

The course of ADAMTS-13 activity and inhibitor titre in the treatment of thrombotic thrombocytopenic purpura with plasma exchange and vincristine

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Summary

The therapeutic efficacy of plasma exchange (PE) in thrombotic thrombocytopenic purpura (TTP) is attributed to the restoration in ADAMTS-13 (a disintegrin and metalloproteinase with thrombospondin motif-13) activity by substitution of the enzyme and removal of ADAMTS-13-neutralizing autoantibodies. We explored this rationale by analysing ADAMTS-13 activity and corresponding inhibitor levels during PE-treatment in 27 episodes from 23 adults with TTP. All patients with an initial episode of TTP ($n = 14$) and nine of 11 patients with a relapse showed severe ADAMTS-13 deficiency. ADAMTS-13 inhibitors were detected in 81% of these patients. Twenty-one patients responded to PE-therapy and two patients died. For patients with severe ADAMTS-13 deficiency, 15 patients (71%) showed a PE-induced recovery in ADAMTS-13 activity and six patients (29%) had persistent severe ADAMTS-13 deficiency despite clinical response. Three patients with recurrent TTP demonstrated a permanent increase in inhibitor titre during therapy. Six patients (43%) with an initial episode of TTP displayed a transient increase in inhibitor titre during PE-therapy, which was associated with deterioration in clinical and haematological symptoms of TTP. Treatment with vincristine induced an immediate increase in platelet count and ADAMTS-13 activity in seven of eight patients. We conclude that ADAMTS-13 activity and inhibitor levels, as measured using current methodology, do not solely determine the clinical course of TTP.

Keywords: thrombotic thrombocytopenic purpura, ADAMTS-13, plasma exchange.

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Thrombotic thrombocytopenic purpura (TTP) is characterized by microvascular platelet clumping, resulting in microangiopathic haemolytic anaemia and consumptive thrombocytopenia. Moake *et al* (1982) found ultra large molecular forms of von Willebrand Factor (UL-VWF) in patients with TTP and proposed them to play a pathogenic role in the formation of microvascular platelet-rich thrombi, which are found in the microcirculation of patients with acute TTP (Moschowitz, 1924). von Willebrand Factor (VWF) is released from endothelial cells as large multimers, which are cleaved by a specific metalloproteinase, recently characterized as a new member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin type I motif) family of metalloproteinases

and denoted as ADAMTS-13 (Fujikawa *et al*, 2001; Gerritsen *et al*, 2001; Levy *et al*, 2001). The majority of patients with TTP show severe deficiency in the VWF-cleaving activity of ADAMTS-13, either caused by missense or frame-shift mutations in the ADAMTS13 locus (Levy *et al*, 2001; Kokame *et al*, 2002; Antoine *et al*, 2003; Assink *et al*, 2003; Schneppenheim *et al*, 2003; Matsumoto *et al*, 2004; Pimanda *et al*, 2004; Veyradier *et al*, 2004) or because of ADAMTS-13-neutralizing autoantibodies (Furlan *et al*, 1998a; Tsai & Lian, 1998; Veyradier *et al*, 2001; Mori *et al*, 2002; Vesely *et al*, 2003; Kremer Hovinga *et al*, 2004; Peyvandi *et al*, 2004; Zheng *et al*, 2004). Plasma exchange (PE), often combined with the administration of glucocorticoids, is the treatment of choice

for acquired TTP, reducing the mortality from 90% to 20% (Rock *et al*, 1991). Until recently, PE was, although very effective, an empirical treatment. It is only since the discovery of ADAMTS-13 deficiency in patients with TTP that the therapeutic efficacy of PE has apparently been understood. PE is believed to supply ADAMTS-13 by plasma replacement and remove ADAMTS-13-neutralizing IgG-autoantibodies by apheresis. This rationale is challenged by the fact that PE is also effective in other subtypes of thrombotic microangiopathy (TMA), e.g. the haemolytic uremic syndrome (HUS), which lack a severe ADAMTS-13 deficiency (Vesely *et al*, 2003). Patients with severe ADAMTS-13 deficiency, on the other hand, do not always respond to PE and some patients respond despite persistent severe ADAMTS-13 deficiency (Zheng *et al*, 2004). Additional methods of treatment in TTP aim to reduce autoantibody levels. These include therapy with glucocorticoids, vincristine, splenectomy, immunoadsorption (Moake, 2002) and, most recently, therapy with rituximab (Yomtovian *et al*, 2004). Measurement of ADAMTS-13 activity and inhibitor levels are proposed to predict clinical outcome (Tsai *et al*, 2001; Mori *et al*, 2002) and to assist in tailoring treatment. However, although the efficacy of plasma treatment in TTP has been demonstrated, the effect of treatment on ADAMTS-13 activity and corresponding inhibitor levels, especially in conjunction with the effect on the clinical course, has not been systematically investigated. The aim of this study was to examine the behaviour of ADAMTS-13 activity and inhibitor levels during treatment with PE and thereby evaluate the possible association between ADAMTS-13 parameters and the clinical course of TTP.

Materials and methods

Patients and treatment

For this study, we recruited consecutive adult patients with a clinical diagnosis of non-familial TTP according to the following criteria: (i) thrombocytopenia ($<100 \times 10^9/l$); (ii) elevated levels of serum lactate dehydrogenase (LDH; >240 U/ml); and (iii) microangiopathic haemolytic anaemia (haemoglobin level <14 g/dl, fragmented red cells in the peripheral blood smear, direct antiglobulin test negative). Patients whose onset of TMA was associated with bone-marrow-transplantation, cancer or predominant renal failure were excluded from the study. Each participating patient or their legal guardian gave informed consent for blood collection and research use. Patients were treated with daily PE therapy (3l fresh frozen plasma/d) combined with glucocorticoids (1–1.5 mg/kg) until normalization of platelet count and LDH level. Thereafter, PE was usually continued with three to six sessions every other day and was terminated if platelet count and LDH-level remained normal. Patients with severe neurological symptoms or delayed response to PE therapy were additionally treated with vincristine (three to four doses of 1 mg/d). Further therapy modulations included

rituximab (375 mg/m²) in three patients and mycophenolatemofetil (1–3 g/d) in two patients.

