BRIEF REPORT

Low ADAMTS-13/VWF ratio and altered gut–liver axis predict complications of advanced chronic liver disease: a pilot study

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Introduction

Liver cirrhosis is characterized by a delicate balance between thrombosis and bleeding. Parenchymal extinction due to microvascular thrombosis is involved in disease progression and anti-coagulant therapy with low-molecular-weight heparin has been shown to reduce the frequency of hepatic decompensation [1]. Dysbiosis and bacterial translocation, which are hallmarks of liver cirrhosis, can influence the coagulation system through inflammation or by the activation of Toll-like receptors on endothelial cells and platelets [2]. However, little is known about the connections between the coagulation system, the occurrence of liver cirrhosis complications, and the gut microbiome.

Patients with advanced chronic liver disease (ACLD) have high serum levels of von Willebrand factor (VWF) due to endothelial activation caused by portal hypertension and inflammation, and low levels of a disintegrin and metalloprotease with thrombospondin 1 repeats number 13 (ADAMTS-13), causing an enzyme–substrate imbalance [3]. ADAMTS-13 deficiency usually results in thrombotic microangiopathy; however, alterations in hemostatic parameters have been associated with outcomes in patients with ACLD rather than with coagulation potential, as recently demonstrated for the increase in the factor VIII/protein C ratio [4]. At present, little is known about the association of the ADAMTS12/VWF ratio with complications of ACLD.

In this pilot study, we investigated the prognostic role of the ADAMTS-13/VWF ratio on the development of decompensated ACLD. We also explored its correlation with the composition of the gut microbiome and its metabolites.

Patients and methods

This study was carried out in the Hepatology Unit of the Fondazione Policlinico Universitario “Agostino Gemelli” IRCCS in Rome, from March 2017 to May 2021; this study included patients with ACLD classified into Child–Pugh A without signs of decompensation, including ascites, hepatic encephalopathy, gastrointestinal bleeding, jaundice, previous portal hypertension-
related gastrointestinal bleeding, or hepatocellular carcinoma (HCC) [5]. The following conditions were considered as exclusion criteria: extrahepatic malignancies, active alcohol intake in the past year, diseases or drugs that can potentially affect the gut microbiota, treatment with anticoagulant/antiplatelet agents, known congenital coagulation disorders, and severe thrombocytopenia (<30,000/μL). Participants underwent clinical evaluation from collection of medical history and demographic data, routine blood tests, determination of ADAMTS-13, VWF, circulating cytokines (e.g. interleukin [IL]-1beta, IL-2, IL-6, interferon [IFN]-gamma, tumor necrosis factor [TNF]-alpha), C-reactive protein (CRP), and lipopolysaccharide (LPS). Stool samples were also obtained for analysing gut microbiome composition and metabolomics profile. These analyses were carried out as previous studies described [6, 7]. Each patient was followed up every 6 months for 1 year to monitor the occurrence of complications (ascites, encephalopathy, portal hypertension-related bleeding, infections, HCC, portal vein thrombosis). The study was approved by the institutional ethics committee (ID 741) and all participants provided written informed consent prior to inclusion.

**Statistical analysis**

Categorical variables were expressed as frequencies and percentages; continuous variables were shown as medians and interquartile ranges. Wilcoxon’s test and Spearman’s coefficient were used to assess differences in the ADAMTS-13/VWF ratio between groups and to evaluate correlations between continuous variables, respectively. The evaluation of the gut microbiome alpha diversity and differential abundance, as well as fecal metabolomics analysis, was performed as previously described [7, 8].

**Results**

Among 79 consecutive patients with ACLD, a total of 18 patients fulfilling the inclusion criteria were enrolled in this study (Supplementary Table 1). There were no statistically significant differences in the ADAMTS-13/VWF ratio with respect to disease etiology (P = 0.778). However, we observed that ADAMTS-13/VWF had negative correlations with the model for end-stage liver disease (MELD; r = −0.57, P = 0.014) and CRP (r = −0.54, P = 0.020), and a positive correlation with platelets count (r = 0.51, P = 0.031), as shown in Supplementary Table 2. No significant correlation between ADAMTS-13/VWF and serum cytokines was found.

