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 gam- mopathy and TTP co-existed as distinct patholog- ical entities.

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, thrombocyto- penia (<10x10^9/L), normal renal function (creatinine 73 µmol/L), and increased total bilirubin (104 µmol/L; conjugated bilirubin 18 µmol/L) and lactate dehydrogenase (5033 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 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 plas- ma exchange was started the following day. The diagno- sis 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 re- attachment without fundoscopic abnormalities, and visu- al acuity had increased to 1.0 in both eyes. She stayed in complete remission, despite the recurrence of the M-com- ponent (Figure 1).

Design and methods
ADAMTS13 activity was determined using a collagen binding assay (CBA) of degraded VWF essentially as described previously. Levels of ADAMTS13 activity in normal individuals range from 28-119% whereas ADAMTS13 activity levels are < 5% in patients with acquired or congenital TTP. Levels of anti-ADAMTS13 antibodies in plasma were measured using Techno- nzym 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. To confirm the presence of anti- bodies to ADAMTS13, we assessed the binding of patient-derived antibodies to a panel of recombinant ADAMTS13 fragments, essentially as described previously. 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 fix- ation) from the electrophoresis gel and subsequent diffu- sion of the M-protein into phosphate buffered saline (PBS). After this procedure 7.7% kappa immunoglobulins and 92.3% lambda immunoglobulins were found. Thus,
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. 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. 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. Alternatively, 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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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