Blood samples

Venous blood was collected in plastic tubes containing 3.2% sodium citrate as anticoagulant. Unless otherwise indicated, blood samples were obtained at admission before plasma therapy was commenced. Blood samples from 18 patients were collected during a series of PE sessions in 22 episodes of TTP. These samples were always drawn immediately before the PE session. Blood was centrifuged for 40 min at 4°C and 2500 g, and plasma was stored at –20°C until analysis.

ADAMTS-13 activity assay

ADAMTS-13 activity (normal range 58–134%) was estimated by measuring the residual ristocetin cofactor activity of the degraded VWF substrate as described previously (Böhm *et al*, 2002) with the following modifications: (i) for assay calibration imidazole buffer was replaced by heat-inactivated normal pooled plasma (30 min at 56°C and centrifuged for 15 min at 15000 g); and (ii) for calculation of ADAMTS-13 activity, the residual ristocetin cofactor activity of the substrate was expressed in change of absorbance instead of percent of normal.

ADAMTS-13 inhibitor assay

Inhibitory activity against ADAMTS-13 was detected by mixing patient plasma, either neat or diluted, with normal pooled plasma and incubating the mixtures for 30 min at 37°C before measuring ADAMTS-13 activity. The inhibitor concentration that neutralized 50% of ADAMTS-13 activity in an equal volume of normal plasma was defined as 1 U/ml. Inhibitors were considered non-detectable (<0.4 U/ml) if residual ADAMTS-13 activity in the mixture was higher than 75% of the reference mixture. Patient plasma was heat inactivated (30 min at 56°C) before performing the inhibitor assay.

Statistics

Normally and not normally distributed parameters were analysed with the unpaired *t*-test and Mann–Whitney *U*-test, respectively. Spearman rank correlation was used to examine the relationship between variables. Values of $P < 0.05$ were considered statistically significant.

Results

Clinical data, treatment and outcome

We investigated 23 patients (16 females, seven males) in 27 episodes of acute, non-familial TTP. The clinical and laboratory data of the patients are summarized in Table I. Of the 23 patients, 21 were Caucasians, one was African American

Table I. Clinical and laboratory data of each patient.

Patient	Episode	Neurologic symptoms	Plts ($\times 10^9/l$) pre-PE	ADAMTS-13 activity (%) pre-PE	Inhibitor titre (U/ml) pre-PE	Treatment	PE courses	ADAMTS-13 activity (%) post-PE	Inhibitor titre (U/ml) post-PE	Inhibitor during PE
1	Initial	Syncope, fatigue	15	<6.25	0.7	PE, C	6	22	<0.4	↓
2	Initial	Seizure, confusion	10	<6.25	<0.4	PE, C	7	67	<0.4	
3	Initial	Aphasia, paraesthesia	29	<6.25	13.3	PE, C	8	<6.25	2.4	↓
4	Initial	Seizure, coma	15	<6.25	4	PE, CV	13	52	<0.4	↓
5	Initial	None	8	<6.25	0.6	PE, C	15	93	<0.4	↓
6	Initial	Seizure, confusion	10	<6.25*	0.8*	PE, CV	17	68	<0.4	↑↓
7	Initial	Seizure, aphasia	20	<6.25	<0.4	PE, CV	20	76	<0.4	↓
8	Initial	Confusion, numbness	10	<6.25	1.6	PE, CV, R	21	70	<0.4	↑↓
9	Initial	Coma, vigilance	29	<6.25	1.4	PE, C	25	30	<0.4	↑↓
10	Initial	Aphasia, dizziness	19	<6.25	1.5	PE, CV	31	<6.25	<0.4	↑↓
11	Initial	Seizure, fatigue	12	<6.25*	4*	PE, CM, V	36	53	<0.4	↑↓
12	Initial	Seizure, hemiparesis	13	<6.25	0.6	PE, CR, V	37	65	<0.4	↑↓
13	Initial	Coma, somnolence	10	<6.25	3.6	PE, C	Died during first session of PE from cerebral bleeding			
14	Initial	Seizure, stroke	17	<6.25	0.6	PE, CV	Died on the 4th day of PE therapy from myocardial infarction			
15	Relapse	Dizziness, paraesthesia	70	<6.25	<0.4	FFP, PE, C	3†	<6.25	4.9	↑
3	Relapse	None	22	<6.25	2.7	PE, C	5	46	<0.4	↓
16	Relapse	Aphasia, dizziness	92	<6.25	2.0	PE, C	5	55	<0.4	↓
17	Relapse	None	12	<6.25	82	PE, C	5	<6.25	6.7	↓
1	Relapse	None	11	<6.25	<0.4	PE, C	6	30	<0.4	
1	Relapse	None	21	<6.25	0.7	PE, C	7	70	<0.4	↓
18	Relapse	Headache	26	23	<0.4	PE, C	6	97	<0.4	
19	Relapse	None	37	<6.25	<0.4	PE, C	7	13	<0.4	
20	Relapse	None	35	<6.25	<0.4	PE, C	8	21	<0.4	
21	Relapse	None	43	<6.25	<0.4	PE, CM	11	<6.25	4	↑
21	Relapse	None	19	<6.25	1.2	FFP, M, C, PE	16†	<6.25	16	↑
22	Relapse	Headache	56	49	<0.4	PE, C	12	45	<0.4	
23	Relapse	None	11	<6.25	5.0	PE, CR	25	<6.25	50	↑

Plts, platelet count; PE, plasma exchange; PE courses, number of plasma exchange sessions; G, glucocorticoids; V, vincristine; R, rituximab; and M, mycophenolatomofetil.

↓ indicates decrease in ADAMTS-13 inhibitor titre; ↑↓, increase in inhibitor titre was eventually followed by decrease in inhibitor titre; and ↑, persistent increase in inhibitor titre.

*ADAMTS-13 activity and inhibitor titre results from plasma samples collected during PE therapy (see Fig 1B, F), because these patients were not analysed before initiation of PE-therapy.

†Patients 15 and 22 received FFP infusion prior to PE therapy.

