Von Willebrand factor-cleaving protease (ADAMTS-13) activity in thrombotic microangiopathies: diagnostic experience 2001/2002 of a single research laboratory

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Summary

Background: Severe deficiency of von Willebrand factor-cleaving protease (ADAMTS-13) activity (<5% of normal) is specific for classical thrombotic thrombocytopenic purpura (TTP), a disorder presenting with thrombocytopenia, microangiopathic haemolytic anaemia and often with organ dysfunction such as neurological symptoms, renal failure, and fever. A certain, though according to several case series, variable percentage of patients with clinically diagnosed TTP and most patients with other forms of thrombotic microangiopathies (TMA) do not show severe ADAMTS-13 deficiency.

Methods: We determined ADAMTS-13 activity in 508 plasma samples of 309 patients referred to our laboratory in 2001 and 2002. Plasma samples with ADAMTS-13 activity <5% were additionally tested for the presence of inhibitory antibodies. Patients were assigned to ten predefined clinical categories according to information provided in the referral letter (TMA not specified; TMA associated with neoplasia or chemotherapy; TMA following haematopoietic stem cell transplantation; TMA with additional disorder; idiopathic TTP; haemolytic-uraemic syndrome (HUS) not specified; HUS with diarrhoea prodrome; atypical HUS; other haematological disorder; no clinical information available).

Results: We detected 50 (16%) patients with severe ADAMTS-13 deficiency. Forty-four (88%) of these patients had been classified as idiopathic TTP, 2 as neoplasia- or chemotherapy-associated, and 4 as non-specified TMA. Among the patients labelled as acute idiopathic TTP, the prevalence of severe ADAMTS-13 deficiency was 63% (44/70). Inhibitory antibodies were found in 31 (62%) patients with ADAMTS-13 activity <5%. Of the 44 patients with acute idiopathic TTP, at initial presentation or at relapse, with ADAMTS-13 activity <5%, 11 were identified to have (probable) constitutional severe ADAMTS-13 deficiency.

Conclusion: Severe ADAMTS-13 deficiency is found in about 60% of patients diagnosed with idiopathic TTP but in none of 111 diagnosed with HUS. Plasma ADAMTS-13 activity <5%, however, does not identify all patients clinically diagnosed with TTP. Detection of inhibitory antibodies against ADAMTS-13 helps to differentiate between acquired and constitutional forms of TTP, which may be important for treatment strategies.

Key words: von Willebrand factor-cleaving protease; thrombotic microangiopathy; ADAMTS-13; thrombotic thrombocytopenic purpura; TTP; haemolytic-uraemic syndrome; HUS; microangiopathic haemolytic anaemia; plasma exchange

The first two authors, J.-D. Studt and J. A. Kremer Hovinga, contributed equally to the study.

Introduction

Thrombotic thrombocytopenic purpura (TTP), first described by Moschcowitz in 1924 [1], is a rare disorder with an annual incidence of 3.7 cases per 10^6 individuals [2], possibly slightly increasing in recent years [3]. It is characterised by microvascular platelet clumping resulting in

thrombocytopenia, microangiopathic haemolytic anaemia, often accompanied by organ dysfunction such as neurological abnormalities, renal failure, and fever [4]. However, the expression of the clinical features is variable, and oligosymptomatic forms may occur. The introduction of plasma exchange (PE) with replacement of fresh frozen plasma (FFP) has reduced mortality from >90% to about 10–20% [3, 5–7], and is now considered the therapy of choice in acute TTP [8].

In the past decade, remarkable advances in understanding the pathogenesis of classical TTP have been made. This condition is often associated with a severe deficiency of the von Willebrand factor (VWF)-cleaving protease [9, 10], now denoted as ADAMTS (A disintegrin and metalloprotease with thrombospondin type 1 domains)-13 [11–15]. Deficiency of ADAMTS-13 activity prevents normal processing of very large VWF multimers, which are synthesised and secreted into plasma by endothelial cells. It is assumed that these unusually large VWF multimers promote in-vivo platelet clumping, leading in turn to microvascular thrombosis with ischaemic organ dysfunction [16]. Congenital TTP, also known as Upshaw-Schul-man syndrome [17, 18], is associated with a severe constitutional deficiency of ADAMTS-13 [19–22] due to compound heterozygous or homozygous defects of the ADAMTS13 gene [23–27], while acquired TTP is caused by circulating autoantibodies inhibiting ADAMTS-13 activity [9, 10].

