Reversal of hypercoagulability in patients with HCV-related cirrhosis after treatment with direct-acting antivirals

Russo, Francesco Paolo; Zanetto, Alberto; Campello, Elena; Bulato, Cristiana; Shalaby, Sarah; Spiezia, Luca; Gavasso, Sabrina; Franceschet, Enrica; Radu, Claudia; Senzolo, Marco

Published in:
Liver International

DOI:
10.1111/liv.13873

Publication date:
2018

Received: 21 February 2018 | Accepted: 20 April 2018

DOI: 10.1111/liv.13873

WILEY Liver INTERNATIONAL

Reversal of hypercoagulability in patients with HCV-related cirrhosis after treatment with direct-acting antivirals

Francesco Paolo Russo[1] | Alberto Zanetto[1] | Elena Campello[2] | Cristiana Bulato[2] | Sarah Shalaby[1] | Luca Spiezia[2] | Sabrina Gavasso[2] | Enrica Franceschet[1] | Claudia Radu[2] | Marco Senzolo[1] | Patrizia Burra[1] | Ton Lisman[3] | Paolo Simioni[2]

[1]Gastroenterology and Multivisceral Transplant Unit, Department of Surgery, Oncology and Gastroenterology, Padua University Hospital, Padua, Italy

[2]Thrombotic and Haemorrhagic Diseases Unit, Department of Medicine, Padua University Hospital, Padua, Italy

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

Correspondence
Paolo Simioni, Thrombosis and Haemorrhagic Diseases Unit, Department of Medicine, Padua University Hospital, Padua, Italy.
Email: paolo.simioni@unipd.it

Funding information
The study was supported by Grant from the MIUR (Italian Ministry of Education, University and Research) and DIMED (Department of Medicine, Padua University Hospital) to PS.

Handling Editor: Alejandro Forner

Abstract

Background & Aims: The long-term impact of sustained virological response (SVR) after direct-acting antivirals (DAAs) on the hypercoagulability associated with HCV cirrhosis is unknown. We longitudinally evaluated the effect of DAAs treatment on cirrhotic coagulopathy.

Methods: Pro- and anticoagulant factor levels and thrombin generation were assessed in patients with HCV-related cirrhosis at baseline, end of therapy (EOT), at 12, 24 and 48 weeks (W) after EOT.

Results: Fifty-eight patients were enrolled (86% Child's A). SVR was 100%. Median factor VIII activity significantly decreased at EOT, 12 weeks and 24 weeks compared with baseline, whereas protein C significantly increased at 24 weeks and 48 weeks. Cirrhotic patients showed a slight but sustained increase in endogenous thrombin potential (ETP) with a statistically significant difference at EOT, 12 weeks, 24 weeks and 48 weeks compared with baseline. Conversely, thrombomodulin-modified ETP was elevated before treatment and decreased over time to normal levels at 24 weeks and 48 weeks. The ETP ratio decreased slowly at EOT and 12 weeks, and was significantly decreased at 24 weeks and 48 weeks compared with baseline ($P < .001$ for both comparisons), being not statistically different from ETP ratio measured in healthy controls. Child's B patients showed a significantly higher ETP ratio compared to Child's A at baseline and did not show any significant improvement in ETP ratio through 12 weeks. Two Child's B patients developed PVT with an incidence rate of 1.1% p-yrs (95%CI, 0.18 to 3.58).

Conclusions: DAAs therapy in HCV-related cirrhotic patients is associated with significant changes in thrombin generation suggesting a reversal of hypercoagulability particularly in Child's A patients.

KEYWORDS
hypercoagulability, cirrhosis, portal vein thrombosis, thrombin generation

Abbreviations: DAAs, direct-acting antiviral agents; EOT, end of treatment; ETP, endogenous thrombin potential; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PPP, platelet-poor plasma; PVT, portal vein thrombosis; RBV, Ribavirin; SOF, Sofosbuvir; SVR, sustained virological response; TGA, thrombin generation assays; TM, thrombomodulin.

Francesco Paolo Russo and Alberto Zanetto equally contributed to the manuscript.

1 | INTRODUCTION

Cirrhosis is characterized by multiple and complex haemostatic alterations that can predispose to both bleeding and thrombotic complications.[1-3] Thrombin generation assay (TGA), when performed in the presence of thrombomodulin (TM), the cofactor in the thrombin-induced activation of the anticoagulant protein C pathway, is increasingly recognized as a valuable tool to assess the haemostatic balance in platelet-poor plasma, as it measures all pro- and anticoagulant protein levels. In patients with complex haemostatic disorders, results from TM-modified TGA therefore sharply contrast with routine coagulation tests such as prothrombin time which is sensitive to procoagulant proteins only.[4] Early studies using TM-modified TGA initially appeared to indicate that the thrombin generating capacity of patients with cirrhosis was comparable to that of healthy controls.[5] In subsequent studies, however, multiple groups demonstrated increased thrombin generation in patients compared with controls using TM-modified TGA.[6-10] The hypercoagulable state of patients with cirrhosis relates, at least in part, to elevated plasma levels of factor VIII and decreased protein C[11,12] which leads to an intrinsic resistance to the anticoagulant effect of TM in cirrhotic patients.[2]

Whether this in vitro hypercoagulability detected by TGA is truly representative of what occurs in vivo has not been clearly demonstrated yet, but it could explain the higher risk of portal vein thrombosis (PVT) observed in cirrhotic patients.[13,14]

The recent introduction of direct-acting antiviral agents (DAAs) has dramatically changed the management of hepatitis C (HCV) infection.[15] Notably, sustained virological response (SVR) has been linked to favourable outcomes such as regression of fibrosis, amelioration of liver synthesis and reduction in portal pressure.[16] Additionally, the improvement in liver function by treating HCV-related cirrhosis patients with DAAs could also theoretically revert their coagulopathy. A recent study by Tripodi et al[17] showed that the treatment with DAAs resulted in an improvement of the individual pro- and anticoagulant factors and the authors hypothesized that this improvement would stabilize haemostatic balance, leading to a reduction in bleeding and thrombotic complications.

