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# Plasma ADAMTS-13 protein is not associated with portal hypertension or hemodynamic changes in patients with cirrhosis

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ABSTRACT

*Background:* Activated hepatic stellate cells synthesize the matrix metalloprotease ADAMTS13, which may be involved in the development of liver cirrhosis and portal hypertension. Plasma ADAMTS13 activity has been reported as both increased and decreased in cirrhosis, but ADAMTS13 protein has not previously been examined.
*Aim:* To evaluate ADAMTS13 protein in the hepatic circulation and the relation to disease severity, portal pressure, and systemic hemodynamics in cirrhotic patients.
*Methods:* Sixty-one cirrhotic patients (Child class: A = 22; B = 21; C = 18) and nine healthy controls underwent a liver vein catheterization with measurement of splanchnic and systemic hemodynamics, and plasma ADAMTS13 protein concentration in a hepatic vein and the femoral artery.
*Results:* ADAMTS13 protein concentrations were increased in cirrhotic patients compared with controls (774 ng/ml [IQR: 585–955] vs. 538 ng/ml [IQR: 484–631], $p < 0.03$). There were no significant correlations to MELD score, Child Pugh score, portal pressure, nor systemic vascular resistance or cardiac output.
*Conclusions:* The increased concentration of ADAMTS13 protein in the hepatic circulation may reflect an increased number of active hepatic stellate cells in cirrhosis. However, ADAMTS13 was unrelated to portal hypertension and systemic hemodynamics. In conclusion, ADAMTS13 does not appear to be associated to disease severity or the hemodynamic derangement in patients with cirrhosis.


## 1. Introduction

Cirrhosis is associated with development of portal hypertension and hemodynamic changes, which lead to severe complications and increased mortality. The cellular mechanisms leading to the generation of fibrosis and cirrhosis are complex. Hepatic stellate cells (HSCs) transdifferentiate into myofibroblasts during liver fibrosis followed by proliferation and deposition of abnormal extracellular matrix. HSCs are believed to be a key contributor in the pathological processes leading to fibrosis and cirrhosis. Activated HSCs have increased expression of several matrix metalloproteases (MMP) and tissue inhibitors of matrix metalloproteases (TIMP). In both experimental and human studies, these proteases have been shown to be involved in the hepatic fibrogenesis and fibrolysis, and it is most likely the TIMP-MMP balance that determinates the degree of fibrogenesis [1–3]. These profound changes of the liver architecture result in the development of portal hypertension. Therefore, research has focused on the possible role of both MMPs and TIMPs in the underlying pathophysiology of portal hypertension. ADAMTS13 is a MMP primarily synthesized in HSCs. In animal models of acute liver injury and hepatic fibrosis ADAMTS13 activity in plasma is increased and correlates positively with the severity of fibrosis [4]. However, in clinical studies of patients with cirrhosis, decreased ADAMTS13 concentrations have been observed and have been attributed to declining organ function or inflammation [5,6]. ADAMTS13 is involved in the regulation of the primary hemostasis by cleaving von Willebrand factor (vWF). Recently, vWF has been associated with the degree of portal hypertension in patients with cirrhosis, and elevated concentrations of vWF

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seem to be an independent predictor of poor outcome [6]. Since vWF is the substrate of ADAMTS13 it has been hypothesized that ADAMTS13 may be involved in the development of portal hypertension and hemodynamic changes in cirrhosis. Previous studies in patients with cirrhosis have investigated ADAMTS13 activity, but this is a suboptimal way of assessing ADAMTS13 in cirrhosis due to biochemical disturbances caused by the liver disease. Especially, increased bilirubin concentrations are known to interfere with ADAMTS13 activity measurements [7]. Therefore the plasma mass levels of ADAMTS13 provide a more detailed understanding of the role of ADAMTS13 in cirrhosis.

The aim of this study was to examine plasma mass concentrations of ADAMTS13 in the hepatic circulation of patients with cirrhosis and to evaluate the relation to portal pressure, liver function, and systemic hemodynamic alterations.

## 2. Methods

### 2.1. Patients

We retrospectively investigated a population of 61 patients (41 men and 20 women) with cirrhosis verified by either liver biopsy or with presence of classical clinical and biochemical characteristics of cirrhosis and presence of portal hypertension, i.e. a hepatic venous pressure gradient (HVPG) above 5 mmHg. According to the modified Child-Turcotte classification, 22 patients belonged to class A, 21 to class B, and 18 to class C. The median age of the patients was 59 years (range 34–78). The etiology of cirrhosis was excessive alcohol intake (i.e., a consumption exceeding 50 g/day for more than 5 years) in 40 of the patients, whereas the remaining 21 patients had post-hepatitic, cryptogenic or autoimmune etiology. 12 patients received beta blockers as prophylaxis against variceal hemorrhage and this treatment was discontinued for 48 hours prior to the hemodynamic investigation. None of the patients had hepatic encephalopathy, alcoholic hepatitis, received antibiotics or had experienced gastrointestinal bleeding within the last week. Our control group consisted of nine individuals (six men and three women) with a median age of 76 (range 53–81) with abdominal pain in whom a catheterization had been performed in order to exclude intestinal ischemia. None of the controls had intestinal ischemia, nor did they show clinical or biochemical signs of liver disease. Portal hypertension was ruled out in all controls by catheterization. Written informed consent was obtained from all study participants prior to inclusion.

