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RESEARCH LETTER

Comparable hemostatic capacity of blood taken from the portal vein compared with systemic blood in patients with cirrhosis

Nontumoral portal vein thrombosis (PVT) is a common complication in patients with cirrhosis, with a incidence ranging from 5% to 26% in liver transplant candidates [1]. The effect of anticoagulation is heterogeneous and PVT progression despite anticoagulation occurs in approximately 9% to 14 % of cases [2,3]. To improve recanalization rates, better insight in the pathogenesis of PVT is required. There is increasing evidence that cirrhotic PVT is a very different disease than, eg, deep vein thrombosis (DVT) of the legs. First, whereas a DVT develops around venous valves, the portal vein (PV) lacks venous valves [4]. Second, whereas ABO blood type is an established risk factor for DVT, a very large study showed no relation between ABO blood type and presence of PVT in patients with cirrhosis [5]. In addition, some studies indicated that factor (F)V Leiden and prothrombin G20210A are not associated with PVT risk in patients with cirrhosis [5,6], and a prospective study showed that genetic and acquired hypercoagulability is not linked to PVT risk in these patients [7]. Third, a recent study using histology showed that the majority of PV thrombi lacked classical clot components. Instead, intimal hyperplasia appeared to be the hallmark of cirrhotic PVT [8]. Thus, the role of the hemostatic system in the pathogenesis of PVT has been questioned, and reduced portal flow appears to be the key mechanism underlying development of cirrhotic PVT [9]. However, increased levels of markers of coagulation activation and von Willebrand factor (VWF) have been shown in the PV relative to the systemic circulation [10–12]. This hypercoagulable state in the PV has been linked to endotoxemia [13]. In contrast, we have recently provided evidence that the PV in patients with cirrhosis is not a particularly hypercoagulable vascular bed [8]. Our study has been criticized [14] which is why we performed a new, prospective study with the aim to confirm and extend previous findings. We compared markers of inflammation, endothelial damage, as well as various hemostatic tests between samples taken from the PV and samples taken from the systemic circulation.

1 | STUDY POPULATION

Patients with cirrhosis with a clinical diagnosis of portal hypertension undergoing transjugular intrahepatic portosystemic shunt (TIPS) placement were prospectively included between January 2020 and June 2022. Criteria for TIPS placement include secondary prevention of variceal bleeding, refractory ascites, and recurrent symptomatic hydrothorax. Exclusion criteria were known hepatocellular carcinoma, pregnancy, previous liver transplantation, refusal to provide informed consent, use of antithrombotic treatment, thrombophilic disorders, PVT, and TIPS placement for acute bleeding. Patients were clinically stable at the time of TIPS. All patients gave written informed consent for participation in this study. Ethical approval was obtained from Hospital Clinic, Barcelona (HCB/2019/0761). The study was funded by Ministry of Science, Innovation, and Universities (PI19/00076). For each patient, baseline clinical and laboratory data were collected. Patients were followed during 6 months after TIPS placement. Eight milliliters of blood (4 mL for laboratory test and 4 mL for rotational thromboelastometry [ROTEM]) were taken from the systemic circulation (jugular vein) before the procedure of TIPS placement, and from the PV right after the puncture, and were collected into a citrate-containing tube (0.129 M, 3.8%; Vacutainer system, Becton Dickinson). After centrifugation at 3000 × *g* for 20 minutes at 4 °C, plasma was aliquoted and stored at −80 °C for subsequent analysis, whereas ROTEM tests were performed in whole fresh blood. The following assays were performed: 1) individual coagulation proteins (plasma levels of FII, FV, FVII, FX, FIX, FXI, FXII, FVIII, antithrombin, fibrinogen, free protein S, protein C, tissue plasminogen activator [tPA], plasminogen activator inhibitor 1, VWF, FXIII, tissue factor plasma inhibitor, thrombin activatable fibrinolysis inhibitor, and soluble tissue factor) [15–17]; 2) markers of inflammation (interleukin, lipopolysaccharide [LPS], malondialdehyde, cell-free DNA, and myeloperoxidase DNA) [18,19]; 3) markers of activation of coagulation (D-dimer and

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TABLE 1 Patient characteristics (*N* = 47).