(patient 11) and one was African (patient 14). The median age was 45 years with a range of 21–71 years. Eleven patients (48%) were obese with a body mass index (BMI) ≥ 30 kg/m². Twenty-one patients (91%) had idiopathic TTP. One patient developed TTP after ticlopidine therapy (patient 21) and one patient developed TTP after fetal death in the 26th week of gestation (patient 11). Fourteen patients were investigated in their initial episode of TTP and 11 patients were investigated in one or more relapses. Neurological abnormalities were present in 13 of 14 patients with an initial episode of TTP, nine of whom suffered coma or seizures (Table I). Seven patients with a relapse of TTP lacked any neurological abnormalities and four patients experienced only minor symptoms, such as headaches, dizziness, paraesthesia and mild aphasia. All patients were treated with PE therapy combined with glucocorticoids until consistent nor-

malization of platelet count and LDH level for at least three consecutive days. Two patients (patients 15 and 21) were initially treated with fresh frozen plasma (FFP) infusions, which did not induce a sufficient response. Eight patients, who had either severe neurological symptoms (seizures) or who exhibited insufficient increase in platelet count during PE therapy were treated with vincristine (Table I; Figs 1 and 2). Patients 8, 11 and 23 were treated with rituximab (one to two doses of 375 mg/m²) (Figs 1 and 2). Two patients received 1–3 g/d mycophenolatomofetil (patient 11 and 21; Table I). Clinical remission was achieved in 21 patients (91%). One patient with an initial episode of TTP died on the day of admission from massive cerebral bleeding during her first PE session (patient 13). Another patient with an initial episode of TTP died 4 d after initiation of PE therapy from myocardial infarction (patient 14).

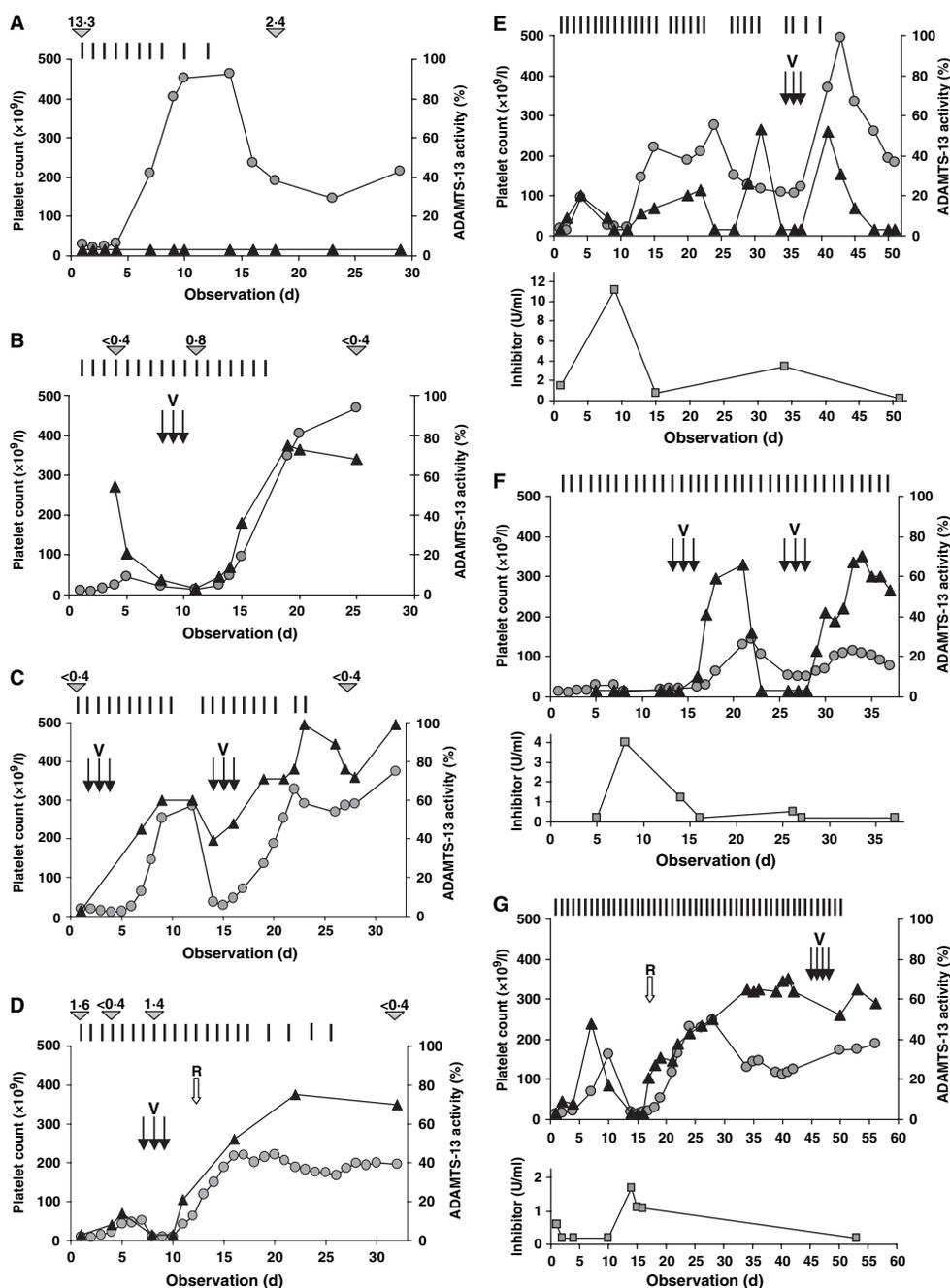


Fig 1. Course of platelet count (grey circles, $\times 10^9/l$), ADAMTS-13 activity (black triangles, expressed as a percentage of normal plasma) and inhibitor titre during PE therapy for patients 3, 6, 7, 8, 10, 11 and 12 (A-G, respectively) with an initial episode of TTP. Each PE session is indicated by a vertical black line. The inhibitor titre expressed in U/ml is depicted on the top of inverted grey triangles, except for patients 10–12, where the inhibitor titre is displayed in an additional graph. Each dose of vincristine (V, 1 mg) and rituximab (R, 375 mg/m²) is indicated by an arrow.

