Enoxaparin Prevents Portal Vein Thrombosis and Liver Decompensation in Patients With Advanced Cirrhosis

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This article has an accompanying continuing medical education activity on page e17. Learning Objective: Upon completion of this exam, successful learners will be able to correctly identify patients with cirrhosis who should be considered for prophylactic anticoagulation, to formulate a correct surveillance protocol, to select the appropriate treatment schedule, and to prevent PVT.

See Covering the Cover synopsis on page 1126; see editorial on page 1138.

BACKGROUND & AIMS: We performed a randomized controlled trial to evaluate the safety and efficacy of enoxaparin, a low-molecular-weight heparin, in preventing portal vein thrombosis (PVT) in patients with advanced cirrhosis. **METHODS:** In a nonblinded, single-center study, 70 outpatients with cirrhosis (Child–Pugh classes B7–C10) with demonstrated patent portal veins and without hepatocellular carcinoma were assigned randomly to groups that were given enoxaparin (4000 IU/day, subcutaneously for 48 weeks; n = 34) or no treatment (controls, n = 36). Ultrasonography (every 3 months) and computed tomography (every 6 months) were performed to check the portal vein axis. The primary outcome was prevention of PVT. Radiologists and hepatologists that assessed outcomes were blinded to group assignments. Analysis was by intention to treat. **RESULTS:** At 48 weeks, none of the patients in the enoxaparin group had developed PVT, compared with 6 of 36 (16.6%) controls ($P = .025$). At 96 weeks, no patient developed PVT in the enoxaparin group, compared with 10 of 36 (27.7%) controls ($P = .001$). At the end of the follow-up period, 8.8% of patients in the enoxaparin group and 27.7% of controls developed PVT ($P = .048$). The actuarial probability of PVT was lower in the enoxaparin group ($P = .006$). Liver decompensation was less frequent among patients given enoxaparin (11.7%) than controls (59.4%) ($P < .0001$); overall values were 38.2% vs 83.0%, respectively ($P < .0001$). The actuarial probability of liver decompensation was lower in the enoxaparin group ($P < .0001$). Eight patients in the enoxaparin group and 13 controls died. The actuarial probability of survival was higher in the enoxaparin group ($P = .020$). No relevant side effects or hemorrhagic events were reported. **CONCLUSIONS: In a small randomized controlled trial, a 12-month course of enoxaparin was safe and effective in preventing PVT in patients with cirrhosis and a Child–Pugh score of 7–10. Enoxaparin appeared to delay the occurrence of hepatic decompensation and to improve survival. www.isrctn.>org: ISRCTN32383354; www.clinicaltrialsregister.eu: EudraCT2007-007890-22.**

Keywords: Prophylaxis; Bacterial Translocation; Anticoagulant Therapy; Portal Hypertension.

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Portal vein thrombosis (PVT) is a critical but frequent event in patients with cirrhosis. Its reported incidence in compensated disease ranges from 0.6% to 5%, which increases up to 40% in advanced disease.[1–3] PVT may result in deterioration of the clinical course,[4] increased complications caused by portal hypertension (PH),[5] and post-transplant mortality.[6,7] Its development is associated inversely with platelet levels.[1,8] Other risk factors for PVT include recurrent liver decompensation[3] and history of

Abbreviations used in this paper: HR, hazard ratio; I-FABP, intestinal-fatty acid binding protein; IL-6, interleukin 6; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; PCR, polymerase chain reaction; PH, portal hypertension; PVT, portal vein thrombosis; RCT, randomized controlled trial; rDNA, ribosomal DNA; sCD14, soluble CD14; SBP, spontaneous bacterial peritonitis.

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2012.07.018

infection,[9] bleeding, endoscopic treatment, and abdominal surgery (eg, splenectomy, and so forth).[10]

The local and systemic factors involved in PVT pathogenesis suggest a procoagulant, multifactorial status culminating in PVT in cirrhotic patients. Local alterations may include changes in the liver cytoarchitecture, including periportal lymphangitis and fibrosis,[11,12] leading to flow reduction and endothelial activation.[13] Systemic factors include altered levels of natural inhibitors of coagulation,[14] inherited coagulation abnormalities,[15] and the presence of antiphospholipid antibodies.[16]

Because anticoagulants can reverse acute PVT in subjects without liver disease,[17] the efficacy of anticoagulation in treating PVT has been tested in cirrhotic patients. Francoz et al[8] showed that sequential anticoagulation with low-molecular-weight heparin and vitamin K antagonists resulted in a 42% complete recanalization (in the period between enrollment and transplant), without bleeding complications. However, no randomized prospective studies devoted to PVT prevention have been performed. We designed a pragmatic, nonprofit, randomized, controlled trial (RCT) comparing the safety and efficacy of enoxaparin with no treatment in patients with advanced cirrhosis, with the primary end point of preventing PVT. Secondary end points were prevention of liver decompensation, overall survival, and transplant-free survival. Because a compromised intestinal mucosal barrier (as found in advanced cirrhosis)[18,19] may lead to increased bacterial translocation and favor inappropriate activation of coagulation, we also evaluated intestinal fatty acid binding protein (I-FABP), a marker of enterocyte damage and 3 markers associated with microbial translocation and the immune response to it (16S ribosomal DNA [rDNA], soluble CD14 [sCD14], interleukin [IL]-6)[20] to evaluate whether enoxaparin, by improving intestinal microcirculation, was able to decrease mucosal ischemia and reduce bacterial translocation.

Materials and Methods

Study Design and Participants

Between April 2008 and November 2010, all consecutive patients seen at a tertiary referral liver unit (Azienda Ospedaliero-Universitaria, Modena) and satisfying predefined inclusion criteria were recruited. Eligible patients were 18 years and older and had cirrhosis of any etiology, a Child–Pugh score between B7 and C10, absence of ascites, spontaneous bacterial peritonitis (SBP), portal hypertensive bleeding or portosystemic encephalopathy for at least 3 months before enrollment, and no evidence of PVT or splenomesenteric thrombosis by ultrasound evaluation and angio-computed tomography. Before enrollment, all patients underwent hepatic, renal, and coagulative evaluations.

Exclusion criteria were as follows: age older than 75 years; history of gastrointestinal bleeding, hepatocellular carcinoma, other intrahepatic/extrahepatic cancers, or thromboembolic disease; ongoing anticoagulation, antiaggregation, or antiphospholipid antibody treatment; pregnancy or breastfeeding; F2 varices with red whale marks or F3 varices unless ligated; platelet count less than 10,000/mm^3; evidence of paroxysmal nocturnal hemoglobinuria (based on CD55–CD59 flow cytometry); or human immunodeficiency virus infection.