Characteristics	Values
Age (y)	58 (49-65)
Female sex (%)	48
BMI (kg/m^2)	25 (23-27)
Etiology (%)	
HCV	60
OH	26
MASH	4
Child score	8 (7-10)
Child class (%)	
A	18
B	52
C	29
MELD	15 (11-20)
SOFA	4 (2-6)
CRP (mg/L)	1.5 (1.0-3.2)
Reason for TIPS (%)	
Refractory ascites	30
Pre-emptive TIPS	20
Prevention of rebleeding	35
Others	15
Hb (g/dL)	9 (8-10)
HVPG before TIPS (mmHg)	16 (14-19)
GPP after TIPS (mmHg)	9 (8-11)
Platelets (×10^9/L)	90 (69-117.5)
Leucocytes (× 10^9/L)	5.5 (3.7-7.5)
Neutrophils (×10^9/L)	76 (69-83)
Bilirubin (mg/dL)	2 (1-6)
Albumin (g/L)	30 (26-34)
INR	1.3 (1.2-1.5)
AST (IU/L)	59 (32-113)
ALT (IU/L)	34 (23-72)
ALP (IUI/L)	116 (79-167)
GGT (IU/L)	77 (44-137)
6-mo follow-up (after TIPS)	
Thrombosis, *n*	1
Previous PVT, *n*	0
Bleeding history, n	2
PHT-related, *n*	1
PHT-unrelated, *n*	1
Sepsis, *n*	2
Survival, *n*	44

Data are given in median and IQR, unless stated otherwise.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; GGT, gamma-glutamyl transpeptidase, sex, either of the two categories (male and female); GPP, gradient portal presure; Hb, hemoglobin; HCV, hepatitis C virus, HVPG, hepatic venous pressure gradient; INR, international normalized ratio; MASH, metabolic dysfunction-associated steatohepatitis; MELD, model for end stage liver disease; OH, alcohol; PHT, portal hypertension; PVT, portal vein thrombosis; SOFA , secuential organ failure assessemenr score; TIPS, transjugular intrahepatic portosystemic shunt.

thrombin-antithrombin [TAT] complexes [18]; 4) global tests (clot lysis time [20] and thrombomodulin-modified thrombin generation [21]); 5) viscoelastic assays with ROTEM device (EXTEM, INTEM, and HEPTEM test) [22]; and 6) prothrombin time, activated partial thromboplastin time, and platelet count [23].

2 | STATISTICAL ANALYSIS

Data are presented as median with IQR or numbers and percentage for continuous or categorical variables. Statistical analysis was performed with IBM SPPS 23.0 (Statistical Package for the Social Sciences) GraphPad Prism 8 with a 2-sided significance level of .05. The matched samples Wilcoxon test was used for comparison between the vascular beds and the Mann–Whitney U-test was used for comparisons between subgroups of patients. Given the multiple comparisons carried out, we performed an adjustment with the Benjamini–Hochberg procedure setting the false discovery rate at a maximum of 0.05.

This study included 47 adult (≥18 years) patients with cirrhosis who underwent TIPS placement. The median age was 58 (49-65) years and 23 (48%) were female. The majority of patients had moderate liver disease (Child class A, 9 [18%]; Child class B, 24 [52%]; Child class C, 14 [30%]), and hepatic venous pressure gradient >12 mmHg. Patient characteristics are summarized in Table 1. With the exception of tPA that was clearly higher in the portal circulation, levels of all proteins assessed were similar in the PV and systemic circulation (<10% difference between levels in the portal and systemic circulation; Table 2). Similarly, plasma levels of markers of inflammation and oxidative stress were similar in samples taken from the PV and the systemic circulation, except for LPS that was somewhat higher in the portal circulation. Markers of coagulation activation were identical (D-dimer) or lower (TAT complexes) in the portal circulation. Finally, global tests of hemostasis (thrombin generation, plasma-based clot lysis times, ROTEM), and routine

TABLE 2 Levels of individual proteins, markers of inflammation, global assays, and standard coagulation test, measured in plasma samples from the systemic circulation and portal vein in patients with cirrhosis.

