ORIGINAL ARTICLE

Annals of Gastroenterology (2026) **39,** 310-318

Liver fibrosis severity evaluated by shear-wave elastography is associated with procoagulant and systemic inflammatory activity in patients with cirrhosis

Georgios Kalambokis[a], Nikolaos Aggelis[a], Ilias Tsiakas[a], Dimitrios Biros[a], Grigorios Despotis[a], Evangelia Papapetrou[b], Christina Tsaousi[b], Georgios S. Markopoulos[c], Georgios Kolios[d], Reveka Konstantopoulou[a], Stavroula Tsiara[e], Gerasimos Baltayiannis[f], Haralampos Milionis[a]

University Hospital of Ioannina, Greece

Abstract

Background Hemostatic and inflammatory pathways may drive liver fibrogenesis. We investigated whether coagulation and systemic inflammatory activities influence liver fibrosis severity in patients with cirrhosis and thrombocytopenia.

Methods Two patient groups were evaluated according to liver stiffness measurement (LSM) through shear wave elastography: ≥25 kPa (n=100) vs. <25 kPa (n=100). Anti-hemostatic parameters (platelet count, factors II, V, VII, IX, X, XI, XII, and XIII, fibrinogen, and a2-antiplasmin), pro-hemostatic parameters (factor VIII [FVIII], protein C [PC], protein S, antithrombin, von Willebrand factor-antigen [vWf-Ag], plasminogen, and plasminogen activator inhibitor-1), the FVIII-to-PC ratio as procoagulant imbalance index, and systemic inflammation markers (serum lipopolysaccharide-binding protein [LBP], tumor necrosis factor [TNF]-α, and interleukin [IL]-6) were measured. Cirrhosis severity was evaluated by model for end-stage liver disease (MELD) score.

Results Patients with LSM≥25 kPa exhibited significantly higher decompensation rates, MELD score, FVIII-to-PC ratio, and levels of FVIII, PC, protein S, vWf-Ag, LBP, TNF-α, and IL-6, and significantly lower levels of anti-hemostatic parameters than those with LSM<25 kPa. In multivariate analysis evaluating coagulation parameters alone or alongside inflammatory markers, FVIII-to-PC ratio (P=0.01/P=0.03) and LBP (P=0.01) were linked to LSM≥25 kPa after adjusting for MELD score and prior decompensation. LSM correlated significantly with FVIII-to-PC ratios, and levels of FVIII, PC, vWf-Ag, LBP, TNF-α and IL-6, in both the total cohort and patients with LSM≥25 kPa. LBP, TNF-α and IL-6 levels correlated significantly with the FVIII-to-PC ratios and vWf-Ag levels in both LSM subgroups.

Conclusion Higher procoagulant and systemic inflammatory activities are associated with greater liver fibrosis severity in cirrhotic patients.

Keywords Liver fibrosis, cirrhosis, systemic inflammation, prothrombotic activity, shear wave elastography

Ann Gastroenterol 2026; 39 (3): 310-318

Conflict of Interest: None

Correspondence to: Georgios N. Kalambokis, MD, PhD, First Division of Internal Medicine, Medical School, University of Ioannina, Ioannina 45500, Greece, e-mail: gkalambo@uoi.gr

Received 26 November 2025; accepted 23 February2026; published online 23 April 2026

DOI: https://doi.org/10.20524/aog.2026.1060

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms

Introduction

Liver fibrogenesis occurs as a result of persistent liver damage from various causes, and is characterized by an overproduction of extracellular matrix (ECM), primarily composed of various collagen types. Activated hepatic stellate cells (HSCs) are the primary source of ECM, by adopting a myofibroblast-like phenotype. Sustained fibrogenesis plays a crucial role in structural liver remodeling, which ultimately results in cirrhosis [1]. Moreover, activated HSCs and liver sinusoidal endothelial cells (LSECs) develop a vasoconstrictive phenotype that, combined with the

© 2026 Hellenic Society of Gastroenterology

structural changes, contributes to the development of portal hypertension [2].

Patients with cirrhosis are increasingly recognized to exhibit a hypercoagulable state, despite indications of bleeding risk from conventional coagulation tests and thrombocytopenia. Indeed, apart from a deficiency of procoagulant factors, these patients commonly present reduced plasma concentrations of major natural anticoagulants such as protein C (PC) and antithrombin (AT) [3]. Additionally, high circulating levels of factor VIII (FVIII) [4] and von Willebrand factor-antigen (vWf-Ag) [5] are commonly detected in patients with advanced cirrhosis, as a result of increased synthesis in activated LSECs. An increased FVIII-to-PC ratio signifies a procoagulant imbalance [6], while the raised vWf levels enhance platelet adhesion and aggregation, even amid thrombocytopenia [7,8]. Consequently, a prothrombotic state is promoted, potentially resulting in intrahepatic activation of coagulation pathways with stimulation of thrombin generation in the portal microvasculature. Thrombin can directly activate HSCs through protease-activated receptors, triggering ECM protein secretion [9]. Furthermore, the procoagulant activity predisposes to the development of intrasinusoidal microthrombi leading to local ischemia and parenchymal extinction, thereby accelerating fibrosis progression [9,10].

The role of inflammatory responses in the progression of hepatic fibrosis has also attracted growing interest [11,12]. In patients with cirrhosis, the liver is constantly exposed to gut-derived bacterial products, including lipopolysaccharide (LPS), commonly referred to as endotoxin. This occurs because of bacterial translocation (BT), which is driven by small intestinal bacterial overgrowth and increased intestinal permeability [13]. Experimental findings indicate that LPS promotes the onset and progression of liver fibrosis by activating HSCs via Toll-like receptors 4 [14]. Moreover, circulating LPS stimulates monocytes and lymphocytes to release proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, which may also trigger liver fibrosis [15].

