Dig Dis Sci (2011) 56:2456–2465
DOI 10.1007/s10620-011-1729-4

ORIGINAL ARTICLE

Idiopathic Noncirrhotic Intrahepatic Portal Hypertension Is Associated with Sustained ADAMTS13 Deficiency

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Received: 26 October 2010 / Accepted: 16 April 2011 / Published online: 15 May 2011

Abstract
Background ADAMTS13 deficiency leading to excess ultralarge von Willebrand factor (VWF) multimers and platelet clumping is typically found in thrombotic thrombocytopenic purpura (a type of thrombotic microangiopathy). Idiopathic noncirrhotic intrahepatic portal hypertension (NCIPH) is a microangiopathy of portal venules associated with significant thrombocytopenia and predisposing gut disorders.

Aim To determine whether the portal microangiopathy in NCIPH is associated with ADAMTS13 deficiency.
Methods Plasma levels of ADAMTS13, anti-ADAMTS13 antibodies, and VWF were compared between cases (NCIPH patients) and controls (with chronic liver diseases of other etiology) matched for severity of liver dysfunction. Eighteen NCIPH patients [median (range) MELD score 12 (7–25)] and 25 controls [MELD score 11 (4–26)] were studied.
Results ADAMTS13 activity was reduced in all 18 NCIPH patients and significantly lower than controls (median, IQR: 12.5%, 5–25% and 59.0%, 44–84%, respectively, $P < 0.0001$) [normal range for plasma ADAMTS13 activity (55–160%)]. ADAMTS13 activity was <5% in 5/18 NCIPH patients (28%) and 0/25 controls ($P = 0.009$). ADAMTS13 antigen levels were also decreased. Sustained low ADAMTS13 levels were seen in four NCIPH patients over 6 weeks to 11 months (highest ADAMTS13 level in each patient: <5%, 6%, 6%, and 25%), despite two patients having MELD score 12. Although nine cases had low titer anti-ADAMTS13 antibodies, there was no significant difference between cases and controls. Abnormally large VWF multimers were observed in 4/11 NCIPH patients (36%) and in 0/22 controls ($P = 0.008$).
Conclusions Sustained deficiency of ADAMTS13 appears characteristic of NCIPH, irrespective of severity of liver disease.

Keywords ADAMTS13 deficiency · Portal venopathy · Noncirrhotic intrahepatic portal hypertension · Enteropathies

Abbreviations
NCIPH Idiopathic noncirrhotic intrahepatic portal hypertension

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ADAMTS13	A disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13
VWF	von Willebrand factor
MELD	Model for end-stage liver disease
TTP	Thrombotic thrombocytopenic purpura

Introduction

Though different terms, mostly describing pathological features, are used to denote idiopathic noncirrhotic intrahepatic portal hypertension (NCIPH), including nodular regenerative hyperplasia, idiopathic portal hypertension, noncirrhotic portal fibrosis, incomplete septal cirrhosis, partial nodular transformation, hepatoportal sclerosis, and benign intrahepatic portal hypertension [1–3], there is increasing consensus that NCIPH is best viewed as a single clinical entity [1, 4, 5]. The primary causative lesion in NCIPH has been shown to be obliteration of portal venous microcirculation on corrosion cast injection studies [6], dissection of intrahepatic vasculature [6], morphometric studies [7], and histological studies of operative wedge liver biopsy samples [8] and of whole liver specimens at autopsy [9].

Microvascular hyaline thrombi of terminal arterioles and capillaries are characteristic of thrombotic thrombocytopenic purpura (TTP) which is associated with deficiency of ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13). von Willebrand factor (VWF), secreted from vascular endothelium as an ultralarge molecular weight form, is normally cleaved into smaller molecular forms by ADAMTS13 [10]. The physiological role of VWF is to facilitate platelet adhesion at sites of endothelial damage. Mutations of the ADAMTS13 gene are seen in congenital TTP, while antibodies to ADAMTS13 are found in 95% of adult acquired idiopathic cases [11]. Decreased ADAMTS13 activity and persistence of ultralarge VWF at the endothelial surface and in circulation is thought to predispose to platelet clumping, causing microvascular occlusion and other features of TTP.

A similar imbalance in VWF and ADAMTS13 levels, if confined to the portal microcirculation at the point where hepatic arterial blood pressures are superimposed, would provide a mechanism for obliteration of terminal portal venules which is characteristic of NCIPH. The hepatic portal microcirculation is the prime target for any low-grade constitutive depletion of ADAMTS13 activity by virtue of its upstream relation to hepatic stellate cells which are the primary [12], perhaps exclusive [13], source of its de novo supply.

Patients with congenital TTP, despite having ADAMTS13 mutations, may present with adult-onset disease. This suggests that some added trigger (such as pregnancy) is required to induce clinical disease [14]. Similarly, ADAMTS13-knockout mice develop TTP only when challenged by infection or elevated VWF levels [15]. Hence, we postulated that, in NCIPH patients, local factors operant in the portal circulation may predispose to selective occlusion of terminal portal venules by platelet-rich thrombi.

We have documented that NCIPH is associated with intestinal pathology such as adult celiac disease and ulcerative colitis [16, 17]. How can inflammation (in the intestine) predispose to thrombotic microangiopathy (of the portal vein branches)? Serum proinflammatory cytokine levels are significantly higher in patients with active celiac disease [18]. Inflammatory cytokines such as interleukin 8 and tumor necrosis factor α stimulate release of ultralarge VWF from endothelial cells, as does interleukin 6 when complexed with its soluble receptor. Interleukin 6 also inhibits ultralarge VWF cleavage by ADAMTS13, under flowing but not static conditions [19]. Inflammatory cytokines also inhibit ADAMTS13 synthesis in hepatic stellate cells and endothelial cells [20]. Accumulation of hyperreactive ultralarge VWF in plasma and on the surface of endothelial cells can induce platelet adhesion and aggregation to the vascular endothelium. Thus, it is plausible that cytokine-stimulated release of ultralarge VWF within the portal circulation in conjunction with nadir concentrations of ADAMTS13 may predispose to microvascular occlusion of portal vein branches characteristic of NCIPH.