All patients provided written informed consent. The study protocol was approved by the Ethics Committee of Azienda Ospedaliero-Universitaria, Modena (ISRCTN32383354, EudraCT 2007-007890-22). The study was conducted according to the guidelines of the Declaration of Helsinki and the applicable provisions of Good Clinical Practice in clinical trials. All authors had access to the study data, reviewed, and approved the final manuscript.

Study Design

The experimental arm received enoxaparin (Clexane; Sanofi Aventis, Milan, Italy) subcutaneously at a prophylactic dose (4000 IU/day) for 48 weeks. The control arm received no treatment. After the first year, both groups continued follow-up evaluation.

Randomization and Masking

Patients were randomized to treatment groups by an independent statistician who prepared sequentially numbered, sealed, opaque envelopes derived from a computer-generated scheme, with a concealed block size of 10. Patients who met the inclusion criteria were assigned randomly, with equal probability, to 1 of 2 treatment arms. Caregivers and patients were not masked to treatment assignments. Radiologists and hepatologists (performing computed tomography and ultrasound, respectively) assessing primary outcome were instead blinded to group assignment.

Efficacy Assessment

The primary end point of the study was the 2-year prevention of portal or mesenteric vein thrombosis. Ultrasound evaluation of the portal vein system was performed at baseline and every 3 months thereafter. Patency of the portal vein system was confirmed at enrollment and at weeks 48, 96, and 144 by angio–computed tomography (which was repeated whenever a thrombotic event was suspected). Secondary end points were as follows: (1) occurrence or recurrence of liver decompensation, defined as development of ascites, portosystemic encephalopathy, SBP, or portal hypertensive bleeding; and (2) overall and transplant-free survival.

Patients were seen regularly in the outpatient clinic every 3 months, unless their clinical condition required more frequent monitoring. At each interval, complete biochemical tests were obtained. Patients who stopped the planned treatment or missed 2 consecutive drug doses at any time, because of subjective intolerance or side effects, would have been considered as withdrawn from treatment. All other patients were followed up until death, liver transplant, or completion of the study. Results were analyzed by intention to treat. Reporting of this RCT was performed according to criteria in the last CONSORT statement.[21]

Safety Assessment

Side effects were recorded according to the World Health Organization grading system of toxicity.[22] Protocol guidelines allowed for dose interruption in patients who had relevant adverse events or important laboratory value abnormalities. If these issues resolved, then the drug was restarted; otherwise, therapy was stopped.

Biomarkers of Microbial Translocation

Bacterial DNA. *DNA extraction and purification.* Bacterial cells were pelleted from 500 μL of serum at 14,000 rpm, 4°C. Pellets were resuspended in 180 μL of enzyme solution (20 mmol/L Tris-HCl, pH 8.0; 2 mmol/L EDTA; 1.2% Triton; lysozyme 20 mg/mL). The mixture was incubated at 37°C for 30 minutes to break the bacterial cell walls. DNA extraction and purification were performed with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Cellular suspensions were resuspended in proteinase K/lysis buffer at 56°C. At

3 hours, DNA was purified by the spin column method and eluted with 30 μL of sterile water.

Microbial DNA amplification by polymerase chain reaction. A 24-μL aliquot of the DNA template was added to polymerase chain reaction (PCR) mixtures that contained 50 pmol of each appropriate primer (oligonucleotide primers, forward: 5′-GATCATGGCT CAGATTGAAC G-3′ and reverse: 5′-CGTAT-TACCG CGGCTGCT-3′) (Eurofins MWG Operon, Ebersberg, German), 200 μmol/L of each deoxynucleoside triphosphate, 1× Taq buffer advanced (5 PRIME, Gaithersburg, MD), 2.5 U of Taq DNA polymerase (5 PRIME), and 16 μL of deionized water. The total reaction volume was 50 μL.

The PCR primers were designed against a conserved region of the bacterial 16S ribosomal RNA gene with Primer3 software (available at: http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and the *Escherichia coli* 16S ribosomal RNA sequence (gene accession number: J01695). The expected amplicon size was approximately 521 bp. In each PCR assay, positive controls (DNA from *E coli* DH5α) and negative controls (sterile water and PCR mixtures, without DNA) were used.

PCR was performed on a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems, Carlsbad, CA). The PCR running conditions were an initial denaturation of 94°C for 6 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with a final extension step at 72°C for 10 minutes. Purified products were separated on 1.8% agarose gel containing ethidium bromide by electrophoresis. The PCR products were visualized by exposure to UV light on a Trans illuminator and photographed with a Molecular Imager Gel Doc XR+ System (Bio-Rad, Hercules, CA). A band of approximately 521 bp was obtained, corresponding to the specific amplification of the prokaryotic 16S ribosomal RNA gene.

Bacterial DNA (16S rDNA) in sera was PCR-amplified at baseline and at 48 and 72 weeks from enrollment.

Determination of serum I-FABP, IL-6, sCD14. I-FABP, IL-6, and sCD14 levels were measured in duplicate with a commercial enzyme-linked immunosorbent assay kit, according to the manufacturer's instructions. Serum I-FABP concentrations were tested with the human I-FABP enzyme-linked immunosorbent assay kit (Hycult Biotechnology, PB Uden, The Netherlands), according to the manufacturer's instructions. Serum IL-6 and sCD14 levels were determined with the quantikine/high-sensitivity enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. Each experiment was performed in duplicate. Absorbances were measured at 450 and 490 nm with an automatic microplate reader (Multiskan EX; Thermo Fisher Scientific, Inc, Waltham, MA), with background subtraction at 570 and 650 nm, respectively.

Statistical Analysis

Prevention of 2-year portal or splenomesenteric vein thrombosis was chosen as the primary outcome to calculate sample size. Assuming a 20% difference in the 2-year rate of portal or splenomesenteric vein thrombosis between the enoxaparin (5%) and control groups (25%), and 5% α error and 20% β error, 34 patients were needed in each group.

Dichotomous or continuous variables were compared with the Fisher exact test with mid-p correction or the nonparametric Mann–Whitney rank-sum test, respectively. The Kaplan–Meier method was used to estimate the cumulative probability of portal or splenomesenteric vein thrombosis development, occurrence or recurrence of liver decompensation, and overall or transplant-free survival. Differences in observed probability were assessed by log-rank test.