Liver biopsy is considered the definitive procedure for evaluating liver fibrosis in chronic liver diseases, but its invasive nature is a major drawback [16]. Liver stiffness measurement (LSM), assessed by ultrasound-based elastography, has been validated for quantifying liver fibrosis [17]. Specifically, 2-dimensional shear-wave elastography (2D-SWE) demonstrates high reproducibility and, unlike other elastographic methods, its applicability is not limited by the presence of ascites [18].

The current prospective study aimed to elucidate the relationship between liver fibrosis severity, evaluated by LSM, and procoagulant and systemic inflammatory activities, as well as the interaction of coagulation imbalance and systemic inflammation in patients with cirrhosis.

Patients and methods

Patients

Patients with liver cirrhosis seen at the outpatient hepatology clinics of the University Hospital of Ioannina, Greece, from May 2020 to May 2025 were prospectively evaluated. Written informed consent was obtained from every participant. The study conformed to the principles of declaration of Helsinki and was approved by the Institutional Ethics Committee. The diagnosis of cirrhosis was based on clinical and laboratory findings, endoscopy, imaging studies, or on liver biopsy. We included patients with thrombocytopenia, defined by a platelet count <150 $x10^9$/L, and age between 18 and 75 years. Exclusion criteria were: a) ascites requiring large volume paracentesis; b) use of anticoagulant or antiplatelet drugs, or other therapy known to interfere with blood coagulation and platelet function; c) known hemostatic disorders other than cirrhosis; d) use of pre-/probiotics or antibiotics in the past 6 weeks prior to enrolment; e) hepatic encephalopathy grade II or higher, variceal bleeding, and spontaneous bacterial peritonitis or other bacterial infection at least 3 months prior to inclusion; f) serum creatinine levels >1.5 mg/dL; g) recent (within 6 months) or active ethanol abuse; h) history of portal vein thrombosis or other episode of thrombosis; i) hepatocellular carcinoma or other malignancy; j) transjugular intrahepatic portosystemic shunt insertion; k) a history of cardiovascular, pulmonary or renal disease; and l) uncontrolled diabetes. Primary prophylaxis for variceal bleeding included a beta-blocker, such as propranolol or carvedilol, endoscopic band ligation, or a combination of the two.

Study design

On the first day of the study, a thorough clinical assessment was performed, and the following demographic and clinical characteristics were recorded for each patient: age, sex, etiology of cirrhosis, previous decompensation, use of beta-blockers, and diabetes. Subsequently, 2D-SWE was performed for assessment of liver fibrosis after patients had fasted for at least 4 h. All eligible patients were consecutively enrolled into one of the following study groups until each group consisted of 100 patients: the first group included patients with LSM≥25 kPa, defined as advanced liver fibrosis [17], while the second group included patients with LSM<25 kPa. At 8:00 am the following day, blood samples were obtained for measurement

[a]First Division of Internal Medicine Department, Medical School, University of Ioannina, Ioannina (Georgios Kalambokis, Nikolaos Aggelis, Ilias Tsiakas, Dimitrios Biros, Grigorios Despotis, Reveka Konstantopoulou, Haralampos Milionis); [b]Hematology Laboratory, University Hospital of Ioannina, Ioannina (Evangelia Papapetrou, Christina Tsaousi); [c]Hematology Laboratory, Unit of Molecular Biology and Translational Flow Cytometry, Medical School, University Hospital of Ioannina, Ioannina (Georgios S. Markopoulos); [d]Laboratory of Clinical Chemistry, University Hospital of Ioannina, Ioannina (Georgios Kolios); [e]Second Division of Internal Medicine Department, Medical School, University of Ioannina, Ioannina (Stavroula Tsiara); [f]Department of Gastroenterology, Medical School, University of Ioannina, Ioannina (Gerasimos Baltayiannis), Greece

of anti-hemostatic factors (platelet count, factors [F] II, V, VII, IX, X, XI, XII, and XIII, fibrinogen, and a2-antiplasmin as a marker of high fibrinolysis), pro-hemostatic factors (FVIII, PC, protein S [PS], AT, vWf-Ag, and markers of low fibrinolysis such as plasminogen and plasminogen activator inhibitor-1 [PAI-1]), and indicators of systemic inflammatory activity (serum levels of lipopolysaccharide-binding protein [LBP], TNF-α, and IL-6). Plasma and serum samples were stored at -80°C until analysis. Routine laboratory examinations, including international normalized ratio, and liver and renal biochemistry were also performed. The severity of cirrhosis was evaluated by the Child-Pugh classification and the model for end-stage liver disease (MELD) score, calculated using laboratory findings.

Hemostatic parameters

The levels of pro- and anti-hemostatic factors were expressed as the percentage of normal pooled plasma, which was arbitrarily set at 100%. The activities of FII, FV, FVII, FVIII, FIX, FX, FXI, FXII and fibrinogen were determined by 1-stage clotting assays, using factor-deficient plasmas (Siemens, Marburg, Germany). The activities of FXIII, PC, and AT were measured using the Berichrom® FXIII, Berichrom® PC and Berichrom® AT chromogenic assays (Siemens, Marburg, Germany). The PS activity was measured by a clotting assay (PS Ac, Siemens, Marburg, Germany). The vWf-Ag levels were determined by an immunoturbidimetric assay (INNOVANCE® VWF Ac and VWF ag; Siemens, Marburg, Germany). The activities of a2 antiplasmin and plasminogen were determined by the Berichrom® a2-antiplasmin and Berichrom® Plasminogen chromogenic assays (Siemens, Marburg, Germany). The normal ranges for the above tests were: 70-120% for FII, FV, FVII, FIX, FX, and FXI; 70-150% for FVIII and FXII; 75-130% for FXIII; 70-140% for FV, PC, and PS; 80-125% for AT; 50-160% for vWf-Ag; 170-400 mg/dL for fibrinogen; 80-120% for a2-antiplasmin; and 75-150% for plasminogen. The PAI-1 activity was measured using the Berichrom® PAI-1 chromogenic assay (Siemens, Marburg, Germany) and the reference interval was 0.3-3.5 U/mL.

