Endotoxemia as a trigger of thrombosis in cirrhosis

Cirrhosis is associated with a so called "coagulopathy", which is underscored by the prolongation of global clotting tests, hyperfibrinolysis and thrombocytopenia.[1] These laboratory changes have been suggested to predispose to bleeding complications, but recent data have challenged this hypothesis.[2]

Analysis of the interplay between bleeding and cirrhosis revealed that spontaneous and provoked bleeding events are not always associated with clotting changes; of note, apart from gastrointestinal bleeding, which is unrelated to clotting changes, spontaneous (serious) bleeding such as cerebral hemorrhage is infrequent amongst patients with cirrhosis.[3]

In contrast with the putative association between "coagulopathy" and bleeding in cirrhosis, recent data indicate that the clinical history of cirrhosis may be complicated by thrombotic events in the peripheral and portal circulation, which worsens clinical outcomes of cirrhotic patients.[3]

Cirrhosis is the underlying cause of portal vein thrombosis (PVT) in 22-28% of all cases.[3] In studies using angiography or surgery, the prevalence of PVT ranged from 0.6% to 16%, whilst ultrasonography studies reported a prevalence as high as 10-25%.[3] The prevalence of PVT increases with the severity of cirrhosis, being approximately 1% in patients with compensated cirrhosis and rising to 8-25% in candidates for liver transplantation.[3] For example, one study reported that the 5-year cumulative incidence of PVT was 10.7% in 1,243 patients with mild to moderate cirrhosis (Child-Pugh classes A and B); prothrombin time and esophageal varices were the only variables associated with PVT.[4]

The detection of a hypercoagulable or prothrombotic state in cirrhosis may account for the increased risk of venous thrombosis associated with this condition. Thus, using highly sensitive tests of a hypercoagulable state, such as pro-thrombin fragment F1+2, D-dimer, high-molecular weight fibrin/fibrinogen complexes or soluble fibrin, clotting activation with secondary hyperfibrinolysis has been detected in about 30% of cirrhotic patients.[3] The concept that cirrhosis is associated with an ongoing pro-thrombotic state has been supported by experiments suggesting that an enhanced ratio of factor VIII/natural anticoagulants is implicated in clotting activation.[1] However, the precise mechanism accounting for hypercoagulation status and eventually venous thrombosis in cirrhosis has not been elucidated.

Endotoxemia and thrombotic risk

There are consolidated experimental and clinical data that bacterial endotoxin such as Lypopolysaccharide (LPS) predisposes to thrombosis by increasing thrombin generation *via* tissue factor (TF) up-regulation.[5] Patients with cirrhosis have increased levels of bacterial endotoxins in the portal and systemic circulation compared to controls.[3,6] This "low-grade" endotoxemia is related to the translocation of bacteria and bacteria products such as endotoxins from the intestinal lumen to the portal circulation, and to endotoxin spillover into the systemic circulation.[7] Accordingly, we and others have previously documented an endotoxemia gradient between the portal and peripheral circulation, with significantly higher levels in the portal compared to the peripheral circulation.[3,6] This would suggest that endotoxemia originating from the intestinal tract may contribute to extra-hepatic manifestations of cirrhosis, including PVT and VTE.[3]

These data lead us to hypothesize that low-grade endotoxemia might favor thrombosis. In support of this, the experimental and clinical studies would demonstrate that in cirrhosis, endotoxemia affects the Virchow's triad, i.e. hypercoagulation, endothelial damage and reduced flow velocity, which are crucial for thrombus formation.

Experimental studies by our group have investigated the interplay between endotoxins and clotting activation in the peripheral and portal circulation of cirrhotic patients undergoing transjugular portosystemic shunt.[3]