The combination of thrombocytopenia and microangiopathic haemolytic anaemia with schistocytes is not specific for TTP. It is also a hallmark of the haemolytic-uraemic syndrome (HUS) [28], a disease predominantly, although not exclusively, diagnosed in children [29, 30], and clinically often indistinguishable from TTP [8]. In addition, thrombocytopenia and schistocytic anaemia may occur, usually to a lesser extent, in disseminated intravascular coagulation, pre-eclampsia/eclampsia and the HELLP (haemolysis, elevated liver enzymes, low platelets) syndrome, severe vasculitis, or dysfunctional prosthetic heart valves.

Here, we describe our experience with measuring ADAMTS-13 activity in relation to the clinical presentation in a large series of patients whose plasma or serum samples were sent to our laboratory during a two-year period.

Materials and methods

Between January 2001 and December 2002 we measured ADAMTS-13 activity in 508 citrated plasma samples of 309 consecutive patients with suspected TTP, HUS, other thrombotic microangiopathies (TMA), aetiologically unclear thrombocytopenias and haemolytic anaemias, or haematological disorders associated with a pathological VWF multimer distribution. As a rule, plasma samples were sent on dry ice. However, about 10% of samples were thawed upon arrival at our laboratory. Due to high enzymatic stability of ADAMTS-13 (for several days at room temperature [12], for years at –20 °C or –80 °C, and after repeated thawing [31]) these samples were analysed and results were judged to be reliable. Beside the 508 plasma samples of 309 patients, we investigated 21 family members of patients with (suspected) hereditary TTP. In addition, 155 serum samples of patients from the Oklahoma TTP/HUS study were analysed. Data on these latter patients have been reported elsewhere [32] and are not considered here.

ADAMTS-13 activity was measured using a quantitative immunoblotting assay as previously described [9, 19, 33]. Briefly, plasma samples were diluted 1:20 in 0.01 M Tris, 0.15 M sodium chloride, pH 7.4 (TBS), containing 1 mM Pefabloc SC (Boehringer, Mannheim, Germany), incubated with 10 mM barium chloride for 5 minutes at 37 °C and added to purified VWF substrate. The reaction mixture was dialysed on the surface of a hydrophilic filter membrane (Millipore, Bedford, MA) at 37 °C against 1.5 M urea, 5 mM Tris, pH 8.0. After 16 to 20 hours the reaction was stopped by the addition of 10mM EDTA. The extent of VWF degradation was analysed by VWF multimer analysis on SDS-1.4% agarose gels and immunoblotting using a peroxidase-conjugated rabbit anti-human VWF antibody (DAKO, Glostrup, Denmark). Dilutions of a normal human plasma pool (NHP) from 42 healthy male volunteers were used for assay calibration of ADAMTS-13 activity in patient samples. For inhibitor detection, patient plasma was heated for 30 min at 56 °C [21].

After centrifugation, the supernatant was mixed 1:1 (v:v) with NHP, incubated for 2 hours at 37 °C, and diluted 1:10 before addition of barium chloride. For inhibitor assay calibration, NHP was mixed 1:1 (v:v) with TBS and incubated for 2 hours at 37 °C before dilution.

Five arbitrary categories of ADAMTS-13 activity were made: <5%, severe deficiency; 5–9%, borderline severe deficiency; 10–25%, moderate deficiency; 26–50%, mild or minimal deficiency; and >50%, normal [9, 32]. Plasma samples with ADAMTS-13 activity <5% were screened for the presence of ADAMTS-13 inhibitory antibodies. One unit of inhibitory autoantibody reduced the ADAMTS-13 activity of an equal volume of NHP by 50%. Semi-quantitative inhibitor levels were determined and three categories of inhibitors were made: no inhibitor detected, uncertain or low titre inhibitory antibody, and definite inhibitor present [31].

Patients were assigned to one of ten predefined clinical categories related to associated conditions based on the information provided by the referring clinicians: (1) TMA not further specified; (2) neoplasia- or chemotherapy-associated TMA; (3) bone marrow or haematopoietic stem cell transplantation-associated TMA; (4) presence of an additional or alternative disorder which may have caused the presenting signs; (5) idiopathic TTP; (6) HUS not further specified; (7) typical HUS with (bloody) diarrhoea prodrome (D+HUS); (8) atypical HUS; (9) other (haematological) disorder; (10) no clinical information available. Patients with idiopathic TTP were subdivided into (A) initial presentation; (B) relapse; (C) follow-up in remission; and (D) follow-up after splenectomy (Table 1). Diagnosis of hereditary TTP was based on a suitable patient and/or family history, the finding of severe ADAMTS-13 deficiency in the absence of inhibitory antibodies, about 50% ADAMTS-13 activity in the parents, and on an adequate recovery of ADAMTS-13 activity upon infusion of a given volume of fresh frozen plasma.