The following variables at baseline were considered for univariate analysis: age, sex, etiology of cirrhosis (viral vs nonviral), Child–Pugh score, Model for End-Stage Liver Disease (MELD) score, esophageal varices size, portal vein diameter, allocation to treatment, platelet level, bilirubin level, albumin level, international normalized ratio (INR), creatinine level, sodium level, and potassium level. To avoid the effect of colinearity, individual components of the MELD score (bilirubin level, INR, and creatinine level) were not included in multivariate models that included the MELD score. Variables with a *P* value of less than .10 in univariate analysis were included in the final multivariate model. The Cox proportional hazards model was used to identify risk factors for portal or splenomesenteric vein thrombosis development, occurrence or recurrence of liver decompensation, and overall or transplant-free survival. The PASW Statistics 18 program (SPSS, Inc, Chicago, IL) was used for analysis.

Results

Supplementary Figure 1 shows the trial profile. A total of 396 patients with cirrhosis were screened for study eligibility: 326 patients were excluded (Supplementary Figure 1), and 70 patients were assigned randomly to the enoxaparin (n = 34) or control group (n = 36). All patients but 1 (withdrawn for thrombocytopenia) completed the treatment. The trial was completed as planned in November 2011. There were no missing values for the primary outcome. There were no significant differences in baseline characteristics between the 2 groups at the time of entry into the study (Table 1). The mean (± standard deviation) follow-up period was 58 ± 37 weeks in the control and 89 ± 57 weeks in the enoxaparin group. Seven patients (4 controls, 3 enoxaparin patients) underwent liver transplantation during the follow-up period.

PVT Incidence

During the year of active treatment, no patient in the enoxaparin group (0 of 34) developed PVT, compared with 6 of 36 (16.6%) controls ($P = .025$). At 2 years, none of the enoxaparin-treated patients (0 of 34) had yet developed PVT, compared with 10 of 36 (27.7%) controls ($P = .001$). Enoxaparin-treated patients developed PVT only at weeks 105, 111, and 121 after enrollment. Overall, 3 of 34 (8.8%) enoxaparin-treated patients and 10 of 36 (27.7%) controls developed PVT ($P = .048$). All patients developing PVT in the enoxaparin group and 9 of 10 patients developing PVT in the control group were symptomatic. The actuarial probability of developing PVT was lower in the enoxaparin-treated group ($P = .006$) (Figure 1*A*). Cox regression analysis showed that enoxaparin treatment (hazard ratio [HR], 0.098; 95% CI, 0.014–0.697; $P = .020$) and higher protein C levels (HR, 0.984; 95% CI, 0.858–0.981; $P = .012$) were associated independently with a decreased risk of developing PVT (Supplementary Table 1).

Occurrence of Decompensation

During the active treatment period, decompensation was more frequent in controls than in enoxaparin-treated patients (21 of 36 [59.4%] vs 4 of 34 [11.7%]; $P <$

Table 1. Baseline Characteristics of Patients

	Enoxaparin (n = 34)	Controls (n = 36)	*P* value
Sex, M/F	25/9	26/10	
Mean age, *y* (±SD)	56 ± 5	57 ± 7	.556
Etiology			
HBV/HCV/alcohol/HCV+alcohol/NASH	2/10/12/8/2	4/16/12/2/2	.347
Child–Pugh			
B7/B8/B9/C10	20/10/2/2	18/11/3/4	.253
Mean ± SD	7.5 ± 0.9	7.7 ± 1.0	.252
MELD score (mean ± SD)	12.8 ± 3.3	13.0 ± 4.0	.210
Varices grading			
F1/F2	16/9	18/12	.580
Gastropathy	20	21	.921
Previous variceal bleeding	3	5	.506
Previous ascites	12	10	.724
Previous SBP	2	4	.674
Previous HE	6	6	.868
β-blockers	11	10	.931
Diuretic treatment	8	9	.887
Chronic rifaximin treatment	2	4	.435
Bilirubin level, *mg/dL*	2.5 ± 1.4	2.6 ± 1.9	.997
Aspartate aminotransferase level, *IU/mL*	84 ± 49	75 ± 50	.453
Alanine aminotransferase level, *IU/mL*	54 ± 47	57 ± 53	.795
γ-glutamyl transpeptidase level, *IU/mL*	143 ± 228	92 ± 60	.241
Platelets, $\times 10^3/mm^3$	91 ± 49	81 ± 50	.124
Creatinine level, *mg/dL*	0.6 ± 0.3	0.7 ± 0.4	.113
Na level, *mEq/L*	135 ± 4.8	136 ± 4.0	.591
K level, *mEq/L*	4.1 ± 0.5	4.1 ± 0.6	.931
Protein C level, *%*	45 ± 18	44 ± 22	.810
Protein S level, *%*	68 ± 25	62 ± 22	.198
Homocysteine level, *mmol/L*	9 ± 3	11 ± 8	.119
Antithrombin III level, *%*	49 ± 15	50 ± 21	.630
Anticardiolipin antibodies IgG level, IgG phospholipid units*/mL*	8.0 ± 5.9	7.3 ± 6.4	.655
Anticardiolipin antibodies IgM level, IgM phospholipid units*/mL*	3.7 ± 3.5	2.5 ± 2.4	.152
Anti-B2-glycoprotein antibodies IgG level, *U/mL*	1.7 ± 1.3	3.1 ± 4.2	.113
Anti-B2-glycoprotein antibodies IgM level, *U/mL*	2.5 ± 3.5	3.3 ± 8.3	.638
Factor V G1691A	2	0	—
Factor II G20210A	0	0	—

HBV, hepatitis B virus; HCV, hepatitis C virus; HE, hepatic encephalopathy; NASH, nonalcoholic steato-hepatitis; SD, standard deviation.

.0001). During the follow-up period, new decompensation occurred in 9 of 31 (29.0%) controls and 9 of 32 (28.0%) enoxaparin-treated patients ($P = .890$). Overall, decompensation occurred in 30 of 36 (83.0%) controls and 13 of 34 (38.2%) enoxaparin-treated patients ($P < .0001$). In the year of active treatment, occurrence of decompensation was significantly lower in enoxaparin-treated patients independently from previous decompensation (previous de-