Procoagulant imbalance index

The ratio of FVIII-to-PC was taken as an index of the procoagulant imbalance (the greater the ratio the higher the procoagulant imbalance).

Inflammatory markers

The LEGENDplex™ Human TNF-α Capture Bead B3, 13X (Cat. No. 740053, Biolegend, USA) and the LEGENDplex™ Human IL-6 Capture Bead A7, 13X (Cat. No. 740044, Biolegend, USA) were used for the measurement of TNF-α and IL-6 serum levels, respectively, according to the manufacturer's instructions. The samples were analyzed in duplicate by Cytometric Bead Array flow cytometry in a BD FACSCalibur flow cytometer using CellQuest V3 Software (BD Biosciences, San Jose, CA, USA) and the data were analyzed using LEGENDplex™ Data Analysis Software V8.0 (BioLegend, USA). The assay sensitivities were 1.97 pg/mL for TNF-α and 2.01 pg/mL for IL-6. A commercially available enzyme-linked immunosorbent assay kit (ALX-850-304-KI01, Enzo Life sciences, Farmingdale, NY, USA) was used to measure serum LBP concentrations in accordance with the manufacturer's instructions. The assay sensitivity was 5 ng/mL. TNF-α, IL-6 and LBP levels below the detection limit of the assay's sensitivity were assigned values of 1.97 pg/mL, 2.01 pg/mL and 5 ng/mL, respectively.

Liver stiffness measurement

2D-SWE was performed using an Aixplorer US system (Supersonic Imagine S.A, Aix-en-Provence, France) equipped with elastographic software and a convex array transducer. All 2D-SWE measurements were made by an experienced sonographer with more than 7 years of experience in real-time elastography studies. The shear wave was generated by a continuously repeated focused ultrasound beam to the target tissue along the direction the longitudinal wave propagated. The velocity of the generated shear wave was measured by performing an ultra-fast ultrasound scan at a very high frame rate (more than 4000 frames/sec), and the stiffness of the corresponding liver tissue was calculated by measuring the shear-wave velocity generated. After grayscale ultrasound, 2D-SWE was performed using the same probe. The curved transducer was placed intercostally at the level of the right lobe of the liver, with the target area located in the right anterior hepatic segment at a depth of more than 2 cm from the hepatic capsule to avoid major vessels. Liver stiffness was measured within a 5-sec breath hold. The measurement was performed 10 times for each patient, and the results were expressed in kilopascals (kPa). The median value was considered representative of the liver stiffness.

Statistical analysis

The baseline characteristics were expressed as absolute and relative frequencies for categorical variables and as mean ± standard error for continuous variables. Pearson's chi-square test and Student's unpaired *t*-test were used to compare categorical and continuous variables, respectively. Variables showing significance in univariate analysis were entered into a forward stepwise multivariate binary logistic regression analysis to determine factors independently associated with the presence of LSM≥25 kPa. Two multivariate models were employed: one including only coagulation variables (model 1) and one including coagulation and inflammation variables (model 2). Both multivariate models were adjusted for MELD score and

decompensation status. The relationship between LSM, coagulation parameters and systemic inflammatory markers was assessed by the Spearman rank correlation coefficient. A P-value of <0.05 was considered statistically significant. All statistical analyses were performed with the SPSS 26.0 statistical package (IBM Corp., Armonk, N.Y., USA).

Results

Clinical characteristics of patients

No significant differences were observed among patient subgroups regarding age, sex, etiology of cirrhosis, use of beta-blockers or presence of diabetes (Table 1). The incidence of decompensation (P=0.004), particularly ascites (P=0.01), and liver disease severity as assessed by MELD score (P=0.01) and Child-Pugh classification (P=0.02) were significantly higher in patients with LSM≥25 kPa. As expected, LSM was considerably higher in patients with LSM≥25 kPa (range: 25-46 kPa) compared to those with LSM<25 kPa (range: 14-24.8 kPa) (P<0.001). Q: Is this correct? Or should the first LSM be something else?

Relationship of liver fibrosis severity with coagulation parameters and systemic inflammatory markers on univariate analysis

Lower platelet counts (P=0.009), as well as levels of FII (P<0.001), FV (P=0.002), FVII (P<0.001), FIX (P<0.001), FX (P=0.001), FXI (P=0.004), FXII (P=0.005) and FXIII (P=0.03), PC (P<0.001), AT (P<0.001) and PS (P=0.001), increased levels of FVIII (P<0.001) and vWf-Ag (P<0.001), and higher FVIII-to-PC ratios (P<0.001) were associated significantly with LSM≥25 kPa on univariate analysis (Table 2). Patients with LSM≥25 kPa had significantly higher serum levels of LBP (P<0.001), TNF-α (P<0.001) and IL-6 (P<0.001) compared to those with LSM<25 kPa.

Table 1 Clinical characteristics of patients

Characteristics	LSM<25 kPa (n=100)	LSM≥25 kPa (n=100)	P-value
Age (years)	60.4±1.2	59±1.4	0.4
Male sex (%)	81	79	0.7
Etiology of cirrhosis (alcohol/viral/other)	77/15/8	72/17/11	0.6
Previous decompensation (%)	43	63	0.004
Ascites (%)	37	55	0.01
Variceal bleeding (%)	6	14	0.06
Hepatic encephalopathy (%)	6	7	0.9
Child-Pugh A/B/C	56/33/11	41/35/24	0.02
MELD score	10.9±0.6	12±0.4	0.01
Beta-blockers (%)	50	62	0.08
Diabetes (%)	20	29	0.1
LSM (kPa)	19.1±0.3	32.9±0.5	<0.001

Data are reported as mean±standard error or absolute (percentage)
LSM, liver stiffness measurement; MELD, model for end-stage liver disease

Factors associated with liver fibrosis severity in multivariate analysis

In multivariate model 1, FVIII (P=0.008), PC (P=0.01), FVIII-to-PC ratio (P=0.002) and vWf-Ag (P=0.007) were independently associated with LSM≥25 kPa (Table 3). FVIII-to-PC ratio (P=0.01) and vWf-Ag (P=0.04) remained the only variable independently associated with LSM≥25 kPa after adjusting for MELD score and decompensation status. In multivariate model 2, independent associations with LSM≥25 kPa were noted for FVIII (P=0.02), PC (P=0.05), FVIII-to-PC ratio (P=0.009), vWf-Ag (P=0.03), LBP (P=0.005), TNF-α (P=0.03) and IL-6 (P=0.04). When multivariate analysis was adjusted for MELD and prior decompensation, FVIII-to-PC ratio (P=0.03) and LBP (P=0.01) were the only significant factors associated with LSM≥25 kPa (Table 3).