Table 1

Relation of ADAMTS-13 activity to clinical categories of 309 consecutive patients referred for ADAMTS-13 activity determination.

Clinical category	No. pts	age median in y (range)	ADAMTS-13 activity (% NHP) n (% of total)				
			<5%	5–9%	10–25%	26–50%	>50%
1 Thrombotic microangiopathy (TMA), not specified	31	43.9 (0–66)	4 (13%)	1 (3%)	4 (13%)	9 (29%)	13 (42%)
2 Neoplasia- or chemotherapy-associated TMA	14	65 (15–78)	2 (14%)	1 (7%)	0	4 (29%)	7 (50%)
3 Haematopoietic stem cell transplantation-associated TMA	9	30 (3–64)	0	0	0	4 (44%)	5 (56%)
4 TMA associated with additional/ alternative disease*	7	43.6 (0–64)	0	1 (14%)	2 (29%)	0	4 (57%)
5 Idiopathic TTP							
– acute, at presentation	57	39.1 (0–78)	34 (60%)	6 (10%)	7 (12%)	5 (9%)	5 (9%)
– acute, at relapse	13	36.2 (19–63)	10 (77%)	1 (7.5%)	1 (7.5%)	0	1 (7.5%)
– follow-up, in remission	16	37.5 (15–56)	0	0	2 (12.5%)	8 (50%)	6 (37.5%)
– follow-up, in remission after splenectomy	6	39.8 (18–67)	0	0	0	2 (33%)	4 (67%)
6 HUS, not specified	59	10.3 (0–80)	0	1 (2%)	5 (8%)	16 (27%)	37 (63%)
7 HUS with diarrhoea prodrome (D + HUS)	31	4.8 (0–73)	0	0	1 (3%)	9 (29%)	21 (68%)
8 HUS, atypical	21	9.1 (0–56)	0	0	0	4 (19%)	17 (81%)
9 Other (haematological) disease**	11	38.8 (0–60)	0	0	0	1 (9%)	10 (91%)
10 No clinical information	34	32.2 (1–86)	0	0	0	13 (38%)	21 (62%)
Total	309 (100%)	30.4 (0–86)	50 (16%)	11 (4%)	22 (7%)	75 (24%)	151 (49%)

*   One patient each with antiphospholipid antibody syndrome; scleroderma, hypertension and renal failure; resuscitation because of cardiogenic shock and consecutive multiorgan failure; haemophilia, HCV and HIV infection after liver transplantation; snake bite (Cerastes cerastes); Kassabach-Merrit syndrome; and a premature infant from a triplet with foeto-foetal transfusion syndrome (donor), respectively.

** One patient each with Evans' syndrome; haemolytic anaemia of unknown origin; autoimmune haemolytic anaemia; congenital dyserythropoietic anaemia; essential thrombocythaemia with bleeding tendency; acute thrombosis of renal transplant artery; and aortic valve stenosis and acquired bleeding tendency with partial loss of high molecular weight von Willebrand factor multimers, respectively; and 4 patients with chronic, therapy-refractory thrombocytopenia.

Results

Between January 2001 and December 2002, we received 529 plasma samples of 309 patients and 21 family members from 90 centres in 15 countries: Austria (2 centres), Croatia (1), Czech Republic (3), France (1), Germany (49), Hungary (1), Italy (1), the Netherlands (1), Norway (2), Portugal (1), Slovenia (1), Sweden (2), Switzerland (21), Turkey (2) and the United States (2) for determination of ADAMTS-13 activity and inhibitory autoantibodies. A severe ADAMTS-13 deficiency (<5% of NHP) was found in the samples of 50 patients: 34 patients with an initial bout and 10 with a relapse of acute idiopathic TTP, 4 patients with not further specified TMA, and 2 patients with neoplasia- or chemotherapy-associated TMA (Table 1). Among the 44 patients with a first manifestation or relapse of idiopathic TTP, we identified 11 patients with possible or probable constitutional severe ADAMTS-13 deficiency. Screening for mutations in the ADAMTS13 gene, consisting of 29 exons, has been set up very recently in our laboratory, and is on the way in several of these patients with hereditary TTP (Upshaw-Schulman syndrome [17, 18]). No case of severe ADAMTS-13 deficiency was found among the 9 patients with TMA following haematopoietic stem cell transplantation, the seven patients with TMA and additional/alternative disorder, nor in any of the 111 patients diagnosed with HUS. Eleven patients had ADAMTS-13 activity values between 5–9%: seven with idiopathic TTP, one patient each with unspecified TMA, neoplasia- or chemotherapy-associated TMA, TMA with additional/alternative disorder (resuscitation because of cardiogenic shock and consecutive multiorgan failure) and unspecified HUS (Table 1).