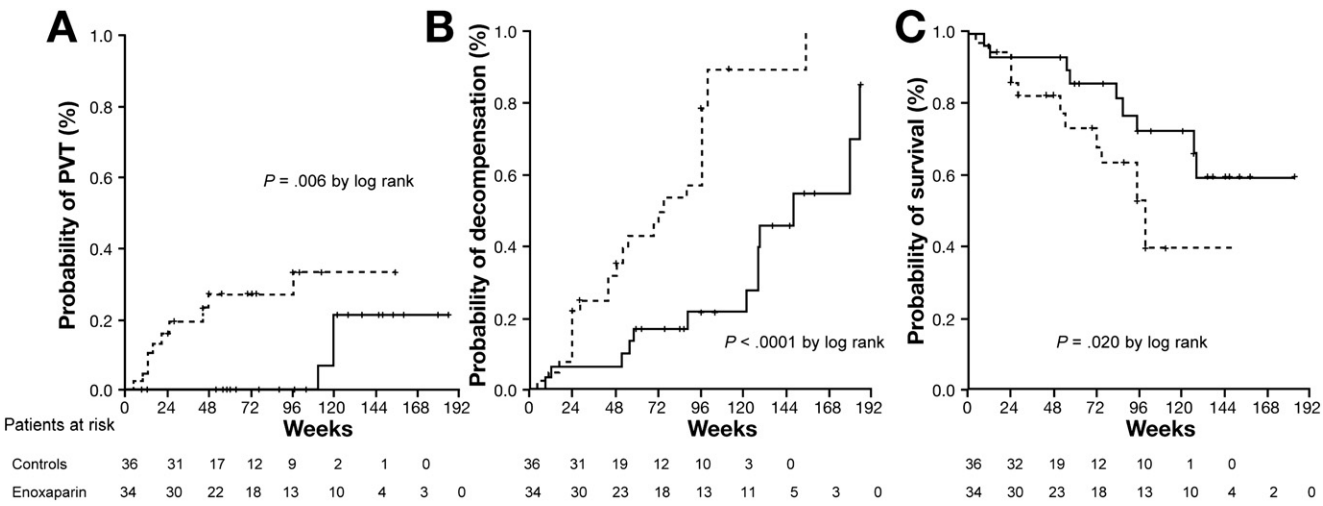

Figure 1. Actuarial probability of developing PVT or hepatic decompensation, and probability of survival according to treatment group. Probability of remaining free from (*A*) PVT, (*B*) hepatic decompensation, and (*C*) probability of survival. *Dashed line*: controls; *continuous line*: enoxaparin-treated patients.

Table 2. Markers of Liver Disease Severity (Albumin, INR, Bilirubin) and Creatinine Levels at Enrollment and at Weeks 48 and 96 in Enoxaparin-Treated and Control Patients

	Bilirubin level, *mg%*	*P*	Albumin level, *g/L*	*P*	INR, *%*	*P*	Creatinine level, *mg%*	*P*
Enoxaparin-treated patients								
Baseline (n = 30)	2.3 ± 1.3	.039	3.2 ± 0.6	.018	1.3 ± 0.2	.004	0.7 ± 0.2	.338
48 weeks	1.7 ± 1.0		3.7 ± 0.4		1.2 ± 0.2		0.7 ± 0.1	
Baseline (n = 25)	2.1 ± 1.0	.845	3.2 ± 0.4	.991	1.3 ± 0.1	1.000	0.7 ± 0.2	.377
96 weeks	2.0 ± 1.3		3.3 ± 0.7		1.3 ± 0.1		0.7 ± 0.1	
Controls								
Baseline (n = 32)	2.4 ± 2.0	.780	3.2 ± 0.6	.614	1.3 ± 0.3	.401	0.7 ± 0.4	.021
48 weeks	2.6 ± 3.4		3.1 ± 0.5		1.4 ± 0.4		0.9 ± 0.5	
Baseline (n = 27)	2.2 ± 1.8	.164	3.1 ± 0.6	.113	1.3 ± 0.3	.880	0.8 ± 0.5	.030
96 weeks	4.4 ± 6.2		2.8 ± 0.7		1.9 ± 1.2		1.5 ± 1.1	

NOTE. Data were analyzed by paired *t* test comparing baseline levels with data at 48 and 96 weeks.

compensation: controls: 11 of 17 [64.7%] vs enoxaparin: 3 of 14 [21.4%]; $P = .029$; no previous decompensation: controls: 10 of 19 [52.6%] vs enoxaparin: 1 of 20 [5%]; $P = .001$). Ascites was the most frequent event (control, 19 of 36 [52.5%] vs enoxaparin, 6 of 34 [17.6%]; $P = .002$), followed by sepsis (control, 6 of 36 [16.6%] vs enoxaparin, 4 of 34 [11.7%]; $P = .558$), variceal bleeding (control, 1 of 36 [2.7%] vs enoxaparin, 2 of 34 [5.8%]; $P = .521$), and encephalopathy (control, 1 of 36 [2.7%] vs enoxaparin, 1 of 34 [2.9%]; $P = .967$). In patients developing PVT, the disease course was mostly complicated by ascites occurrence (control, 7 of 10 [70.0%] vs enoxaparin, none). Bleeding and sepsis developed equally (1 and 2 for each group, respectively).

Paired comparison within enoxaparin-treated patients and control groups of markers of disease severity (albumin level, INR, bilirubin level) and of renal function (creatinine) between baseline and 48 weeks (before stopping treatment) or 96 weeks showed a significant improvement of all liver tests but renal function in enoxaparin-treated patients at 48 weeks. In contrast, in controls, paired comparison showed worsening of renal function both at 48 ($P = .021$) and 96 weeks ($P = .030$) whereas liver function tests showed a trend toward deterioration, although the trend was nonsignificant (Table 2).

The actuarial probability of developing decompensation was lower in enoxaparin-treated patients than in controls ($P < .0001$) (Figure 1*B*). Cox regression analysis showed that enoxaparin treatment (HR, 0.331; 95% CI, 0.148–0.741; $P = .007$), baseline bilirubin level (HR, 1.478; 95% CI, 1.074–2.033; $P = .017$), portal vein diameter (HR, 1.216; 95% CI, 1.010–1.464; $P = .026$), and previous encephalopathy (HR, 3.196; 95% CI, 1.282–7.964; $P = .013$) were independent risk factors for decompensation (Supplementary Table 2). Actuarial probability during follow-up evaluation of not changing or worsening by 2 or more points in the Child–Pugh score was different between enoxaparin-treated and control patients ($P < .0001$) (Figure 2). The improvement of clinical conditions was paralleled by a significant difference both in the number of patients requiring hospital admission (enoxaparin-treated patients vs controls: 11 of 34 [32.3%] vs 22 of 36 [55.5%], respectively; $P = .016$) and in the length of stay in hospital (enoxaparin-treated patients vs controls: 6.1 ± 14.6 vs 15.2 ± 17.6 days, respectively; $P = .045$).

