

## Antibodies against the CUB1-2 domains of ADAMTS13 in a patient with benign monoclonal gammopathy: no causal relationship

We present a patient with a history of benign monoclonal gammopathy, who developed thrombotic thrombocytopenic purpura (TTP), initially presenting as bilateral serous retinal detachment. Plasma of the patient contained high titers of anti-ADAMTS13 antibodies that were directed towards the disintegrin/TSR1/cysteine-rich/spacer and CUB1-2 domains. ADAMTS13 activity was undetectable. Total IgG purified from plasma of the patient partially inhibited ADAMTS13 activity. In contrast, the isolated M-protein did neither bind to, nor inhibit activity of ADAMTS13. We conclude that in this patient the monoclonal gammopathy and TTP co-existed as distinct pathological entities.

*Haematologica* 2007; 92(6):e74-e76

Idiopathic thrombotic thrombocytopenic purpura (TTP) is a rare disorder associated with the presence of unusually large von Willebrand factor (VWF) multimers in plasma, which induce platelet aggregation and consumption, tissue ischaemia, and microangiopathic haemolytic anaemia.<sup>1</sup> Unusually large VWF multimers arise as a consequence of severely reduced activity of the VWF-cleaving protease ADAMTS13 (a disintegrin and metalloprotease, with thrombospondin-1-like domains).<sup>1</sup> In most patients with acquired TTP, antibodies directed to the cysteine-rich/spacer domains of ADAMTS13 are present.<sup>2,5</sup>

In the current study, we describe a patient with a history of benign monoclonal gammopathy who developed TTP, and initially presented with bilateral serous retinal detachment. We hypothesized that in this patient the monoclonal immunoglobulin (M-protein) was directed against ADAMTS13, and we conducted a series of in vitro experiments to test this hypothesis.

### Case report

A 43-year-old woman was referred to the department of ophthalmology of our tertiary care university hospital. She was known in a different hospital with chronic discoid lupus erythematoses and monoclonal gammopathy of undetermined significance (MGUS) for four years (most recent values: lambda immunoglobulins 28 g/L, 2.2 % of lambda monoclonal plasma cells in bone marrow). Since three days she experienced progressive bilateral loss of vision with nausea and vomiting. On admission her visual acuity was 1/60 in both eyes. Fundoscopic examination revealed bilateral serous retinal detachment and two small peripheral retinal haemorrhages in the left eye. The department of internal medicine was consulted to search for potential underlying disease. She appeared ill and anaemic, and she had icteric sclerae. Her blood pressure was 110/60 mmHg, there was no fever, and there were no neurological abnormalities. Laboratory studies showed a haemoglobin concentration of 5.6 mmol/L, thrombocytopenia ( $<10 \times 10^9/L$ ), normal renal function (creatinine 73  $\mu\text{mol/L}$ ), and increased total bilirubin (104  $\mu\text{mol/L}$ ; conjugated bilirubin 18  $\mu\text{mol/L}$ ) and lactate dehydrogenase (3033 U/L) concentrations. The Coombs test was negative, and the M-protein concentration was 31.9 g/L. A peripheral blood smear revealed schistocytes, and bone marrow examination demonstrated 6% plasma cells and an increased number of megakaryocytes. The diagnosis of TTP was considered, and she was treated initially with

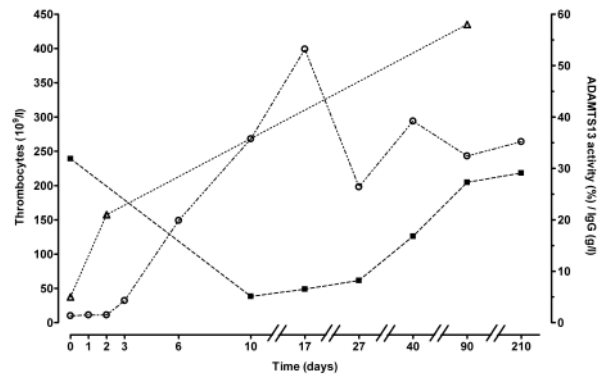


Figure 1. Plasma concentrations of thrombocytes and immunoglobulins, and ADAMTS13 activity. The course of the number of circulating thrombocytes (open circles), plasma immunoglobulin concentration (filled squares), and activity of ADAMTS13 (open triangles) in time (days after first plasma exchange).

