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A patient with SLE-associated thrombotic microangiopathy and non-neutralizing antibodies against ADAMTS13

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Abstract

In this case report, we describe for the first time a patient with thrombotic thrombocytopenic purpura (TTP) accompanying highly active systemic lupus erythematosus (SLE) that was associated with non-neutralizing antibodies against the plasma metalloprotease ADAMTS13. Those non-neutralizing antibodies could have been the cause of ADAMTS13 depletion and consecutive TTP. More extensive analyses are required to determine the prevalence as well as the clinical relevance of non-neutralizing antibodies against ADAMTS13 in SLE patients.

Keywords: ADAMTS13; autoantibodies; SLE; TTP

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening syndrome of acute mechanical haemolytic anaemia, thrombocytopenia and visceral ischaemic manifestations related to the formation of platelet thrombi in the microcirculation [1]. TTP can be of congenital or acquired origin with TTP occurring in the context of predisposing conditions such as stem cell transplantation or treatment with calcineurin inhibitors being considered as 'secondary'. Recent research suggests that the functional deficiency of von Willebrand factor-cleaving protease ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 repeats), either congenital due to genomic mutations or acquired due to autoantibodies directed against ADAMTS13, plays an important role in the pathogenesis of primary TTP. The lack of ADAMTS13 may lead to the accumulation of unusually large von Willebrand factor (ULVWF) multimers, platelet clumping and thrombotic occlusions within the microvasculature [2,3].

Secondary TTP accompanying systemic lupus erythematosus (SLE) has been well reported in the literature but the pathogenic mechanism that accounts for their coexistence is incompletely understood. In SLE patients,

TTP seems to be of autoimmune, i.e. acquired nature because low levels of ADAMTS13 were usually associated with the presence of autoantibodies that inhibit the enzyme activity [1,3]. However, more recently, non-neutralizing antibodies, i.e. anti-ADAMTS13 immunoglobulin G (IgG) and/or IgM antibodies associated with normal ADAMTS13 activity, have been detected in 13–18% of SLE patients using an enzyme-linked immunosorbent assay (ELISA) [4] suggesting an additional mechanism for acquired ADAMTS13 deficiency in SLE [4,5]. However, the significance of this observation, i.e. non-neutralizing IgG anti-ADAMTS13 antibodies in SLE patients, remains to be determined. Here, for the first time, we describe a patient in whom active SLE and active TTP occur simultaneously, with severely reduced ADAMTS13 activity and in the presence of non-neutralizing anti-ADAMTS13 IgG antibodies.

Case report

In January 2007, a 20-year-old female patient was admitted to our hospital. She reported shortness of breath and increase in body weight from 84 to 103 kg within 2 weeks. Two weeks before, the patient suffered from an upper respiratory tract infection with fatigue, malaise and rhinitis, which was treated symptomatically. On admission, she was afebrile, and her blood pressure was 160/115 mm Hg with a pulse rate of 120/min. She presented a malar rash and anasarca. The patient also reported blurry vision of the left eye, which normalized before being confirmed by an ophthalmologist. No further focal neurological deficits could be found. Apart from reduced pulmonary respiratory sounds, lung and heart examination was normal.

A chest X-ray revealed cardiomegaly and bilateral pleural effusions. The trans-thoracic echocardiogram showed a pericardial effusion and global hypokinesia with a left ventricular ejection fraction of 45%.

Blood tests showed haemolytic anaemia with a haemoglobin of 60 g/L; total bilirubin was 30 $\mu\text{mol/L}$ (norm: 5–18 $\mu\text{mol/L}$), and lactate dehydrogenase was 774 U/L (norm: 135–214 U/L). Peripheral blood smear revealed 5 to 10 schistocytes per visual field. The platelet count was decreased to $17 \times 10^9/\text{L}$ (norm: $150\text{--}450 \times 10^9/\text{L}$). On admission, serum creatinine was 214 $\mu\text{mol/L}$ (norm: 45–93 $\mu\text{mol/L}$), and urinalysis revealed >50 dysmorphic and non-dysmorphic erythrocytes and one to two leucocytes per high-power field. The urine protein/creatinine ratio was 276 mg/mmol (norm <11 mg/mmol). Two days after admission, haemodialysis had to be started due to anuric renal failure.