In this study, we prospectively evaluated the long-term effect of HCV clearance by DAAs on the coagulopathy in a group of HCV-related cirrhotic patients by standard coagulation tests as well as TGA. In addition, the incidence rate of PVT was evaluated during a follow-up period of 1 year after cessation of DAAs treatment.

Key points

- Direct-acting antivirals (DAAs) therapy improved liver synthesis of pro- and anticoagulant factors and reduced systemic inflammation.
- Coagulation factors liver synthesis improvement occurred soon after the end of DAAs treatment.
- A late complete reversal of HCV-related cirrhotic hypercoagulability was highlighted 24 and 48 weeks after the end of treatment by thrombomodulin-modified thrombin generation.
- The correction of coagulopathy in Child's B did not seem to occur as early as in Child's A.

2 | MATERIALS AND METHODS

2.1 | Study cohort

All consecutive HCV-related cirrhosis patients who were referred to the Multivisceral Transplant Unit and to the Gastroenterology Unit of Padua University Hospital for anti-HCV treatment with DAAs from September 2015 to March 2016 were enrolled. The diagnosis of cirrhosis was based on clinical, laboratory and imaging data as established by the European Association for the Study of the Liver (EASL) Guidelines.[18,19]

The exclusion criteria were hepatocellular carcinoma (HCC), extrahepatic neoplastic disease, haematological diseases, ongoing anticoagulant or antiplatelet treatments, history of venous thromboembolism and/or PVT, active infection, recent (3 months) or active variceal bleeding and HBV and/or HIV co-infection.

Antiviral therapy was administered following the EASL Guidelines for HCV treatment[20,21] and therapeutic regimens were as follows: Sofosbuvir (SOF) + Ribavirin (RBV), SOF + Ledipasvir ± RBV, SOF + Daclatasvir ± RBV, Ombitasvir + Paritaprevir + Ritonavir + D asabuvir (3D) ± RBV.

Blood samples for virological (HCV-RNA values), biochemical and coagulation assessments were collected at baseline, at the end of treatment (EOT), 12-weeks (12 wk), 24-weeks (24 wk), and 48 weeks after EOT (48 wk). Liver fibrosis was non-invasively assessed by Transient Elastography (TE, FibroScan). TE was performed before antiviral therapy, 24 and 48 weeks after the EOT.

The study was conducted in compliance with the Declaration of Helsinki and all patients gave written consent before enrolment. The Padua University Hospital Ethical Committee approved the study (3103/A0/14).

2.2 | Biochemical and virological markers

Liver panel tests included aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), serum albumin and serum bilirubin. Successful achievement of sustained virological response 12 weeks after the end of therapy (SVR12) was defined as serum HCV-RNA levels below the lower limit of quantification (LLOQ, 15 IU/mL).[21]

2.3 | Blood sampling

Nine millilitre of venous blood were drawn from the antecubital vein with a light tourniquet, using a butterfly device with 21-gauge

TABLE 1 Baseline characteristics of the study cohort

	Patients (n = 58)
Age, years	59 (41-66)
Sex male/female, n	38/20
Child Class A/B, n	50/8
Previous decompensation, n (%)	7 (12%)[a]
Presence of oesophageal varices, n (%)	14 (24%)[b]
MELD	9 (6-14)
INR	1 (0.9-1.1)
Creatinine, mg/dL (n.v. 0.6-1.2)	0.8 (0.7-0.9)
Total serum bilirubin, mg/dL (n.v. 0.2-1.1)	0.8 (0.7-1.4)
Platelet count, 10^3/UL (n.v. 150-450)	137 (125-158)
AST, UI/L (n.v. 10-45)	75 (54-110)
ALT, UI/L (n.v. 10-50)	72 (42-100)
GGT, UI/L (n.v. 3-65)	81 (50-131)
ALP, UI/L (n.v. 56-128)	104 (82-103)
αFP, μg/L (n.v. 0-8.8 μg/L)	5 (2-8)
HCV genotype 1/2/3/4 (%)	69/12/10/9
Baseline HCV-RNA, UI/mL	1 256 000 (754 000-1 540 000)
Previous antiviral therapy, n (%)	49 (84%)
Antiviral therapeutic regimens, n (%)	
SOF + RBV	12 (20%)
SOF + LED ± RBV	6 (11%)
SOF + DAC ± RBV	10 (17%)
3D	30 (52%)
SVR12, n (%)	58 (100%)

Each value is expressed as median (interquartile range).
3D, ombitasvir, paritaprevir, dasabuvir; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DAC, daclatasvir; GGT, gammaglutamil transpeptidase; INR, international normalized ratio; LED, ledipasvir; MELD, model for end stage liver disease; n.v., normal values; RBV, ribavirin; SOF, sofosbuvir; SVR12, sustained virological response 12 wk after end of therapy; αFP, alfafetoprotein.
[a]All Child's B patients; none of the Child's A patients had previously experienced a decompensation episode.
[b]Six (43%) of them were Child's B patients.

needle. Blood was collected directly into 3.2% sodium citrate (9/1, v/v) vacuum tubes. The first few millilitres were discarded. Platelet-poor plasma (PPP) was prepared within 1 hour by double centrifugation (2 × 10 minutes at 1500 *g*) at room temperature. Aliquots (1.5 mL) were immediately frozen and then stored at −80°C until use.