N = 47	Systemic[a]	Portal[b]	*P*
Individual coagulation proteins			
FII (%)	42 (33-56)	44 (35-56)	.01
FV (%)	40 (28-55)	41 (29-53)	.04
FVII (%)	40 (23-53)	41 (23-56)	<.01
FX (%)	51 (37-61)	55 (40-64)	<.01
FIX (%)	57 (45-74)	60 (47-69)	.04
FXI (%)	40 (29-68)	41 (28-64)	.25
FXII (%)	69 (52-88)	70 (50-94)	.63
FVIII (%)	164 (126-233)	169 (123-262)	.45
AT (%)	49 (32-71)	51 (35-69)	.60
Fibrinogen (mg/mL)	2.18 (1.56-3.06)	2.20 (1.48-3.25)	.15
Free protein S (%)	67 (55-77)	65 (55-80)	.22
Protein C (%)	40 (24-60)	40 (25-59)	.44
VWF (%)	403 (306-533)	384 (310-531)	.39
FXIII (%)	37 (30-51)	38 (28-47)	.23
TFPI (pg/mL)	59 (44-83)	55 (41-81)	.25
TAFI (%)	41 (35-66)	44 (35-62)	.64
tPA (ng/mL)	12.7 (7.5-27.1)	15.4 (8.2-29.7)	<.01
PAI-1 (ng/mL)	2.65 (0.69-6.71)	2.68 (0.78-6.34)	.91
sTF (ng/mL)	0 (0-12.33)	0 (0-11.85)	.39
Markers of inflammation			
IL-6 (ng/mL)	10.9 (6.4 -29.6)	12.3 (6.1 -30.8)	.89
LPS (pg/mL)	156 (107-311)	176 (116-330)	.04
MDA (mm/L)	3.80 (2.43-7.30)	4.16 (2.75-7.39)	.23
Cell-free DNA (μg/mL)	0.93 (0.81-1.16)	0.96 (0.79-1.16)	.43
MPO-DNA (AU)	0.1830 (0.1150-0.3450)	0.1830 (0.1280-0.4480)	.18
Markers of coagulation activation			
D-dimer (μg/mL)	3.8 (2.7-12.1)	3.9 (2.6-9.5)	.47
TAT (%)	45 (25-102)	35 (22-67)	.02
Global test			
CLT min	46 (33-66)	49 (36-81)	.02
Thrombin generation assay			
Lag time (min)	1.67 (1.67-2.33)	2.00 (1.67-2.33)	.11
ETP (nM IIa · min)	605 (517-709)	633 (544-722)	.13
Peak (nM)	149 (110-168)	153 (123-180)	.23
Velindex (nM/min)	75 (57-101)	83 (59-104)	.56

(Continues)

TABLE 2 (Continued)

N = 47	Systemic[a]	Portal[b]	*P*
Viscoelastic assay			
CT EXTEM (s)	68 (62-74)	73 (65-79)	.07
CFT EXTEM (s)	95 (69-150)	90 (67-146)	.33
MCF EXTEM (mm)	53 (47-59)	53 (47-58)	.97
CT INTEM (s)	165 (153-182)	164 (142-193)	.79
CFT INTEM (s)	105 (74-160)	100 (72-158)	.24
MCF INTEM (mm)	50 (46-57)	50 (45-57)	.44
CT HEPTEM (s)	164 (145-179)	163 (139-188)	.93
CFT HEPTEM (s)	104 (79-169)	113 (76-168)	.43
FIBTEM (mm)	14 (11-21)	14 (11-19)	.18
Standard coagulation test			
PT (%)	56 (44-65)	57 (41-66)	.38
aPTT (s)	30 (28-38)	30 (25-37)	.80
Platelets ($\times 10^9$/L)	90 (69-117)	74 (44-91)	.84

[a]Wilcoxon test.