Survival

Thirteen controls and 8 enoxaparin-treated patients died ($P = .251$). Causes of death were sepsis (4 enoxaparin, 6 controls), progressive liver failure (5 controls), hepatocellular carcinoma (2 enoxaparin, 1 control), esophageal variceal bleeding (1 in each group), and hemoperitoneum from intra-abdominal hepatocellular carcinoma rupture (1 enoxaparin). Kaplan–Meier curve analysis revealed a higher survival rate in the enoxaparin-treated group than in controls (Figure 1*C*) ($P = .020$). Independent factors related to survival were enoxaparin treatment (HR, 0.366; 95% CI, 0.082–0.795; $P = .018$) and portal vein diameter (HR, 1.349; 95% CI, 1.051–1.731; $P = .019$) (Supplementary Table 3).

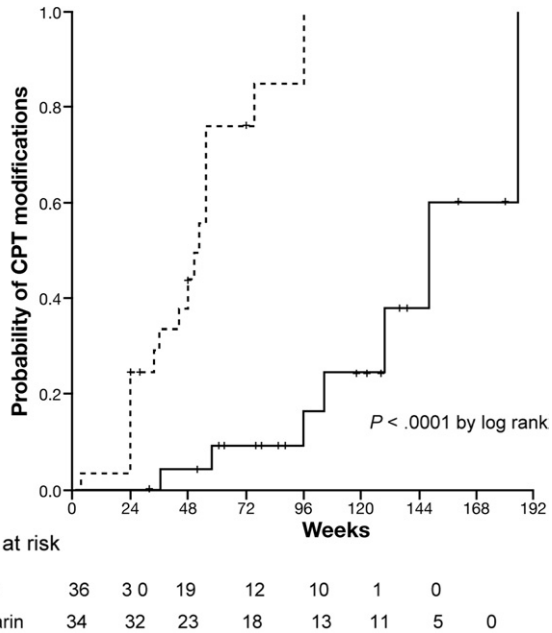

Figure 2. Actuarial probability of not changing or worsening by 2 or more points in the Child–Pugh score. *Dashed line*: controls; *continuous line*: enoxaparin-treated patients.

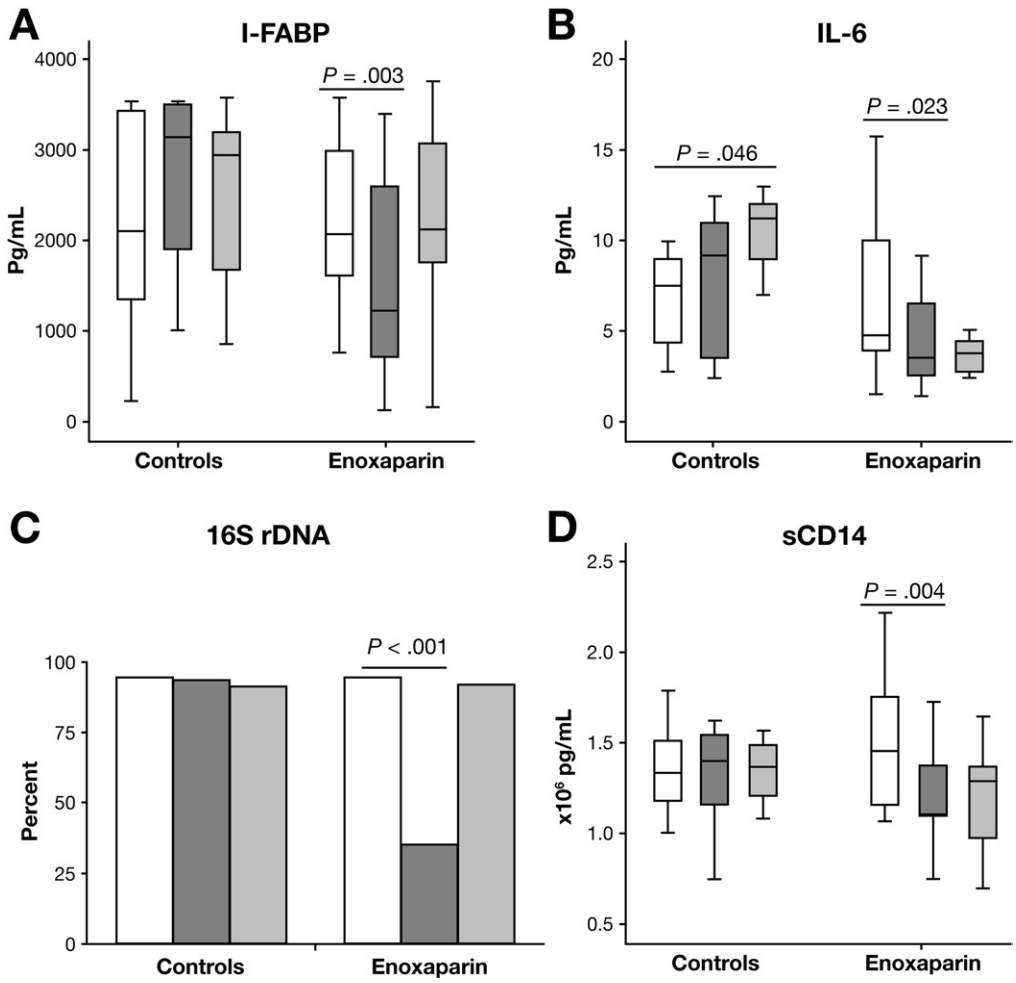

Figure 3. Baseline levels of markers of (*A*) enterocyte death, (*B*) inflammatory reaction, (*C*) microbial translocation, and (*D*) host response to microbial products were increased in both groups. All 4 biomarkers were decreased significantly at week 48 (end of treatment) in the enoxaparin group but not in controls. IL-6 levels steadily increased in controls throughout the observation period. *Bars*, standard deviation; *horizontal lines*, median value and 5%–95% range. *P* values were calculated with the Mann–Whitney *U* test.

Rate of Bacterial Infections and Biomarkers of Microbial Translocation

A significantly different rate of bacterial infections (either as SBP or bacteremia) occurred during the year of active treatment between enoxaparin-treated patients and controls (3 of 34 [8.8%] and 12 of 36 [33.3%], respectively; $P = .019$). Therapy with intravenous antibiotics was strictly related to occurrence of bacterial infections and was therefore less used in enoxaparin-treated patients. Use of poorly absorbed antibiotics was not significantly different between cases and controls.

To explore a possible counteracting effect of enoxaparin on bacterial translocation as a factor determining the observed prevention of PVT and decompensation in our cohort of cirrhotic patients, we evaluated circulating biomarkers of intestinal integrity and immune activation to bacterial products.