Table 2 Coagulation parameters and systemic inflammation markers

Parameters	LSM<25 kPa (n=100)	LSM≥25 kPa (n=100)	P-value
Anti-hemostatic parameters			
Platelet count (×10^3/μL)	102±25	91±36	0.009
Factor II (%)	92.8±3.8	68.5±3.9	<0.001
Factor V (%)	96±3.6	79.6±3.7	0.002
Factor VII (%)	91.4±3.7	70.2±3.7	<0.001
Factor IX (%)	103.5±2.9	86.8±3.2	<0.001
Factor X (%)	95.3±3.3	79.9±2.9	0.001
Factor XI (%)	89.2±2.6	77.3±3	0.004
Factor XII (%)	97.9±2.4	87±2.8	0.005
Factor XIII (%)	116.9±3.6	105.9±3.5	0.03
Fibrinogen (mg/dL)	375.6±10.1	353.4±12.1	0.1
a2-antiplasmin (%)	89.2±2.2	88.4±2.2	0.7
Pro-hemostatic parameters			
Protein C (%)	100.5±3.1	70.5±3	<0.001
Antithrombin (%)	89.5±2.3	75.8±2.5	<0.001
Protein S (%)	88.5±2.6	75.8±2.6	0.001
Factor VIII (%)	131.5±2.9	174.5±3.2	<0.001
Factor VIII-to-Protein C ratio	1.49±0.07	3.04±0.16	<0.001
von Willebrand factor antigen (%)	176.6±8.7	326.5±14.6	<0.001
Plasminogen (%)	91.3±2.5	87.1±2.6	0.2
Plasminogen activator inhibitor-1 (U/mL)	1.75±0.25	1.52±0.23	0.5
Systemic inflammation markers			
Lipopolysaccharide binding protein (ng/mL)	6.12±0.06	7.1±0.07	<0.001
Tumor necrosis factor-α (pg/mL)	9.87±0.85	27.8±2.6	<0.001
Interleukin-6 (pg/mL)	11.6±0.8	43.9±4.1	<0.001

Data are reported as mean±standard error
LSM, liver stiffness measurement

Table 3 Variables independently associated with liver stiffness measurement ≥25 kPa

Variables	OR	95%CI	P*	OR	95%CI	P**
Multivariate model 1: coagulation parameters						
Factor VIII	1.243	1.122-1.1408	0.008			
Protein C	0.943	0.889-1.072	0.01			
Factor VIII-to- Protein C ratio	2.952	1.866-4.947	0.002	2.049	1.481-3.771	0.01
von Willebrand factor antigen	1.375	1.117-1.715	0.007	1.109	1.004-1.404	0.04
Multivariate model 2: coagulations parameters and systemic inflammation markers						
Factor VIII	1.132	1.090-1.348	0.02	1.645	1.309-2.932	0.03
Protein C	0.976	0.932-1.163	0.05			
Factor VIII-to-Protein C ratio	2.235	1.466-4.385	0.009			
von Willebrand factor antigen	1.186	1.072-1.499	0.03			
Lipopolysaccharide binding protein	2.849	1.456-6.102	0.005	2.350	1.038-5.573	0.01
Tumor necrosis factor-α	1.144	1.042-1.337	0.03			
Interleukin-6	1.094	1.023-1.208	0.04			

*Multivariate analysis not including MELD and previous decompensation; **Multivariate analysis including MELD and previous decompensation
OR, odds ratio; CI, confidence interval; MELD, model for end-stage liver disease

Correlation of LSM with coagulation parameters and systemic inflammation markers

LSM was significantly correlated with the levels of FVIII (r=0.608; P<0.001), PC (r=0.497; P<0.001) and vWf-Ag (r=0.621; P<0.001), and with the FVIII-to-PC ratios (r=0.640; P<0.001) in the total cohort of 200 patients (Fig. 1). Additional significant correlations were identified between LSM and the serum levels of LBP (r=0.606; P<0.001), TNF-α (r=0.600; P<0.001) and IL-6 (r=0.557; P<0.001) in these patients (Fig. 2). When the LSM subgroups were analyzed, the correlations of LSM with both the coagulation parameters and the systemic inflammation markers remained significant for patients with LSM≥25 kPa.

Correlation of coagulation parameters with systemic inflammation markers

Serum concentrations of LBP, TNF-α and IL-6 showed significant correlations with the FVIII-to-PC ratios (r=0.523; P<0.001, r=0.493; P<0.001, and r=0.578; P<0.001, respectively) as well as with the vWf-Ag levels (r=0.539; P<0.001, r=0.538; P<0.001, and r=0.439; P<0.001) across the whole patient cohort (Table 4). The above correlations were also significant in both LSM subgroups.

Discussion

Liver fibrosis represents an important pathophysiological feature and key prognostic indicator in advanced chronic liver disease [1,2]. The activation of HSCs is the primary pathogenic mechanism behind the onset of liver fibrosis and its progression to cirrhosis [1]. The potential contribution of the intrasinusoidal procoagulant and inflammatory processes to the progression of liver fibrosis is currently a topic of increasing interest [9-12]. Consequently, the present study investigated whether coagulation balance and systemic inflammation are associated with the severity of liver fibrosis in a large cohort of prospectively recruited patients with clinically stable cirrhosis. 2D-SWE is an effective method for detecting and staging liver fibrosis in patients with chronic liver diseases [19]. An LSM cutoff of 25 kPa was established to identify advanced fibrosis in our study and to subgroup the enrolled patients, acknowledging the clinical importance of this threshold in defining clinically significant portal hypertension [17,20].