All 50 patients with severe ADAMTS-13 deficiency were tested for the presence of inhibitory antibodies, which were found in 31 (62%) patients. In additional 10 (20%) cases, including 4 patients with probably hereditary TTP, the result of the inhibitor assay was judged to be uncertain and the presence of a low titre inhibitor could not be excluded (Table 2). Nine patients had no inhibitor.

Selected case descriptions

1. Acute idiopathic TTP due to inhibitory ADAMTS-13 autoantibodies.

A previously healthy 37-year-old woman developed general illness with fatigue, weakness, headache, tachycardia and she reported dark reddish-brown urine. She was admitted to a peripheral hospital because of jaundice, petechial skin bleeding, haematoma and general malaise. Laboratory investigations revealed severe anaemia (haemoglobin 58 g/L, reticulocytes of 101×10^9/L) with many schistocytes on the peripheral blood smear, severe thrombocytopenia (5×10^9/L), hyperbilirubinaemia (86.3 μmol/L) and elevation of LDH (1876 U/L), leading to the clinical diagnosis of acute idiopathic TTP. Focal neurological signs or renal dysfunction (creatinine 73 μmol/L) were not observed. The patient was transferred to our University hospital for further treatment. Plasma exchange (PE) therapy of about one patient plasma volume using fresh frozen plasma (FFP) for replacement was started immediately, and prednisolone was given in addition. Daily PE was continued until the platelet count was in the normal range requiring a total of 10 PE therapy sessions (Figure 1). The clinical condition improved gradually and the patient was discharged on the 17th day after admission. Follow-up was uneventful, haemoglobin values normalised within the next three weeks, and prednisolone was tapered and finally withdrawn after four months. The patient was last seen at our outpatient clinic ten months after initial presentation. She was doing well and laboratory values for haemoglobin, platelets, bilirubin, LDH and creatinine were within the normal range. At presentation, severe ADAMTS-13 deficiency in association with a mild inhibitor was found in her plasma. After ten PE therapy sessions ADAMTS-13 activity values had normalised, and the inhibitor had disappeared (Figure 1).

2. Chronic relapsing TTP due to high titre ADAMTS-13 autoantibody, effect of splenectomy

A French woman developed classical TTP with thrombocytopenia and microangiopathic haemolytic anaemia at the age of 25 years. She was treated by PE with FFP replacement, FFP infusions, and corticosteroids. During the next four years she experienced several relapses, which were treated essentially in the same way. She remained PE- and FFP-dependent between 10/2001 and 2/2002, when splenectomy was performed. Retrospective analysis of stored serial plasma samples showed a severe acquired ADAMTS-13 deficiency (Figure 2). Seven months after splenectomy, the inhibitor against ADAMTS-13 had disappeared (Figure 2, panel B, lane 9) in parallel with a full recovery of ADAMTS-13 activity (Figure 2, panel A, lane 15), normalisation of the platelet count and haemoglobin values. (Communicated by Dr. P. Hénon, Mulhouse, France).

3. Severe congenital ADAMTS-13 deficiency causing hereditary TTP (Upshaw-Schulman syndrome)

A newborn Norwegian girl of non-consanguineous parents developed severe thrombocytopenia and hyperbilirubinaemia within 24 hours after birth. Severe anaemia was noted on the second day of life. She was treated with several blood transfusions during her first weeks of life and also received intravenous immunoglobulins. At the age of nine months she was readmitted because of petechiae. The platelet count was 6×10^9/L and she was given intravenous immunoglobulins. During the ensuing months the girl was repeatedly seen at the paediatric outpatient clinic for petechial bleeding, anaemia and/or jaundice. Evans' syndrome was suspected but no platelet or red blood cell autoantibodies were found. Focal neurological signs or renal insufficiency had never been observed. At the age of 20 months, ADAMTS-13 activity was found to be <5%. No inhibitor was detected in her plasma. ADAMTS-13 activity one hour after infusion of 10 ml FFP/kg body weight was 10% of normal, definitely ruling out the presence of a circulating inhibitor which might not have been detected by our assay. Both parents had ADAMTS-13 activity of 50% establishing the diagnosis of severe congenital ADAMTS-13 deficiency in the child. The girl is now treated with FFP infusions at regular intervals (every 12–14 days). She is doing well, and no relapses of thrombocytopenia or microangiopathic haemolytic anaemia have occurred since this prophylactic regimen was established. Determination of ADAMTS-13 activity in citrated cord blood of her newborn baby sister ruled out hereditary TTP as ADAMTS-13 activity was 50% (Figure 3A). Therefore, regular FFP infusions could be withheld. Screening of the ADAMTS13 gene for mutations was initiated in this family. (Communicated by Dr. E. Siebke, Ålesund, Norway).