One confounder in studying ADAMTS13 deficiency as a possible pathogenic mechanism for NCIPH is that advanced severity of liver disease per se is associated with markedly reduced plasma ADAMTS13 activity. In one study, mean plasma ADAMTS13 activity was decreased to 79%, 63%, and 31% in patients with Child score A, B, and C cirrhosis, respectively. Of 109 patients with cirrhosis studied, ADAMTS13 activity of <3% (severe ADAMTS13 deficiency) was seen only in 5 patients with Child's C cirrhosis [21].

To address this issue, we conducted this study to look for ADAMTS13 deficiency in NCIPH cases and disease controls (with chronic liver disease with other etiology) matched for severity of liver disease.

Materials and Methods

We conducted this case–control study to compare plasma levels of ADAMTS13, anti-ADAMTS13 antibodies, and VWF between cases (NCIPH patients) and controls (patients with chronic liver diseases of other etiology) matched for severity of liver dysfunction. Patients were informed about the study, and consent was obtained from them prior to recruitment into the study.

NCIPH Cases: Case Selection, Demography, Liver Histology, and Laboratory Parameters

The idiopathic NCIPH population consisted of 18 patients (8 F, 10 M), aged 44 (24–64) years [median (range)], who had previously been diagnosed and were under long-term follow-up in the Liver Unit at Queen Elizabeth Hospital, Birmingham. Study inclusion criteria were (1) evidence of portal hypertension, (2) patent hepatic and portal veins on Doppler ultrasound at time of diagnosis of NCIPH, (3) absence of cirrhosis or bridging fibrosis on liver biopsy, (4) exclusion of conditions causing cirrhosis by conventional diagnostic criteria (such as chronic viral hepatitis, alcoholic hepatitis, etc.), and (5) exclusion of conditions that may cause portal venous lesions similar to NCIPH on histology such as congenital hepatic fibrosis and sarcoidosis. All NCIPH study cases met these five criteria. Study exclusion criteria were (1) predominant histological features of another disease process in addition to portal venous insufficiency, (2) NCIPH that developed after liver transplantation, and (3) hepatic malignancy. Fifty-four healthy normal subjects were also studied.

Initial presentation was variceal bleed (10 patients), splenomegaly and thrombocytopenia detected on routine investigation (4), investigation of bruising (2), and thrombocytopenia detected during pregnancy (2). Associated medical diagnoses included adult celiac disease (two patients, one of whom also had ulcerative colitis), and common variable immunodeficiency, Felty's syndrome, and previous pre-eclamptic toxemia (one patient each).

Of the 18 NCIPH patients, liver biopsy showed nodular regenerative hyperplasia in 10 patients. Focal abnormalities in portal vein branches within the portal tracts were seen in 13 NCIPH patients: atretic portal veins (5 patients), absent portal veins (8 patients), ectatic portal veins (4 patients), thickened walls of portal vein (2 patients), portal vein thrombosis (1 patient). The other findings noted were small, incomplete portal tracts (3 patients), focal mild sinusoidal dilatation (3 patients), and mild focal perisinusoidal/pericellular fibrosis (4 patients). Mild biliary changes (in five patients), were deemed to be secondary to portal venous insufficiency. In addition, moderate siderosis within the sinusoidal cells was seen in one patient, and moderate fatty change with some features of steatohepatitis was seen in one patient. No patient had cirrhosis or advanced fibrosis.

At the time of assaying plasma ADAMTS13 levels, other laboratory parameters in the 18 NCIPH cases were as follows: hemoglobin 13.6 (8.8–17.6) g/dl [median (range)], white blood cell count 4.3 (1.1–8.9) $\times$ 10^9/l, platelet count 74 (21–436) $\times$ 10^9/l, serum bilirubin 41 (4–133) μmol/l, serum albumin 37 (29–50) g/dl, international normalized ratio (INR) 1.2 (1.0–1.8), serum creatinine 88 (38–130) μmol/l, and MELD score 12 (7–25).

Disease Controls: Patients with Chronic Liver Disease

The 25 disease controls (14 F, 11 M) consisted of consecutive patients seen in a follow-up clinic for patients with chronic liver disease unrelated to chronic viral hepatitis B and C. Etiology of liver disease was alcohol (10), autoimmune hepatitis (3), primary sclerosing cholangitis (2), primary biliary cirrhosis (2), fibropolycystic disease (2), Budd Chiari (1), and miscellaneous cases with a diagnosis of cryptogenic liver disease (5). These 25 control patients had median (range) MELD score of 11 (4–26) at time of ADAMTS13 assay.

Assays for ADAMTS13 and Its Inhibitors and Antibodies in Plasma

Citrated (0.105 M) whole blood was taken by clean venepuncture with minimal stasis using the Vacutainer system (Becton–Dickinson, Oxford, UK). Platelet-poor plasma was prepared by double centrifugation at 2,000*g* for 15 min with the top two-thirds of the supernatant removed after each step. The plasma was then aliquoted and frozen at −80°C until assay.