high dose glucocorticoids and transfusions of fresh frozen plasma. Because of a rapid decline of the Hb-concentration, she received red blood cell transfusions. Daily plasma exchange was started the following day. The diagnosis of TTP was confirmed later by ADAMTS13 activity levels that fell below 5%. Plasma exchange therapy induced a rapid increase in thrombocytes, haemoglobin, and ADAMTS13 activity (Figure 1). The frequency of plasma exchange was tapered after ten days and stopped twenty days after the first day of treatment. Three weeks after initial presentation, all laboratory values had returned to normal, and ADAMTS13 activity had increased to 57%. Both eyes showed complete retinal reattachment without fundoscopic abnormalities, and visual acuity had increased to 1.0 in both eyes. She stayed in complete remission, despite the recurrence of the M-component (Figure 1).

### Design and methods

ADAMTS13 activity was determined using a collagen binding assay (CBA) of degraded VWF essentially as described previously.<sup>6</sup> Levels of ADAMTS13 activity in normal individuals range from 28-119% whereas ADAMTS13 activity levels are  $< 5\%$  in patients with acquired or congenital TTP.<sup>6</sup> Levels of anti-ADAMTS13 antibodies in plasma were measured using Technozym inhibitor ELISA (Technoclone, Vienna, Austria) according to the instructions provided by the manufacturer. Values of  $> 15$  U/mL were considered to reflect the presence of anti-ADAMTS13 antibodies in accordance with the instructions provided by the manufacturer. The ADAMTS13 antibody assay has produced very high values in occasional individuals without TTP or severe ADAMTS13 deficiency.<sup>7</sup> To confirm the presence of antibodies to ADAMTS13, we assessed the binding of patient-derived antibodies to a panel of recombinant ADAMTS13 fragments, essentially as described previously.<sup>4</sup> Total immunoglobulins (IgG) were purified from plasma of the patient using protein G affinity chromatography (Amersham Biosciences, Uppsala, Sweden). Using gel electrophoresis, partial purification of the M-protein was performed by excision of the band (without immune fixation) from the electrophoresis gel and subsequent diffusion of the M-protein into phosphate buffered saline (PBS). After this procedure 7.7% kappa immunoglobulins and 92.3% lambda immunoglobulins were found. Thus,

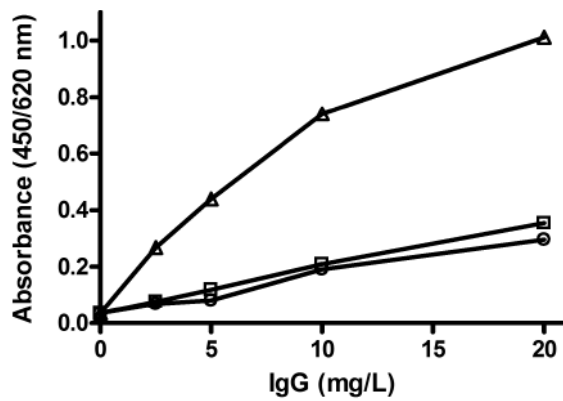


Figure 2. Binding of IgG to ADAMTS13. Binding of the isolated M-protein (open squares), total IgG purified from plasma of the patient (open triangles), and IgG purified from NHP (open circles), to immobilized ADAMTS13.

taking into account the normal kappa/lambda distribution of 2/1, there was 88.4% M-protein in the sample and 11.6% polyclonal immunoglobulins. To determine the effect of the isolated immunoglobulins on FRETSS-VWF73 substrate (Peptides International, Louisville, KY, USA) cleavage by plasma ADAMTS13, thorough dialysis was required to remove all traces of salts and buffer components. IgG purified from normal pooled plasma (NHP) was included as control. Purified IgG was pre-incubated for 1 hour with NHP, prior to the addition of FRETSS-VWF73 substrate and start of measurement. Residual ADAMTS13 activity was calculated as described previously.<sup>8</sup> Binding of immunoglobulins to ADAMTS13 was determined using a modification of the Technozym inhibitor ELISA; instead of serial plasma dilutions, several dilutions of IgG in incubation buffer were used.

#### Results and Discussion

Our patient presented with subacute loss of vision due to bilateral serous retinal detachment. This is a rare ocular complication of TTP, which can develop, mostly late, in the course of the disease.<sup>9-11</sup> Retinal detachment is thought to occur due to localized ischaemic injury to the retinal pigment epithelium, caused by microthrombi in the small choroidal vessels, which inhibits adequate transport of fluid from the subretinal space.<sup>11</sup> Our patient regained complete vision by treatment with plasma exchange therapy.