A direct antiglobulin (Coombs) test was weakly positive for IgG. In addition, the patient had an antinuclear antibody (ANA) titer positive at 1:1280 (norm: <1:80) and antibodies against double-stranded DNA (727 IU/ml, Farr assay, norm: <7 U/ml). Complement C3 and C4 levels were low at 14 (norm: 50–90 mg/dl) and 2.7 mg/dl (norm: 10–40 mg/dl), respectively. No antibodies against cardiolipin (IgG and IgM) could be detected.

A renal biopsy showed a diffuse proliferative glomerulonephritis (GN) with characteristics of an endocapillary as well as a membranoproliferative GN. The glomeruli showed a number of mesangiolytic lesions and intravascular protein thrombi. In addition, renal infarction with acute tubular necrosis of unknown origin could be observed in some areas. Immunofluorescence was positive for IgG, IgM and IgA as well as for complement C1q, C4, C3 and C5b-9. Electron microscopy showed subendothelial deposits with finger print-like structures. The findings were compatible with severe type IV lupus nephritis but were not typical of thrombotic microangiopathy.

In a magnetic resonance imaging of the neurocranium, small vessels had a pearl cord-like appearance with ischaemias in the right cerebellum and the right frontal cortex. Radiologically, the findings were considered to be compatible with cerebral vasculitis.

Based on the initial findings, the diagnosis of SLE was established. In addition, due to features of thrombotic microangiopathy, ADAMTS13 activity was determined. Severely reduced ADAMTS13 activity (<5%) and antigen levels (0.130 $\mu\text{g/ml}$, normal antigen range 0.740–1.420 $\mu\text{g/ml}$) were found but no inhibitory anti-ADAMTS13 antibodies. Since the coincidence of SLE with hereditary ADAMTS13 deficiency appeared unlikely, further blood tests were initiated. Using an ELISA-based method, low levels of IgG anti-ADAMTS13 antibodies (titer 1/50) binding to ADAMTS13, but not inhibiting its function *in vitro*, were found. IgM and IgA anti-ADAMTS13 antibodies were negative. Subclass characterization of the IgG anti-ADAMTS13 antibodies showed a prevalence of IgG1 (89%) and very low levels of IgG3 (4%) and IgG2 (7%) antibodies. No IgG4 anti-ADAMTS13 antibodies could be detected.

Immunosuppression consisting of high-dose prednisone (methylprednisolone 1 g/day for 3 days followed by prednisone 150 mg/day, tapered during the following weeks) and cyclophosphamide pulses (500 mg every 2 weeks for 3 months, i.e. six doses) as well as daily plasma ex-

change (PEX) were started (3.5 L plasma were exchanged with fresh frozen plasma). In addition, due to the severe systemic involvement of SLE, anti-CD20 antibodies (rituximab) were given (two doses of 1 g administered 2 weeks apart). With this combined treatment, improvement of renal function, haemoglobin levels, platelet counts and normalization of ADAMTS13 activity were achieved. Haemodialysis could be stopped after 10 days. PEX was tapered and could be ceased after a total of 18 procedures. The patient became asymptomatic and could be discharged 5 weeks after admission. Under a maintenance therapy with mycophenolate and hydroxychloroquine, the patient remained asymptomatic. Upon last follow-up, 22 months later, serum creatinine was 69 $\mu\text{mol/L}$.

Methods

Determination of ADAMTS13 activity, ADAMTS13 antigen and ADAMTS13 inhibitors

ADAMTS13 activity and inhibitor determination were performed as described [4,6]. ADAMTS13 antigen levels were analysed by an ELISA according to Rieger *et al.* [6]. Briefly, a purified polyclonal rabbit anti-human ADAMTS13 IgG fraction was used for both the capture and the detection step of human plasmatic ADAMTS13 (reference interval: 0.740–1.420 $\mu\text{g/ml}$). The intra- and inter-assay coefficients of variation (%CV) were found to be 2.5% and 11.1%, respectively.

Determination of anti-ADAMTS13 antibodies

Total IgG, IgG subclasses, IgM and IgA anti-ADAMTS13 antibodies were analysed by ELISA as described [4,7,8]. The ELISA uses an anti-His tag antibody to capture recombinant His-tagged ADAMTS13. Diluted plasma samples are added, and bound total IgG, IgM, IgA or IgG subclasses are detected using an alkaline phosphatase-conjugated goat anti-human IgG, IgM or IgA antibody (Sigma, St. Louis, MO, USA) or mouse monoclonal anti-human IgG1, IgG2, IgG3 and IgG4 antibodies (Zymed Laboratories, San Francisco, CA, USA). The results are expressed either as a titer (total IgG, IgM or IgA) or as percentage (IgG subclasses). The inter-assay CV of the ELISA was found to be <20%.