In the current study, all hemostatic parameters—except for fibrinogen and factors linked to fibrinolysis—as well as the systemic inflammation markers demonstrated significant associations with increasing severity of liver fibrosis in univariate analysis. Nonetheless, only changes in FVIII, PC and vWf-Ag levels, and the FVIII-to-PC ratio, all linked to thrombotic potential, were independently related to LSM≥25 kPa. Notably, high FVIII-to-PC ratios and vWf-Ag levels emerged as the only factors significantly associated with advanced liver fibrosis after adjustment for liver disease severity and history decompensation. Alongside thrombophilic factors, high systemic inflammatory activity was associated with LSM≥25 kPa. Importantly, only elevated LBP levels and the FVIII-to-PC ratio were independently linked to advanced liver fibrosis when both coagulation parameters and systemic inflammatory markers were analyzed after adjustment for MELD score and history of decompensation. LBP was utilized as a surrogate marker of LPS in the present study, since the latter has a half-life of only 2-4 min as compared to 12-24 h for LBP [21].

Our results in patients with cirrhosis align with the concept that hypercoagulable forces contribute to liver fibrogenesis [22]. In support of our findings, Brodard *et al* [23] demonstrated that patients with LSM≥21 kPa had considerably greater thrombin generation than those with lower LSM values. In this regard, the high FVIII levels observed in our study enhance thrombin generation [24,25],

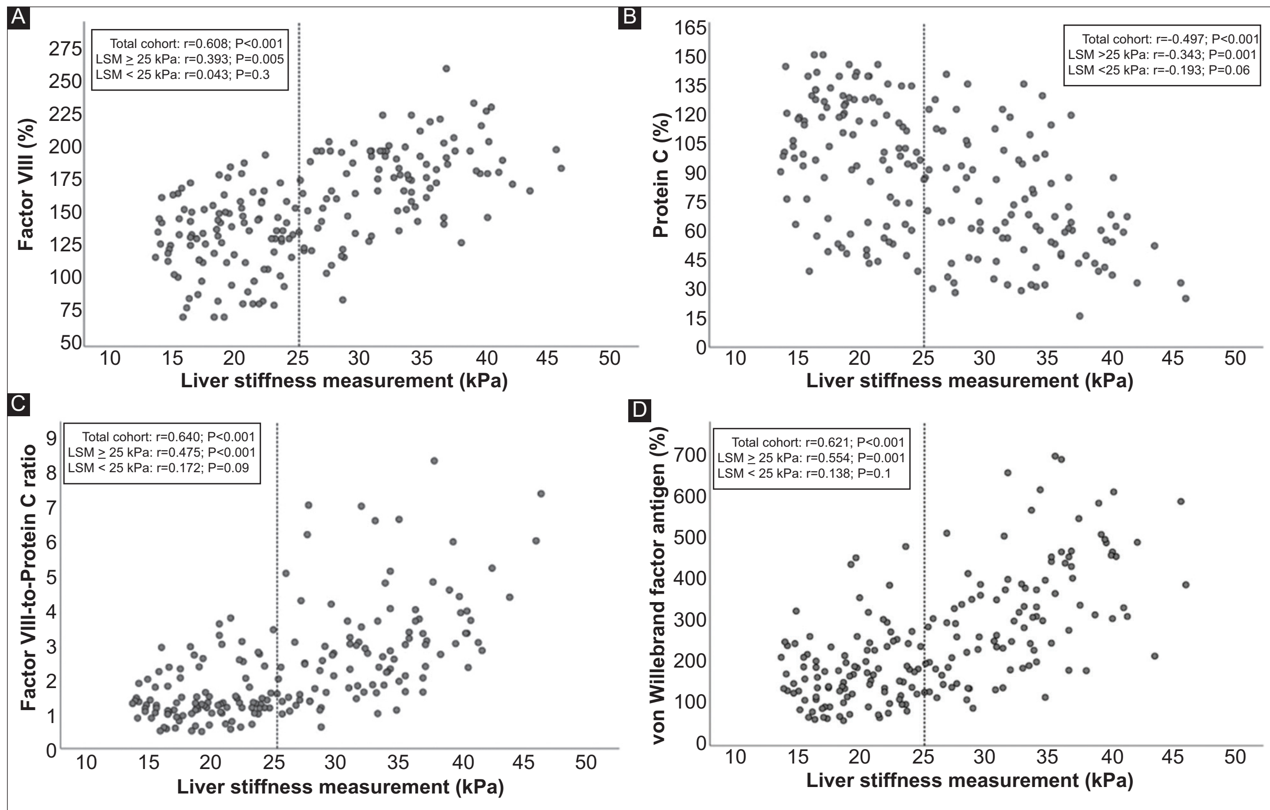

Figure 1 Correlations between liver stiffness measurements and factor VIII levels (panel A), protein C levels (panel B), factor VIII-to-Protein C ratio (panel C), and von Willebrand factor-antigen levels (panel D) in patients with cirrhosis

Table 4 Correlations between coagulation parameters and systemic inflammation markers

Parameters	LBP (ng/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)
Total cohort (n=200)			
Factor VIII-to-Protein C ratio	r=0.523; P<0.001	r=0.493; P<0.001	r=0.578; P<0.001
Von Willebrand factor antigen (%)	r=0.539; P<0.001	r=0.538; P<0.001	r=0.439; P<0.001
LSM≥25 kPa (n=100)			
Factor VIII-to-Protein C ratio	r=0.304; P=0.002	r=0.408; P<0.001	r=0.342; P=0.001
von Willebrand factor antigen (%)	r=0.310; P=0.002	r=0.410; P<0.001	r=0.308; P=0.04
LSM<25 kPa (n=100)			
Factor VIII-to-Protein C ratio	r=0.282; P=0.005	r=0.188; P=0.06	r=0.261; P=0.009
Von Willebrand factor antigen (%)	r=0.301; P=0.003	r=0.279; P=0.005	r=0.221; P=0.03