2.4 | Coagulation parameters

Prothrombin time (PT, normal values [n.v.] 70%-100%), factor VIII (n.v. 60%-160%) and factor II activities (n.v. 80%-120%), antithrombin (n.v. 80%-120%), protein C chromogenic (n.v. 70%-130%) and coagulometric (n.v. 80%-120%) activities and protein S coagulometric activity (n.v. 70%-130%) were measured as previously described.[22,23]

Reference values were obtained from 100 healthy volunteers of both genders, age 20-70 years. Pregnant women, women on oral contraceptives and subjects taking any medication were excluded. Values were normally distributed and reference ranges were calculated as mean ± 2 standard deviations.

2.5 | Thrombin generation assay (TGA)

TGA was determined in PPP with the calibrated automated thrombogram method (Thrombinoscope BV, Maastricht, the Netherlands), as previously described.[24,25] Briefly, 80 μL of PPP were dispensed into the wells of a 96-well microtitre plate and coagulation was triggered with 20 μL of PPP-Reagent Low (Thrombinoscope BV), a mixture of TF (1 pmol/L final concentration) and synthetic phospholipids (4 μmol/L final concentration). The reaction was initiated by adding 20 μL of a mixture composed of a thrombin fluorogenic substrate and CaCl2 (FluCa-Kit, Thrombinoscope BV). Thrombin calibrator (Thrombinoscope BV) was used to correct each curve for inner-filter effects and substrate consumption. TGA was run in parallel with the addition of 1.5 nmol/L rabbit thrombomodulin (TM; Sekisui Diagnostics, Stamford, CT, USA). The concentration of TM was chosen to reduce the endogenous thrombin potential (ETP) value by approximately 75% in normal pool plasma (resulting in an ETP ratio of approximately 0.25). Plasma from 25 normal healthy subjects was also tested to evaluate the effect of TM on ETP and acted as control group for TGA. This control group consisted of 14 males and 11 females, median age 54 [39-58] years, without a history of cardiovascular, autoimmune and acute diseases and not taking antithrombotic, antibiotic or hormonal therapy. Healthy controls were enrolled simultaneously with the cirrhotic patients, in the same ambulatory, among volunteer non-relative companions of the patients. Fluorescence was read in a Fluoroscan Ascent® reader (Thermo Labsystems, Helsinki, Finland) and TGA curves were calculated using the Thrombinoscope Software version 5.0.0.742 (Thrombinoscope BV). All tests were performed in duplicate.

TGA parameters considered were lag-time, peak height and ETP. The ETP ratio was calculated by dividing "ETP with TM" by "ETP without TM"; the ETP ratio reflects the "resistance" to the effect of protein C after activation by TM. The ETP reduction after the addition of TM was also expressed as a percentage [(ETP)−(ETP + TM)] / (ETP)%.

2.6 | Follow-up and diagnosis of portal vein thrombosis

After the EOT, each patient was followed up to 48 weeks at our outpatient clinic. Liver ultrasound was performed every 6 months to exclude the presence of asymptomatic PVT or earlier in the presence of any clinical symptom. Additionally, if a patient was admitted to the hospital for a complication of cirrhosis, diagnostic tests for PVT

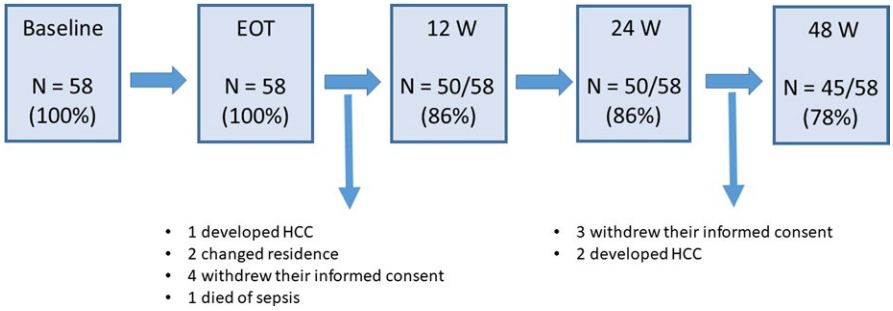

FIGURE 1 Flow chart of patients' enrolment. DAAs, direct-acting antivirals; EOT, end of therapy; HCC, hepatocellular carcinoma; W, weeks

were routinely performed. Subsequently, in the presence of either a positive Doppler ultrasound or an uncertain test for PVT, angio-computed tomography scan or angio-magnetic resonance imaging of the abdomen was performed.[26]

2.7 | Statistical analysis

Qualitative data were described as frequencies, while quantitative as median and interquartile range (IQR). Quantitative variables were compared using Kruskal-Wallis test with Dunn's correction for multiple comparisons among different groups. Shapiro-Wilk Test was used to compare continuous variables. Follow-up duration and time to PVT development were expressed as median (IQR). Time to PVT was calculated from the EOT until the time of objectively documented thrombosis. The incidence rate of PVT (95% CI) was calculated. Statistical significance was set at $P < .05$ (SPSS version 22.0.0, Chicago, IL, USA).

3 | RESULTS

3.1 | Study cohort

Out of 86 eligible patients with HCV cirrhosis, 58 were enrolled in the study (M/F 38/20, mean age 59 years). Patients with ongoing anticoagulant therapy (n.7), HCC (n.15), previous PVT (n.4) and concurrent infection (n.2) were excluded. Detailed clinical characteristics of the study cohort are presented in Table 1. Among the enrolled patients, 50 (86%) were classified as Child's A and the remaining 8 (14%) as Child's B. No Child's C patients were included. Seven (12%) patients experienced previous liver decompensation and were all Child's B. Moreover, 14 (6 Child's B) patients presented with small (F1-F2) oesophageal varices. Genotype 1 was the most common (40/58, 69%) and antiviral therapy was mainly based on "3D" combo (30/58, 52%). Eighty-four percent of patients had previously received PEG-interferon-based antiviral therapy.