ADAMTS-13 activity, inhibitor titre, platelet count and the response to PE-therapy

Severe ADAMTS-13 deficiency was present in 14 of 14 patients with an initial episode of TTP and in nine of 11 patients with a relapse of TTP (Table 1). Inhibitory activity against ADAMTS-13 could be detected in 20 of 25 episodes (80%) from 21 patients with severe ADAMTS-13 deficiency (Table 1). The

inhibitor titre before initiation of PE therapy is illustrated in Fig 3 and ranged from 0.6 to 13.3 U/ml (median = 1.5 U/ml) and from 0.7 to 82 U/ml (median = 2.3 U/ml) in patients with an initial episode and in patients with a relapse, respectively. There was no statistical difference for the inhibitor titre between the two groups (Fig 3, $P > 0.05$, Mann-Whitney U -test). Patients with an initial episode had significantly lower platelet counts at admission

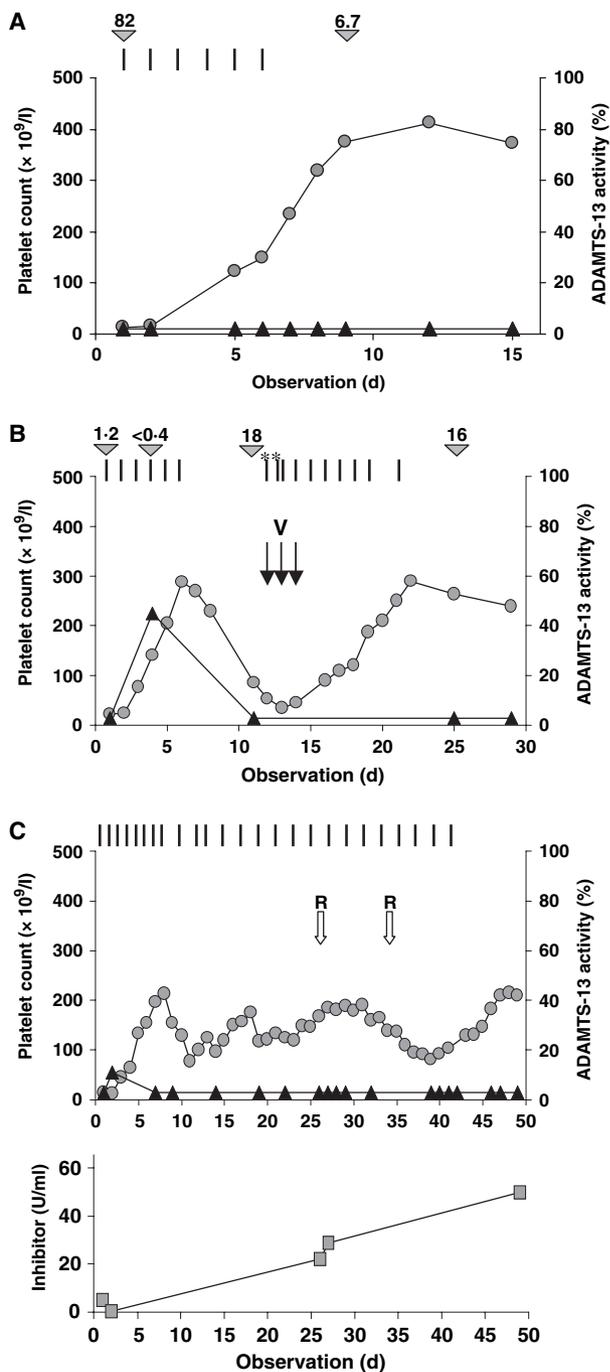


Fig 2. Course of platelet count (grey circles, $\times 10^9/l$), ADAMTS-13 activity (black triangles, expressed as a percentage of normal plasma) and inhibitor titre during PE therapy for patients 17, 21 and 23 (A–C, respectively) with a relapse of TTP. Each PE session is indicated by a vertical black line. Patient 21 received 1 l FFP instead of PE, twice (marked by an asterisk on the top of the vertical black line). The inhibitor titre expressed in U/ml is depicted on the top of inverted grey triangles, except for patient 23, where inhibitor titre is shown in an additional graph. Each doses of vincristine (V; 1 mg) and rituximab (R; 375 mg/m²) is indicated by an arrow.

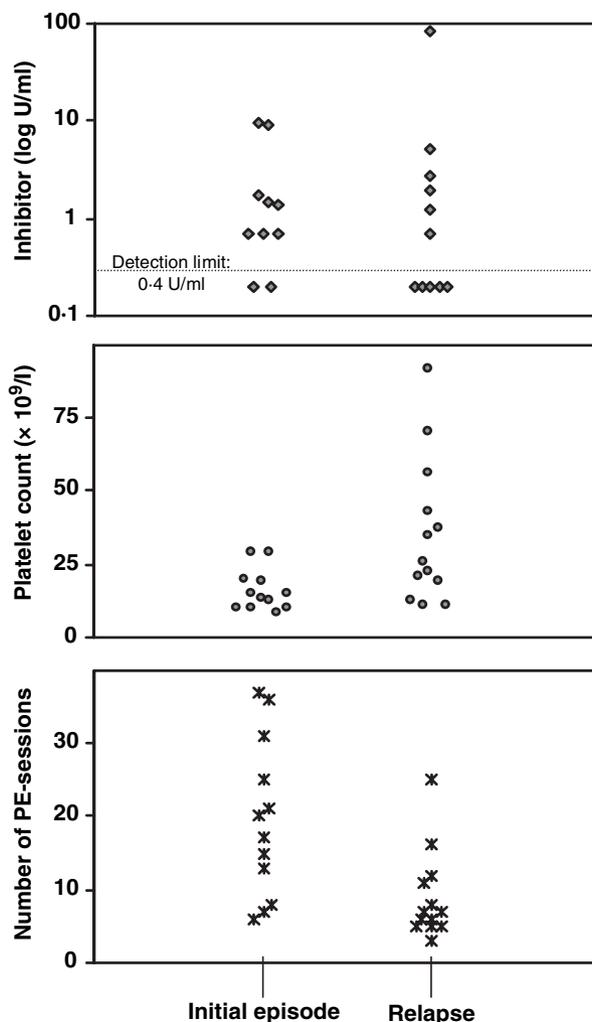


Fig 3. Inhibitor titre (log U/ml) and platelet count ($\times 10^9/l$) at admission and number of required PE sessions for 21 surviving patients in initial episodes ($n = 10$) and/or in relapses of TTP ($n = 13$). The initial inhibitor titre was not available for two patients with initial episodes and is only shown for episodes with severe ADAMTS-13 deficiency. The broken horizontal line indicates the detection limit of the ADAMTS-13 inhibitor assay (0.4 U/ml).