During the follow-up, complications occurred in three participants. Two of them developed HCC, portal hypertension-related gastrointestinal bleeding, and infections requiring hospitalization; one of these two patients also developed ascites, hepatic encephalopathy, and portal vein thrombosis. The third patient presented with portal hypertension-related gastrointestinal bleeding. The ADAMTS-13/VWF ratio was significantly lower in patients who developed complications than in patients without complications (0.25 [0.22–0.33] vs 0.71 [0.57–1.13], P = 0.009), whereas LPS serum level was higher in patients with complications than in patients without complications (207.58 [132.49–211.78] vs 29.95 [14.20–45.12] EU/mL, P = 0.048) (Figure 1A and B). Among the differential abundant gut bacterial taxa in patients with a low ADAMTS-13/VWF, Akkermansia was significantly depleted (log2-fold change [log2FC] = −5.30, false discovery rate [FDR] = 0.03), as shown in Figure 1.

**Figure 1.** (A)–(C) ADAMTS-13/VWF, LPS serum levels, and Akkermansia abundance (plotted as log count) according to the development of complications. (D) PLSDA plot showing metabolic features of patients who developed ACLD complications (green) and those who did not (red). (E) VIP scores plot highlighting the main metabolites involved in the discrimination between groups (score of >1 was considered significant). (F) Metabolic enriched pathways in patients who developed complications. ADAMTS-13, a disintegrin and metalloprotease with thrombospondin 1 repeats number 13; VWF, von Willebrand factor; LPS, lipopolysaccharide; PLSDA, partial least squares-discriminant analysis; ACLD, advanced chronic liver disease; VIP, variable importance in projection.
Supplementary Table 3 (Figure 1C). A peculiar fecal metabolic profile was also observed in patients with complications compared with those without (Figure 1D). 3-Hexanone, hexadecane, arabinose, 4-hydroxyphenylacetate, and valine were important metabolites in differentiating the two groups (Figure 1E) and the main metabolic pathways enriched in patients with complications were related to the biosynthesis of pantothenate and coenzyme A (CoA); the interconversion of pentoses and glucuronate; the biosynthesis and degradation of valine, leucine, and isoleucine; and the biosynthesis of aminoacyl-tRNA (Figure 1F).

Discussion

The progression of liver cirrhosis has been associated with intra-parenchymal microvascular thrombosis, so recent attention has been paid to the predictive value of the hemostatic balance markers for complication development and survival. During chronic liver damage, ADAMTS-13 activity is reduced and VWF release increased, leading to microcirculatory disturbance [3]; however, it is not clear whether this change in the ADAMTS/VWF ratio may be related to worsening portal hypertension and the development of ACLD complications. In this study, we used the ADAMTS-13/VWF ratio as a prognostic factor in patients with compensated ACLD and showed that its reduction precedes the development of complications. This supports the hypothesis that imbalance in the hemostatic system is not simply a consequence of liver disease decompensation, but rather may act as a promoting factor at the parenchymal level.

Interestingly, patients with complications presented high LPS serum levels, low abundance of Akkermansia in the gut microbiota, and low ADAMTS-13/VWF ratios. The stimulatory effect of LPS on the release of VWF is well known [9]; the lack of Akkermansia may contribute to increase intestinal permeability and enhance gut-derived inflammation. Thus, leaky gut may be involved directly or indirectly in the modulation of ADAMTS-13/VWF and in the development of ACLD complications. Besides, two participants developed HCC. Akkermansia depletion and ADAMTS-13/VWF ratio reduction have been associated with HCC in patients with AILD [10, 11]. Taken together, these data highlight a possible link between inflammation arising from the gut microbiota, coagulation profile, and hepatocarcinogenesis that needs further and deep investigation.

Finally, the fecal metabolomic profile of patients who developed complications was characterized by the differential expression of metabolites and the activation of pathways involved in the production of amines, further producing ammonia [12]. This implies that an already compromised metabolic function of the gut microbiome was present in these patients, which was a prelude to the development of decompensation.

In conclusion, although this study was limited by the small number of participants, it was able to give a preliminary demonstration of the association between the ADAMTS-13/VWF ratio and the development of ACLD complications in patients who were previously compensated. Our findings shed further light and open a debate on the interconnection between the gut-liver axis, inflammation, and the development of complications in patients with ACLD.

Supplementary Data

Supplementary data is available at Gastroenterology Report online.

Authors’ Contributions

F.R.P. conceived the study; M.S., M.T., F.R.P., F.S., A.M., and S.L. collected the data; F.R.P., M.S., M.T., and S.L. analysed and interpreted the data; F.R.P., A.M., R.D.C., and M.P. drafted the manuscript; R.D.C. and M.P. supervised the study. All authors read and approved the final manuscript.

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Conflict of Interest

None declared.

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