### 2.2. Catheterization

All participants underwent a hemodynamic investigation in the morning after an overnight fast. Hepatic veins, right atrium, and femoral artery were catheterized as described elsewhere [8]. The HVPG was determined as the wedged minus free hepatic venous pressure. The hepatic blood flow, postsinusoidal resistance, and indocyanine green clearance (ICG clearance, a test, that reflects the metabolic/excretory liver function) were determined as previously described [8,9]. The mean arterial pressure (MAP) was determined by electronic integration of the pressure signal. Cardiac output (CO) was measured by the indicator dilution method, as previously described [8]. Systemic vascular resistance (SVR) expressed in dyn s $cm^5$ was assessed as 80 × (mean arterial pressure − right atrial pressure/CO); pressures were expressed in mmHg, and CO in liters per minute. An indwelling polyethylene catheter was placed in the femoral artery, and the arterial blood pressures were measured directly by a capacitance transducer. Heart rate was determined by ECG monitoring.

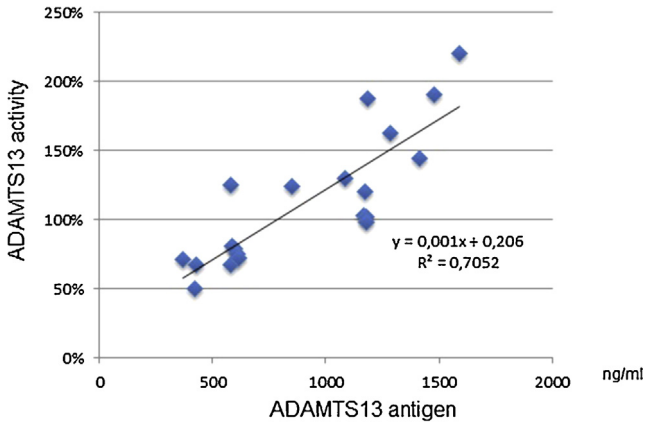


**Fig. 1.** Comparison of plasma protein ADAMTS13 concentration and ADAMTS13 activity by linear regression in 10 cirrhotic patients and 9 controls.

### 2.3. ADAMTS-13

During the catheterization procedure, blood samples (10 ml) were collected simultaneously from the hepatic vein and the femoral artery, discharging the content of the catheter dead space. The samples were centrifuged at 3000 × *g* immediately at 4 °C and heparinized plasma added 10,000 KIU/mL aprotinin were stored at −80 °C until assayed. ADAMTS13 antigen concentration was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (American Diagnostica, Pfungstadt, Germany) performed according to the manufactures instructions. Inter assay CV ($n = 8$) = average of control CV = 8.4%. Intra assay CV ($n = 140$) = average % CV = 4.1%. The enzymatic activities of ADAMTS13 were measured with a fluorescence resonance energy transfer (FRET) assay [10] by use of the fluorescence-quenching substrate FRETS-VWF73 peptide (Pepta Nova, Sandhausen, Germany). In brief, the VWF peptide fragment used in the assay contains the ADAMTS13 cleavage-site (Tyr1605-Met1606). On either site of the cleavage-site, an amino acid (Gln1599 and Asn 1610) is substituted by a fluorescence probe (2-(N-methylamino)benzyl) and a quencher (2,4-dinitrophenyl), respectively. The fluorescent probe is exited at 340 nm and cleavage of the substrate emits light at 440 nm. The enzymatic activities of the plasma samples were measured as a function over time for 60 min at an Enspire® multimode plate reader (Perkin Elmer, Skovlunde, Denmark). To determine the correlation between ADAMTS13 mass concentration and activity both analyses were carried out in a subgroup of 10 patients with cirrhosis together with 9 healthy controls. Linear correlation was performed as shown in Fig. 1.

### 2.4. Ethics

Patients participated after giving their informed consent in accordance with the Helsinki II Declaration and the study was approved by the local Ethics Committee for Medical Research in Copenhagen and Danish Data Protection Agency (J-No.2008-41-2020) and registered Pathophysiological Biobank at Hvidovre Hospital (HVH-2011-02).

### 2.5. Statistical analysis

Results are given as medians and interquartile range (IQR). The Mann–Whitney test was used to compare variables in patients and controls. The non-parametric Kruskal–Wallis one-way analysis of variance (ANOVA) was used to test differences in ADAMTS13 between Child-Turcotte classes. The Wilcoxon test for paired data