(mean = $16 \times 10^9/l$) than patients with a relapse (mean = $35 \times 10^9/l$, Fig 3, $P < 0.0001$, unpaired *t*-test). The treatment of initial episodes required significantly more PE sessions to achieve remission (median = 19) than the treatment of relapses (median = 7, Fig 3, $P < 0.007$, Mann–Whitney *U*-test). There was no correlation between the number of PE sessions necessary and inhibitor titre, platelet count, age or BMI.

The effect of PE-therapy on ADAMTS-13 activity, inhibitor titre and platelet count in patients with an initial episode of TTP

To investigate the effect of PE on ADAMTS-13 activity and corresponding inhibitor titre, serial blood samples during

PE-therapy were obtained from all surviving patients with an initial episode of TTP ($n = 12$). Two patients (patients 1 and 2, Table I) showed immediate increases in platelet count and ADAMTS-13 activity, requiring only six and seven PE sessions, respectively. Patient 3 (Table I) showed persistent severe ADAMTS-13 deficiency (her inhibitor titre decreased from 13.3 to 2.6 U/ml) but also responded with a prompt increase in platelet count as illustrated in Fig 1A. Patients 4–12 (Table I) responded less quickly to PE therapy, requiring 13–37 PE-sessions (mean = 21). Increase in platelet count was highly associated with recovery of ADAMTS-13 activity ($r > 0.6$, $P < 0.01$, Spearman rank correlation) in seven of these patients (patients 4–8, 11 and 12). Patients 9 and 10 did not show a correlation between ADAMTS-13 activity and platelet count; patient 9 displayed delayed increase in ADAMTS-13 activity and patient 10 showed only transient increase in ADAMTS-13 activity during PE-therapy. Six patients (patients 6, 8–12) showed a temporary drop in ADAMTS-13 activity to undetectable levels and a corresponding boost in inhibitor titre during PE therapy. The increase in inhibitor titre was associated with a decrease in platelet count, as illustrated in Fig 1B–G, and a recurrence of clinical symptoms in five of these patients.

The effect of PE therapy on ADAMTS-13 activity, inhibitor titre and platelet count in patients with a relapse of TTP

Severe ADAMTS-13 deficiency was found in nine patients with one or two relapses of TTP. PE therapy induced rapid restoration in platelet count and in ADAMTS-13 activity in five of these patients (patients 1, 3, 16, 19 and 20, Table I). Four patients (patients 15, 17, 21 and 23, Table I) displayed persistent severe ADAMTS-13 deficiency despite a sufficient clinical response. The course of platelet count, ADAMTS-13 activity and inhibitor titre is exemplary described for patients 18, 21 and 23 in Fig 2A–C. PE therapy induced a decline in inhibitor titre from 82 to 6.7 U/ml in patient 17. Patients 15, 21 and 23 showed an approximate 10-fold increase in inhibitor titre during PE therapy (Table I, Fig 2). These patients tended to require more PE sessions than the other patients with a relapse of TTP (Table I). The increase in inhibitor titre in patients with a relapse of TTP was, in contrast to patients with an initial episode of TTP, permanent and not associated with a decrease in platelet count or the occurrence of clinical symptoms.

Therapeutic effects of vincristine and rituximab

Eight patients with an initial episode of TTP were treated with vincristine (1 mg/d), as indicated in Table I and Fig 1B–G. Vincristine induced an immediate increase in ADAMTS-13 activity as well as in the platelet count in seven of eight patients (Fig 1). Patient 13 received one dose of vincristine 1 d before she died from myocardial infarction. Two patients (patients 8 and 12) with an initial episode received one dose of rituximab

(375 mg/m²; Fig 1D and G). Treatment with rituximab was followed by normalization of platelet counts and increase in ADAMTS-13 activity. However, it is unclear whether rituximab contributed to the recovery from thrombocytopenia and ADAMTS-13 deficiency, since both parameters had already started to increase in both patients prior to the infusion of rituximab. One patient, with a relapse of TTP and an increasing inhibitor titre during PE therapy (patient 23), received two doses of rituximab (375 mg/m²; Fig 2C). Consistent clinical remission was achieved 3 weeks after the first dose of rituximab, but again it is unclear if normalization in platelet count was because of rituximab-therapy. The inhibitor titre in this patient increased further within 3 weeks of the first rituximab-infusion from 22 to 52 U/ml (Fig 2C).

Discussion

The prevalence of severe ADAMTS-13 deficiency in patients with TTP reported in the literature ranges from 48% to 100% (Furlan *et al*, 1998a; Tsai & Lian, 1998; Veyradier *et al*, 2001; Mori *et al*, 2002; Remuzzi *et al*, 2002; Fujimura, 2003; Kremer Hovinga *et al*, 2004; Peyvandi *et al*, 2004; Zheng *et al*, 2004). In this study, 91% of the patients with TTP demonstrated severe ADAMTS-13 deficiency. The variable prevalence of severe ADAMTS-13 deficiency within the published reports might be caused, at least in part, by different diagnostic criteria used by the various groups. However, the two patients with measurable ADAMTS-13 activity in our cohort were clinically indistinguishable from patients who were admitted with severe ADAMTS-13 deficiency. We thus confirmed the observation of most studies, that a significant proportion of patients with a definite clinical diagnosis of idiopathic TTP have measurable ADAMTS-13 activity. Tsai (2003) proposed a re-classification of thrombotic microangiopathies, by which a TMA associated with hereditary or acquired ADAMTS-13 deficiency (usually labelled as TTP) should be clearly distinguished from TMA without severe ADAMTS-13 deficiency. One patient of our cohort (patient 18) interestingly showed undetectable ADAMTS-13 activity during his first relapse (data not shown), whereas his second relapse was accompanied by a decline in ADAMTS-13 activity from 100% to 23%. Fontana *et al* (2004) described a similar patient who showed severe ADAMTS-13 deficiency in his initial episode of TTP and measurable protease activity of 15% during relapse. We conclude that ADAMTS-13 deficiency can be quite variable during a given patient's history and should thus not be used to classify the disease. It is moreover not possible to exclude a pathogenic role of ADAMTS-13, even though ADAMTS-13 activity might be detectable *in vitro*. Dong *et al* (2002) provided evidence that proteolysis of VWF by ADAMTS-13 takes place on the endothelial surface. In this regard, it is noteworthy that Scheiflinger *et al* (2003) reported a TTP-patient with high titres of IgG and IgM autoantibodies that bound to ADAMTS-13, but which did not neutralize ADAMTS-13 activity *in vitro*. It could very well be possible that ADAMTS-13 activity is

blocked *in vivo*, e.g. by inefficient docking of the enzyme to the endothelial surface, which would not be detectable by commonly used static *in vitro* assays. In summary, we suggest that diagnosis of TMA should primarily be a clinical diagnosis and not yet depend on ADAMTS-13 activity as measured using current methodology.