Paired levels of I-FABP showed no difference at weeks 0, 48, or 72 in controls. In enoxaparin-treated subjects, paired levels of I-FABP were decreased at week 48 ($P = .003$) (Figure 3*A*). Baseline I-FABP levels correlated with greater portal vein diameter ($r = 0.638$, $P = .006$). In controls, paired levels of IL-6 showed no difference at weeks 0 and 48, but levels at week 72 were higher than at baseline ($P = .046$). During enoxaparin treatment, levels at week 48 were decreased compared with baseline ($P = .012$) (Figure 3*B*). Higher baseline IL-6 level positively correlated with baseline CD14 levels ($r = 0.525$, $P = .006$) and survival (nonsurvivors, 8.7 ± 5.5 pg/mL vs survivors, 5.3 ± 4.1 pg/mL; $P = .022$).

To identify the presence of bacterial DNA, 16S rDNA was amplified from patient sera at baseline (0 weeks; n = 68 samples), 48 weeks (end of the active treatment period; n = 60), and 72 weeks (end of follow-up period; n = 55). At baseline, all but 4 serum samples (2 per group) were positive for bacterial DNA. All but 2 controls gave consistently positive results at 48 and 72 weeks. At 48 weeks, 64.5% of serum samples from surviving enoxaparin-treated patients (21 of 32) were negative for bacterial DNA ($P < .0001$); they reverted to positive at week 72 (Figure 3*C*).

To evaluate host response to microbial products, we measured basal levels of serum CD14. Baseline and follow-up levels did not differ significantly in controls, whereas enoxaparin treatment was associated with a decrease in sCD14 levels at week 48 ($P = .004$) (Figure 3*D*). Baseline sCD14 was associated with occurrence of PVT ($r = 0.417$, $P = .030$).

Safety

Enoxaparin treatment was very well tolerated. Only 1 patient stopped treatment at week 36 for marked

thrombocytopenia. On the whole, platelet count significantly decreased in enoxaparin-treated patients during treatment (baseline vs week 48: $100 \pm 42 \times 10^3/mm^3$ vs $56 \pm 78 \times 10^3/mm^3 \times 10^3/mm^3$, $P = .002$, paired t test) to return to baseline after stopping the drug (baseline vs week 96: $97 \pm 38 \times 10^3/mm^3$ vs $103 \pm 63 \times 10^3/mm^3 \times 10^3/mm^3$, $P = .850$, paired t test). No difference was observed in controls.

There were 3 bleeding episodes from ruptured esophageal varices, 2 in treated patients and 1 in the control group ($P = .521$). They were controlled by conservative endoscopic therapy. Three episodes of epistaxis (1 in the control group, 2 in the enoxaparin-treated group) were also encountered. No significant difference from baseline was observed in hemoglobin levels during the year of active treatment and during the follow-up period in each of the groups.

Discussion

The results of this RCT in a cohort of patients with advanced cirrhosis showed that anticoagulant treatment with enoxaparin is safe and effective, significantly reducing risk of PVT development and liver decompensation, markedly improving liver function and Child–Pugh score, and increasing overall survival. Our findings are consistent with data showing that successful PVT recanalization is accompanied by improvement of the Child–Pugh score.[23]

It is plausible that PVT prevention has a protective role in liver disease progression. Nevertheless, taken alone, PVT prevention by enoxaparin cannot explain the observed improvement in terms of liver decompensation and survival. Enoxaparin treatment was associated with a definite improvement of liver function and a striking decrease of occurrence or recurrence of decompensation (mostly ascites), a favorable effect that was much greater than the mere prevention of PVT. Although PVT prevention may be attributed to the anticoagulant action of enoxaparin, its effect on decompensation is less obvious. Although this study was not designed to clarify the pathogenetic link between enoxaparin treatment and the prevention of PVT or improvement of the course of liver disease, some hypotheses can be made.

PH has severe consequences for the upper and lower gastrointestinal tracts.[24] In the colon, PH is associated with profound changes in the intestinal microcirculation, leading to microthrombosis.[25] It can lead to ischemic damage of enterocytes, followed by a marked increase in permeability of the intestinal mucosa, a key factor for bacterial translocation,[26] progression of liver damage,[20] and decompensation.[27] Our patients displayed extremely high I-FABP serum levels that correlated positively on ultrasound with the portal vein diameter (a well-known surrogate for PH). The protein I-FABP is made exclusively by enterocytes, and it is released into the circulation upon enterocyte necrosis. A high percentage of subjects were positive for circulating 16S rDNA. Patients also displayed extremely high baseline levels of CD14, a marker of immune activation against lipopolysaccharide,[20] and showed markedly increased levels of IL-6, a biomarker of inflammation associated with endotoxemia and sepsis.[28] Therefore, the protective effect of enoxaparin on the course of liver disease might be mediated by an improvement of intestinal microcirculation able to improve enterocytic damage and therefore reducing bacterial translocation. In this view, enoxaparin could act on microthrombosis, facilitating blood flow and improving endothelial function by interfering with the hypercoagulable state through tissue factor, a molecule that plays an extremely relevant role[29] in initiating septic coagulopathy after activation by bacterial endotoxins, lipopolysaccharide, and Toll-like receptor 4.[30] As might be expected, the positive effect was bound to drug administration and maintained only for some time after its discontinuation; this suggests the opportunity of performing larger double-blind RCTs encompassing longer treatment periods and evaluating, at the same time, modifications of portal hypertension through protocolized endoscopies and hepatic venous pressure gradient measurement.

Taken together, our data suggest that cirrhotic patients are at increased risk for thrombotic events, particularly in the portal venous system. In our view, PVT and decompensation are similar expressions of a rapidly worsening PH. By damaging the intestinal mucosal barrier, severe PH leads to systemic dissemination of enteric-borne infection, inappropriate activation of the coagulation system (which is delicately balanced in the cirrhotic patient),[31] and strong up-regulation of systemic inflammation.

The main limitation of this study was in the relatively low number of patients enrolled. A further methodologic question is the potentially limited external validity of the results for different populations and settings. Our study included a cohort of Italian patients enrolled at a tertiary care center, with advanced cirrhosis arising from different etiologies. Lastly, we did not test for anti-Xa activity: this is a relevant issue that should be addressed in future trials together with evaluation of novel tests that work independently of antithrombin III concentrations.[32]

In conclusion, we showed that enoxaparin treatment is safe in patients with advanced cirrhosis, as it has been shown in the treatment of established PVT.[17] No potentially life-threatening side effects occurred, and the occurrence of bleeding episodes did not differ between the control (1 episode) and enoxaparin groups (2 episodes) ($P = .521$). Future therapeutic interventions should aim to reinforce prevention of the microvascular disturbances associated with PVT progression, acting well before establishment of end-stage liver disease.[24]

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2012.07.018.

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Received April 15, 2012. Accepted July 9, 2012.

