

Von Willebrand and Factor VIII Portosystemic Circulation Gradient in Cirrhosis: Implications for Portal Vein Thrombosis

Michael Praktijn¹, Jonel Trebicka, MD^{2,3,4}, Roberto Carnevale, PhD^{5,6}, Daniele Pastori, MD, PhD⁷, Alexander Queck, MD², Evaristo Ettore, MD⁸ and Francesco Violi, MD^{5,7}

- OBJECTIVES:** Portal vein thrombosis seems to be dependent on local hypercoagulation and venous stasis; data regarding endothelial damage are lacking.
- METHODS:** von Willebrand factor, a marker of endothelial damage/perturbation, factor VIII, and lipopolysaccharides (LPS) were studied in the portal and systemic circulation of 20 cirrhotic patients undergoing transjugular intrahepatic portosystemic procedure.
- RESULTS:** von Willebrand factor, factor VIII, and LPS were higher in the portal compared with systemic circulation, with a significant correlation between LPS and the other 2 variables.
- DISCUSSION:** Endothelial damage and hypercoagulation coexist in the portal tree of patients with cirrhosis, and both could contribute to portal vein thrombosis. LPS may be a potential trigger of endothelial damage.

Clinical and Translational Gastroenterology 2020;11:e00123. <https://doi.org/10.14309/ctg.0000000000000123>

INTRODUCTION

There is a growing body of evidence to suggest that liver cirrhosis is associated with portal vein thrombosis (PVT), which may be detected in approximately 17% of the patients (1,2). The mechanisms accounting for PVT have not been fully elucidated. Risk factors such as factor V Leiden, prothrombin G20210 mutations, and splenectomy may account for PVT (3). According to the Virchow triad, local factors such as hypercoagulation, venous stasis, and endothelial damage may be also determinants for venous thrombosis; 2 of them, namely venous stasis and hypercoagulation, have been detected in the portal circulation of cirrhotic patients. In particular, Stine et al. (4) have recently reported that a portal flow <15 cm/s predisposes to PVT and its recurrence. We previously reported enhanced thrombin generation in the portal compared with systemic circulation; such a change was suggested to be dependent on an elevated concentration of lipopolysaccharides (LPS) in the portal circulation (5). Conversely, it is not known whether endothelial damage is also detectable in the portal tree. To address this issue, we measured blood levels of von Willebrand factor (vWF), which is a recognized marker of endothelial damage/perturbation (6), in the portal and systemic circulation of cirrhotic patients undergoing transjugular intrahepatic portosystemic shunt (TIPS) procedure.

METHODS

Twenty patients with decompensated cirrhosis diagnosed by clinical, imaging, or histologic finding and undergoing TIPS procedure were included in the study. Among them, 8 had ascites and 12 had variceal bleeding. Blood from the portal and the hepatic vein was collected as previously described (7). Briefly, first central venous blood was taken, and thereafter, blood sample from the portal vein was taken immediately after puncture of the vein, but before dilatation of the tract or insertion of the TIPS-stent portal, the venous samples were taken. After discarding, 5 mL blood was collected in tube with or without anticoagulant (3.8% sodium citrated) and centrifuged at 300 g for 10 min at room temperature. The supernatants were collected and stored at -80°C up to 5 years. The concentration of vWF, factor VIII, and LPS from *Escherichia Coli* was determined as previously described (8) by enzyme-linked immunosorbent assay (Abnova, KA0512, Taipei, Taiwan; Lifespan Bioscience, LS-F10415, Washington and Cusabio, CSB-E09945h, Houston, TX; respectively). The enzyme-linked immunosorbent assay kit is designed for the detection of monoclonal antibody specific for vWF and factor VIII and *E. Coli*-LPS expressed in units per deciliter for vWF and factor VIII and pg/mL for LPS. Inter- and intra-assay coefficients of variations were <10%.