LBP, lipopolysaccharide-binding protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; LSM, liver stiffness measurement

while at the same time the downregulation of thrombin generation through FVIII inhibition is limited by low PC activity [26]. Higher FVIII-to-PC ratios and lower PC levels were also independently associated with higher LSM in patients with metabolic-associated steatotic liver disease [27]. On the other hand, the circulating levels of vWf-Ag have been directly correlated with the histological stage of liver fibrosis in patients with chronic viral hepatitis [28]. Furthermore, different anticoagulants have been found to reduce liver fibrosis in preclinical cirrhosis models [29], while a recent study involving patients with advanced cirrhosis revealed a notable decrease in LSM following the administration of rivaroxaban [30]. Other reports demonstrated that patients with chronic viral hepatitis and thrombophilic conditions resulting from PC or ATIII deficiencies [31,32], increased expression of FVIII [32], and heterozygote carriage of the factor V Leiden mutation [33], experienced accelerated liver fibrosis progression.

Our findings are also in line with experimental research showing that inflammatory responses upon exposure to BT and LPS promote liver fibrogenesis [13,34]. However, there are only limited data that specifically address whether systemic inflammation is connected with the severity of liver fibrosis in patients with established cirrhosis. In a recent study, LBP and IL-6 were significantly associated with histological markers of liver fibrosis [35].

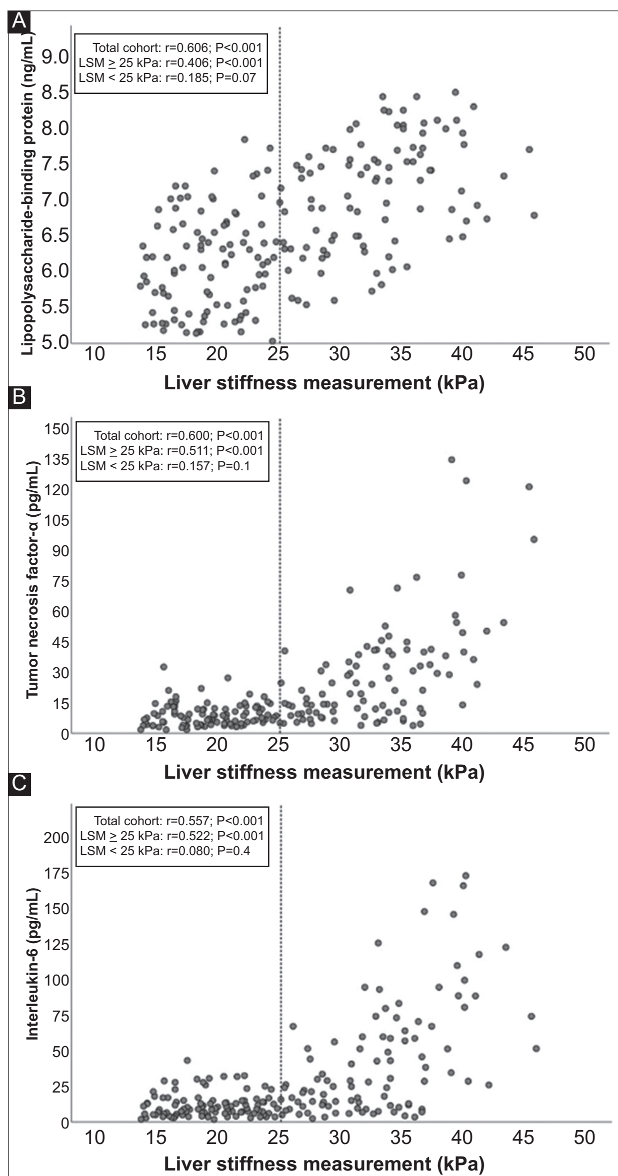

Figure 2 Correlations between liver stiffness measurements and levels of lipopolysaccharide-binding protein (panel A), tumor necrosis factor-α (panel B), and interleukin-6 (panel C), in patients with cirrhosis

Similarly, a link between systemic inflammation and liver fibrogenesis has been reported in patients with acute-on-chronic liver failure [36]. In addition, the use of rifaximin, a non-absorbable antibiotic that reduces gut-derived toxins, ameliorated liver fibrosis in experimental models of metabolic steatohepatitis [37,38] and humans with compensated alcoholic cirrhosis [39].

Notably, our study showed a consistent association of procoagulant activity and systemic inflammation with LSM in the overall cohort. However, when subgroups were examined, this link was significant only in patients with advanced liver fibrosis. The higher prevalence of decompensation in the latter subgroup might point to elevated portal pressure, resulting in more marked BT and associated hemostatic and inflammatory reactions [13]. However, the presence of decompensation at entry was not significantly associated with advanced liver fibrosis in our multivariate analyses, corroborating recent observations showing a robust correlation of systemic inflammation with liver fibrogenesis, regardless of portal hypertension severity, in patients with cirrhosis [35]. An alternative explanation might be that the patients with LSM≥25 kPa exhibit the greatest procoagulant and inflammatory responses, potentially leading to an increased incidence of microthrombi in the portal circulation and more pronounced liver fibrogenesis.

A noteworthy finding of our study is the strong relationship between prothrombotic and inflammatory markers, irrespective of liver fibrosis severity, which could amplify the fibrotic potential. Abundant evidence suggests that BT-related inflammatory responses may upregulate coagulation within the cirrhotic liver. Violi *et al* confirmed a link between endotoxemia and thrombin generation markers in the portal circulation of patients with cirrhosis [40]. Furthermore, LPS has been shown to stimulate LSECs to secrete FVIII [41] and vWf-Ag [41,42,43]. Proinflammatory cytokines, such as TNF-α and IL-6, may also trigger the release of vWf-Ag from activated LSECs [43,44], inhibit ADAMTS13, a protease that degrades VWf multimers into less thrombogenic fragments [45], and reduce the hepatic production of PC [46]. Conversely, a marked decrease in vWf-Ag levels, concomitantly with amelioration of endotoxemia, was noted in patients with cirrhosis after treatment with non-absorbable antibiotics [42].