[b]Mann–Whitney U-test.Data are given in median and IQR. The results are presented as median(IQR) and *n* (%) for continuous and categorical variables of available data. Assuming a false discovery rate of 5% or less, only contrasts with a *P* value lower than .003 were considered significant after a multiple-comparison adjustment.

aPTT, activated partial thromboplastin time; AT, antithrombin; CFT, clot formation time; CLT, clot lysis time; CT, clotting time; ETP, endogenous thrombin potential; F, factor; IL-6, interleukin-6; LPS, lipopolysaccharide; MCF, maximum clot firmness; MDA, malondialdehyde; MPO-DNA, complexes of myeloperoxidase and DNA; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin time; sTF, soluble tissue factor; TAFI, thrombin activatable fibrinolysis inhibitor; TAT, thrombin-antithrombin; TFPI, tissue factor plasma inhibitor; tPA, tissue plasminogen activator; VWF, von Willebrand factor.

hemostasis tests were similar between the portal and systemic circulation (Table 2). In the following 6 months, 1 patient experienced thrombosis of the TIPS, and 1 patient with dysfunctional TIPS experienced upper gastrointestinal bleeding. Two patients had sepsis no related to TIPS.

In this study, we confirmed and extended our previous findings on the hemostatic status of the PV in patients with cirrhosis [8]. Using an extensive panel of tests including levels of individual coagulation proteins, markers of activation of coagulation, markers of inflammation, and functional tests of coagulation and fibrinolytic activity, we demonstrate a remarkably similar hemostatic profile in the PV compared to the systemic circulation. These results align with the concept that PVT is not driven by (local) hypercoagulability, but rather by changes in the PV wall that are likely driven by portal hypertension [7,8]. Even though we have demonstrated very little differences in the hemostatic status between the systemic and portal circulation in 2 independent studies of approximately 50 patients each, others have reported clear difference in selected markers [11,13,24,25]. Differences in etiology of disease, clinical status, blood sampling techniques or the timing during the TIPS procedure, may explain this finding. Importantly, in previous and in the present study we demonstrate that our conclusions remain consistent when analyzing patients with better or worse clinical status separately [8]. In the present study, the hemostatic status was similar between the portal and systemic circulation in patients with model for end stage liver disease scores <15 and >15 separately, in patients with hepatic venous pressure gradient <17 or >17, and in patients who received a TIPS prophylactically, (Supplementary Tables S1 and S2). Although most analytes had a <10% difference between the PV and systemic circulation, levels of LPS and tPA are clearly higher in the PV, as it has been reported previously [11,13,24]. Elevated LPS levels are likely explained by the "leaky gut" that is well-described in patients with cirrhosis. Previously, LPS has been shown to lead to endothelial cell activation [22]. However, in our study, levels of VWF were not different between the PV and systemic circulation, which suggests that the net effect of PV endotoxemia on portal hemostasis is clinically irrelevant. Whereas our previous study found somewhat elevated levels of TAT and D-dimer in the PV compared to the systemic circulation, the present study found no difference in D-dimer, and lower TAT levels in the PV, which may be explained by differences in patient characteristics.

Our results argue against the PV as a hypercoagulable or inflammatory vascular bed, in basal conditions. These results deviate from data published by others but are in line with a previous study from our group. However, given the wealth of evidence that coagulation activation is not central to the pathogenesis of PVT, we argue against the concept of PV hypercoagulability as the driver of PVT.

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AUTHOR CONTRIBUTIONS

All authors contributed to the work. A.B. designed the study, analyzed data, and wrote the manuscript. R.M. analyzed data and approved the final manuscript. M.A.T., A.C., F.T., J.C.G.-P., V.H.-G., and D.T. performed cases, collected cases, and approved the final manuscript. J.C.R. and T.L. designed study and collaborated in writing the manuscript.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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SUPPLEMENTARY MATERIAL

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