¹Department of Medicine I, University of Bonn, Bonn, Germany; ²Department of Medicine I, University of Frankfurt, Frankfurt, Germany; ³European Foundation for the Study of Chronic Liver Failure—EF Clif, Barcelona, Spain; ⁴Department of Gastroenterology, Odense Hospital, University of Southern Denmark, Odense, Denmark; ⁵Department of Medico-Surgical Sciences and Biotechnologies Sapienza University of Rome, Latina, Italy; ⁶Mediterranea Cardiocentro, Napoli, Italy; ⁷Department of Internal Medicine and Medical Specialties, Sapienza University of Rome, Rome, Italy; ⁸Division of Gerontology, Department of Cardiovascular, Respiratory, Nephrologic, Anesthesiologic, and Geriatric Sciences, Sapienza University, Rome, Italy. **Correspondence:** Francesco Violi. E-mail: francesco.violi@uniroma1.it. **Received September 4, 2019; accepted October 8, 2019; published online February 5, 2020**

© 2020 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of The American College of Gastroenterology

The local ethics committee of the University of Bonn approved the study (029/13), and all patients signed an informed written consent in accordance with the Helsinki Declaration for the procedures they underwent.

Statistical analysis

Comparisons between vWF, LPS, and factor VIII in the portal and systemic circulation were carried out by Mann–Whitney test for unpaired samples, and data are expressed as median and interquartile ranges (IQR). The Spearman rank correlation coefficient (rS) was used for bivariate correlation.

RESULTS

The median age was 57 (IQR 41–74) years and 13 patients were men. Median model for end-stage liver disease was 14 (IQR 8–34); 4 patients were staged Child Turcotte Pugh (CTP) A, 14 were CTP B, and 2 were CTP C. Eleven patients had alcoholic etiology of liver cirrhosis. Indication for TIPS was variceal bleeding in 9 and refractory ascites in 11 patients. Although there was no significant correlation with the scores of liver disease severity (model for end-stage liver disease, CTP), we found a portal/systemic circulation gradient for all the 3 variables investigated: vWF (250.0 [IQR 214.0–267.0] vs 180.0 [IQR 161.0–204.0] U/dL, $P < 0.001$), factor VIII (164.0 [IQR 152.0–180.0] vs 112.0 [IQR 97.0–127.0] U/dL, $P < 0.001$), and LPS (56.5 [IQR 50.5–65.0] vs 43.0 [IQR 36.3–45.0] pg/mL, $P < 0.001$) were higher in the portal compared with systemic circulation (Figure 1a–c). Furthermore, LPS was significantly correlated with vWF ($rS:0.400$, $P = 0.01$) and factor VIII ($rS:0.360$, $P = 0.02$) (Figure 1d,e). Interestingly, we found a higher porto-systemic gradient of vWF in patients with ascites as indication for TIPS compared with variceal bleeding (hepatic–portal: 81.1 ± 38.4 vs -41.3 ± 48.5 U/dL, $P < 0.05$).

DISCUSSION

This study reports for the first time the existence of a portosystemic vWF gradient in cirrhotic patients, suggesting that the portal tree encompasses local factors predisposing to endothelial damage/perturbation are detectable in cirrhosis. We focused our attention on LPS because previous studies reported that LPS is elevated in the portal circulation of cirrhosis (5) and elicits endothelial perturbation with vWF secretion (8). The significant association between LPS and vWF is in favor of the hypothesis that LPS is one factor eliciting endothelial damage in the portal circulation of cirrhosis.

Another interesting finding of the study is the presence of a portosystemic factor VIII gradient in cirrhosis, which reinforces a previous study reporting an increased concentration of factor VIII in the portal circulation of cirrhotic patients compared with systemic circulation of healthy subjects (9). This finding supports and extends our previous report showing the existence of a hypercoagulation state in the portal circulation of cirrhosis (5) suggesting enhanced factor VIII as an important mechanism predisposing to the hypercoagulation state. LPS may also be relevant to this clotting change because it elicits endothelial secretion of factor VIII at concentrations commonly found in the venous circulation of cirrhosis (8). Thus, LPS could represent a unique mechanism which is implicated in enhancing either vWF or factor VIII in the portal circulation, thus favoring endothelial damage and hypercoagulation. However, we cannot exclude that other mechanisms such as increase of ADAMS 13 or microvesicles, both possessing procoagulant properties (10), may contribute to hypercoagulation in the portal circulation of cirrhosis (10,11). Changes in the gut permeability with consequent translocation of LPS into systemic circulation may be a factor accounting for the enhanced LPS concentration in the portal tree, but further study is necessary to explore this hypothesis. In conclusion, here, we provide evidence in