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Acknowledgments

E.V., M.L., R.C., B.L., V.B., R.V., A.K., M.D.B., E.T., and A.F. on behalf of the Research Network "Women_in_Hepatology."

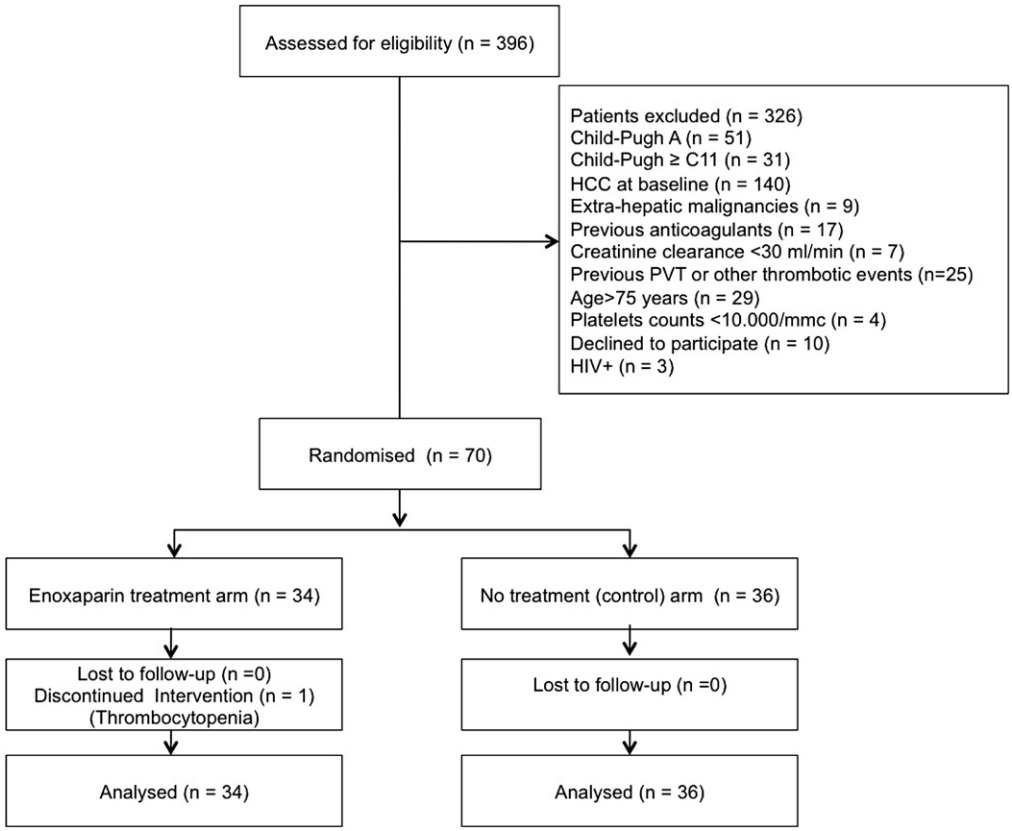
The corresponding author had full access to all of the data and takes full responsibility for the veracity of the data and statistical analysis.

Conflicts of interest

The authors disclose no conflicts.

Funding

This study was independently designed and was not supported by any pharmaceutical company. The National Health System provided enoxaparin. This study adheres to the standards of accountability, access to data, and control of publications of the International Committee of Medical Journal Editors.[33] The study protocol was approved by the Ethics Committee of Azienda Ospedaliero-Universitaria, Modena (ISRCTN32383354, EudraCT 2007-007890-22).



Supplementary Figure 1. Screening and randomization of patients.

Supplementary Table 1. Univariate and Multivariate Analysis of the Risk Factors for Portal or Splenomesenteric Vein Thrombosis Development

	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, *y*	0.973 (0.912–1.038)	.410	—	—
Sex[a]	1.051 (0.329–3.357)	.933	—	—
Etiology	0.867 (0.593–1.266)	.460	—	—
Mean Child–Pugh score	1.022 (0.609–1.713)	.935	—	—
Mean MELD score	1.049 (0.901–1.222)	.538	—	—
Enoxaparin treatment	0.179 (0.047–0.682)	.012	0.098 (0.014–0.697)	.020
Size of esophageal varices	2.143 (0.923–4.979)	.076	1.957 (0.453–8.460)	.368
Gastropathy	0.926 (0.320–2.678)	.887	—	—
Portal vein diameter, *mm*	1.038 (0.776–1.388)	.804	—	—
Mean portal velocity, *cm/s*	0.970 (0.893–1.054)	.472	—	—
History of ascites	0.707 (0.221–2.259)	.558	—	—
History of variceal bleeding	1.893 (0.526–6.818)	.329	—	—
History of SBP	1.942 (0.426–8.858)	.391	—	—
History of HE	0.416 (0.054–3.186)	.399	—	—
Platelet count, $\times 10^3/mm^3$	0.975 (0.959–0.992)	.003	0.970 (0.940–1.002)	.065
INR	0.650 (0.116–3.647)	.624	—	—
Bilirubin level, *mg%*	1.259 (0.972–1.631)	.082	1.209 (0.761–1.923)	.421
Creatinine level, *mg%*	0.768 (0.154–3.836)	.748	—	—
Albumin level, *g/L*	1.817 (0.696–4.746)	.223	—	—
Aspartate aminotransferase level, *IU/L*	0.995 (0.982–1.009)	.495	—	—
Alanine aminotransferase level, *IU/L*	0.998 (0.986–1.010)	.765	—	—
γ-glutamyl transpeptidase level, *IU/L*	1.000 (0.996–1.003)	.778	—	—
Na level, *mEq/L*	1.012 (0.879–1.165)	.867	—	—
K level, *mEq/L*	1.449 (0.524–4.007)	.475	—	—
Protein C level, *%*	0.959 (0.915–1.005)	.083	0.917 (0.858–0.981)	.012
Protein S level, *%*	0.984 (0.959–1.010)	.215	—	—
Antithrombin III level, *%*	0.957 (0.916–0.999)	.045	0.970 (0.906–1.037)	.370
Homocysteine level, *mmol/L*	0.945 (0.820–1.089)	.437	—	—
Anticardiolipin Ab IgG level, IgG phospholipid units*/mL*	1.006 (1.000–1.011)	.055	1.005 (0.999–1.011)	.095
Anticardiolipin Ab IgM level, IgM phospholipid units*/mL*	0.976 (0.753–1.263)	.851	—	—
Anti-β2 glycoprotein Ab IgG level, *U/mL*	0.986 (0.764–1.273)	.913	—	—
Anti-β2 glycoprotein Ab IgM level, *U/mL*	0.909 (0.723–1.143)	.415	—	—

HE, hepatic encephalopathy.
[a]Male sex is used as the reference sex.