4. Fatal course of hereditary TTP diagnosed post mortem

A Turkish boy, the only child of a probably consanguineous couple, had been suffering from repeated attacks of thrombocytopenia and (Coombs'-negative) haemolytic anaemia since infancy. He had been frequently treated with packed red blood cells and platelet concentrates, and occasionally received intravenous immunoglobulins. FFP was only given at the terminal stage. He died at the age of 7 years of fulminant and widespread occlusion of the microcirculation, without an established diagnosis. Autopsy findings were suggestive of TTP and determination of ADAMTS-13 activity in a pre-mortem serum sample confirmed severe ADAMTS-13 deficiency post-hoc. The hereditary nature of severe ADAMTS-13 deficiency was established by family analysis (Figure 3B). Molecular analysis of the ADAMTS13 gene is underway. (Communicated by Dr. M. Hermann, Tübingen, Germany)

Table 2

Detection of inhibitory autoantibodies in patients with severe ADAMTS-13 deficiency (<5% of NHP).

	no. pts	ADAMTS-13 inhibitor*		
		none	uncertain/ low titre	definite
Thrombotic microangiopathy (TMA) not specified	4	0	0	4
Neoplasia- or chemotherapy-associated TMA	2	1	0	1
Idiopathic TTP:				
– acute, at presentation	34	7	9	18
– acute, at relapse	10	1	1	8
Total	50	9	10	31

* Categories according to semi-quantitative inhibitor assay

Discussion

We here describe our experience with the measurement of ADAMTS-13 activity in a large group of patients whose plasma or serum samples were sent to our laboratory from 90 centres in 15 countries in 2001–2002.

A severe ADAMTS-13 deficiency (defined as an activity <5% of that in NHP) was found in only 50 (16%) of 309 patients investigated, including 11 patients with confirmed or suspected constitutional ADAMTS-13 deficiency and 33 patients with acquired TTP, either at initial disease manifestation or at relapse. Thus, 44 (88%) of the 50

Figure 1

Time course of platelet count and ADAMTS-13 activity in relation to treatment with plasma exchange and prednisolone in a patient with acute idiopathic TTP due to an inhibitory autoantibody.
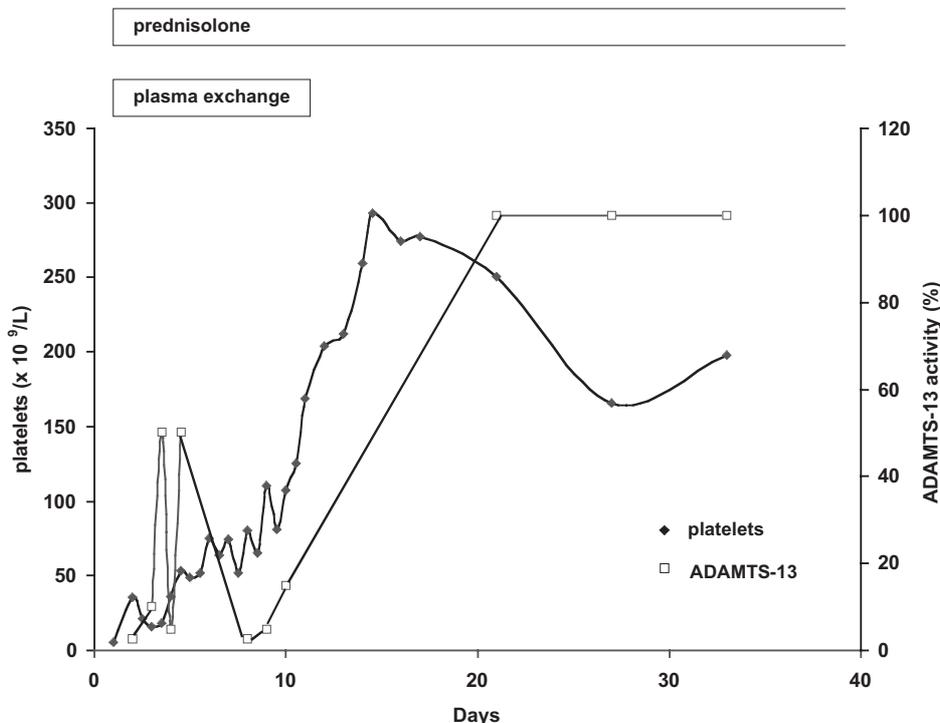
Figure 2

