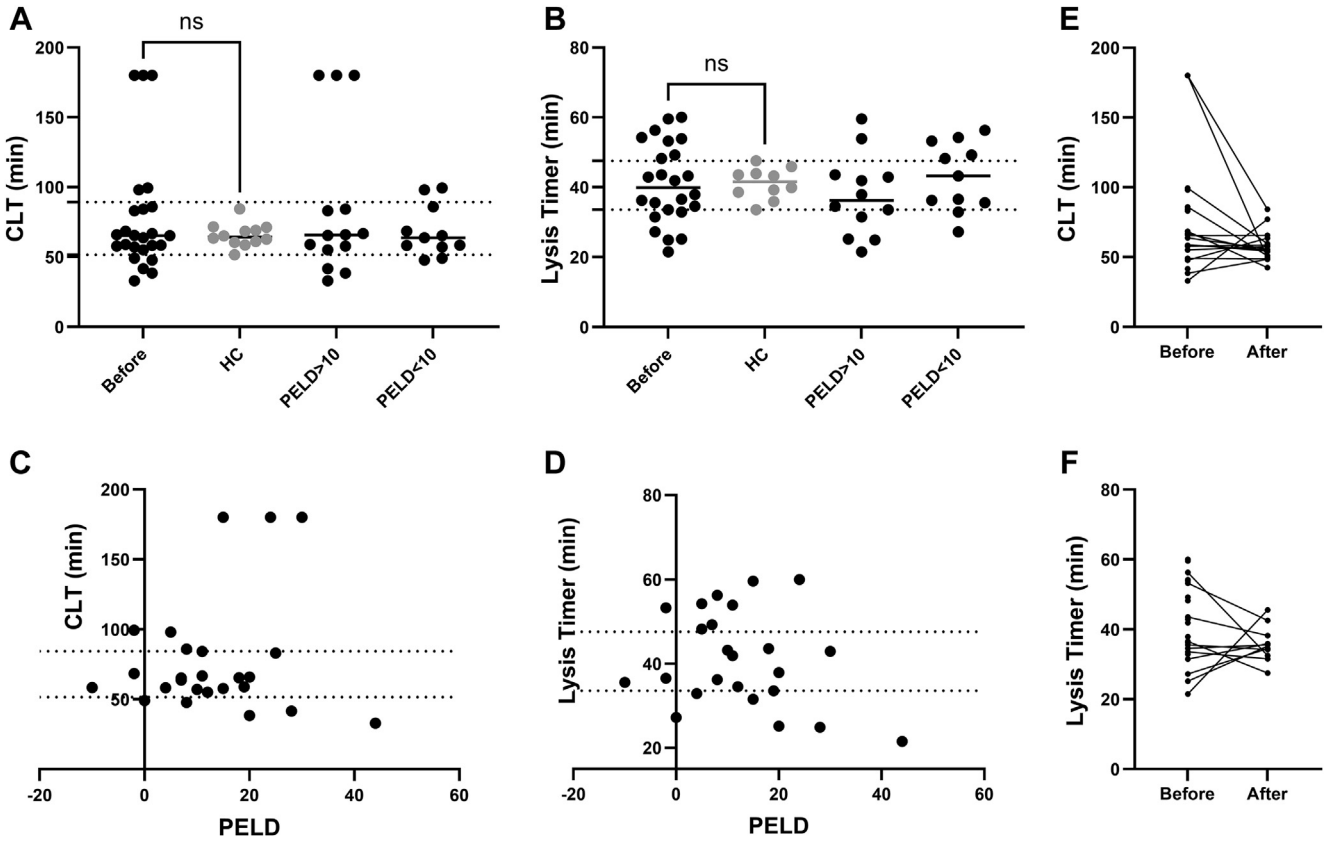

FIGURE 1 Clot lysis time (CLT) and Lysis Timer results in patients and controls. (A) CLT results in patients before liver transplantation compared with age-matched controls. Note the presence of severe hypofibrinolysis for Pediatric End-Stage Liver Disease (PELD) score of >10. (B) Lysis Timer results in patients before compared with age-matched controls. (C) Correlation between CLT and PELD score. (D) Correlation between Lysis Timer and PELD score. (E, F) Spaghetti plots comparing patients before and 3 months after liver transplantation for (E) CLT and (F) Lysis Timer (F). HC, healthy controls; ns, not significant.