A main limitation of our study is that the procoagulant imbalance was assessed using the FVIII-to-PC ratio, since data regarding thrombin generation were not accessible. However, a good correlation between these parameters has been noted in patients with cirrhosis [47]. The lack of objective evidence concerning the association of hypercoagulability markers with increased intrahepatic thrombotic potential poses an additional limitation. In addition, LSM was obtained by 2D-SWE instead of transient elastography, which was utilized in the Baveno VII guidelines. Nonetheless, the overall accuracy and prognostic value of 2D-SWE are comparable to those of transient elastography, and appear to be even superior when assessing the early stages of liver fibrosis [48]. We also have to acknowledge that our findings cannot prove that systemic inflammation and coagulation pathways directly cause HSCs activation.

In conclusion, this study suggests that the interplay between thrombogenic and systemic inflammatory mechanisms may contribute to the severity of liver fibrosis in patients with cirrhosis. Our findings warrant the design of interventional clinical trials assessing whether therapeutic approaches that modulate thrombotic potential and systemic inflammation could ameliorate liver fibrogenic activity in these patients.

Summary Box

What is already known:

- Liver fibrosis is an important pathophysiological feature and a major prognostic indicator of advanced chronic liver disease
- A prothrombotic state has frequently been reported in patients with cirrhosis
- Increased procoagulant activity may contribute to the development and progression of liver fibrosis
- Experimental evidence has demonstrated that systemic inflammation could promote liver fibrogenesis via activation of hepatic stellate cells

What the new findings are:

- Advanced liver fibrosis, indicated by a liver stiffness measurement ≥25 kPa assessed by shear wave elastography, is strongly linked to higher prothrombotic and systemic inflammatory activities, regardless of the severity of cirrhosis
- Prothrombotic activity is closely related to systemic inflammation, irrespective of the severity of liver fibrosis

References

1. Akkız H, Gieseler RK, Canbay A. Liver fibrosis: from basic science towards clinical progress, focusing on the central role of hepatic stellate cells. *Int J Mol Sci* 2024;**25**:7873.
2. Iwakiri Y, Trebicka J. Portal hypertension in cirrhosis: Pathophysiological mechanisms and therapy. *JHEP Rep* 2021;**3**:100316.
3. Rautou PE, Caldwell SH, Villa E. Bleeding and thrombotic complications in patients with cirrhosis: a state-of-the-art appraisal. *Clin Gastroenterol Hepatol* 2023;**21**:2110-2123.
4. Pradhan-Sundd T, Gudapati S, Kaminski TW, Ragni MV. Exploring the complex role of coagulation factor VIII in chronic liver disease. *Cell Mol Gastroenterol Hepatol* 2021;**12**:1061-1072.
5. Elhence A, Shalimar. Von Willebrand factor as a biomarker for liver disease - an update. *J Clin Exp Hepatol* 2023;**13**:1047-1060.
6. Tripodi A, Primignani M, Chantarangkul V, et al. An imbalance of pro- vs anti-coagulation factors in plasma from patients with cirrhosis. *Gastroenterology* 2009;**137**:2105-2111.
7. Lisman T, Bongers TN, Adelmeijer J, et al. Elevated levels of von Willebrand Factor in cirrhosis support platelet adhesion despite reduced functional capacity. *Hepatology* 2006;**44**:53-61.
8. Kalambokis GN, Oikonomou A, Christou L, et al. Von Willebrand factor and procoagulant imbalance predict outcome in patients with cirrhosis and thrombocytopenia. *J Hepatol* 2016;**65**:921-928.
9. Bitto N, Liguori E, La Mura V. Coagulation, microenvironment and liver fibrosis. *Cells* 2018;**7**:85.
10. Anstee QM, Dhar A, Thursz MR. The role of hypercoagulability in liver fibrogenesis. *Clin Res Hepatol Gastroenterol* 2011;**35**:526-533.
11. Hammerich L, Tacke F. Hepatic inflammatory responses in liver fibrosis. *Nat Rev Gastroenterol Hepatol* 2023;**20**:633-646.
12. de Zawadzki A, Leeming DJ, Sanyal AJ, et al. Hot and cold fibrosis: the role of serum biomarkers to assess immune mechanisms and ECM-cell interactions in human fibrosis. *J Hepatol* 2025;**83**:239-257.
13. Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol* 2014;**60**:197-209.
14. Yang L, Seki E. Toll-like receptors in liver fibrosis: cellular crosstalk and mechanisms. *Front Physiol* 2012;**3**:138.
15. Nakadate K, Saitoh H, Sakaguchi M, et al. Advances in understanding lipopolysaccharide-mediated hepatitis: mechanisms and pathological features. *Curr Issues Mol Biol* 2025;**47**:79.
16. Neuberger J, Patel J, Caldwell H, et al. Guidelines on the use of liver biopsy in clinical practice from the British Society of Gastroenterology, the Royal College of Radiologists and the Royal College of Pathology. *Gut* 2020;**69**:1382-1403.
17. Castera L, Rinella ME, Tsochatzis EA. Noninvasive assessment of liver fibrosis. *N Engl J Med* 2025;**393**:1715-1729.
18. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis - 2021 update. *J Hepatol* 2021;**75**:659-689.
19. Jeong JY, Cho YS, Sohn JH. Role of two-dimensional shear wave elastography in chronic liver diseases: A narrative review. *World J Gastroenterol* 2018;**24**:3849-3860.
20. de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C; Baveno VII Faculty. Baveno VII - renewing consensus in portal hypertension. *J Hepatol* 2022;**76**:959-974.
21. Opal SM, Scannon PJ, Vincent JL, et al. Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock. *J Infect Dis* 1999;**180**:1584-1589.
22. Airola C, Pallozzi M, Cerrito L, et al. Microvascular thrombosis and liver fibrosis progression: mechanisms and clinical applications. *Cells* 2023;**12**:1712.
23. Brodard J, Calzavarini S, Quarroz C, Berzigotti A, De Gottardi A, Angelillo-Scherrer A. Resistance to thrombomodulin correlates with liver stiffness in chronic liver disease a prospective single-center cohort study. *Thromb Res* 2021;**207**:40-49.
24. Mann KG, Orfeo T, Butenas S, Undas A, Brummel-Ziedins K. Blood coagulation dynamics in haemostasis. *Hamostaseologie* 2009;**29**:7-16.
25. Ryland JK, Lawrie AS, Mackie IJ, Machin SJ. Persistent high factor VIII activity leading to increased thrombin generation - a prospective cohort study. *Thromb Res* 2012;**129**:447-452.
26. Dahlbäck B. Progress in the understanding of the protein C anticoagulant pathway. *Int J Hematol* 2004;**79**:109-116.
27. Valenti L, Tripodi A, La Mura V, et al. Clinical and genetic determinants of the fatty liver-coagulation balance interplay in individuals with metabolic dysfunction. *JHEP Rep* 2022;**4**:100598.
28. Takaya H, Kawaratani H, Tsuji Y, et al. Von Willebrand factor is a useful biomarker for liver fibrosis and prediction of hepatocellular carcinoma development in patients with hepatitis B and C. *United European Gastroenterol J* 2018;**6**:1401-1409.
29. Zhang R, Huang X, Jiang Y, Wang J, Chen S. Effects of anticoagulants on experimental models of established chronic liver diseases: a systematic review and meta-analysis. *Can J Gastroenterol Hepatol* 2020;**2020**:8887574.
30. Puente Á, Turón F, Martínez J, et al. Rivaroxaban to prevent complications of portal hypertension in cirrhosis: The CIRROXABAN study. *J Hepatol* 2025;**83**:1069-1076.
31. Papatheodoridis GV, Papakonstantinou E, Andrioti E, et al. Thrombotic risk factors and extent of liver fibrosis in chronic viral hepatitis. *Gut* 2003;**52**:404-409.
32. Poujol-Robert A, Rosmorduc O, Serfaty L, Coulet F, Poupon R, Robert A. Genetic and acquired thrombotic factors in chronic hepatitis C. *Am J Gastroenterol* 2004;**99**:527-531.