Discussion

Deficiency of von Willebrand factor-cleaving protease ADAMTS13 was reported to be a specific finding of acute TTP [9]. Frequently, the acquired form of ADAMTS13 deficiency is associated with the presence of circulating autoantibodies (inhibitors) that neutralize ADAMTS13 activity [2,3,10]. However, in our patient with SLE-associated TTP, the severe reduction in ADAMTS13 activity was not associated with neutralizing anti-ADAMTS13 antibodies but with the presence of non-neutralizing antibodies that were missed by the functional inhibitor assay. These non-neutralizing antibodies were detected by a newly developed ELISA method where they bind to immobilized recombinant ADAMTS13.

Considering the very low levels of ADAMTS13 on admission, the severe thrombocytopenia and the haemolytic anaemia that was not fully explained by the weakly positive Coombs test, the non-neutralizing anti-ADAMTS13 antibodies observed in our patient are likely to be of clinical relevance. However, we did not observe thrombotic microangiopathy in the kidney biopsy, and no definite neurological abnormality could be detected. Thus, more stud-

ies will be required to clarify the potential pathogenic role of non-neutralizing anti-ADAMTS13 antibodies.

Recent reports could also demonstrate the presence of non-neutralizing antibodies in a patient with TTP and in patients with SLE without TTP [4,5], suggesting that mechanisms other than the direct inhibition of protease activity of ADAMTS13 by autoantibodies may be involved in TTP. One explanation could be that non-neutralizing antibodies bind to regions of ADAMTS13 which are not necessary for exerting protease activity under the conditions of the *in vitro* assay. *In vivo*, however, they could lead to an increased clearance of the ADAMTS13–antibody complexes and/or interfere with shear stress-dependent cleavage of ULVWF by ADAMTS13.

In conclusion, for the first time, we report a patient with TTP accompanying highly active SLE that was associated with non-neutralizing antibodies against ADAMTS13. Those non-neutralizing anti-bodies could have been the cause of ADAMTS13 depletion and consecutive TTP. More extensive analyses are required to determine the prevalence as well as the clinical relevance of non-neutralizing antibodies against ADAMTS13 in SLE patients.

References

1. Moake JL. Thrombotic microangiopathies. *N Engl J Med* 2002; 347: 589–600
2. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med* 1998; 340: 1585–1594
3. Furlan M, Robles R, Galbusera M *et al.* von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *N Engl J Med* 1998; 339: 1578–1584
4. Rieger M, Mannucci PM, Kremer Hovinga JA *et al.* ADAMTS13 autoantibodies in patients with thrombotic microangiopathies and other immuno mediated diseases. *Blood* 2005; 106: 1262–1267
5. Scheiflinger F, Knöbl P, Trattner B *et al.* Non neutralising IgM and IgG antibodies to von Willebrand factor-cleaving protease (ADAMTS-13) in a patient with thrombotic thrombocytopenic purpura. *Blood* 2003; 102: 3241–3243
6. Rieger M, Ferrari S, Kremer Hovinga JA *et al.* Relation between ADAMTS13 activity and ADAMTS13 antigen levels in healthy donors and patients with thrombotic microangiopathies (TMA). *Thromb Haemost* 2006; 95: 212–220
7. Ferrari S, Scheiflinger F, Rieger M *et al.* Prognostic value of anti-ADAMTS13 antibody features (Ig isotype, titer, and inhibitory effect) in a cohort of 35 adult French patients undergoing a first episode of thrombotic microangiopathy with undetectable ADAMTS13 activity. *Blood* 2007; 109: 2815–2822
8. Ferrari S, Mudde GC, Rieger M *et al.* IgG subclass distribution of anti-ADAMTS13 antibodies in patients with acquired thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2009; 7: 1703–1710
9. Bianchi V, Robles R, Alberio L *et al.* Von Willebrand factor-cleaving protease (ADAMTS13) in thrombocytopenic disorders: a severe deficient activity is specific in thrombotic thrombocytopenic purpura. *Blood* 2002; 100: 710–713
10. Furlan M, Robles R, Solenthaler M *et al.* Acquired deficiency of von Willebrand factor-cleaving protease in a patient with thrombotic thrombocytopenic purpura. *Blood* 1998; 91: 2839–2846

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