ADAMTS13 activity was determined by the residual collagen binding activity of pure VWF after incubation with plasma samples as described by Gerritsen et al. [22] and modified by Yarranton et al. [23]. For inhibitor detection, patient plasma was mixed 1:1 with pooled normal plasma and incubated at 37°C for 1 h. Residual ADAMTS13 activity was measured by collagen binding activity assay. Presence of an inhibitor was defined as reduction of ADAMTS13 activity by >50%. Some samples were also assayed for ADAMTS13 activity using either the fluorescence resonance transfer assay (FRET) method (Actifluor activity assay; American Diagnostica Inc., Stamford, CT, USA) or a peptide substrate chromogenic assay (Technozym activity ELISA; Technoclone, Vienna, Austria). ADAMTS13 antigen was measured using Imubind® ADAMTS13 enzyme-linked immunosorbent assay (ELISA) kit (American Diagnostica Inc.). ADAMTS13 activity-to-antigen ratios were calculated after converting the antigen concentration from ng/ml to a percentage of normal plasma.

IgG anti-ADAMTS13 antibodies were measured using Imubind® ADAMTS13 Autoantibody ELISA kit (American Diagnostica Inc.). IgA and IgM anti-ADAMTS13 were measured by a similar method, using microplates coated with r-ADAMTS13 (American Diagnostica Inc.), a 1/20 dilution of test sample, and polyclonal α- or μ-chain specific antibodies conjugated to horseradish peroxidase (Dakopatts and Sigma–Aldrich, respectively). Results were calculated by comparison of optical density of patient samples with that of healthy normal subjects and by reference to known positive samples.

The normal values were: ADAMTS13 activity by collagen binding assay (55–160%), FRET assay (60–140%), and chromogenic assay (40–130%); ADAMTS13 antigen (485–1,242 ng/ml); IgG ADAMTS13 antibody (≤11 AU/ml).

Assays for VWF

VWF antigen (VWF:Ag) was measured on an automated coagulation analyzer (CA-1500; Sysmex, Kobe, Japan) using an immunoturbidimetric assay (VWF:Ag reagent; Siemens Healthcare Diagnostics, Marburg, Germany). VWF collagen binding (VWF:CB) activity was measured using a method similar to that for ADAMTS13 activity assay, but without prior incubation with pure VWF, inhibitors, and urea [24]. Normal values were: VWF antigen (50–150 IU/dl) and VWF:CB activity (50–150 IU/dl). Multimeric structure of VWF was determined by agarose gel electrophoresis (Phast System; GE Healthcare, Amersham) followed by Western blotting [25]. The results were assessed subjectively by comparison of the migration distances of HMW multimers in patients with a pooled normal plasma tested on each gel. Fibrinogen was measured by a modified von Clauss method, using reagents from Siemens Healthcare Diagnostics (Marburg, Germany) on a CA-1500 analyzer.

Statistical Methods

Data were assessed for normality using Shapiro–Wilk test, and nonparametric data were compared using the Mann–Whitney *U* test and Spearman rank correlation, using a statistical software package (Analyse-it Software Ltd., Leeds) for Microsoft Excel. To facilitate statistical analysis, values of ADAMTS13 activity of <5% were taken as 5%. We compared plasma ADAMTS13 activity with severity of liver disease (assessed by MELD scores) in NCIPH cases and disease controls. Clinical associations were tested using Fisher's exact test (FET).

Results

Plasma ADAMTS 13 Activity and Antigen Levels Were Markedly Reduced in NCIPH Patients Compared with Disease Controls

Thirty-three samples from 18 NCIPH patients who had not received recent treatment with blood products or undergone liver transplant were investigated. Median ADAMTS13 activity (Table 1), as measured by the collagen binding assay, was decreased in all 18 NCIPH patients (median, IQR: 12%,

Table 1 Plasma ADAMTS13 and VWF in patients with NCIPH

Case	MELD	ADAMTS13			VWF		
		Activity (%)	Antigen (ng/ml)	IgG Ab (AU/ml)	VWF:Ag (IU/dl)	VWF:CB (IU/dl)	VWF multimers
1	12	<5	232	17	126	110	Slight increase in HMW forms
2	7	26	215	19	172	143	Normal distribution
3	19	26	278	11	298	159	Normal distribution
4	14	22	235	6	280	229	Normal distribution
5	15	12	239	12	302	231	Normal distribution
6	12	36	461	13	158	125	na
7	10	31	353	16	924	46	na
8	10	11	328	27	210	89	Normal distribution
9	21	<5	123	11	332	275	Increased HMW forms
10	12	<5	181	9	325	263	Slight increase in HMW forms
11	8	26	556	10	242	155	Normal distribution
12	13	21	344	12	240	199	Slight increase in HMW forms
13	14	<5	110	7	675	497	Normal distribution
14	10	18	452	23	255	239	na
15	25	7	162	6	na	na	na
16	12	28	433	29	na	na	na
17	16	<4	188	9	na	na	na
18	12	18	233	11	na	na	na
Normal values		55–160	485–1,242	≤11	50–150	50–150	

Median results for each case

na result not available, *HMW* high molecular weight

Table 2 Plasma ADAMTS13 and VWF in disease controls (chronic liver disease of varying etiology)