Supplementary Table 2. Univariate and Multivariate Analysis of the Baseline Risk Factors for Decompensation

	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, *y*	1.017 (0.979–1.057)	.381	—	—
Sex[a]	0.856 (0.427–1.718)	.662	—	—
Etiology	0.716 (0.547–0.937)	.015	0.721 (0.517–1.006)	.055
Mean Child–Pugh score	1.477 (1.125–1.940)	.005	—	—
Mean MELD score	1.143 (1.044–1.252)	.004	—	—
Enoxaparin treatment	0.339 (0.172–0.668)	.002	0.331 (0.148–0.741)	.007
Size of esophageal varices	1.139 (0.725–1.790)	.572	—	—
Gastropathy	0.926 (0.320–2.678)	.887	—	—
Portal vein diameter, *mm*	1.220 (1.026–1.450)	.024	1.216 (1.010–1.464)	.026
Mean portal velocity, *cm/s*	1.039 (1.004–1.076)	.030	1.043 (0.975–1.116)	.220
History of ascites	1.336 (0.707–2.523)	.372	—	—
History of variceal bleeding	1.893 (0.526–6.818)	.329	—	—
History of SBP	1.942 (0.426–8.858)	.391	—	—
History of HE	2.366 (1.151–4.862)	.019	3.196 (1.282–7.964)	.013
Platelets count, $\times 10^3/mm^3$	0.995 (0.988–1.002)	.180	—	—
INR	2.769 (0.825–9.294)	.099	1.183 (0.217–6.438)	.846
Bilirubin level, *mg%*	1.359 (1.133–1.630)	.001	1.478 (1.074–2.033)	.017
Creatinine level, *mg%*	1.018 (0.417–2.484)	.969	—	—
Albumin level, *g/L*	0.691 (0.395–1.208)	.195	—	—
Aspartate aminotransferase level, *IU/L*	1.004 (0.998–1.010)	.198	—	—
Alanine aminotransferase level, *IU/L*	1.000 (0.994–1.006)	.931	—	—
γ-glutamyl transpeptidase level, *IU/mL*	0.999 (0.997–1.002	.580	—	—
Na level, *mEq/L*	0.955 (0.873–1.046)	.323	—	—
K level, *mEq/L*	1.365 (0.701–2.657)	.361	—	—
Protein C level, *%*	0.986 (0.966–1.006)	.169	—	—
Protein S level, *%*	0.987 (0.973–1.002)	.083	0.984 (0.963–1.005)	.144
Antithrombin III level, *%*	0.973 (0.951–0.994)	.014	1.008 (0.970–1.048)	.678
Homocysteine level, *mmol/L*	1.030 (0.986–1.076)	.186	—	—
Anticardiolipin antibodies IgG level, IgG phospholipid units*/mL*	1.000 (0.995–1.005)	.967	—	—
Anticardiolipin antibodies IgM level, IgM phospholipid units*/mL*	0.998 (0.976–1.020)	.847	—	—
Anti-β2-glycoprotein antibodies IgG level, *U/mL*	1.037 (0.950–1.132)	.410	—	—
Anti-β2-glycoprotein antibodies IgM level, *U/mL*	1.044 (1.004–1.085)	.029	0.744 (1.015–1.106)	.744

HE, hepatic encephalopathy.

[a]Male sex is used as the reference sex.

Supplementary Table 3. Univariate and Multivariate Analysis of the Baseline Risk Factors for Survival

	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, *y*	1.025 (0.971–1.082)	.371	—	—
Sex[a]	0.777 (0.284–2.126)	.623	—	—
Etiology	0.795 (0.574–1.100)	.166	—	—
Mean Child–Pugh score	1.113 (0.736–1.681)	.612	—	—
Mean MELD score	1.083 (0.950–1.235)	.234	—	—
Enoxaparin treatment	0.347 (0.134–0.896)	.029	0.256 (0.082–0.795)	.018
Size of esophageal varices	1.485 (0.750–2.941)	.256	—	—
Gastropathy	1.764 (0.683–4.554)	.241	—	—
Portal vein diameter, *mm*	1.223 (0.963–1.553)	.098	1.349 (1.051–1.731)	.019
Mean portal velocity, *cm/s*	1.022 (0.987–1.058)	.219	—	—
History of ascites	1.614 (0.684–3.809)	.274	—	—
History of variceal bleeding	1.817 (0.595–5.552)	.295	—	—
History of SBP	2.602 (0.859–7.881)	.091	1.766 (0.503–6.181)	.374
History of HE	2.087 (0.799–5.456)	.133	—	—
Platelet count, $\times 10^3/mm^3$	0.997 (0.987–1.007)	.527	—	—
INR	3.097 (0.528–18.154)	.210	—	—
Bilirubin level, *mg%*	1.263 (1.025–1.556)	.029	1.183 (0.912–1.535)	.205
Creatinine level, *mg%*	1.748 (0.484–6.313)	.394	—	—
Albumin level, *g/L*	0.755 (0.360–1.583)	.457	—	—
Aspartate aminotransferase level, *IU/L*	1.002 (0.993–1.010)	.727	—	—
Alanine aminotransferase level, *IU/L*	0.998 (0.988–1.008)	.694	—	—
γ-glutamyl transpeptidase level, *IU/mL*	0.999 (0.996–1.002)	.572	—	—
Na level, *mEq/L*	0.996 (0.897–1.107)	.947	—	—
K level, *mEq/L*	1.400 (0.642–3.053)	.398	—	—
Protein C level, *%*	0.992 (0.964–1.021)	.579	—	—
Protein S level, *%*	0.990 (0.970–1.011)	.354	—	—
Antithrombin III level, *%*	0.967 (0.935–1.000)	.050	1.003 (0.968–1.038)	.309
Homocysteine level, *mmol/L*	0.992 (0.898–1.095)	.867	—	—
Anticardiolipin Ab IgG level, IgG phospholipid units*/mL*	1.005 (1.000–1.011)	.067	0.965 (0.902–1.033)	.965
Anticardiolipin Ab IgM level, IgM phospholipid units*/mL*	0.937 (0.757–1.160)	.550	—	—
Anti-β2 glycoprotein Ab IgG level, *U/mL*	0.990 (0.813–1.204)	.917	—	—
Anti-β2 glycoprotein Ab IgM level, *U/mL*	1.015 (0.968–1.063)	.542	—	—

HE, hepatic encephalopathy.

[a]Male sex is used as the reference sex.