Our data illustrate that the clinical course differs significantly between initial episodes and relapses of TTP. The treatment of initial episodes required significantly more PE-sessions to achieve remission than the treatment of relapses. Ninety-three per cent of the patients with initial episodes developed neurological disturbances, the majority suffered from life-threatening symptoms, such as seizures and coma. In contrast, patients with a relapse of TTP lacked any neurological symptoms or suffered only minor symptoms, such as headaches, dizziness, paraesthesia or mild aphasia. Patients with initial episodes of TTP showed significantly lower platelet counts and higher mortality than patients with relapses. We assume that clinical manifestations during a relapse were less severe because most relapses were diagnosed during a routine check-up. We thus confirmed the observation of most investigators, that patients with a delayed diagnosis and a delayed initiation of plasma therapy experienced a more severe course of illness with slow or even no response to PE therapy. Our study shows that it is necessary to clearly differentiate between patients with relapse and those with an initial episode of TTP. This has not always been done in the past and might explain some discrepancies in various studies.

The prevalence of ADAMTS-13 neutralizing inhibitors in patients with severe ADAMTS-13 deficiency ranges between 53% and 100% (Furlan *et al.*, 1998a; Tsai & Lian, 1998; Veyradier *et al.*, 2001; Mori *et al.*, 2002; Vesely *et al.*, 2003; Kremer Hovinga *et al.*, 2004; Peyvandi *et al.*, 2004; Zheng *et al.*, 2004). In our patient cohort, 17 of 21 patients (81%) with severe ADAMTS-13 deficiency showed ADAMTS-13-neutralizing activity, with an inhibitor level ranging between 0.6 and 82 U/ml (median = 1.5 U/ml). Tsai *et al.* (2001) found that patients with high inhibitor titres (≥ 2 U/ml) had a lower platelet count, a higher prevalence of neurological abnormalities and a delayed response to PE therapy compared with patients with low inhibitor titres (≤ 2 U/ml). Our study did not support these findings, since we found no correlation between the inhibitor titre at admission and the severity of clinical symptoms, the response to PE-therapy nor the initial platelet count. The response to PE-therapy was also not related to other features, such as age and BMI.

For patients with severe ADAMTS-13 deficiency, PE therapy led to a recovery in ADAMTS-13 activity in 64% of the investigated episodes. Six patients showed persistent severe ADAMTS-13 deficiency after PE therapy despite a clinical response. PE therapy induced a decline in inhibitor titre in three of these patients. In contrast, three patients with recurrent disease showed an approximate 10-fold increase in inhibitor titre. The course of inhibitor titre in these patients was highly suggestive of an anamnestic response to FFP. A rise

in inhibitor titre during PE-therapy has been described in some individuals (Furlan *et al.*, 1998b; Tsai, 2000; Knöbl *et al.*, 2003; Zheng *et al.*, 2004). According to our data, anamnestic response to FFP is a frequent event, since it occurs in 20% of the patients with recurrent disease, but, interestingly, in none of the patients with an initial episode of TTP. It is significant that these patients responded to PE therapy. This draws the current supposition into question, that the therapeutic efficacy of PE is the result of PE-induced recovery in ADAMTS-13 activity. In theory, this alleged conflict might be resolved by one or more of the following possibilities: (i) PE eliminates or adds an hitherto unidentified factor; (ii) static *in vitro* measurement of ADAMTS-13 activity does not portray ADAMTS-13 activity *in vivo*; and/or (iii) therapeutic proteolysis of VWF occurs *in vivo* during the PE session. We favour the latter possibility, since Dong *et al.* (2002) found that ADAMTS-13-catalysed proteolysis of the VWF occurs within seconds on the surface of endothelial cells. In patients with a persistently high inhibitor titre, a short-term recovery in ADAMTS-13 activity during the PE session might therefore result in sufficient proteolysis of ultralarge VWF before the inhibitor neutralizes endogenous or added ADAMTS-13.

We found a temporary drop of ADAMTS-13 activity to undetectable levels and a corresponding rise in inhibitor titre during PE therapy in six of 12 surviving patients (50%) with an initial episode of TTP. The increase in inhibitor titre for these patients was, in contrast to the elevation in inhibitor titre for patients with a relapse of TTP, always transient and, for five of these patients, was associated with a drop in platelet count and a recurrence of clinical symptoms. The elevation in inhibitor titre might not be responsible for the worsening in the clinical condition, since the increase in inhibitor titre does not lead to a decrease in platelet count in patients with a relapse of TTP. We thus assume that elevation in inhibitor titre in patients with an initial episode of TTP is not a cause, but rather a symptom, of other disease-determining factors.

Treatment with vincristine induced a rapid increase in platelet count and in ADAMTS-13 activity in seven of eight patients. The efficacy of vincristine in the treatment of TTP has been shown in the past (Ferrara *et al.*, 2002). However, our study shows, for the first time, a prompt response in both the platelet count and ADAMTS-13 activity. Vincristine is believed to restrain VWF-platelet interaction and to inhibit the production of autoantibodies, presumably against ADAMTS-13. Considering the half-life of IgG-autoantibodies, a rapid increase in ADAMTS-13 activity within 1–2 d after administration of vincristine would not be expected. The vincristine-induced increase in ADAMTS-13 activity might, therefore, rather be a consequence of vincristine-induced inhibition of VWF-platelet interaction. Binding of platelet glycoprotein Ib α to VWF stimulates ADAMTS-13-mediated VWF-proteolysis (Nishio *et al.*, 2004), which might result in enhanced clearance of ADAMTS-13.

