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LETTER TO THE EDITOR

“The portal vein in patients with cirrhosis is not an excessively inflammatory or hypercoagulable vascular bed, a prospective cohort study”: comment from Violi et al.

Dear Editor,

We read with interest the study reported by Driever et al. [1], who explored the mechanism that potentially accounts for portal vein thrombosis (PVT) by investigating whether a hypercoagulation status is detectable in the portal circulation of patients with liver cirrhosis (LC) and whether endotoxemia has a role. This issue is of great relevance because PVT is a frequent complication of LC. PVT occurs in ∼10% to 25% of patients, as determined using an ultrasonography series, depending on the severity of the disease, hepatocellular carcinoma, prior PVT, and elderly population [2]. The occurrence of PVT is associated with poor outcomes such as intestinal bleeding and infarction [2]. Prospective studies have reported an incidence of 2% to 4% per year depending on the severity of LC, with a rate of 18.9% in case of patients with PVT at admission [2]. The mechanism accounting for PVT is still debated upon. We and others consistently reported that low-grade endotoxemia by lipopolysaccharides (LPSs) is detectable in patients with LC, with a portosystemic gradient, suggesting that in patients with LC, LPSs translocate into the systemic circulation as a consequence of gut barrier dysfunction [2]. LPSs are a glycolipid component of the outer membrane of gram-negative gut bacteria, which, in the absence of infections or sepsis, circulate in very low concentrations, usually <20 pg/mL [2]. In case of gut barrier dysfunctionality, LPSs may across the epithelial barrier by eluding the intrinsic detoxification mechanism of the epithelial barrier, including alkaline phosphatase and high-density lipoprotein 3 [2]; once they reach the portal circulation, LPSs are also detoxified by specific liver enzymes, such as acyloxyaclyhydroxylase and alkaline phosphatase, or excreted into the bile via scavenger receptors [2]. In patients with liver disease, LPS clearance is likely to be impaired, as evidenced by enhanced LPS localization in the liver cells of patients with nonalcoholic liver steatosis or steatohepatitis [3]. Consequent to impaired liver catabolism, LPSs may reach the systemic circulation, localize at the level of vessel walls, and favor the occurrence of the thrombotic process by acting at the level of several cell lines via their receptor, toll-like receptor 4 [4]. In particular, LPSs elicit endothelial cell (EC) perturbation by the release of von Willebrand factor and factor VIII via formation and secretion of Weibel-Palade bodies or a shift toward a prothrombotic phenotype because of tissue factor overexpression at the level of ECs and monocytes or via the release of neutrophil extracellular traps [2]. The relevance of low-grade endotoxemia as a mechanism favoring thrombosis has been documented in patients with myocardial infarction, in whom LPSs localize within the coronary thrombus in association with leucocyte infiltration and toll-like receptor 4 overexpression, and in animals injected with LPSs (0.5 mg/kg), resulting in low-grade endotoxemia with a systemic concentration of 40 pg/mL and accelerated thrombus growth [5]. Low-grade endotoxemia may accomplish 3 factors that, according to the Virchow theory, are necessary for the occurrence of venous thrombosis, ie, hypercoagulability, endothelial perturbation, and venous stasis. Thus, enhanced levels of the prothrombin fragment F1+2, a marker of thrombin generation, coincidentally with high levels of circulating LPSs, have been detected in the portal circulation compared with those in the systemic circulation of patients with LC [6]; in addition, Shalaby et al. [7] reported a portosystemic endotoxemia gradient in patients with LC with a higher concentration of microparticles from an endothelial origin, suggesting EC perturbation by LPSs. Endotoxemia may also affect the third component of the Virchow triad, ie, venous stasis. Thus, experimental and clinical studies of LC have demonstrated overproduction of nitric oxide, a powerful vasodilator molecule, with a correlation with endotoxemia, an effect putatively attributable to upregulation of endothelial nitric oxide synthase or inducible nitric oxide synthase by LPSs [2]; interestingly, oral administration of a nonabsorbable antibiotic elicited simultaneous reduction of nitric oxide and endotoxemia [8]. Even if, altogether, these experimental and clinical studies provide a biologically plausible role of LPSs as a factor potentially favoring PVT, a cause-effect relationship *in vivo* is yet to be demonstrated. The study by Driever et al. [1] is, however, in contrast with this hypothesis because they did not find a portosystemic gradient of LPS levels, which would negate the role of LPSs as prothrombotic molecules in the portal circulation of patients with LC, and suggested that portal hypertension per se, but not hypercoagulability, is a major determinant of PVT. Despite the interest in this novel interpretation of PVT in patients with LC, we believe that the study by Driever et al. [1] has methodologic and clinical issues that limit such a conclusion. The analysis of LPSs in human blood is a cumbersome assay that requires an accurate technology to exclude blood endotoxin contamination. Thus, we previously reported that blood sampling and the endotoxin assay should be performed in a laminar flow bank, and all laboratory material should be sterile. In addition, in order to be certain that the procedure is performed using an aseptic technique, 2 blank

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values were to be prepared for each test sample by adding endotoxin-free water instead of the same volume of diluted plasma or Limulus amoebocyte lysate [9]. Although Driever et al. [1,10–14] reported that the blood LPS levels were measured using the enzyme-linked immunosorbent assay, they did not report how the LPS analysis was performed or whether they accurately avoided any form of endotoxin contamination during blood sampling and manipulation; in accordance with this, none of the 4 studies [10–14] cited to substantiate LPS level measurement reported the LPS assay among the biomarkers examined. Therefore, we suspect that Driever et al. [1] overlooked the accuracy necessary to exclude blood endotoxin contamination during blood sampling and manipulation, which likely precluded the observation of a portosystemic gradient of the LPS levels. In accordance with this, the LPS values were 79 pg/mL in healthy subjects, ie, 4 times higher than the values commonly found in healthy subjects [2], and 130 to 160 pg/mL in patients with LC, ie, ~3 times higher than blood LPS levels previously reported in patients with LC [10,11]. Apart from this methodologic issue, an alternative explanation for these high values may be to consider the clinical status of patients with LC as concomitant infections, which could have enhanced the systemic LPS levels, thus precluding—also in this case—an accurate separate analysis of the venous districts; however, they did not report whether infections were an exclusion criterion. The consequence of these arguments is that Driever et al. [1] did not have sufficient elements to exclude endotoxemia as a factor favoring hypercoagulability and, eventually, PVT in patients with LC; therefore, further studies are necessary to support or deny this hypothesis.

AUTHOR CONTRIBUTIONS

F.V. conceived and wrote the manuscript. R.C. and P.P. discussed and revised the manuscript.

DECLARATION OF COMPETING INTEREST

There are no competing interests to disclose.

Francesco Violi[1,2]
Roberto Carnevale[2,3]
Pasquale Pignatelli[1,2]

[1]*Department of Clinical Internal, Anaesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy*
[2]*Mediterranea Cardiocentro-Napoli, Naples, Italy*
[3]*Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy*

Correspondence
Francesco Violi, Department of Clinical Internal, Anaesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Viale del Policlinico155, 00161 Rome, Italy.
Email: francesco.violi@uniroma1.it

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