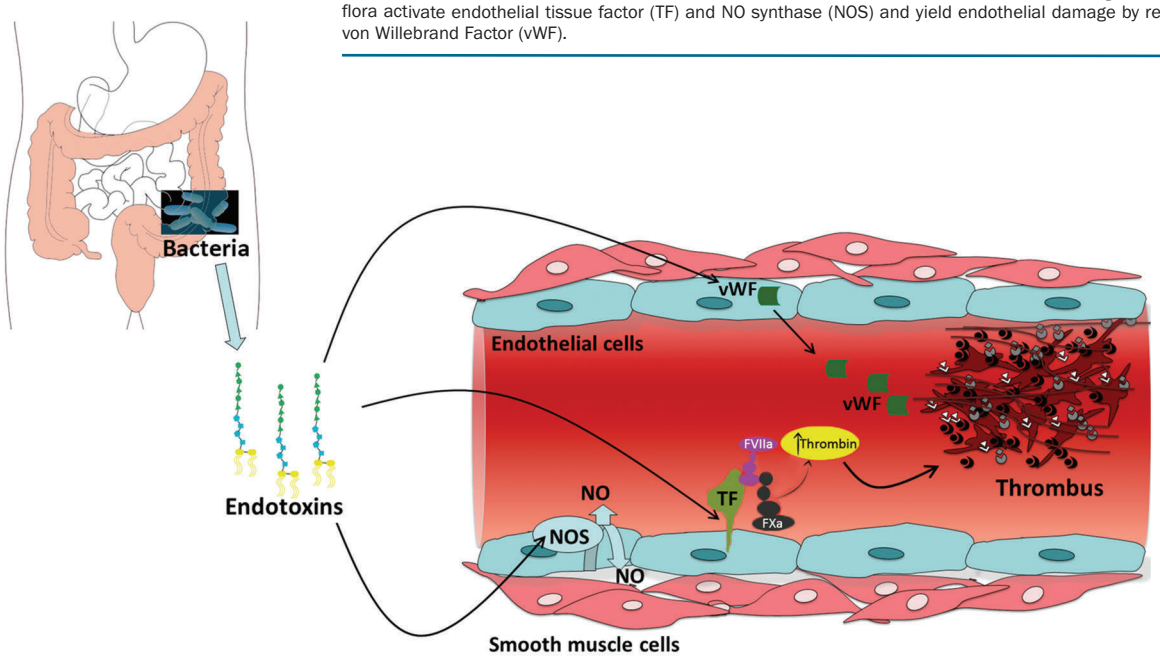

Figure 1. Hypothetic mechanism accounting for venous thrombosis in cirrhosis. Endotoxins originated by gut flora activate endothelial tissue factor (TF) and NO synthase (NOS) and yield endothelial damage by releasing von Willebrand Factor (vWF).

Thrombin generation, as assessed by plasma levels of prothrombin fragment F1+2 and D-dimer, were increased in the peripheral circulation of cirrhotic patients compared to controls; also, both thrombin generation and hyperfibrinolysis were elevated in the portal compared to peripheral circulation, and correlated with endotoxemia. These findings suggest that endotoxins generated by gut flora yield an ongoing pro-thrombotic state in the portal and (eventually) systemic circulation of cirrhotic patients.[3]

Based on the fact that endotoxins up-regulate tissue factor (TF) at levels of endothelial cells, thus triggering factor VII activation and coagulation cascade,[5] we measured TF expression by monocytes taken from the peripheral circulation of cirrhotic patients. This experiment compared TF mRNA expression in monocytes of cirrhotic patients with normal or elevated endotoxemia, and demonstrated that TF mRNA was expressed only in patients with elevated values of endotoxemia.[8] Together these findings indicate that, in cirrhosis, endotoxemia is a plausible mechanism accounting for a hypercoagulation state *via* overexpression of TF (see Figure 1).

Endotoxemia may also be a determinant for splanchnic vasodilatation, which is a key factor for portal circulation venous stasis. Experimental and clinical studies documented that endotoxins enhance the expression of inducible nitric oxide (NO) *via* NO synthase up-regulation. Thus, cirrhotic patients show raised levels of serum endotoxins and NO bio-products, such as nitrite and nitrate.[7] This suggests that NO overproduction is responsible for portal and systemic vasodilatation[7] (Figure 1).

Endothelial dysfunction or damage is representative of the third component of Virchow's triad, which may be affected by endotoxins. Indeed, von Willebrand factor (vWF) levels are elevated in cirrhosis (particularly in patients with severe liver failure), correlate with portal hypertension and are independently predictive of mortality.[9]

Thus we suggest that endotoxemia could be implicated in endothelial dysfunction because of a significant correlation between circulating vWF and endotoxemia. Further support for this hypothesis has been provided by an experimental study showing that human umbilical vein endothelial cells incubated with 125 to 500 pg/mL LPS released vWF antigen into the medium in a dose-dependent manner.[3]

Such endothelial activation by endotoxins may account for the increase of factor VIII, as the enhanced plasma vWF levels observed in cirrhosis could reduce hepatic clearance of factor VIII and, ultimately, determine its elevation[10] (Figure 1).

Perspectives and conclusions

Increased endotoxin levels in the portal and systemic circulation represent a plausible biological mechanism accounting for the increased risk of thrombosis in the portal and systemic circulation of cirrhotic patients. Prospective studies are needed to assess if endotoxin blood levels are associated with an enhanced risk of PVT and VTE.

The treatment of PVT in cirrhosis is becoming a new and challenging scenario, particularly in the era of non-Vitamin K antagonist oral anticoagulants, but planning such trials with anticoagulants might be difficult because the perception of "coagulopathy in cirrhosis" is likely to be a barrier against the use of anticoagulants. For this reason, thrombosis prevention would be a more realistic and easier to approach objective by lowering endotoxemia, for example, with non-absorbable antibiotics or probiotics. This approach may be of interest as these drugs reduce endotoxemia and clotting activation[3] and could perhaps lower the thrombosis risk.

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