TABLE 2 Liver function tests, HCV-RNA levels and transient elastography during and after DAAs treatment

	At baseline (n = 58)	EOT (n = 58)	12 wk (n = 50)	24 wk (n = 50)	48 wk (n = 45)
AST, UI/L (n.v. 10-45)	75 (54-110)	29*** (21-33)	28*** (20-35)	30*** (20-44)	27*** (20-41)
ALT, UI/L (n.v. 10-50)	72 (42-100)	22*** (13-31)	26*** (15-32)	26*** (15-30)	25*** (15-28)
GGT, UI/L (n.v. 3-65)	81 (50-131)	26*** (20-39)	40*** (16-58)	34*** (15-41)	33*** (15-40)
ALP, UI/L (n.v. 56-128)	104 (82-130)	93* (77-197)	97* (71-112)	92* (80-98)	94* (81-96)
Total bilirubin, mg/dL (n.v. 0.2-1.1)	1 (0.7-1.4)	0.7* (0.5-1.1)	0.6** (0.4-0.9)	0.7* (0.4-1.1)	0.6* (0.4-1.1)
Serum Albumin, g/dL (n.v. 38-44)	39 (37-42)	41 (38-43)	42* (40-44)	42* (39-45)	44* (40-46)
Presence of ascites[a], n(%)	6 (10%)	6 (10%)	4 (8%)	4 (8%)	3 (7%)
Presence of hepatic encephalopathy[b], n(%)	6 (10%)	6 (10%)	4 (8%)	4 (8%)	3 (7%)
Child class, A/B	50/8	50/8	43/7	43/7	42/3
HCV-RNA, UI/mL	1 256 000 (754 000-1 540 000)	<12***	Undetectable	Undetectable	Undetectable
FibroScan, kPA[c]	20.9 [12-66]	17 [8-65]*	15 [5-66]**	14.5 [4-69]**	14 [5-66]**

Each value is expressed as median (interquartile range). *P* values are calculated versus baseline values: *<.05, **<.01, ***<.001.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; EOT, end of treatment; GGT, gammaglutamil transpeptidase; n.v., normal values; W, weeks.
[a]Ascites in Child B patients was "diuretic responsive" in all the cases.
[b]Hepatic encephalopathy in Child B patients was "mild to moderate" in all the cases.
[c]The cut-off between F3 and F4 (cirrhosis) was 12.5 kPA.

TABLE 3 Longitudinal trend of INR, platelet count and levels of pro- and anticoagulant factors in the study population

	At baseline (n = 58)	EOT (n = 58)	12 wk (n = 50)	24 wk (n = 50)	48 wk (n = 45)
INR (n.v. 0.8-1.2)	1.15 [0.90-1.18]	1.10 [0.90-1.20]	1.12 [0.84-1.18]	1.10 [0.90-1.20]	1.12 [0.90-1.18]
Platelet count, 10^3/μL (n.v. 150-450)	137 [82-152]	153** [90-187]	169** [94-162]	146 [100-209]	140 [98-188]
Factor VIII, % (n.v. 60-160)	187 [146-223]	154** [123-183]	156* [113-182]	140** [120-175]	182 [147-234]
Factor II, % (n.v. 80-120)	76 [70-102]	90* [80-114]	99 [66-121]	112*** [99-121]	126*** [98-142]
Antithrombin act, % (n.v. 80-120)	82 [64-92]	87 [70-93]	86 [68-94]	91** [81-100]	91* [82-97]
Protein C chromogenic act, % (n.v. 70-130)	71 [53-88]	81 [65-94]	84 [61-97]	93*** [75-103]	87*** [77-103]
Protein C coagulometric act, % (n.v. 80-120)	65 [45-79]	75 [60-87]	76 [58-90]	84*** [72-96]	78*** [70-106]
Protein S coagulometric act, % (n.v. 70-130)	79 [63-93]	84 [69-102]	87 [78-112]	92** [79-110]	91** [83-115]

Data are expressed by median and interquartile range. *P* values are calculated versus baseline values: *<.05, **<.01, ***<.001.
12 wk, 12 weeks after EOT; 24 wk, 24 weeks after EOT; 48 wk, 48 weeks after EOT; act, activity; EOT, end of treatment; n.v., normal values.

Eight patients dropped out before the 12-week follow-up timepoint (1 patient developed HCC, 2 patients changed residence, 4 patients withdrew their informed consent, 1 patient died of sepsis) and 5 patients after 24 weeks (3 withdrew their informed consent, 2 developed HCC). Overall, follow-up was completed for 45 out of 58 patients (78%) (Figure 1).

3.2 | Biochemical and virological markers

Transaminases and bilirubin levels significantly decreased at EOT and throughout the follow-up compared to baseline. Serum albumin significantly increased at 12 weeks and throughout the follow-up compared to baseline (Table 2). Child classification statuses remained unchanged for all patients throughout the follow-up. HCV-RNA was undetectable in all patients at EOT. Sustained virological response was confirmed 12 weeks, 24 weeks and 48 weeks post treatment with 100% (58/58) rate of SVR12, SVR24 and SVR48. No late relapses were detected. At SVR24, TE values significantly decreased compared to baseline (20.9 [12-66] vs 14.5 [4-69] kPa; $P < .001$), and remained stable at SVR48 ($P = .8$) (Table 2).

3.3 | Coagulation tests

PT-INR values remained unchanged throughout the follow-up. A slight increase in platelet count was observed at EOT and 12 weeks compared to baseline ($P < .001$ for both comparisons). However, platelet count reverted nearly to baseline levels at 24 weeks and 48 weeks (Table 3).