Code	MELD	ADAMTS13			VWF		
		Activity (%)	Antigen (ng/ml)	IgG Ab (AU/ml)	VWF:Ag (IU/dl)	VWF:CB (IU/dl)	VWF multimers
1	7	43	469	31	na	na	na
2	7	44	308	7	na	na	na
3	8	52	460	11	304	234	Normal distribution
4	16	12	364	21	297	227	Normal distribution
5	4	90	784	12	108	105	Normal distribution
6	10	81	505	14	244	235	Slight reduction in no. of HMW forms
7	14	48	612	11	343	254	Normal distribution
8	18	80	651	18	312	241	Normal distribution
9	15	68	997	13	230	223	Normal distribution
10	12	63	584	9	301	292	Normal distribution
11	12	52	479	10	329	303	Normal distribution
12	8	91	858	14	152	159	Slight reduction in no. of HMW forms
13	7	26	494	6	248	237	Normal distribution
14	6	107	1,054	56	476	428	Normal distribution
15	10	18	382	10	230	206	Normal distribution
16	24	57	392	26	344	311	Normal distribution
17	10	75	1,000	44	435	320	Slight reduction in no. of HMW forms
18	14	109	817	129	252	255	Normal distribution
19	26	51	407	25	513	457	Normal distribution
20	15	95	862	9	na	na	na
21	8	43	551	9	231	226	Normal distribution
22	18	59	628	7	603	628	Normal distribution
23	23	25	405	14	390	432	Normal distribution
24	5	98	677	15	120	123	Normal distribution
25	Warfarin	66	1,049	8	146	127	Normal distribution
Normal values		55–160	485–1,242	≤11	50–150	50–150	

na result not available, *HMW* high molecular weight

5–25%), with 10 patients having levels <20%, and was significantly lower than in disease controls (median, IQR: 59%, 44–84%, $P < 0.0001$), among whom activity was reduced in only 11/25 subjects, with only two having a level <20% (Table 2). Five NCIPH patients (28%) and none of the disease controls had ≤5% ADAMTS13 activity ($P = 0.009$, FET). Only one NCIPH patient, on a single occasion, had normal (66%) ADAMTS13 activity level (Fig. 1).

A corresponding reduction in ADAMTS13 antigen (Tables 1, 2; Fig. 1) was also observed in cases and disease controls (236, 184–391 versus 584, 442–831 ng/ml, $P < 0.0001$), although the NCIPH patients showed an excess of ADAMTS13 antigen over activity compared with disease controls ($P < 0.0005$) and normal subjects ($P < 0.0001$) (Fig. 2) (median, IQR for ratio of activity to antigen: 0.34, 0.28–0.55 in cases, 0.75, 0.51–0.87 in controls, and 1.05, 0.87–1.27 in normal subjects).

ADAMTS13 activity (studied in all 18 cases and 25 disease controls) was also reduced in NCIPH using other types of activity assay utilizing peptide substrates derived from VWF, although the absolute values were slightly higher than those in the collagen binding assay, in both cases and disease controls: FRET assay (47%, 30–74% versus 101%, 62–137%, $P = 0.0002$) and chromogenic assays (36%, 31–55% versus 63%, 55–92%, $P < 0.0001$) (Fig. 1). The median activity-to-antigen ratios for the FRET and chromogenic assays were 1.03 and 1.13, respectively.

Platelet count was reduced in 15 of 18 patients with NCIPH (median, IQR: 74, 46–113 $\times 10^9$/l) and lower than in disease controls (123, 85–193, $P = 0.0153$), showing an inverse correlation with MELD score in the controls ($rs = -0.53$, $P < 0.01$), but not in cases.

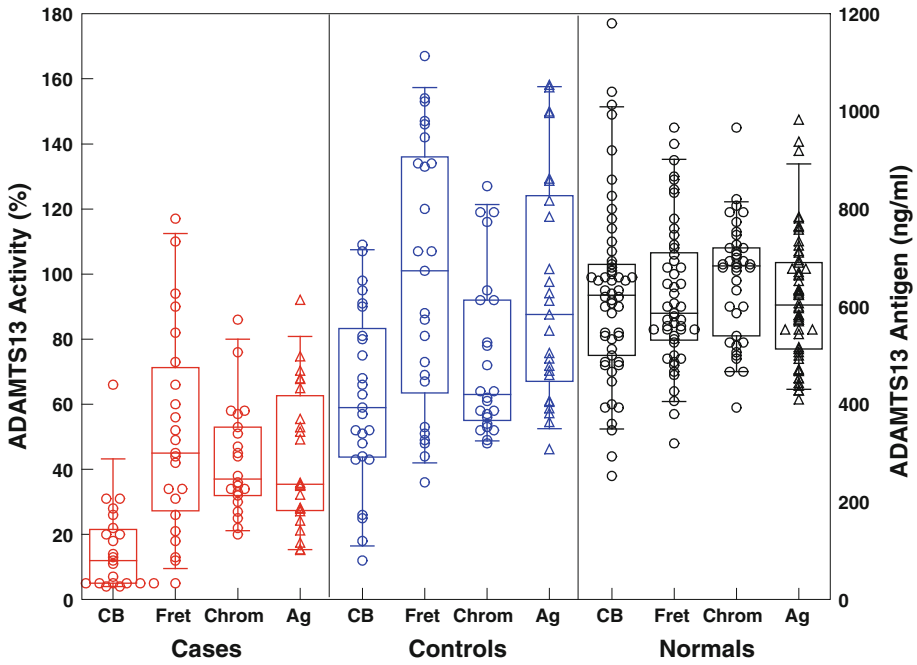

Fig. 1 ADAMTS13 activity and antigen data in all blood samples from NCIPH cases, liver disease controls, and healthy normal subjects. *Left panel* NCIPH results, *middle panel* liver disease controls, *right panel* healthy normal subjects. *CB* collagen binding assay, *FRET* fluorescence resonance transfer assay (American Diagnostica), *Chrom* chromogenic assay (Technozym), *Ag* ADAMTS13 antigen (right-hand *Y*-axis for scale). The *box* shows the 25–75th percentile, the *bar* indicates the median value, and the *whiskers* indicate the 5–95th percentile