One-year follow-up in a patient with relapsing acquired TTP due to an inhibitory autoantibody. Quantitative immunoblotting of purified von Willebrand factor substrate degraded by barium chloride-activated ADAMTS-13 in dilutions of a normal human plasma pool (NHP) and serial patient plasma samples. Panel A and B constitute separate immunoblotting assays with individual standard curves to adjust for inter-assay variability.
Panel A. Determination of ADAMTS-13 activity. Lanes 1–7 = assay calibration with NHP dilutions of 1:20 [100% activity], 1:40 [50%], 1:80 [25%], 1:160 [12.5%], 1:320 [6.25%], 1:640 [3%], and buffer control [0%]. Lanes 8–15 = patient plasma diluted 1:20. The date of blood withdrawal of the earliest preserved plasma sample from the last TTP episode before splenectomy was arbitrarily defined as day 1 (d1). Further samples were dated in relation to this sample. Severe ADAMTS-13 deficiency (<3% of NHP) was present on day 1 through day 69. On day 349, seven months after splenectomy, ADAMTS-13 activity was 50%.
Panel B. Detection of inhibitory autoantibodies against ADAMTS-13. Lanes 1–4 = assay calibration with NHP dilutions of 1:20 [100% activity], 1:40 [50%], 1:80 [25%], and buffer control [0%]. Lanes 5–9 = 1:1 mixtures of NHP with patient plasma (final NHP dilution 1:20). Semi-quantitative inhibitory autoantibody assays gave 1–2 units/ml on days 1, 9 and 24 and >>2 units/ml on day 69. No inhibitor was found after splenectomy (d349).
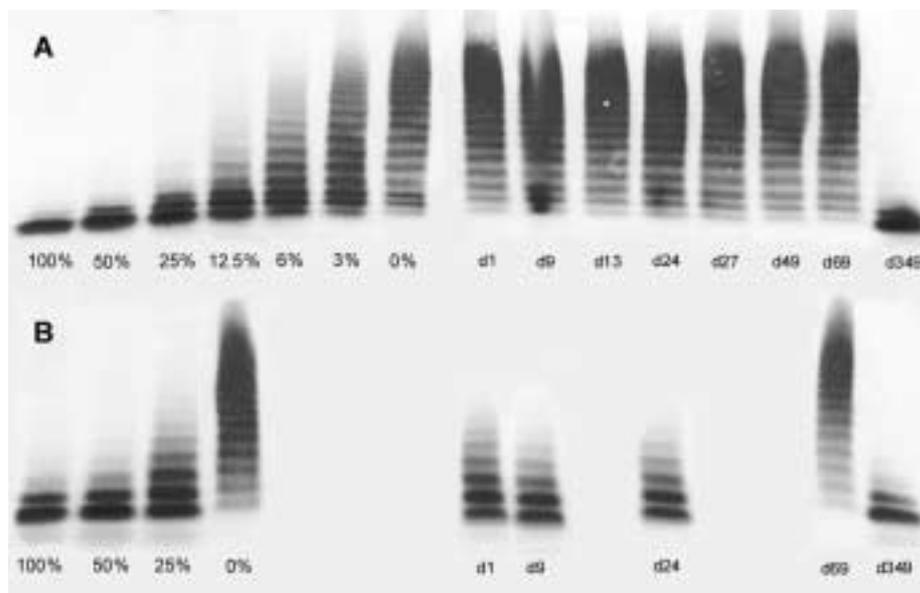
Figure 3

Pedigrees of two families with hereditary TTP due to constitutional ADAMTS-13 deficiency. Rectangles symbolise male and circles female family members, open symbols indicate healthy and filled symbols affected subjects. Individual ADAMTS-13 activity (% of normal human plasma pool) at diagnosis and present age (y) are indicated.
A. Norwegian family. The affected girl was diagnosed to have severe constitutional ADAMTS-13 deficiency at the age of 20 months after several bouts of severe thrombocytopenia and/or anaemia. She is currently treated with regular plasma infusions and doing well.
* indicates the newborn girl whose ADAMTS-13 activity in cord blood permitted to exclude hereditary TTP.
B. Turkish family. The patient had been treated for idiopathic thrombocytopenia and/ or haemolytic anaemia since infancy. Diagnosis of hereditary TTP was first suggested by autopsy findings and confirmed by ADAMTS-13 activity measurement post-hoc as well as family analysis.