A progressive quantitative improvement of pro- and anticoagulant factor levels was observed after treatment. Particularly, median factor VIII activity was 187% (146-223) at baseline and significantly decreased at EOT ($P < .01$), 12 weeks ($P < .05$) and 24 weeks ($P < .01$). However, 48 weeks after EOT, FVIII activity reverted nearly to baseline values. We observed progressively higher median activity of factor II at EOT ($P < .05$) as well as at 24 weeks ($P < .001$) and 48 weeks ($P < .001$), compared to baseline. Levels of antithrombin significantly increased at 24 weeks and 48 weeks ($P < .01$ and <.5 respectively). Finally, protein C (both chromogenic and coagulometric activities) and protein S significantly increased at 24 weeks and 48 weeks (Table 3).

3.4 | Thrombin generation assay

Thrombin generation data are shown in Figure 2. Endogenous thrombin potential (ETP) without thrombomodulin was slightly lower in patients at baseline compared to healthy controls ($P < .05$). In contrast, ETP in the presence of thrombomodulin was substantially higher in patients than in healthy controls at baseline ($P < .001$). Cirrhotic patients showed a slight but sustained increase in ETP without TM with a statistically significant difference at EOT, 12 weeks, 24 weeks and 48 weeks compared to baseline. In particular, ETP at EOT was similar to that measured in healthy controls (1168 [1007-1269] vs 1092 [973-1242], respectively, $P = .54$). Conversely, we observed a more pronounced and progressive reduction in the ETP + TM over time that reached statistical significance at 24 weeks compared to baseline (Figure 2). At 48 weeks, the median levels of ETP + TM were approximately halved. ETP + TM at 24 weeks (331 [248-469] nmol/L*min, $P = .76$) and 48 weeks (269 [159-359] nmol/L*min, $P = .14$) was not statistically different from ETP + TM measured in healthy controls (330 [221-511] nmol/L*min).

The baseline ETP ratio was significantly higher in patients (0.50 [0.33-0.67]) than in controls (0.29 [0.23-0.40], $P < .0001$). The ETP ratio decreased slowly at EOT and 12 weeks, and was significantly decreased at 24 weeks and 48 weeks compared to baseline ($P < .001$ for both comparisons) (Figure 2). ETP ratio at 12 weeks

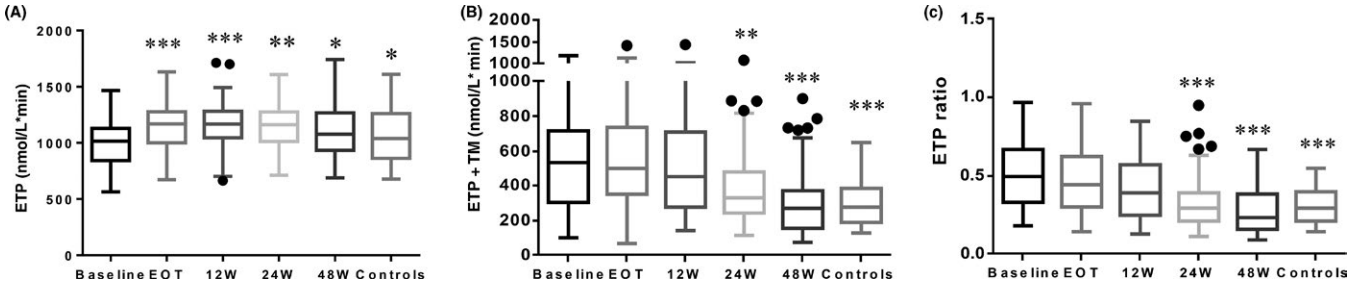

FIGURE 2 Trend of TGA parameters in the study population. A, ETP; B, ETP with thrombomodulin; C, ETP ratio. *P* values are calculated versus baseline: **P* < .05; ***P* < .01; ****P* < .001. EOT, end of therapy; TM, thrombomodulin; TGA, thrombin generation assay; W, weeks. Thrombin generation parameters measured in healthy controls: ETP 1092 (973-1242) nmol/L*min, ETP + TM 330 (221-511) nmol/L*min, ETP ratio 0.29 [0.23-0.40]

(0.39 [0.35-0.57], *P* = .08), 24 weeks (0.29 [0.21-0.39], *P* = .67) and 48 weeks (0.24 [0.16-0.38], *P* = .08) was not statistically different from ETP ratio measured in healthy controls. Finally, the percentage of ETP reduction caused by the addition of TM (50% at baseline) increased slowly at EOT (56%) and 12 weeks (60%), and the difference was statistically significant at 24 weeks (71%) and 48 weeks (76%) compared to baseline (*P* < .0001 for both comparisons).

3.5 | Pro- and anticoagulant factors and thrombin generation parameters according to child score

Child's B patients (8/58, 14%) presented with higher FVIII baseline levels (190% [145-238]) than Child's A patients (184% [147-216]; *P* = .05). The achievement of SVR in Child's B patients was associated with a non-significant decrease in FVIII (163% [145-201]) at EOT. Baseline coagulometric protein C was lower in Child's B (43% [33-44]) than in Child's A patients (69% [53-79], *P* < .05) and no significant increase was observed after EOT (43% [38-52]), at 12 weeks (37% [33-43]), as well as 24 weeks (39% [37-49]) and 48 weeks (48% [43-54]) (only 3 Child's B patients were available at 24 weeks and 48 weeks). Levels of antithrombin followed the same trend as protein C (Table 4).