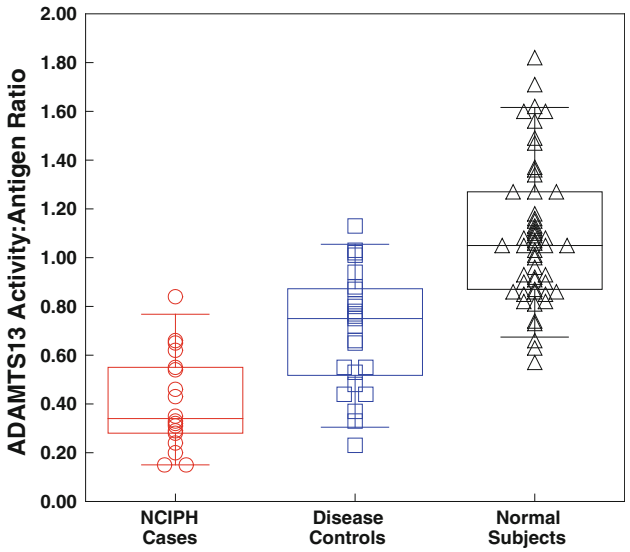

Fig. 2 ADAMTS13 activity-to-antigen ratio in NCIPH cases, disease controls, and healthy normal subjects. Cases = NCIPH patients; Controls = liver disease controls; Normals = healthy normal subjects. The *box* and *whiskers* are defined as in Fig. 1

Deficient Plasma ADAMTS13 Activity Noted in NCIPH Patients Irrespective of Severity of Liver Disease (Even at Low MELD Scores)

ADAMTS13 activity was deficient at all degrees of severity of liver disease (assessed by MELD scores) in NCIPH cases. ADAMTS13 activity and antigen in NCIPH cases showed a trend towards inverse correlation with MELD score ($rs = -0.46$, $P = 0.06$ & $rs = -0.54$, $P = 0.02$, respectively), but this was not seen in disease controls, despite MELD scores being similar in both groups.

NCIPH Patients Lack Inhibitors or Significant Antibodies to ADAMTS13 in Plasma

An inhibitor to ADAMTS13 could not be detected using mixing tests with normal plasma, although IgG antibody to ADAMTS13 was detected in 9 of the cases (Table 1) and 14 disease controls (Table 2). Overall median IgG antibody level in cases was only 13.5 AU/ml, and values were mostly only borderline positive. IgA anti-ADAMTS13 was detected in 3/17 cases and 16/23 controls tested, while IgM anti-ADAMTS13 was detected in 2/7 cases and 3/10 controls tested (data not shown). There was no association between decreased ADAMTS13 activity and presence of IgG, IgM, or IgA anti-ADAMTS13.

One-Third of NCIPH Patients Have Ultralarge VWF Multimers in Plasma

Increased high-molecular-weight VWF multimers, larger than those seen in normal plasma, were detected in plasma in 4/11 NCIPH patients tested (36%) and in none of 22 disease controls tested ($P = 0.008$, FET); rather, 3 control patients had a slight reduction in the higher-molecular-weight multimers.

VWF:Ag and VWF:CB activity (Tables 1, 2) were normal or increased in NCIPH patients (median, IQR: 255, 168–312 IU/dl and 159, 119–243 IU/dl, respectively) and disease controls (299, 230–348 IU/dl and 239, 222–312

IU/dl, respectively). Although the VWF parameters showed good correlation with each other ($r = 0.95$, $P < 0.0001$), neither correlated with ADAMTS13 activity. There was a correlation between MELD score and VWF activity in cases ($rs = -0.55$, $P < 0.05$) and in controls ($rs = -0.59$, $P < 0.01$). There was a trend towards higher VWF activity in patients with lower platelet counts, although this did not reach significance in the cases ($rs = -0.49$, $P = 0.075$ and $rs = -0.63$, $P = 0.002$, in cases and controls, respectively).

Fibrinogen levels (data not shown) were generally normal in patients and did not correlate with ADAMTS13 activity, confirming that hepatic synthetic capacity was not grossly impaired.

Sustained Deficiency of ADAMTS13 Activity in Plasma Documented in All Four NCIPH Patients Studied

Four NCIPH patients had serial assays of plasma ADAMTS13 levels: Case 1 was studied four times over 11 months, and the highest plasma ADAMTS13 activity was 6%, with IgG ADAMTS13 antibody levels of 16, 17, and 55 AU/ml during these 11 months. Case 9 was studied three times over 6 weeks and consistently had <5% activity of ADAMTS13 in plasma. Case 13 was studied three times over 9 months with plasma ADAMTS13 activity levels up to 6%, while case 14 was studied three times over 3 months and plasma ADAMTS13 activity was 14–25%. These four patients had median (range) MELD scores of 12 (10–21) (Table 1).

Plasma ADAMTS13 Levels in NCIPH Patients Were Not Significantly Increased in Hepatic Vein Compared with Peripheral Vein

In five patients, blood samples obtained from the hepatic vein at time of hepatic venous pressure studies were assayed for ADAMTS13 and compared with paired samples obtained simultaneously from peripheral veins. ADAMTS13 activity was identical in four of the paired samples, while the fifth case showed a slight increase (<5% versus 9%). IgG anti-ADAMTS13 was higher in hepatic vein samples from three of the patients (median, IQR: 12, 3 versus 25, 38 AU/ml).

Correction of Plasma ADAMTS13 Deficiency in NCIPH by Infusion of Fresh Frozen Plasma and After Liver Transplantation

One NCIPH patient (case 1) received infusions of fresh frozen plasma on two occasions and demonstrated a rise in ADAMTS13 activity and antigen levels of approximately 50%, despite having IgG anti-ADAMTS13 levels of 55 and 34 AU/ml. Activity levels remained elevated 72 h post infusion. A further patient (case 9) was studied before and after liver transplant, and ADAMTS13 levels were normal post transplant and remained normal at 15 months.

