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.

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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