A

30 y
~ 50%

30 y
~ 50%

*

3 y
<1%

1 day
~ 50%

B

31 y
~ 50%

28 y
~ 50%

7 y (†)
<3%
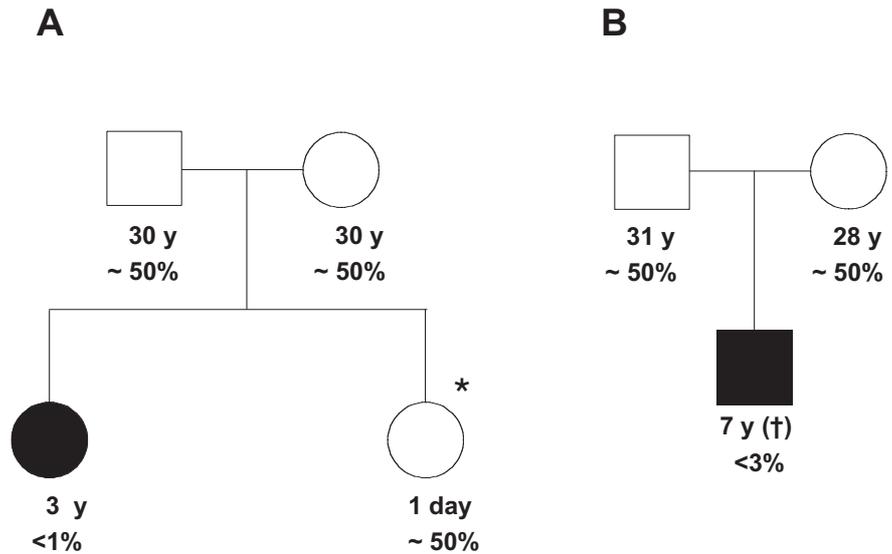
patients severely deficient in ADAMTS-13 activity belonged to the clinical category "TTP" according to the diagnoses made by the referring clinicians. Of the remaining 6 patients with severe ADAMTS-13 deficiency the diagnosis of TMA was not further specified in four and was neoplasia- or chemotherapy-associated in two patients. In one of the latter, chemotherapy included mitomycin C, a drug known to be associated with TMA [34, 35] although an association of mitomycin C-mediated TMA with severe ADAMTS-13 deficiency has not been reported so far. None of the patients with haematopoietic stem cell transplantation-associated TMA (n = 9) or a clinical diagnosis of HUS (n = 111) had ADAMTS-13 activity <5%. This is consistent with earlier reports [9, 25, 36–38], even though Hunt et al. [39] found 1 out of 29 children with E. coli 0157:H7–associated D+HUS to have severe acquired ADAMTS-13 deficiency while Veyradier et al. [40] and Remuzzi et al. [41] found absence of ADAMTS-13 activity not only in TTP but also in some cases diagnosed with (atypical) HUS. We believe that this disagreement reported in the literature is related to the fact that TTP and (at least atypical) HUS are not unequivocally defined, clinically overlap sometimes, and are therefore not clearly distinguishable [8, 32].

Nevertheless, recent claims that severe ADAMTS-13 deficiency is an unspecific finding, being present in various disease states with thrombocytopenia different from TMA [42, 43], have been refuted by a study from our laboratory [33] showing that none of 68 patients with thrombocytopenia of various aetiologies except TTP or HUS had severely deficient ADAMTS-13 activity. Thus, severe ADAMTS-13 deficiency is an abnormality specific for a TMA most often diagnosed clinically as TTP [33], in rare instances, however, labelled as HUS [40, 41]. The sensitivity of this laboratory finding for the diagnosis of TTP remains questionable. In retrospective studies

acute TTP was associated with severe ADAMTS-13 deficiency in 66–100% of patients [9, 10, 37]. A prospective multicentre study by Veyradier et al. [40] found a similar prevalence (71%) of severe ADAMTS-13 deficiency among patients clinically diagnosed with idiopathic or secondary TTP, while a recent inception cohort study on 142 consecutive adult patients by Vesely et al. [32] reported a prevalence of only 33% (16/48 patients) in acute idiopathic TTP-HUS, a diagnosis established on the basis of thrombocytopenia and microangiopathic haemolytic anaemia without another apparent aetiology.