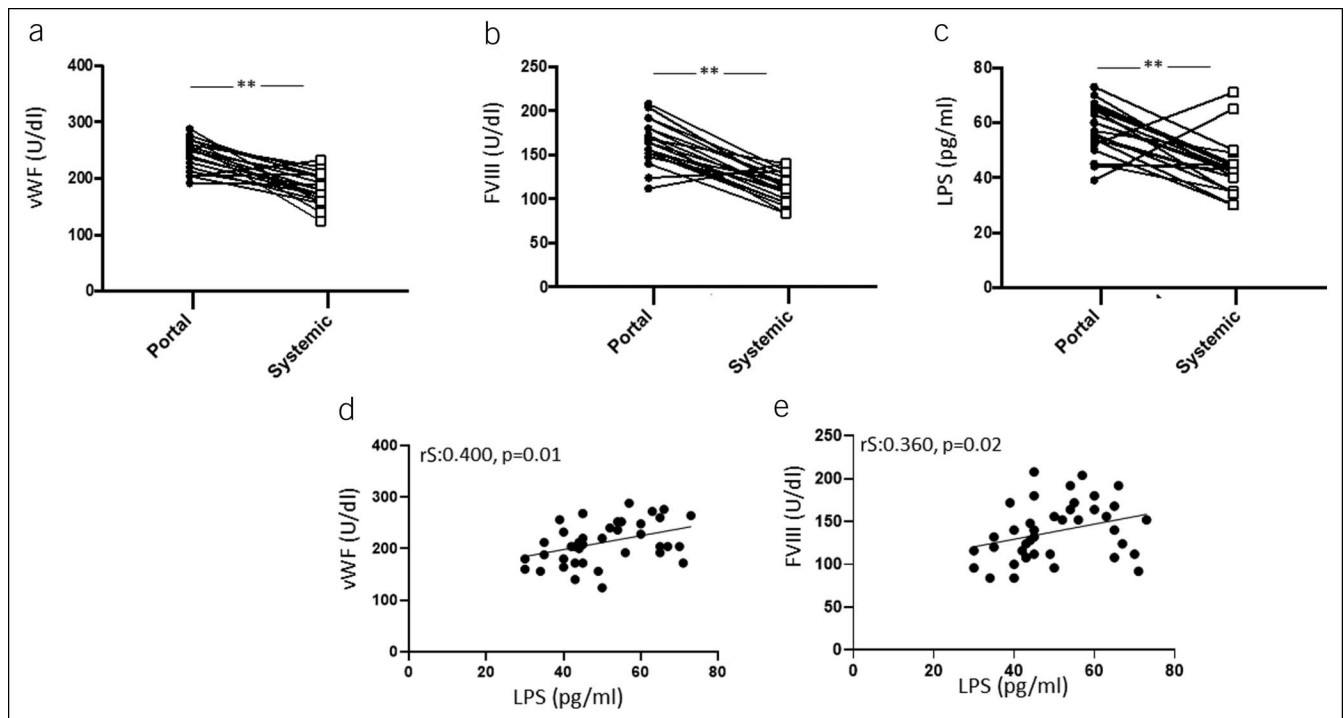


Figure 1. Differences in coagulation and inflammation biomarkers in the portal and systemic circulation. Levels of vWF (a), FVIII (b), and LPS (c) in the portal and systemic circulation of patients with cirrhosis ($n = 20$, $**P < 0.001$). LPS, lipopolysaccharides; vWF, von Willebrand factor.

support that endothelial damage is detectable in the portal circulation of cirrhosis and suggest LPS as a mechanism implicated in this phenomenon; modulation of LPS concentration may be a tool to reduce the thrombotic risk in cirrhosis (12).