Results were expressed as the time at which maximal lysis speed occurred (peak of the first derivative). Where indicated, PAI-1 antigen levels were measured with the Asserachrom PAI-1 Kit (Stago). Details on the plasma-based fibrinolysis assays are provided in Supplementary Table S1.

3 | RESULTS

Twenty-five pediatric patients with cirrhosis were prospectively included just before liver transplantation. From this group, we also collected samples 3 months after liver transplantation for 16 patients. For comparison, a group of 12 age-matched pediatric healthy controls was sampled. Median Pediatric End-Stage Liver Disease (PELD) score was 11 (IQR, 6-20). Median age was 20 months (IQR, 11-48 months) for patients and 28 months (IQR, 11-52 months) for healthy controls. Indications for liver transplantation were biliary atresia in 72%, progressive familial intrahepatic cholestasis in 12%, other causes (α-1 antitrypsin; drug-related) in 8%, and unknown etiology in 12% of patients.

The results for the CLT and Lysis Timer in our study population are shown in Figure 1. The CLT (median [IQR]) was 64.3 minutes (60.6-70.6 minutes) in the controls and 65.2 minutes (56.1-85.1 minutes) in patients ($P = .82$). Although results were not significantly different, a higher variation was seen for CLT in patients. After exclusion of severe hypofibrinolytic results (ie, samples that were not fully lysed or not lysed at all after 3 hours), changes in CLT correlated with the severity of liver disease (PELD; $r = -0.42$; $P = .05$). Five patients (20%) were identified as having hypofibrinolysis, defined as a value above the highest value of the healthy controls. Three of these patients (12%) had severe hypofibrinolysis with CLT higher than 180 minutes (the limit of the assay). All the patients with severe hypofibrinolysis had PELD scores of >10. Hyperfibrinolysis was also demonstrated in 5 patients (20%), identified as a result below the lowest value of the controls, of which 4 patients had PELD score of >10. Similar results were seen with Lysis Timer. Lysis Timer results (median [IQR]) were 41.6 minutes (37.9-47.6 minutes) in controls and 37.9 minutes (32.9-49.3 minutes) in patients ($P = .79$). However, 1 patient with severe hypofibrinolysis with CLT had a normal Lysis Timer result (42 minutes). PAI-1 level in this patient was 101 arbitrary units/mL (reference, <16 arbitrary units/mL), which is concordant with hypofibrinolysis. After exclusion of patients with extreme hypofibrinolysis with CLT (at the limit of the assay), a strong correlation ($r = 0.91$; $P < .001$) was seen between the 2 assays (Figure 2)

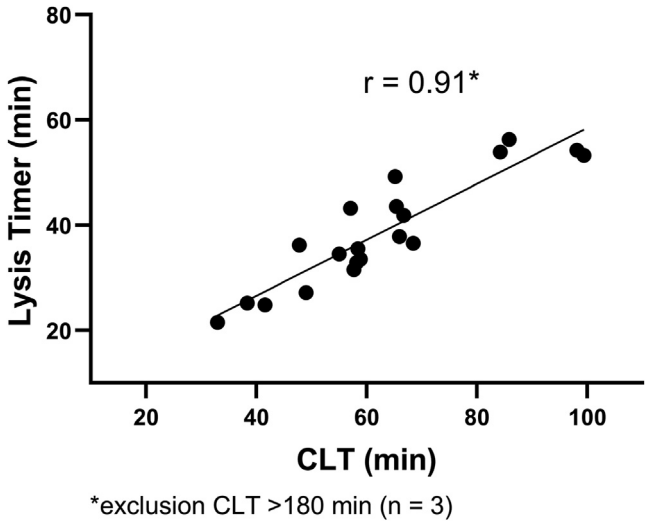

FIGURE 2 Correlation between clot lysis time (CLT) and Lysis Timer after exclusion of CLT of >180 minutes.

for patients. A more moderate correlation between the 2 assays (r = 0.73; $P < .05$) was seen for healthy controls. Most patients with aberrant fibrinolysis profiles before transplantation demonstrated CLT and Lysis Timer results similar to those of healthy controls 3 months after liver transplantation.