Rituximab treatment was followed by sustained remission in all three cases. As reviewed by Yomtovian *et al.* (2004),

Rituximab, a chimaeric monoclonal antibody against CD20, has been successfully used in the treatment of TTP. A rituximab-induced decrease in ADAMTS-13 inhibitor titre has been observed in six of eight patients, but the influence of concurrent therapies could not always be excluded. In our study, the therapeutic benefit of rituximab therapy could not be conclusively determined. One patient with recurrent TTP showed an increase in inhibitor titre from 22 to 52 U/ml within 3 weeks after the first rituximab infusion. This patient received only two doses of rituximab, which might not be sufficient to inhibit production of ADAMTS-13-autoantibodies. In addition, our patients underwent PE on the day after infusion of rituximab, which could have led to premature removal of the antibodies (Yomtovian *et al*, 2004).

This study demonstrated that ADAMTS-13 activity and corresponding inhibitor levels, as measured by current non-physiological static methodology, relate to the clinical course of TTP during PE treatment, but are, however, not solely responsible for the therapeutic efficacy of PE. Future research on (i) *in vitro* measurement of ADAMTS-13 parameters; and (ii) other disease-determining factors are warranted. Our data verify the clinical usefulness of vincristine therapy in acute TTP and hint at a direct effect of vincristine on ADAMTS-13 metabolisms. However, further studies are necessary to verify the specific mechanism of action.

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References

- Antoine, G., Zimmermann, K., Plaimauer, B., Grillowitz, M., Studt, J.D., Lämmle, B. & Scheiflinger, F. (2003) ADAMTS13 gene defects in two brothers with constitutional thrombotic thrombocytopenic purpura and normalization of Von Willebrand factor-cleaving protease activity by recombinant human ADAMTS13. *British Journal of Haematology*, **120**, 821–824.
- Assink, K., Schiphorst, R., Allford, S., Karpman, D., Etzioni, A., Brichard, B., Van de Kar, N., Monnens, L. & Van den Heuvel, L. (2003) Mutation analysis and clinical implications of Von Willebrand factor-cleaving protease deficiency. *Kidney International*, **63**, 1995–1999.
- Böhm, M., Vigh, T., Scharrer, I. (2002) Evaluation and clinical application of a new method for measuring activity of Von Willebrand factor-cleaving metalloprotease (ADAMTS13). *Annals of Hematology*, **81**, 430–435.
- Dong, J.F., Moake, J.L., Nolasco, L., Bernardo, A., Arceneaux, W., Shrimpton, C.N., Schade, A.J., McIntire, L.V., Fujikawa, K. & López, J.A. (2002) ADAMTS-13 rapidly cleaves newly secreted ultralarge Von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood*, **100**, 4033–4039.
- Ferrara, F., Annunziata, M., Ollio, F., Palmieri, S., Copia, C., Mele, G., Pocal, B. & Schiavone, E.M. (2002) Vincristine as treatment for recurrent episodes of thrombotic thrombocytopenic purpura. *Annals of Hematology*, **81**, 7–10.
- Fontana, S., Kremer Hovinga, J.A., Studt, J.D., Alberio, L., Lämmle, B. & Taleghani, B.M. (2004) Plasma therapy in thrombotic thrombocytopenic purpura: review of the literature and the Bern experience in a subgroup of patients with severe acquired ADAMTS-13 deficiency. *Seminars in Haematology*, **41**, 48–59.
- Fujikawa, K., Suzuki, H., McMullen, B. & Chung, D. (2001) Purification of human Von Willebrand factor-cleaving protease and its identification as a new member of the metalloproteinase family. *Blood*, **98**, 1662–1666.
- Fujimura, Y. (2003) Is Upshaw–Schulman syndrome congenital thrombotic thrombocytopenic purpura or hemolytic-uremic syndrome? Yes to both. *Journal of Thrombosis and Haemostasis*, **1**, 2457–2458.
- Furlan, M., Robles, R., Galbusera, M., Remuzzi, G., Kyrle, P.A., Brenner, B., Krause, M., Scharrer, I., Aumann, V., Mittler, U., Solenthaler, M. & Lämmle, B. (1998a) Von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic Syndrome. *New England Journal of Medicine*, **339**, 1578–1584.
- Furlan, M., Robles, R., Solenthaler, M. & Lämmle, B. (1998b) Acquired deficiency of Von Willebrand factor-cleaving protease in a patient with thrombotic thrombocytopenic purpura. *Blood*, **91**, 2836–2846.
- Gerritsen, H.E., Robles, R., Lämmle, B. & Furlan, M. (2001) Partial amino acid sequence of purified Von Willebrand factor-cleaving protease. *Blood*, **98**, 1654–1661.
- Knöbl, P., Haas, M., Laczika, K., Varadi, K. & Turecek, P.L. (2003) Immunoabsorption for the treatment of a patient with severe thrombotic thrombocytopenic purpura resistant to plasma exchange: kinetics of an inhibitor of ADAMTS13. *Journal of Thrombosis and Haemostasis*, **1**, 187–189.
- Kokame, K., Matsumoto, M., Soejima, K., Yagi, H., Ishizashi, H., Funato, M., Tamai, H., Konno, M., Kamide, K., Kawano, Y., Miyata, T. & Fujimura, Y. (2002) Mutations and common polymorphisms in ADAMTS13 gene responsible for Von Willebrand factor-cleaving protease activity. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 11902–11907.
- Kremer Hovinga, J.A., Studt, J.D., Alberio, L. & Lämmle, B. (2004) Von Willebrand factor-cleaving protease (ADAMTS-13) activity determination in the diagnosis of thrombotic microangiopathies: the Swiss experience. *Seminars in Hematology*, **41**, 75–82.
- Levy, G.G., Nichols, W.C., Lian, E.C., Foroud, T., McClintick, J.N., McGee, B.M., Yang, A.Y., Siemieniak, D.R., Stark, K.R., Gruppo, R., Sarode, R., Shurin, S.B., Chandrasekaran, V., Stabler, S.P., Sabio, H., Bouhassira, E.E., Upshaw, J.D., Ginsburg, D. & Tsai, H.M. (2001) Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature*, **413**, 488–494.
- Matsumoto, M., Kokame, K., Soejima, K., Miura, M., Hayashi, S., Fujii, Y., Iwai, A., Ito, E., Tsuji, Y., Takeda-Shitaka, M., Iwade, M., Umeyama, H., Yagi, H., Ishizashi, H., Banno, F., Nakagaki, T., Miyata, T. & Fujimura, Y. (2004) Molecular characterization of ADAMTS13 gene mutations in Japanese patients with Upshaw–Schulman syndrome. *Blood*, **103**, 1305–1310.
- Moake, J.L. (2002) Thrombotic thrombocytopenic purpura: the systemic clumping “plague”. *Annual Review of Medicine*, **53**, 75–88.