We found that ADAMTS13 activity was undetectable in plasma. Anti-ADAMTS13 antibody levels were high (1632 U/mL). Because our patient had a history of a benign monoclonal gammopathy, we hypothesized that the M-protein might be directed against ADAMTS13. It is known that M-proteins can possess antibody activity,<sup>12</sup> but M-proteins directed against ADAMTS13 have never been described, although the possibility has been postulated in 1991, when the pathophysiology of TTP was not yet elucidated.<sup>13</sup> We isolated the M-protein to determine whether this immunoglobulin was directed against ADAMTS13. The isolated M-protein, at a concentration of 50 µg/mL did not inhibit VWF-cleavage activity of ADAMTS13 in a FRETSS-VWF73 assay, whereas 400 µg/mL of total IgG inhibited ADAMTS13 activity with approximately 40% (data not shown). Binding assays demonstrated that the isolated M-protein did not bind to ADAMTS13, in contrast to total IgG purified from plasma of the patient (Figure 2). This experiment demonstrates

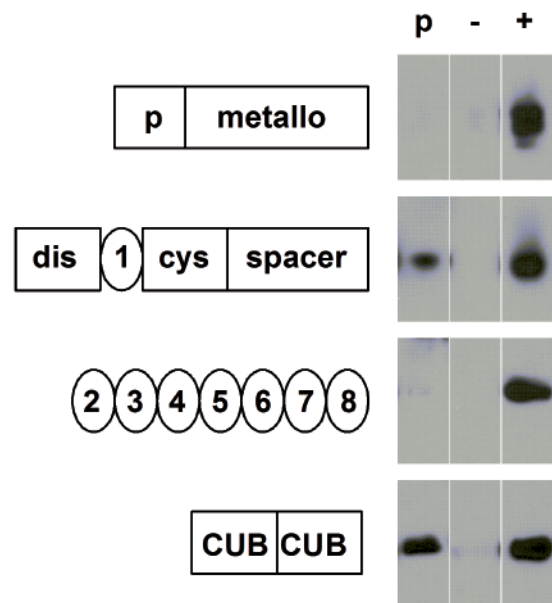


Figure 3. Epitope-mapping of anti-ADAMTS13 antibodies by immuno precipitation. Antibodies present in 30 µL of plasma of the patient (p) were immobilized on protein G sepharose and incubated with recombinant ADAMTS13 protein fragments. Antibodies present in 30 µL of NHP were included as negative control (-), as a positive control a monoclonal antibody directed against the c-terminal V5-tag on the recombinant fragments was included (+). ADAMTS13 fragments that bound specifically to the immobilized antibodies were detected on immunoblot.

that in this patient the M-protein is not involved in the pathogenesis of the TTP.

In most patients with acquired TTP, circulating anti-ADAMTS13 antibodies are present that neutralize ADAMTS13 activity. Recently, it was demonstrated that in all presently characterized patients with anti-ADAMTS13 antibodies, these antibodies are directed against the cysteine-rich/spacer domains.<sup>2,5</sup> To confirm the presence of anti-ADAMTS13 antibodies we determined the binding of patient derived IgG with a series of recombinant ADAMTS13 fragments. These experiments revealed predominant binding of antibodies to the carboxy-terminal CUB1-2 domains. In addition, less prominent, but significant binding to a fragment containing the disintegrin/TSR1/cysteine-rich/spacer domains was observed (Figure 3). Antibodies directed towards the CUB domains have previously been observed by Klaus *et al.*<sup>3</sup> The CUB domains are dispensable for VWF cleavage activity in vitro (2;14), although in vivo the CUB-1 domain may serve as the docking site for ADAMTS13 to bind unusually large VWF under flow.<sup>15</sup> Alternatively, antibodies directed towards the CUB domains may accelerate clearance of ADAMTS13 from the circulation.<sup>16</sup> Further studies are needed to determine whether antibodies directed towards the CUB domains of ADAMTS13 contribute to the pathogenesis of acquired TTP.

In summary, we report a patient with MGUS, with bilateral serous retinal detachment as the first presenting sign of TTP, and we studied the possible relationship between these conditions. The study emphasizes that ocular symptoms may be the first sign of TTP and that early diagnosis and treatment can induce complete recovery of vision. In addition, we demonstrated that MGUS and TTP can co-exist in one patient as distinct pathophysiological entities.