4 | DISCUSSION

In pediatric patients with cirrhosis, hypofibrinolysis, normal fibrinolysis, and hyperfibrinolysis, compared with healthy controls, were demonstrated with 2 plasma-based fibrinolysis assays. The largest variation in fibrinolytic capacity was observed in patients with more severe liver disease. Werner et al. [4] also demonstrated higher variation in results for CLT in patients compared with controls, with CLT results ranging from hypofibrinolysis to hyperfibrinolysis. In a cohort of adult patients with acute decompensation and acute-on-chronic liver failure, patients presented a mixed fibrinolytic phenotype with both clearly prolonged and shortened fibrinolysis [8]. In this adult population, hypofibrinolysis was associated with sepsis, organ failure, and mortality. Although our control group was rather small, our pediatric population had similar results compared with the pediatric healthy controls in the paper by Werner et al. [4] for CLT and compared with the adult healthy population tested by Bareille et al. [9] for Lysis Timer. Roullet et al. [10] defined a Lysis Timer threshold of 31 minutes for the detection of hyperfibrinolysis in adult patients undergoing liver transplantation by comparing results with euglobulin CLT. A limitation of this study is that we could not sample all patients 3 months after liver transplantation. Specifically, 2 patients had deceased, 1 patient experienced a clinical complication, and 6 patients returned to their country of origin shortly before sampling.

Overall concordance between the 2 fibrinolysis assays was excellent, with 1 exception: a patient who exhibited normal fibrinolysis with Lysis Timer but hypofibrinolysis with CLT. This discrepancy might be due to the lower tPA concentration used in CLT, making it more sensitive to hypofibrinolysis. The higher tPA concentrations used in the Lysis Timer, however, result in shorter assay time, making it more suitable for clinical applications. The CLT assay initiates coagulation with tissue factor, whereas the Lysis Timer uses human α-thrombin. Since tissue factor acts upstream in the coagulation pathway, it allows for the detection of coagulation-mediated fibrinolysis defects, such as impaired activation of the thrombin activatable fibrinolysis inhibitor due to hypocoagulability. In contrast, the Lysis Timer's use of excess exogenous thrombin ensures the conversion of a maximum amount of fibrinogen to fibrin, enhancing fibrin clot formation and structure [11]. This makes the assay more focused on the fibrinolytic system without being dependent on thrombin generation. As a commercially available test, the Lysis Timer could also offer better standardization of reagents and reference values. Conversely, the CLT assay has undergone extensive validation for clinical purposes [5].

In conclusion, our results are in line with previous publications in the pediatric cirrhotic population, confirming both hypofibrinolysis and hyperfibrinolysis in this patient population, with 2 plasma-based fibrinolysis assays, of which Lysis Timer has never been tested before in a pediatric population. Aberrant fibrinolytic profiles (hypofibrinolysis and hyperfibrinolysis) were seen in severe liver disease patients. After liver transplantation, these severe liver disease patients demonstrated fibrinolytic profiles similar to healthy controls.

ACKNOWLEDGMENTS

We would like to thank Jelle Adelmeijer of the University Medical Centre Groningen and Sandrine Desmet of Cliniques Universitaires Saint-Luc for expert technical assistance. We also would like to thank Nodia (Boom, Belgium) for providing the Lysis Timer and the accompanying reagents.

FUNDING

Nodia (Boom, Belgium) provided the Lysis Timer and the accompanying reagents.

AUTHOR CONTRIBUTIONS

M.-A.v.D., T.L., and X.S. did the conception and design of the study; M.-A.v.D., T.L., and X.S. acquired, analyzed, and interpreted the data; M.-A.v.D. and X.S. drafted the article; X.S., T.L., K.Z.B., and M.R. revised the article critically for important intellectual content. All authors approved the final version of the article before submission.

RELATIONSHIP DISCLOSURE

Please refer to the accompanying ICMJE disclosure forms for further details.

