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ORIGINAL ARTICLE

Portal vein thrombosis in cirrhosis is not associated with intestinal barrier disruption or increased platelet aggregability

Piotr Wosiewicz[a], Michał Żorniak[a], Marek Hartleb[a,*], Kamil Barański[b], Maciej Hartleb[a], Magdalena Onyszczuk[a], Joanna Pilch-Kowalczyk[c], Sławomira Kyrcz-Krzemień[d]

[a] *Department of Gastroenterology and Hepatology, School of Medicine in Katowice, Medical University of Silesia, 40-752 Katowice, Poland*
[b] *Department of Epidemiology, School of Medicine in Katowice, Medical University of Silesia, 40-752 Katowice, Poland*
[c] *Department of Radiology and Nuclear Medicine, School of Medicine in Katowice, Medical University of Silesia, 40-752 Katowice, Poland*
[d] *Department of Hematology and Bone Marrow Transplantation, School of Medicine in Katowice, Medical University of Silesia, 40-752 Katowice, Poland*

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Summary
Objective: Portal vein thrombosis (PVT) is a common complication of cirrhosis, but its pathogenesis is unclear. We tested the hypotheses that PVT is the result of platelet hyperactivity or intestinal barrier disruption.
Methods: This study included 49 patients with cirrhosis (15 females) of mixed etiology. Based on spiral computed-tomography, the patients were divided into two groups: with PVT ($n = 16$) and without PVT ($n = 33$). Serum biomarkers of intestinal barrier integrity were endotoxins and zonulin, and platelet activity was assessed with multiple electrode aggregometry.
Results: The levels of endotoxin (43.5 ± 18.3 ng/ml vs. 36.9 ± 7.5 ng/ml; $P = 0.19$) and zonulin (56.3 ± 31.1 ng/ml vs. 69.3 ± 63.1 ng/ml; $P = 0.69$) were not different between the patients with and without PVT. Moreover, endotoxin and zonulin did not correlate with the coagulation and platelet parameters. The platelet aggregability measured with the TRAP and the ADP tests was decreased in PVT patients. In the logistic regression analysis the PVT incidence was related to the levels of D-dimer and bilirubin as well as the TRAP test results. Patients with PVT presented with significantly higher levels of D-dimer (4.45 ± 2.59 vs. 3.03 ± 2.97 mg/l; $P < 0.05$)

Abbreviations: PVT, portal vein thrombosis; vWf, Villebrand factor; LPS, bacterial lipopolysaccharides; INR, international normalized ratio; CRP, c-reactive protein; APTT, activated partial thromboplastin time.

* Corresponding author. Centralny Szpital Kliniczny im. prof. K. Gibińskiego, Oddział Gastroenterologii i Hepatologii, ul. Medyków 14, 40-752 Katowice, Poland.
E-mail address: mhartleb@sum.edu.pl (M. Hartleb).

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and prothrombin levels (175 ± 98.8 μg/ml vs. 115 ± 72.9 μg/ml; $P < 0.05$) than patients without thrombosis. PVT could be excluded with a 90% negative predictive value when the D-dimer level was below 1.82 mg/l.
Conclusions: Endotoxemia and platelet activity are not determinants of PVT in patients with cirrhosis. The D-dimer measurement has diagnostic significance for PVT in patients with liver cirrhosis.

Introduction

Portal vein thrombosis (PVT) is defined as the narrowing or occlusion of the portal vein, with or without involvement of the intrahepatic portal veins or other segments of the splanchnic venous axis [1]. PVT rarely occurs in the general population, as estimated from autopsy-based studies, and is usually the result of myeloproliferative disease, genetic or acquired thrombophilia or inflammation in the abdominal cavity [2]. Conversely, PVT is a relatively common complication of liver cirrhosis, and according to different studies, it has an estimated frequency of 5—28% [3—5].

The impact of PVT on short-term clinical outcomes in cirrhosis is a matter of debate; however, it may promote variceal bleeding, diuretic-resistant ascites or encephalopathy, and it increases the risk of bowel infarction [6,7]. PVT may also complicate surgical abdominal procedures including liver transplantation [7]. Recent studies in patients with end-stage cirrhosis have shown that the primary prevention of PVT by antithrombotic drugs decreases the prevalence of life-threatening complications and pre-transplant mortality [8—10].

In general, PVT is more commonly found in more clinically advanced cirrhosis than in the early stages of the disease; however, its development is unpredictable, and the risk factors for PVT are not well recognized. According to Virchow's triad principle, a venous thrombosis is the result of the coexistence of blood flow disturbances, endothelial injury and hypercoagulation. For these reasons, PVT in cirrhosis could be linked with endotoxemia, thrombophilia and portal hypertension, or it may have no definite association to any of these factors.

Advanced cirrhosis is associated with profound and complex coagulation defects, with concomitant defective fibrinolysis and impaired synthesis of thromboxane A2 and serotonin by platelets [11]. The net result of all of these defects may be a prothrombotic state, which is likely related to the increased endothelial synthesis of von Villebrand factor (vWf) and an increased level of factor VIII, combined with low levels of hepatic anticoagulation agents such as antithrombin III, protein C and S [12,13].

In experimental and clinical settings, cirrhosis is associated with overgrowth of the intestinal microbiome and decreased intestinal transit, as well as impaired gastric, pancreatic and biliary secretions. The intestinal bacterial overgrowth and portal hypertension may contribute to ''leaky gut'' and lead to systemic endotoxemia [14,15]. Endotoxins are lipopolysaccharides (LPS) present in the outer membrane of Gram-negative bacteria, and their biological activity is related to the lipid A component [16]. The serum levels of LPS in cirrhotic patients are elevated in both the portal and the systemic circulation [17]. The biological consequences of systemic endotoxemia are low-grade inflammation, peripheral vasodilatation and disturbances in lipid and carbohydrate metabolism. LPS also have detrimental effects on immunity, liver fibrosis and hepatic encephalopathy [17].

In vitro studies have revealed that LPS, even in low concentrations, may stimulate vWf release from the endothelium [13]. Moreover, Violi et al. provided evidence of a direct correlation between endotoxemia and the ongoing prothrombotic state in the portal venous system [14]. Therefore, it is plausible that endotoxemia, in combination with the coexisting increased vWf release frequently found in cirrhosis, may trigger prothrombotic mechanisms.

Human zonulin is a physiological regulator of gut permeability that disassembles intestinal tight junctions [18]. Fasano et al. have shown that zonulin induces polymerization of G-actin to F-actin in a protein kinase C α (PKCα) dependent process, suggesting that actin reorganization of the cytoskeleton is involved in the permeability-regulating effects [19]. A more recent study demonstrated that zonulin also operates through activation of the proteinase-activated receptor 2 (PAR2) and transactivation of the epidermal growth factor receptor (EGFR) [20]. Jayashree et al. proposed that human zonulin (pre-haptoglobin 2) could be a biomarker of disrupted integrity of tight junctions in patients with type 2 diabetes [21].

We hypothesized that the main determinant of PVT may be increased intestinal permeability resulting in endotoxemia, which exerts prothrombotic effects on coagulation factors, platelets and endothelium. Therefore, the aim of the study was to examine endotoxemia in cirrhotic patients who developed PVT and its relationship to zonulin serum level and laboratory parameters defining coagulation system.

Patients and methods

Ethics statement

The study protocol was approved by the Local Bioethical Committee of the Medical University of Silesia. All clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki. All patients gave signed informed consent.