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Key words: ADAMTS13, TTP, MGUS, antibodies, CUB domain, retinal detachment

Acknowledgements: The authors thank Corrie de Kat Angelino for the isolation of the monoclonal immunoglobulins. NP Riksen is a recipient of a grant of the Netherlands Organisation for Scientific Research (ZonMw). Part of these studies was supported by a grant from the Dutch Thrombosis Foundation (TSN) (grant 2004.003).

Author contribution: NPR, NC, and MvD were treating physicians of the patient, initiated further *in vitro* studies, and wrote the paper. BML designed research, performed experiments, analysed data and wrote the paper. JV designed experiments and wrote the paper. IK designed research, performed experiments, and wrote the paper.

## References

1. Moake JL. Thrombotic microangiopathies. *N Engl J Med* 2002;347:589-600.
2. Soejima K, Matsumoto M, Kokame K, Yagi H, Ishizashi H, Maeda H et al. ADAMTS-13 cysteine-rich/spacer domains are functionally essential for von Willebrand factor cleavage. *Blood* 2003;102:3232-7.
3. Klaus C, Plaimauer B, Studt JD, Dörner F, Lammler B, Mannucci PM et al. Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. *Blood* 2004;103:4514-9.
4. Luken BM, Turenhout EA, Hulstein JJ, van Mourik JA, Fijnheer R, Voorberg J. The spacer domain of ADAMTS13 contains a major binding site for antibodies in patients with thrombotic thrombocytopenic purpura. *Thromb Haemost* 2005;93:267-74.
5. Zhou W, Dong L, Ginsburg D, Bouhassira EE, Tsai HM. Enzymatically active ADAMTS13 variants are not inhibited by anti-ADAMTS13 autoantibodies: a novel therapeutic strategy? *J Biol Chem* 2005;280:39934-41.
6. Gerritsen HE, Turecek PL, Schwarz HP, Lammler 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;82:1386-9.
7. Rieger M, Mannucci PM, Kremer Hovinga JA, Herzog A, Gerstenbauer G, Konetschny C et al. ADAMTS13 autoantibodies in patients with thrombotic microangiopathies and other immunomediated diseases. *Blood* 2005;106:1262-7.
8. Luken BM, Kaijen PH, Turenhout EA, Kremer Hovinga JA, van Mourik JA, Fijnheer R et al. Multiple B-cell clones producing antibodies directed to the spacer and disintegrin/thrombospondin type-1 repeat 1 (TSP1) of ADAMTS13 in a patient with acquired thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2006;4:2355-64.
9. Percival SP. Ocular findings in thrombotic thrombocytopenic purpura (Moschcowitz's disease). *Br J Ophthalmol* 1970;54:73-8.
10. Nanayakkara P, Gans RO, Reichert-Thoen J, ter Wee PM. Serous retinal detachment as an early presentation of thrombotic thrombocytopenic purpura. *Eur J Intern Med* 2000;11:286-8.
11. Lambert SR, High KA, Cotlier E, Benz EJ, Jr. Serous retinal detachments in thrombotic thrombocytopenic purpura. *Arch Ophthalmol* 1985;103:1172-4.
12. Dighiero G, Guilbert B, Ferman J, Lymberi P, Danon F, Avrameas S. Thirty-six human monoclonal immunoglobulins with antibody activity against cytoskeleton proteins, thyroglobulin, and native DNA: immunologic studies and clinical correlations. *Blood* 1983;62:264-70.
13. Back H, Nilsson G, Hansson GK, Rodjer S, Tarkowski A. M-component with reactivity against actin associated with thrombotic thrombocytopenic purpura. *Am J Med* 1991;91:429-33.
14. Zheng X, Nishio K, Majerus EM, Sadler JE. Cleavage of von Willebrand factor requires the spacer domain of the metalloprotease ADAMTS13. *J Biol Chem* 2003;278:30136-41.
15. Tao Z, Peng Y, Nolasco L, Cal S, Lopez-Otin C, Li R et al. Recombinant CUB-1 domain polypeptide inhibits the cleavage of ULVWF strings by ADAMTS13 under flow conditions. *Blood* 2005;106:4139-45.
16. Shelat SG, Smith P, Ai J, Zheng XL. Inhibitory autoantibodies against ADAMTS-13 in patients with thrombotic thrombocytopenic purpura bind ADAMTS-13 protease and may accelerate its clearance *in vivo*. *J Thromb Haemost* 2006;4:1707-17.