Discussion

Significantly low plasma ADAMTS13 activity in NCIPH patients compared with patients with chronic liver disease (of different etiologies) matched for severity of liver disease is the main finding of our study. ADAMTS13 activity of ≤5% was noted in 28% of NCIPH patients and in none of the disease controls. ADAMTS13 functions as a VWF cleaving protease, and deficient ADAMTS13 activity is characterized by noncleaved, ultralarge molecular weight VWF multimers with increased platelet binding activity. We found abnormally large VWF multimers in 36% of NCIPH patients tested, but not in disease controls.

Though the measurement of ADAMTS13 in several series of patients with chronic liver disease has yielded inconsistent findings [26–28], Uemura et al. [21] convincingly demonstrated that ADAMTS13 activity was reduced progressively with increasing severity of liver disease. However, this does not explain the low ADAMTS13 levels seen in NCIPH patients without severe liver disease (as per MELD score); in our study, five NCIPH patients with MELD scores ≤12 had <20% ADAMTS13 activity, and two of these patients had ≤5% ADAMTS13 activity (Table 1). While the liver slowly atrophies in NCIPH, functional reserve is initially well maintained [29] and 5-year survival following endotherapy for variceal bleed reaches 100% [30].

ADAMTS13 assays are currently poorly standardized with no internationally recognised reference materials available [31, 32], so the choice of assay technology remains controversial. However, we confirmed ADAMTS13 deficiency in NCIPH using other types of activity assay based on small peptide substrates modeled on the VWF cleavage site as well as ADAMTS13 antigen assays. The degree of deficiency in peptide substrate assays was not as great as seen in our assay based on full-length VWF substrate, but remained significant. Although plasma bilirubin concentration can cause overestimation of ADAMTS13 activity in assays based on fluorescent substrates [33], this did not account for the observations in our patients. The discrepancy between assays might be explained by in vivo degradation of ADAMTS13 protein, or altered concentrations of other substances that bind to ADAMTS13 or VWF. This is supported by the fact that ADAMTS13 antigen levels were decreased in NCIPH, with lower ADAMTS13 activity-to-antigen ratios than in disease controls and normal subjects (Fig. 2).

Our observations are compatible with several different explanations linking NCIPH with ADAMTS13 deficiency. ADAMTS13 deficiency could be due to ADAMTS13 mutations and polymorphisms, as in congenital TTP [34], but this has not been investigated in NCIPH. Repeated estimation of ADAMTS13 activity in four of our NCIPH patients with low MELD score (in the absence of overt symptoms of TTP) yielded consistently low results in the absence of ADAMTS13 autoantibodies, suggesting that reduced ADAMTS13 activity in NCIPH is not the consequence of either advanced liver disease or of a fluctuating TTP-like illness. Our inability to detect an incremental rise in ADAMTS13 concentrations within hepatic vein blood in four of five NCIPH patients tested is consistent with low secretory rates. Similarly, our half-life studies of infused ADAMTS13 in NCIPH patients also support the notion that its deficiency is not due to excessively rapid clearance. It is likely, therefore, that low levels of ADAMTS13 may have been a lifelong and primary characteristic of our patients, putting them at risk of developing NCIPH.

In the presence of nadir concentrations of ADAMTS13 in hepatic portal microcirculation, the formation of platelet strings on ultralarge VWF multimers at the endothelial surface would account for microvascular occlusion beyond the site of admixture of portal venous and hepatic arterial blood, the pressure of the latter being a requirement for unfolding of VWF multimers which attracts adherent platelets. These localizing factors would account for the lobule-to-lobule variation in patency or otherwise of portal tract vessels and corresponding variability between adjacent lobules which characterizes hepatic nodular regenerative hyperplasia. Studies in animal models with vascular stenosis or endothelial stimulation by histamine suggest that VWF and ADAMTS13 may have roles in venous thrombosis and inflammation [35, 36], hence high shear rates are not always essential for VWF unfolding, and ultralarge VWF-mediated platelet attachment may play a role in hepatic pathology in lower-shear vessels.

Our current series includes two patients with celiac disease, one of whom also has ulcerative colitis. In a previous series of NCIPH patients, we reported that 9% patients had ulcerative colitis and 16% had celiac disease. We also noted that survival in NCIPH patients was significantly worse in those with undiagnosed and untreated celiac disease [16]. Accordingly, the trend towards inverse correlation of ADAMTS13 levels with MELD ($rs = -0.46$, $P = 0.06$) in our current series of patients would support the view that hepatic atrophy from longstanding NCIPH may produce an accelerated phase of hepatic decompensation as progressively lower levels of ADAMTS13 complete a vicious cycle. In India, where it has been reported to account for 7.9–46% of all cases of portal hypertension, NCIPH is more prevalent in populations from the lower socioeconomic strata in whom tropical sprue is common, and its incidence declines as hygiene improves [37]. Having confirmed this association of NCIPH with intestinal pathology [16], we measured IgA anti-ADAMTS13 antibodies as well as IgG and IgM classes. We did not find anti-ADAMTS13 antibodies in sufficient frequency or concentration to account for the reduction in ADAMTS13 protein concentration and activity observed in NCIPH.

We also observed a disparity in activity-to-antigen ratio for ADAMTS13 between NCIPH cases, disease controls, and normal subjects, which may indicate the presence of proteolytically degraded forms of ADAMTS13 [38]. Abnormalities of glycosylation could potentially contribute to disparity between various activity and antigen assays. *O*-fucosylation and *N*-glycosylation are necessary for efficient secretion of ADAMTS13 [39, 40], while conversion of *N*-glycans from oligomannose to complex types enhances proteolytic activity for VWF [40]. The status of N-linked glycans of VWF has also been shown to modulate cleavage of VWF by ADAMTS13 [41]. Since abnormalities of glycosylation have been noted in many forms of liver disease [42], these could contribute to ADAMTS13 deficiency seen in NCIPH.