CONFLICTS OF INTEREST

Guarantor of the article: Francesco Violi, MD.

Specific author contributions: Michael Praktiknjo, Jonel Trebicka, and Roberto Carnevale equally contributed to this work. M.P.: patients' recruitment, analysis and interpretation of data, and drafting of the manuscript. J.T.: patients' recruitment, analysis and interpretation of data, and critical revision of the manuscript. R.C.: acquisition of data, analysis and interpretation of data, and drafting of the manuscript. D.P.: statistical analysis. A.Q.: patients' recruitment and analysis and interpretation of data. E.E.: critical revision of the manuscript. F.V.: study conception, analysis and interpretation of data, and critical revision of the manuscript.

Financial support: None to report.

Potential competing interests: None to report.

Study Highlights

WHAT IS KNOWN

- ✓ Cirrhosis is associated with portal vein thrombosis.
- ✓ A hypercoagulation status is detected in the portal circulation.

WHAT IS NEW HERE

- ✓ Endothelial damage, as assessed by vWF and factor VIII, is detectable in the portal circulation of cirrhosis.
- ✓ Serum lipopolysaccharides correlate with endothelial damage suggesting a cause-effect relationship.

TRANSLATIONAL IMPACT

- ✓ This finding provides further insight into the pathogenesis of portal vein thrombosis and reinforces the concept the hypercoagulation status does exist in cirrhosis.

REFERENCES

1. Violi F, Ferro D. Clotting activation and hyperfibrinolysis in cirrhosis: Implication for bleeding and thrombosis. *Semin Thromb Hemost* 2013; 39:426–33.
2. Violi F, Corazza RG, Caldwell SH, et al. Portal vein thrombosis relevance on liver cirrhosis: Italian venous thrombotic events registry. *Intern Emerg Med* 2016;11:1059–66.
3. Qi X, Li H, Liu X, et al. Novel insights into the development of portal vein thrombosis in cirrhosis patients. *Expert Rev Gastroenterol Hepatol* 2015; 9:1421–32.
4. Stine JG, Wang J, Shah PM, et al. Decreased portal vein velocity is predictive of the development of portal vein thrombosis: A matched case-control study. *Liver Int* 2018;38:94–101.
5. Violi F, Ferro D, Basili S, et al. Ongoing prothrombotic state in the portal circulation of cirrhotic patients. *Thromb Haemost* 1997;77: 44–7.
6. Horvath B, Hegedus D, Szapary L, et al. Measurement of von Willebrand factor as the marker of endothelial dysfunction in vascular diseases. *Exp Clin Cardiol* 2004;9:31–4.
7. Berres M-L, Asmacher S, Lehmann J, et al. CXCL9 is a prognostic marker in patients with liver cirrhosis receiving transjugular intrahepatic portosystemic shunt. *J Hepatol* 2015;62:332–9.
8. Carnevale R, Raparelli V, Nocella C, et al. Gut-derived endotoxin stimulates factor VIII secretion from endothelial cells: Implications for hypercoagulability in cirrhosis. *J Hepatol* 2017;67:950–6.
9. Delahousse B, Labat-Debelleix V, Decalonne L, et al. Comparative study of coagulation and thrombin generation in the portal and jugular plasma of patients with cirrhosis. *Thromb Haemost* 2010;104: 741–9.
10. Lemoine S, Thabut D, Housset C, et al. The emerging roles of microvesicles in liver diseases. *Nat Rev Gastroenterol Hepatol* 2014;11(6): 350–61.
11. Lisman T, Bongers TN, Adelmeijer J, et al. Elevated levels of von Willebrand factor in cirrhosis support platelet adhesion despite reduced functional capacity. *Hepatol* 2006;44(1):53–61.
12. Violi F, Pastori D, Farcomeni A, et al. Reply. *Gastroenterology* 2018;154: 761–2.e1.

Open Access This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work, provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.