Marie-Astrid van Dievoet[1,2]
Karim Zouaoui Boudjeltia[3]
Madeleine Rousseaux[2]

Jonathan Douxfils[4,5]
Ton Lisman[6]
Xavier Stephenne[1,7]

[1]*Laboratory of Pediatric Hepatology and Cell Therapy, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium*
[2]*Laboratory Department, Cliniques Universitaires Saint-Luc, Brussels, Belgium*
[3]*Laboratory of Experimental Medicine (Univeristé libre de Bruxelles, 222 Unit), Faculty of Medicine, Centre Hospitalier Universitaire de Charleroi, André Vésale Hospital, Université Libre de Bruxelles, Montigny-le-Tilleul, Belgium*
[4]*Clinical Pharmacology and Toxicology Research Unit, Faculty of Medicine, Namur Research Institute for Life Sciences (NARILIS), Namur Thrombosis and Hemostasis Center, University of Namur, Namur, Belgium*
[5]*Research and Development Deparment, QUALIblood s.a., QUALIresearch, Liège, Belgium*
[6]*Surgical Research Laboratory and Section of Hepatobiliary Surgery and Liver Transplantation, Deparment of Surgery, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*
[7]*Division of Pediatric Gastroenterology and Hepatology, Department of Pediatrics, Cliniques Universitaires Saint-Luc, Rare Liver European Reference Network, TransplantChild European Reference Network, Brussels, Belgium*

Handling Editor: Dr Kristen Sanfilippo

Correspondence
Marie-Astrid van Dievoet, Laboratory Department, Cliniques Universitaires Saint-Luc, 54 Avenue Hippocrate, B-1200 Brussels, Belgium.
Email: marie-astrid.vandievoet@saintluc.uclouvain.be

ORCID

Marie-Astrid van Dievoet https://orcid.org/0000-0001-7056-7123

REFERENCES

[1] Brodsky I, Dennis LH. Evaluation of fibrinolysis in hepatic cirrhosis. Relation of serial thrombin time and euglobulin lysis time. *Am J Clin Pathol*. 1966;45:61–9.
[2] Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. *N Engl J Med*. 2011;365:147–56.
[3] von Meijenfeldt FA, Lisman T. Fibrinolysis in patients with liver disease. *Semin Thromb Hemost*. 2021;47:601–9.
[4] Werner MJM, de Meijer VE, Adelmeijer J, de Kleine RHJ, Scheenstra R, Bontemps STH, et al. Evidence for a rebalanced hemostatic system in pediatric liver transplantation: a prospective cohort study. *Am J Transplant*. 2020;20:1384–92.
[5] Lisman T. Decreased plasma fibrinolytic potential as a risk for venous and arterial thrombosis. *Semin Thromb Hemost*. 2017;43:178–84.
[6] Lisman T, de Groot PG, Meijers JCM, Rosendaal FR. Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis. *Blood*. 2005;105:1102–5.
[7] Amiral J, Laroche M, Seghatchian J. A new assay for global fibrinolysis capacity (GFC): investigating a critical system regulating hemostasis and thrombosis and other extravascular functions. *Transfus Apher Sci*. 2018;57:118–26.
[8] Blasi A, Calvo A, Prado V, Reverter E, Reverter JC, Hernández-Tejero M, et al. Coagulation failure in patients with acute-on-chronic liver failure and decompensated cirrhosis: beyond the international normalized ratio. *Hepatology*. 2018;68:2325–37.
[9] Bareille M, Hardy M, Chatelain B, Lecompte T, Mullier F. Laboratory evaluation of a new integrative assay to phenotype plasma fibrinolytic system. *Thromb J*. 2022;20:73. https://doi.org/10.1186/s12959-022-00435-6
[10] Roullet S, Labrouche S, Mouton C, Quinart A, Nouette-Gaulain K, Laurent C, et al. Lysis Timer: a new sensitive tool to diagnose hyperfibrinolysis in liver transplantation. *J Clin Pathol*. 2019;72:58–65.
[11] Wolberg AS, Campbell RA. Thrombin generation, fibrin clot formation and hemostasis. *Transfus Apher Sci*. 2008;38:15–23.

SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at https://doi.org/10.1016/j.rpth.2024.102551