In typical nodular regenerative hyperplasia, portal venopathy and hepatic lobular atrophy are singularly accompanied by absence of an inflammatory response and fibrosis. The major source of ADAMTS13 is the hepatic stellate cell, which typically responds to hepatic injury by transforming into a collagen secreting fibroblast. Thus, ADAMTS13 deficiency and lack of hepatic fibrosis in NCIPH may be manifestations of abnormally unresponsive hepatic stellate cells.

We therefore believe our series represents a novel paradigm of pathogenesis in diseases caused by ADAMTS13 deficiency. Constitutively low levels of expression expose the afferent portal venous circulation to risk of ADAMTS13 deficiency, causing gradual attrition of terminal portal venules within the liver, potentially exacerbated at times by a leaky bowel as in inflammatory bowel disease and celiac disease. This contrasts with the abrupt systemic manifestations characteristic of TTP where low ADAMTS13 activity has been associated with severe systemic consequences due to microthrombi in brain and kidneys, in particular. In addition, liver dysfunction was not reported in two large registries of TTP patients from the UK and USA [11, 32].

When ultralarge VWF is exposed at the surface of damaged vascular endothelium, the shear stress provided by arterial blood exposes epitopes to which platelets avidly adhere, a process which explains rapid and widespread systemic capillary occlusion in TTP. By contrast, the reduced blood pressure at the confluence of hepatic arterial

and portal venous blood flow may lower the shearing force and explain the indolent time course of several decades of NCIPH pathogenesis. The mechanism which we postulate as a primary cause of NCIPH may conceivably contribute to the advancement of numerous other liver diseases which culminate in cirrhosis even when another causative agent such as alcohol has been clearly recognized. If so, and in accordance with Wanless's theory [43], a tendency to ADAMTS13 deficiency may predispose certain individuals to portal microvascular occlusions and parenchymal collapse promoting cirrhosis although the primary cause of hepatic inflammation is unrelated.

Our study is compatible with the notion that, in NCIPH, persistently low levels of ADAMTS13, either congenital or acquired, confer susceptibility to progressive occlusion of portal microvasculature in the absence of any other hepatic pathology and that this susceptibility is more likely to be unmasked when it coincides with pathological conditions affecting the gastrointestinal tract.

Conflicts of interest None.

References

1. Krasinskas AM, Eghtesad B, Kamath PS, Demetris AJ, Abraham SC. Liver transplantation for severe intrahepatic noncirrhotic portal hypertension. *Liver Transpl*. 2005;6:627–634.
2. Hillaire S, Bonte E, Denninger MH, et al. Idiopathic non-cirrhotic intrahepatic portal hypertension in the West: a re-evaluation in 28 patients. *Gut*. 2002;2:275–280.
3. Sarin SK, Kapoor D. Non-cirrhotic portal fibrosis: current concepts and management. *Gastroenterol Hepatol*. 2002;5:526–534.
4. Henderson JM. Liver transplantation for severe intrahepatic noncirrhotic portal hypertension. *Liver Transpl*. 2005;6:610–611.
5. Madhu K, Ramakrishna B, Zachariah U, Eapen CE, Kurian G. Non-cirrhotic intrahepatic portal hypertension. *Gut*. 2008;57:1529.
6. Boyer JL, Hales MR, Klatskin G. "Idiopathic" portal hypertension due to occlusion of intrahepatic portal veins by organized thrombi. A study based on postmortem vinylite-injection corrosion and dissection of the intrahepatic vasculature in 4 cases. *Medicine (Baltimore)*. 1974;1:77–91.
7. Wanless IR, Godwin TA, Allen F, Feder A. Nodular regenerative hyperplasia of the liver in hematologic disorders: a possible response to obliterative portal venopathy. A morphometric study of nine cases with an hypothesis on the pathogenesis. *Medicine (Baltimore)*. 1980;5:367–379.
8. Kingham JG, Levison DA, Stansfeld AG, Dawson AMl. Non-cirrhotic intrahepatic portal hypertension: a long term follow-up study. *Q J Med*. 1981;199:259–268.
9. Nakanuma Y, Hoso M, Sasaki M, et al. Histopathology of the liver in non-cirrhotic portal hypertension of unknown aetiology. *Histopathology*. 1996;3:195–204.
10. Moake JL. Thrombotic microangiopathies. *N Engl J Med*. 2002;347:589–600.
11. Scully M, Yarranton H, Liesner R, et al. Regional UK TTP Registry: correlation with laboratory ADAMTS 13 analysis and clinical features. *Br J Haematol*. 2008;142:819–826.
12. Uemura M, Tatsumi K, Matsumoto M, et al. Localization of ADAMTS13 to the stellate cells of human liver. *Blood*. 2005;106:922–924.
13. Zhou W, Inada M, Lee TP, et al. ADAMTS13 is expressed in hepatic stellate cells. *Lab Invest*. 2005;85:780–788.
14. Lotta LA, Garagiola I, Palla R, Cairo A, Peyvandi F. ADAMTS13 mutations and polymorphisms in congenital thrombotic thrombocytopenic purpura. *Hum Mutat*. 2010;31:11–19.
15. Motto DG, Chauhan AK, Zhu G, et al. Shigatoxin triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice. *J Clin Invest*. 2005;115:2752–2761.
16. Eapen CE, Nightingale P, Hubscher SG, et al. Non cirrhotic intrahepatic portal hypertension: associated gut diseases and prognostic factors. *Dig Dis Sci*. 2011;56:227–235.
17. Austin A, Campbell E, Lane P, Elias E. Nodular regenerative hyperplasia of the liver and coeliac disease: potential role of IgA anticardiolipin antibody. *Gut*. 2004;7:1032–1034.
18. Manavalan JS, Hernandez L, Shah JG, et al. Serum cytokine elevations in celiac disease: association with disease presentation. *Hum Immunol*. 2010;71:50–57.
19. Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood*. 2004;104:100–106.
20. Cao WJ, Niiya M, Zheng XW, Shang DZ, Zheng XL. Inflammatory cytokines inhibit ADAMTS13 synthesis in hepatic stellate cells and endothelial cells. *J Thromb Haemost*. 2008;6:1233–1235.
21. Uemura M, Fujimura Y, Matsumoto M, et al. Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis. *Thromb Haemost*. 2008;99:1019–1029.
22. Gerritsen HE, Turecek PL, Schwarz HP, Lämmle B, Furlan M. Assay of von Willebrand factor (vWF)-cleaving protease based on decreased collagen binding affinity of degraded vWF: a tool for the diagnosis of thrombotic thrombocytopenic purpura (TTP). *Thromb Haemost*. 1999;82:1386–1389.
23. Yarranton H, Lawrie AS, Mackie IJ, Pinkoski L, Corash L, Machin SJ. Coagulation factor levels in cryosupernatant prepared from plasma treated with amotosalen hydrochloride (S-59) and ultraviolet A light. *Transfusion*. 2005;45:1453–1458.
24. Brown JE, Bosak JO. An Elisa test for the binding of von Willebrand antigen to collagen. *Thromb Res*. 1986;43:303–311.
25. Lawrie AS, Hoser MJ, Savidge GF. Phast assessment of vWf:Ag multimeric distribution. *Thromb Res*. 1990;59:369–373.
26. Mannucci PM, Canciani MT, Forza I, Lussana F, Lattuada A, Rossi E. Changes in health and disease of the metalloprotease that cleaves von Willebrand factor. *Blood*. 2001;98:2730–2735.
27. Lisman T, Bongers TN, Adelmeijer J, et al. Elevated levels of von Willebrand Factor in cirrhosis support platelet adhesion despite reduced functional capacity. *Hepatology*. 2006;44:53–61.
28. Feys HB, Canciani MT, Peyvandi F, Deckmyn H, Vanhoorelbeke K, Mannucci PM. ADAMTS13 activity to antigen ratio in physiological and pathological conditions associated with an increased risk of thrombosis. *Br J Haematol*. 2007;138:534–540.
29. Madhu K, Avinash B, Ramakrishna B, et al. Idiopathic non-cirrhotic intrahepatic portal hypertension: common cause of cryptogenic intrahepatic portal hypertension in a Southern Indian tertiary hospital. *Indian J Gastroenterol*. 2009;28:83–87.
30. Sarin SK, Kumar A. Noncirrhotic portal hypertension. *Clin Liver Dis*. 2006;10:627–651.
31. Peyvandi F, Palla R, Lotta LA, Mackie I, Scully MA, Machin SJ. ADAMTS-13 assays in thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2010;8:631–640.
32. Kremer Hovinga JA, Vesely SK, Terrell DR, Lammle B, George JN. Survival and relapse in patients with thrombotic thrombocytopenic purpura. *Blood*. 2010;115:1500–1511.