- Moake, J.L., Rudy, C.K., Troll, J.H., Weinstein, M.J., Colanino, N.M., Azocar, J., Seder, R.H., Hong, S.L. & Deykin, D. (1982) Unusually large plasma factor VIII: Von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. *New England Journal of Medicine*, **307**, 1432–1435.
- Mori, Y., Wada, H., Gabazza, E.C., Minami, N., Nobori, T., Shiku, H., Yagi, H., Ishizashi, H., Matsumoto, M. & Fujimura, Y. (2002) Predicting response to plasma exchange in patients with thrombotic thrombocytopenic purpura with measurement of VWF-cleaving protease activity. *Transfusion*, **42**, 572–580.
- Moschcowitz, E. (1924) Hyaline thrombosis of the terminal arterioles and capillaries: a hitherto undescribed disease. *Proceedings of the New York Pathology Society*, **24**, 21–24.
- Nishio, K., Anderson, P.J., Zheng, X.L. & Sadler, J.E. (2004) Binding of platelet glycoprotein Ib α to von Willebrand factor domain A1 stimulates the cleavage of the adjacent domain A2 by ADAMTS13. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 10578–10583.
- Peyvandi, F., Ferrari, S., Lavoretano, S., Canciani, M.T. & Mannucci, P.M. (2004) von Willebrand factor cleaving protease (ADAMTS-13) and ADAMTS-13 neutralizing autoantibodies in 100 patients with thrombotic thrombocytopenic purpura. *British Journal of Haematology*, **127**, 433–439.
- Pimanda, J.E., Maekawa, A., Wind, T., Paxton, J., Chesterman, C.N. & Hogg, P.J. (2004) Congenital thrombotic thrombocytopenic purpura in association with a mutation in the second CUB domain of ADAMTS13. *Blood*, **130**, 627–629.
- Remuzzi, G., Galbusera, M., Noris, M., Canciani, M.T., Daina, E., Bresin, E., Contaretti, S., Caprioli, J., Gamba, S., Ruggenti, P., Perico, N. & Mannucci, P.M. (2002) Von Willebrand factor cleaving protease (ADAMTS13) is deficient in recurrent and familial thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *Blood*, **100**, 778–785.
- Rock, G.A., Shumak, K.H., Buskard, N.A., Blanchette, V.S., Kelton, J.G., Nair, R.C. & Spasoff, R.A. (1991) Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian Apheresis Study Group. *New England Journal of Medicine*, **325**, 393–397.
- Scheiflinger, F., Knöbl, P., Trattner, B., Plaimauer, B., Mohr, G., Dockal, M., Dorner, F. & Rieger, M. (2003) Nonneutralizing IgM and IgG antibodies to Von Willebrand factor-cleaving protease (ADAMTS-13) in a patient with thrombotic thrombocytopenic purpura. *Blood*, **102**, 3241–3243.
- Schneppenheim, R., Budde, U., Oyen, F., Angerhaus, D., Aumann, V., Drewke, E., Hasenpflug, W., Häberle, J., Kentouche, K., Kohne, E., Kurnik, K., Mueller-Wiefel, D., Obser, T., Santer, R. & Sykora, K.W. (2003) Von Willebrand factor cleaving protease and ADAMTS13 mutations in childhood TTP. *Blood*, **101**, 1845–1850.
- Tsai, H.M. (2000) High titers of inhibitors of Von Willebrand factor-cleaving metalloproteinase in a fatal case of acute thrombotic thrombocytopenic purpura. *American Journal of Hematology*, **65**, 251–255.
- Tsai, H.M. (2003) Advances in the pathogenesis, diagnosis, and treatment of thrombotic thrombocytopenic purpura. *Journal of the American Society of Nephrology*, **14**, 1072–1081.
- Tsai, H.M. & Lian, E.C. (1998) Antibodies to Von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *New England Journal of Medicine*, **339**, 1585–1594.
- Tsai, H.M., Li, A., Rock, G. (2001) Inhibitors of Von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura. *Clinical Laboratory*, **47**, 387–392.
- Vesely, S.K., George, J.N., Lämmle, B., Studt, J.D., Alberio, L., El-Harake, M.A. & Raskop, G.E. (2003) ADAMTS13 activity in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: relation to presenting features and clinical outcomes in a prospective cohort of 142 patients. *Blood*, **102**, 60–68.
- Veyradier, A., Obert, B., Houllier, A., Meyer, D. & Girma, J.P. (2001) Specific Von Willebrand factor-cleaving protease in thrombotic microangiopathies: a study of 111 cases. *Blood*, **98**, 1765–1772.
- Veyradier, A., Lavergne, J.M., Ribba, A.S., Obert, B., Loirat, C., Meyer, D. & Girma, J.P. (2004) Ten candidate ADAMTS13 mutations in six French families with congenital thrombotic thrombocytopenic purpura (Upshaw–Schulman syndrome). *Journal of Thrombosis and Haemostasis*, **2**, 424–429.
- Yomtovian, R., Niklinski, W., Silver, B., Sarode, R. & Tsai, H.M. (2004) Rituximab for chronic recurring thrombotic thrombocytopenic purpura: a case report and review of the literature. *British Journal of Haematology*, **124**, 787–795.
- Zheng, X.L., Kaufman, R.M., Goodnough, L.T. & Sadler, J.E. (2004) Effect of plasma exchange on plasma ADAMTS13 metalloprotease activity, inhibitor level, and clinical outcome in patients with idiopathic and nonidiopathic thrombotic thrombocytopenic purpura. *Blood*, **103**, 4043–4049.