33. Wright M, Goldin R, Hellier S, et al. Factor V Leiden polymorphism and the rate of fibrosis development in chronic hepatitis C virus infection. *Gut* 2003;**52**:1206-1210.
34. Pradere JP, Troeger JS, Dapito DH, Mencin AA, Schwabe RF. Toll-like receptor 4 and hepatic fibrogenesis. *Semin Liver Dis* 2010;**30**:232-244.
35. Simbrunner B, Villesen IF, Königshofer P, et al. Systemic inflammation is linked to liver fibrogenesis in patients with advanced chronic liver disease. *Liver Int* 2022;**42**:2501-2512.
36. Kerbert AJC, Gupta S, Alabsawy E, et al. Biomarkers of extracellular matrix formation are associated with acute-on-chronic liver failure. *JHEP Rep* 2021;**3**:100355.
37. Nishimura N, Kaji K, Nishimura N, et al. Rifaximin attenuates liver fibrosis and hepatocarcinogenesis in a rat MASH model by suppressing the gut-liver axis and epiregulin-IL-8-associated angiogenesis. *Int J Mol Sci* 2025;**26**:6710.
38. Qiu T, Zhu X, Wu J, Hong W, Hu W, Fang T. Mechanisms of rifaximin inhibition of hepatic fibrosis in mice with metabolic dysfunction associated steatohepatitis through the TLR4/NFκB pathway. *Sci Rep* 2025;**15**:9815.
39. Israelsen M, Madsen BS, Torp N, et al; MicrobLiver Consortia. Rifaximin-α for liver fibrosis in patients with alcohol-related liver disease (GALA-RIF): a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Gastroenterol Hepatol* 2023;**8**:523-532.
40. Violi F, Ferro D, Basili S, et al. Association between low-grade disseminated intravascular coagulation and endotoxemia in patients with liver cirrhosis. *Gastroenterology* 1995;**109**:531-539.
41. 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**:950-956.
42. Ferro D, Quintarelli C, Lattuada A, et al. High plasma levels of von Willebrand factor as a marker of endothelial perturbation in cirrhosis: relationship to endotoxemia. *Hepatology* 1996;**23**:1377-1383.
43. Mandorfer M, Schwabl P, Paternostro R, et al; Vienna Hepatic Hemodynamic Lab. Von Willebrand factor indicates bacterial translocation, inflammation, and procoagulant imbalance and predicts complications independently of portal hypertension severity. *Aliment Pharmacol Ther* 2018;**47**:980-988.
44. Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood* 2004;**104**:100-106.
45. Cao WJ, Niiya M, Zheng XW, Shang DZ, Zheng XL. Inflammatory cytokines inhibit ADAMTS13 synthesis in hepatic stellate cells and endothelial cells. *J Thromb Haemost* 2008;**6**:1233-1235.
46. Yamamoto K, Shimokawa T, Kojima T, Loskutoff DJ, Saito H. Regulation of murine protein C gene expression in vivo: effects of tumor necrosis factor-alpha, interleukin-1, and transforming growth factor-beta. *Thromb Haemost* 1999;**82**:1297-1301.
47. Tripodi A, Primignani M, Lemma L, Chantarangkul V, Mannucci PM. Evidence that low protein C contributes to the procoagulant imbalance in cirrhosis. *J Hepatol* 2013;**59**:265-270.
48. Lai JC, Liang LY, Wong GL. Noninvasive tests for liver fibrosis in 2024: are there different scales for different diseases? *Gastroenterol Rep (Oxf)* 2024;**12**:goae024.