33. Meyer SC, Sulzer I, Lammle B, Kremer Hovinga JA. Hyperbilirubinemia interferes with ADAMTS-13 activity measurement by FRETS-VWF73 assay: diagnostic relevance in patients suffering from acute thrombotic microangiopathies. *J Thromb Haemost*. 2007;5:866–867.
34. Plaimauer B, Fuhrmann J, Mohr G, et al. Modulation of ADAMTS13 secretion and specific activity by a combination of common amino acid polymorphisms and a missense mutation. *Blood*. 2006;107:118–125.
35. Brill A, Fuchs T, Yang J, et al. VWF-mediated platelet adhesion is required for deep vein thrombosis in a flow restriction model. *Blood* 2009; 114: abstract 473.
36. Chauhan AK, Kisucka J, Brill A, Walsh MT, Scheiflinger F, Wagner DD. ADAMTS13: a new link between thrombosis and inflammation. *J Exp Med*. 2008;205:2065–2074.
37. Sarin SK, Kumar A, Chawla YK, et al. (APASL consensus) Noncirrhotic portal fibrosis/idiopathic portal hypertension: APASL recommendations for diagnosis and treatment. *Hepatol Int*. 2007;1:398–413.
38. Crawley JT, De Groot R, Luken BM. Circulating ADAMTS-13-von Willebrand factor complexes: an enzyme on demand. *J Thromb Haemost*. 2009;7:2085–2087.
39. Ricketts LM, Dlugosz M, Luther KB, Haltiwanger RS, Majerus EM. O-fucosylation is required for ADAMTS13 secretion. *J Biol Chem*. 2007;282:17014–17023.
40. Zhou W, Tsai H-M. N-glycans of ADAMTS13 modulate its secretion and von Willebrand factor cleaving activity. *Blood*. 2009;113:929–935.
41. McKinnon TA, Chion ACK, Millington AJ, Lane DA, Laffan MA. N-linked glycosylation of VWF modulates its interaction with ADAMTS13. *Blood*. 2008;111:3042–3049.
42. Blomme B, van Steenkiste C, Callewaert N, van Vlierberghe H. Alteration of protein glycosylation in liver diseases. *J Hepatol*. 2009;50:592–603.
43. Wanless IR, Wong F, Blendis LM, Greig P, Heathcote EJ, Levy G. Hepatic and portal vein thrombosis in cirrhosis: possible role in development of parenchymal extinction and portal hypertension. *Hepatology*. 1995;5:1238–1247.