Background and Aim: Lymphatic vessels (LVs) are crucial for maintaining abdominal fluid homeostasis and immunity. Dilated and dysfunctional gut lymphatic vessels (LVs) have been reported in experimental cirrhosis. Here, we studied LVs in duodenal (D2)-biopsies of liver cirrhosis patients and investigated the prognostic role of a LV marker, podoplanin (PDPN), in predicting the mortality of patients with cirrhosis. Also, Given the established role of VEGF-C in improving LVs, we hypothesized that VEGF-C treatment could ameliorate the functions of mLVs in cirrhosis.

Method: A prospective, single-center cohort study was performed in liver cirrhosis patients (n = 31) and matched healthy controls (n = 9). D2-biopsies were obtained during the endoscopy procedure, immunostained with PDPN, and scored based on 1) intensity and 2) density of positively-stained LVs per high power field. Gut and systemic inflammation in cirrhotic patients were estimated by quantifying duodenal $CD3^+$ intraepithelial lymphocytes (IELs), $CD68^+$ macrophages, and serum TNF-α and IL-6 levels, respectively. Gut permeability and inflammation as assessed by quantifying gene expression of *TJP1*, *OCLN*, *TNF-α,* and *IL-6* in D2-biopsies. Cox regression analysis was performed with PDPN score. In experimental rat models of liver cirrhosis, we targeted the gut LVs orally by recombinant human VEGF-C(Cys156Ser) protein (E-VEGF-C). Cirrhotic rats were given formulation without VEGF-C served as vehicles. Drainage of mLVs in models was analyzed using tracer dye. Portal and systemic physiological assessments and computed tomography were performed in animal models to measure portal pressures and ascites. Gene expression and permeability of primary mesenteric lymphatic endothelial cells (LyECs) from models were studied. Immune cells in mesenteric lymph nodes (MLNs) of animal models were quantified by flow cytometry. Endogenous and exogenous gut bacterial translocation to MLNs was examined.

Results: Gene expression of LV markers, *PDPN* (8-fold), and *LYVE1* (3-fold) was enhanced in D2-biopsies of cirrhosis patients compared to control ($p < 0.0001$). The mean PDPN score in decompensated cirrhosis patients (6.91 ± 1.26, $p < 0.0001$) was significantly increased as compared to those with compensated (3.25 ± 1.60). PDPN score positively and significantly correlated with the number of IELs (r = 0.33), serum TNF-α (r = 0.35), and IL-6 (r = 0.48) levels, while inversely correlated with *TJP1* expression (r = -0.46, $p < 0.05$ each). In Cox regression, the PDPN score was a significant and independent 3-month-mortality predictor in patients (HR: 5.61; 1.08-29.109; $p = 0.04$). The area under the curve for the PDPN score was 84.2, and the cutoff value for predicting mortality was ≥6.5 with 100% sensitivity and 75% specificity. In cirrhotic rats, we reported the same observation of dilated and leaky mLVs with impaired drainage. Treatment with E-VEGF-C in experimental models induced the proliferation of mLVs, reduced their diameter and improved functional drainage. Ascites and portal pressures were significantly reduced in E-VEGF-C rats compared to vehicle rats. In MLNs of E-VEGF-C rats, CD8+CD134+ T-cells were increased while CD25+Treg-cells were decreased. Both endogenous and exogenous bacterial translocation was limited to MLNs in E-VEGF-C treated rats with reduced levels of endotoxins in ascites and blood in comparison to vehicle rats. E-VEGF-C treatment in rats upregulated the expression of VE-Cad in LyECs of mesentery and functionally improved the permeability of these cells.

Conclusion: Collectively, dilated LVs with high PDPN expression in D2-biopsies are a characteristic feature of patients with decompensated cirrhosis. PDPN score correlates with enhanced gut and systemic inflammation and is associated with 3-month mortality in cirrhosis. E-VEGF-C treatment in experimental cirrhosis models ameliorates mesenteric lymph drainage, and portal pressure and strengthens cytotoxic immunity in MLNs. It may thus serve as a promising therapy to manage ascites and reduce pathogenic gut bacterial translocation in cirrhosis.

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