With regard to TGA in Child's A patients, we observed a slight but significant increase in the ETP from EOT throughout the follow-up, combined with a significant decrease in the ETP + TM starting at 24 weeks. Interestingly enough, we observed a significant decrease in ETP ratio at 12 weeks which steadily dropped through 48 weeks (Table 4). ETP ratio at 12 weeks, 24 weeks and 48 weeks was not statistically different from ETP ratio measured in healthy controls. On the contrary, Child's B patients showed a significantly higher ETP ratio compared to Child's A at baseline (0.68 vs 0.47, *P* < .05) and did not show any significant improvement in ETP ratio through 12 weeks. Child's B patients showed a significantly higher ETP ratio at 12 weeks than healthy controls (0.69 vs 0.29, *P* < .01). It is worth mentioning that data at 24 weeks and 48 weeks were available for only 3 Child's B patients (Table 4).

3.6 | Incidence of portal vein thrombosis

During the 1-year follow-up, 2 cases of PVT were diagnosed (both in Child's B patients) with an overall PVT incidence rate of 1.1% p/y (95%CI, 0.18-3.58). PVT was detected incidentally and occurred 1 and 3 months after EOT respectively. In both cases, PVT was extended to the main trunk and in 1 case, the superior mesenteric vein was also occluded. The 2 patients who developed PVT did not show any significant variation in ETP ratio from baseline (0.8) to W12 (0.85), W24 (0.8) and W48 (0.7). Finally, no deep vein thrombosis episodes occurred during the follow-up.

4 | DISCUSSION

The achievement of SVR has a significant impact on the natural course of HCV-related liver disease by halting the progression of the disease and, most importantly, inducing the regression of fibrosis.[27,28] Although the benefits of DAAs on liver function have not been extensively studied yet, it is plausible nonetheless to hypothesize an improvement of clinical outcome through SVR.[29] This study shows that the improvement of liver function following the administration of DAAs and the successful eradication of HCV in cirrhotic patients is linked to a resolution of the hypercoagulable state by bringing coagulation factor levels within the normal range, thus improving TM-modified TGA.

The most significant coagulation changes observed were the reduction in factor VIII and the increase in protein C plasma levels, both towards the normal range in the majority of patients. We also noted increased levels of factor II, antithrombin and protein S, suggesting a clear amelioration of the liver synthesis of coagulation factors and inhibitors following DAAs treatment. ETP increased slightly in the absence of TM (10%-15% increase at 48 weeks vs baseline). However, ETP dropped significantly (about 50% at 48 weeks vs baseline) in the presence of TM, which is a much better reflection of the overall coagulation potential. Interestingly, the ETP without TM showed early improvement and reached the values measured in healthy controls at EOT, mirroring a similar trend in the liver synthesis of pro- and anticoagulant factors. Conversely, ETP + TM showed late improvement starting at 12 weeks after EOT and reaching healthy control values at 24 weeks. In other words, HCV-related cirrhosis coagulopathy, mainly because of an imbalance of the anticoagulant protein C system, was reversed late by DAAs. Indeed, we

TABLE 4 Coagulation factors, TGA parameters and PVT occurrence in Child A and Child B

	Baseline		EOT		12 wk
	Child A (n.50)	*Child B (n.8)*	Child A (n.50)	*Child B (n.8)*	Child A (n.43)
Factor VIII, %	184 [147-216]	190 [145-238]	147** [121-181]	163 [145-201]	155** [112-180]
PC, %	69 [53-79]	43 [33-44]	79 [67-87]**	43 [38-52]	79 [68-90]**
Antithrombin, %	83 [71-93]	50 [41-59]	88 [80-96]*	58 [46-62]	87 [75-94]
ETP, nmol/L*min	1008 [850-1116]	1096 [843-1136]	1164*** [994-1268]	1171 [1148-1340]	1160*** [1028-1266]
ETP + TM, nmol/L*min	490 [287-651]	766 [535-845]	437 [335-622]	837 [687-992]	375 [271-550]
ETP ratio	0.47 [0.31-0.65]	0.68 [0.59-0.81]	0.42 [0.29-0.52]	0.70 [0.63-0.77]	0.37* [0.25-0.47]
rETP, %	53 [34-69]	31 [19-41]	58 [48-71]	30 [23-37]	63 [52-65]*
PVT, n(%)	-	-	-	-	-

Data are expressed by median and interquartile range. *P* values are calculated versus baseline values: *<.05, **<.01, ***<.001. Note that at time point 24 wk and 48 wk only 3 patients were available.
Thrombin generation parameters measured in healthy controls: ETP 1092 (973-1242) nmol/L*min, ETP + TM 330 (221-511) nmol/L*min, ETP ratio 0.29 (0.23-0.40).
12 wk, 12 weeks after EOT; EOT, end of treatment; ETP, endogenous thrombin potential; PVT, portal vein thrombosis; rETP, reduction of ETP; TM, thrombomodulin.

found that the ETP ratio, the parameter that reflects the cirrhotic haemostatic imbalance,[2,3] decreased from 0.49 at baseline to 0.23 at 48 weeks with a percentual reduction in ETP of 76% similar to that measured in the healthy population.

Only limited data are available in the literature on the effect of DAAs therapy on HCV-related cirrhosis coagulopathy. Recently, Tripodi et al reported data on coagulation parameters, TGA and thromboelastography of 28 patients with HCV-related cirrhosis who received DAAs with a 12-week follow-up after the EOT.[17] They reported higher levels of procoagulant and anticoagulant factors after treatment, as well as a lower ETP ratio compared to baseline, in line with our findings. However, Tripodi et al found that the reduction in ETP ratio had more to do with the increase in ETP without TM rather than the improvement of ETP + TM, as was the case in our study. They studied patients for 12 weeks after EOT and therefore we could not compare data on the amelioration of ETP ratio caused by a late improvement of ETP + TM. Generally speaking, both our studies suggest nonetheless that the treatment with DAAs results in an early improvement of the liver synthesis of factor II and anticoagulants, starting soon after the EOT. Additionally, we can surmise that the achievement of SVR leads to a late reversal of cirrhotic coagulopathy with subsequent improvement of the protein C system imbalance.