Of the 70 patients diagnosed with idiopathic TTP, at initial presentation (n = 57) or at relapse (n = 13), who were investigated in the present study, 44 (63%) had severe and 7 (10%) borderline severe ADAMTS-13 deficiency. The prevalence of 63% is slightly lower than in several previous studies [9, 10, 37, 40] but higher than that reported by Vesely et al. [32], possibly reflecting referral of selected cases in the present study. Reported sensitivities of severe ADAMTS-13 deficiency for TTP between 33–100% [9, 10, 32, 37, 40] clearly indicate, that many but certainly not all patients diagnosed clinically with acute TTP have severe ADAMTS-13 deficiency. Other pathogenic factors may lead to a clinical condition indistinguishable from that seen in severe ADAMTS-13 deficiency [4, 44, 45] and would not necessarily be detected by current assays of plasma ADAMTS-13 activity. Ultra-large VWF multimers secreted into the circulation are cleaved by ADAMTS-13 probably directly on the endothelial surface [46]. Therefore, defective binding of ADAMTS-13 to endothelial cells due to hypothetical structural defects or autoantibodies against putative endothelial receptors of ADAMTS-13 [4] could result in defective processing of ultra-large VWF multimers leading to microvascular platelet thrombosis. In this context, it is noteworthy that anti-glycoprotein IV (CD36) antibodies have been found in

patients with TTP [45], glycoprotein IV being an endothelial thrombospondin receptor and ADAMTS-13 having 8 thrombospondin modules [13, 14, 23]. Endothelial cell-based assays of ADAMTS-13 will be necessary to unravel whether some of the TTP cases without severe plasma ADAMTS-13 deficiency may be explained by such impaired binding of ADAMTS-13 to endothelial cells.

In order to avoid further debates whether severe ADAMTS-13 deficiency distinguishes TTP from HUS or not, we propose a new system to classify thrombotic microangiopathies: 1.) TMA due to severe ADAMTS-13 deficiency, either constitutional or autoantibody-mediated, encompassing patients that are most often diagnosed as hereditary or acquired TTP. 2.) TMA due to infection with enterohaemorrhagic E. coli, encompassing all patients, mainly but not exclusively children, with typical HUS (D+HUS). 3.) Other TMA, eg, associated with complement factor H deficiency [30], neoplasia, chemotherapy, other drugs, haematopoietic stem cell transplantation, and others. The pathogenetic mechanisms of these TMAs still need to be elucidated.

Today, PE with replacement of FFP, often used in combination with corticosteroids, is the therapy of choice in acute acquired TMA, since it has reduced mortality rates substantially [5–7]. At present, all patients with acute TMA (except E. coli-associated TMA of childhood) should be treated by this standard procedure. Nevertheless, there is a notion, that patients with acute idiopathic TMA without severe ADAMTS-13 deficiency may have higher mortality rates than those with severe ADAMTS-13 deficiency [37], despite appropriate treatment regimens, suggesting that PE might not be the optimal treatment for the former patients. In the absence of knowledge on the underlying pathophysiology – and hence specific therapy – standard treatment with PE and FFP replacement remains mandatory until improved and pathophysiology-based therapeutic measures become available. Of major importance, however, is the distinction between acquired and constitutional severe ADAMTS-13 deficiency, as patient management is different. Patients with hereditary TTP respond dramatically to simple FFP infusion [18–22] and can be maintained for many years in good health by regular FFP application every 2–3 weeks [22, 44]. PE is often not needed and steroids or splenectomy should be avoided in these patients.

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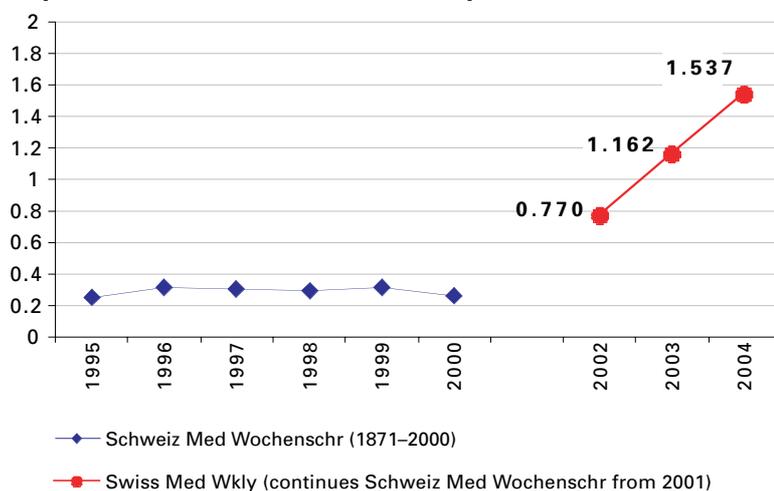- No page charges and attractive colour offprints at no extra cost

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