PVT is the most common complication in cirrhosis with an incidence of up to 13%-15%[30] and its risk increases progressively with the severity of cirrhosis. A high ETP ratio has previously been associated with an increased risk of PVT in cirrhotic patients, particularly Child's B and C.[10] In our cohort, only 2 Child's B patients developed PVT during the follow-up with an overall absolute incidence of 1.1% p/y. Interestingly, neither patient showed any change in their in vitro hypercoagulable state after antiviral therapy, as reflected by a consistently elevated ETP ratio. No Child's A developed either PVT or thrombosis in other sites during the follow-up. The fact that ETP + TM showed a slower recovery compared to the ETP recovery itself means that between EOT and 12 weeks after EOT, patients appeared more shifted towards a hypercoagulable state which in turn was completely reversed starting 6 months from EOT. Interestingly, the 2 PVT episodes occurred exactly within this lapse of time (1 and 3 months after EOT). However, the small number of thrombotic complications does not allow us to draw definitive conclusions.

Are these findings the same regardless of the severity of cirrhosis? Unfortunately, our cohort comprised 50 Child's A, only 8 Child's B and no Child's C cirrhosis and therefore we cannot provide a definitive answer to said question. In particular, in the clinical practice, patients with Child's C cirrhosis do not receive antiviral therapy; they are evaluated for liver transplantation and then treated for HCV recurrence after being transplanted. When we analysed TGA data separately for Child's A and Child's B subgroups, 2 distinct trends emerged: Child's A patients treated with DAAs showed a progressive marked reduction in TM-modified TGA; in contrast, Child's B patients showed an increase in TM-modified TGA with no significant modification of the ETP ratio which remained elevated up to 12 weeks. However, these are preliminary findings and the number of Child's B patients included in our study is clearly too small to draw any definitive conclusion.

Factor VIII is primarily synthesized by hepatic sinusoidal cells[31] and its plasma concentration may increase because of chronic inflammation associated with various chronic liver diseases.[32] As expected, in our patients, baseline FVIII levels were high and significantly decreased after the EOT, possibly reflecting an abatement of the hepatic inflammation. One notable observation worthy of further investigation is the fact that FVIII returned to the higher baseline levels at 48 weeks even though HCV was still undetectable and liver parameters had clearly improved compared to baseline.

The main limitations of this study pertain to the small sample size, albeit very homogeneously and longitudinally followed; the lack of Child's C cirrhotic patients, intrinsically owing to the current clinical practice that exclude those patients from DAAs therapy; platelet

	24 wk		48 wk	
Child B (n.7)	Child A (n.43)	*Child B (n.7)*	Child A (n.42)	*Child B (n.3)*
157 [153-167]	139*** [121-175]	157 [152-161]	191 [152-227]	172 [165-174]
37 [33-43]	87 [80-94]***	39 [37-49]	85 [74-99]**	48 [43-54]
40 [39-44]	93 [82-103]***	40 [38-50]	92 [80-113] **	47 [47-58]
1185 [1142-1389]	1168*** [1009-1271]	1145 [1105-1186]	1200** [1027-1312]	1122 [1094-1143]
954 [639-1003]	300** [237-428]	818 [609-953]	273** [159-291]	480 [441-576]
0.69 [0.57-0.81]	0.28*** [0.19-0.38]	0.67 [0.52-0.81]	0.22*** [0.14-0.29]	0.43* [0.40-0.50]
31 [19-43]	72 [62-81]***	33 [19-48]	78 [71-86]***	57 [50-60]*
2 (28.6)	-	-	-	-

function and the fibrinolytic pathway were not investigated, the inclusion of both would allow for the overall assessment of the longitudinal haemostatic status of cirrhotic patients after DAAs therapy; finally, given the relatively small sample size including mostly Child's A patients, as well as the short follow-up, this study is not able to give a definitive answer as to whether HCV eradication has an impact on the natural incidence of PVT.

In summary, we were able to demonstrate that DAAs therapy improved liver synthesis of pro- and anticoagulant factors and reduced systemic inflammation (factor VIII) with both improvements occurring soon after the end of DAAs treatment. We also observed a late complete reversal of HCV-related cirrhotic hypercoagulability 24 weeks and 48 weeks after the EOT. Finally, the correction of coagulopathy in Child's B did not seem to occur as early as in Child's A though we cannot exclude the possibility of a partial reduction in hypercoagulability at a later stage (48 weeks).

Ultimately, our findings appear to indicate that the possibility to improve coagulation after DAAs treatment is inversely proportional to severity of cirrhosis; the earlier the DAAs treatment is administered the better chance to reverse the coagulopathy and, possibly, even reduce the risk of thrombosis. Furthermore, thorough studies are needed to assess the real benefits of such an approach and evaluate the effect of DAAs in the more severe Child's C patients.

CONFLICT OF INTEREST

The authors do not have any disclosures to report.

ORCID

Alberto Zanetto http://orcid.org/0000-0002-6734-7178
Elena Campello http://orcid.org/0000-0002-0377-3741
Sarah Shalaby http://orcid.org/0000-0002-8700-6282
Patrizia Burra http://orcid.org/0000-0002-8791-191X
Ton Lisman http://orcid.org/0000-0002-3503-7140
Paolo Simioni http://orcid.org/0000-0002-6744-383X

REFERENCES

1. Tripodi A, Primignani M, Mannucci PM, Caldwell SH. Changing concepts of cirrhotic coagulopathy. *Am J Gastroenterol*. 2017;112:274-281.
2. Lisman T, Caldwell SH, Burroughs AK, et al. Hemostasis and thrombosis in patients with liver disease: the ups and downs. *J Hepatol*. 2010;53:362-371.
3. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. *N Engl J Med*. 2011;365:147-156.
4. Lisman T, Porte RJ. Value of preoperative hemostasis testing in patients with liver disease for perioperative hemostatic management. *Anesthesiology*. 2017;126:338-344.
5. Tripodi A, Salerno F, Chantarangkul V, et al. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. *Hepatology*. 2005;41:553-558.
6. Gatt A, Riddell A, Calvaruso V, Tuddenham EG, Makris M, Burroughs AK. Enhanced thrombin generation in patients with cirrhosis-induced coagulopathy. *J Thromb Haemost*. 2010;8:1994-2000.
7. Lebreton A, Sinegre T, Pereira B, Lamblin G, Duron C, Abergel A. Plasma hypercoagulability in the presence of thrombomodulin but not of activated protein C in patients with cirrhosis. *J Gastroenterol Hepatol*. 2017;32:916-924.
8. Lisman T, Bakhtiari K, Pereboom IT, Hendriks HG, Meijers JC, Porte RJ. Normal to increased thrombin generation in patients undergoing liver transplantation despite prolonged conventional coagulation tests. *J Hepatol*. 2010;52:355-361.
9. Senzolo M, Rodriguez-Castro KI, Rossetto V, et al. Increased anticoagulant response to low-molecular-weight heparin in plasma from patients with advanced cirrhosis. *J Thromb Haemost*. 2012;10:1823-1829.
10. 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.

11. Duron C, Lebreton A, Sinegre T, et al. Low sensitivity to thrombomodulin in patients with cirrhosis; Impact of factor VIII increase and protein C deficiency. *J Hepatol*. 2017;66:S143-S144.
12. 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.
13. La Mura V, Tripodi A, Tosetti G, et al. Resistance to thrombomodulin is associated with de novo portal vein thrombosis and low survival in patients with cirrhosis. *Liver Int*. 2016;36:1322-1330.
14. Rossetto V, Spiezia L, Senzolo M, et al. Whole blood rotation thromboelastometry (ROTEM(R)) profiles in subjects with non-neoplastic portal vein thrombosis. *Thromb Res*. 2013;132:e131-e134.
15. Pawlotsky JM, Hepatitis C. Drugs: is next generation the last generation? *Gastroenterology*. 2016;151:587-590.
16. Afdhal N, Everson GT, Calleja JL, et al. Effect of viral suppression on hepatic venous pressure gradient in hepatitis C with cirrhosis and portal hypertension. *J Viral Hepat*. 2017;24:823-831.
17. Tripodi A, D'Ambrosio R, Padovan L, et al. Evaluation of coagulation during treatment with directly acting antivirals in patients with hepatitis C virus related cirrhosis. *Liver Int*. 2017;37:1295-1303.
18. European Association for Study of Liver; Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: non-invasive tests for evaluation of liver disease severity and prognosis. *J Hepatol*. 2015; 63: 237-264.
19. National Guideline Centre (UK). Cirrhosis in over 16s: assessment and management. NICE 2016.
20. European Association for Study of Liver. EASL recommendations on treatment of hepatitis C 2015. *J Hepatol*. 2015; 63:199-236.
21. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. *J Hepatol*. 2017;66:153-194.
22. Bulato C, Radu CM, Campello E, et al. New prothrombin mutation (Arg596Trp, Prothrombin Padua 2) associated with venous thromboembolism. *Arterioscler Thromb Vasc Biol*. 2016;36:1022-1029.
23. Campello E, Spiezia L, Radu CM, et al. Circulating microparticles and the risk of thrombosis in inherited deficiencies of antithrombin, protein C and protein S. *Thromb Haemost*. 2016;115:81-88.
24. Hemker HC, Giesen P, AlDieri R, et al. The calibrated automated thrombogram (CAT): a universal routine test for hyper- and hypocoagulability. *Pathophysiol Haemost Thromb*. 2002;32:249-253.
25. Campello E, Zabeo E, Radu CM, et al. Hypercoagulability in overweight and obese subjects who are asymptomatic for thrombotic events. *Thromb Haemost*. 2015;113:85-96.
26. Piscaglia F, Gianstefani A, Ravaioli M, et al. Criteria for diagnosing benign portal vein thrombosis in the assessment of patients with cirrhosis and hepatocellular carcinoma for liver transplantation. *Liver Transpl*. 2010;16:658-667.
27. Bachofner JA, Valli PV, Kroger A, et al. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. *Liver Int*. 2017;37:369-376.
28. Knop V, Hoppe D, Welzel T, et al. Regression of fibrosis and portal hypertension in HCV-associated cirrhosis and sustained virologic response after interferon-free antiviral therapy. *J Viral Hepat*. 2016;23:994-1002.
29. Nahon P, Bourcier V, Layese R, et al. Eradication of hepatitis C virus infection in patients with cirrhosis reduces risk of liver and non-liver complications. *Gastroenterology*. 2017;152:142-156: e2.
30. Zanetto A, Senzolo M, Vitale A, et al. Thromboelastometry hypercoagulable profiles and portal vein thrombosis in cirrhotic patients with hepatocellular carcinoma. *Dig Liver Dis*. 2017;49:440-445.
31. Hollestelle MJ, Geertzen HG, Straatsburg IH, van Gulik TM, van Mourik JA. Factor VIII expression in liver disease. *Thromb Haemost*. 2004;91:267-275.
32. Kerr R. New insights into haemostasis in liver failure. *Blood Coagul Fibrinolysis*. 2003;14(Suppl 1):S43-S45.

How to cite this article: Russo FP, Zanetto A, Campello E, et al. Reversal of hypercoagulability in patients with HCV-related cirrhosis after treatment with direct-acting antivirals. *Liver Int*. 2018;38:2210–2218. https://doi.org/10.1111/liv.13873