Refactory thrombotic thrombocytopenic purpura and membranoproliferative glomerulonephritis successfully treated with rituximab: a case associated with hepatitis C virus infection

Plasmapheresis remains the main treatment modality for patients with thrombotic thrombocytopenic purpura. We report a patient who had simultaneous onset of membranoproliferative glomerulonephritis and thrombotic thrombocytopenic purpura. She did not improve after 48 plasmapheresis sessions. A 6-week course of weekly intravenous doses of rituximab was then given. This achieved complete remission of her nephrotic syndrome and improvement in her renal function, so plasmapheresis was ceased. She had a low ADAMTS13 antigen level and a positive ADAMTS13 antibody, both of which reverted to normal after treatment with rituximab. This coincided with a rise in her hepatitis C virus RNA and liver transaminases. Liver biopsies did not reveal active fibrosis. Her hepatitis C virus RNA titre dropped afterwards, and she had no relapses of her thrombotic thrombocytopenic purpura and nephrotic syndrome, for more than 2 years after remission. The simultaneous onset and successful outcomes of both the membranoproliferative glomerulonephritis and thrombotic thrombocytopenic purpura illustrate the usefulness of rituximab. We discuss its use and risks, in the context of chronic hepatitis C infection.

Case report

A 30-year-old woman was first seen in our clinic in 1997 for mild proteinuria (0.53 g/d). She had been given a blood transfusion during an appendicectomy at the age of 27 years. On presentation, she was normotensive and had no urinary symptoms. Her full blood count, urine microscopy, and renal function tests were unremarkable. Her creatinine clearance was 97 mL/min/1.73 m² and her serological markers were all negative, except for a persistently low complement level (C3, 0.7-0.78 g/L; reference range, 0.86-1.84 g/L). She was HCV antibody-positive but her serum aminotransferase levels were normal. An ultrasound examination revealed a normal liver and kidneys. She was started on an angiotensin-converting enzyme inhibitor and remained asymptomatic for the next 9 years, with proteinuria ranging from 0.19 to 0.77 g/d, and normal renal function. Renal biopsy was not offered.

She was hospitalised in March 2006 for a 10-day history of bilateral ankle swelling, facial puffiness and lethargy. There were no preceding symptoms and she had not taken any medications, including oral contraceptives. On admission, she was pale and had bilateral
血漿提取是血栓血小板減少紫斑症一個主要的診治方法。本文報告一名同時患上血栓血小板減少紫斑症及膜增生性腎小球腎炎的病人，在接受48次血漿提取後病情未見改善。後來病人連續6星期接受rituximab靜脈注射,不但腎病徵狀得以完全舒緩,腎功能亦有改善，於是停止了血漿提取。病人原本的ADAMTS13抗原量低,並對ADAMS13抗體呈陽性,接受rituximab注射後,兩者的水平回復正常,但丙形肝炎病毒RNA水平及血清轉氨酶同時上升。肝臟活組織檢查未發現纖維化。其後病人的丙形肝炎病毒RNA水平下降,兩年後,血栓血小板減少紫斑症及腎病徵狀皆無復發。本病例中,血栓血小板減少紫斑症及腎小球腎炎同一時間出現,在施行rituximab後,兩種病同時得以治理,顯示rituximab的果效。本文討論在丙形肝炎感染的情況下rituximab的使用及風險。
Essentially, the biopsy had typical features of both TTP and MPGN, with the presence of immune complexes suggesting a secondary process, probably her chronic hepatitis C infection, behind the pathology.

On day 24 her platelet count dropped to 64×10^9/L, after we attempted to reduce the plasmapheresis frequency from daily to every 2 or 3 days (Fig 3). Her serum creatinine crept up slowly to a peak of 343 μmol/L and her urine output dropped. She required three sessions of haemodialysis, mainly for ultrafiltration. She had been started on prednisone 1 mg/kg/d on day 8, but did not respond so intravenous cyclophosphamide pulses (500 mg every 2 to 3 weeks for 4 doses) were given from day 23 till day 93. This did not have a sustained effect, so she was given intravenous Ig (400 mg/kg/d for 5 days) on day 62.

On day 32, she had two brief generalised tonic-clonic seizures. Investigations including a lumbar puncture, electroencephalogram and serum electrolytes were unremarkable. Magnetic resonance imaging of her brain revealed bilateral multiple small infarcts affecting the occipital, parietal, posterior frontal gyri and posterior basal ganglia. Clinically, she had no neurological deficit after the seizures, apart from lethargy. On day 74, when her platelet count had fallen to 76×10^9/L so the plasmapheresis was resumed for three sessions. Two more doses of rituximab were given, 2 weeks after the last dose of the first course, and the platelet count normalised within a week. Plasmapheresis was stopped on day 128, after a total of 70 sessions had been given. During the next 29 months of follow-up, the patient had a normal platelet count, stable haematocrit, normal haptoglobin and LDH levels, and no schizocytes in her peripheral blood. Her daily prednisone dose was decreased to 5 mg 3 months after discharge. Her proteinuria ceased, her serum creatinine was 145 μmol/L, and creatinine clearance 39 mL/min/1.73m² (Fig 4). She has had no more seizures.

Her liver function remained normal until around 10 weeks after her presentation, when she started to have elevated aminotransferase levels (Fig 4). This started before the use of rituximab and the enzyme levels fluctuated between two to 15 times the upper limit of normal. Her serum HBsAg, hepatitis A virus antibody, cytomegalovirus pp65Ag and other viral titres were negative but her serum HCV RNA was 1.99×10^6 IU/mL (genotype 1b). When her platelet count and haemoglobin level had stabilised, 4 months after presentation, we did a transjugular liver biopsy.

**Liver biopsy findings**

The liver tissue had normal portal tracts with scanty lymphohistiocytic infiltrates, intact bile ducts, and no interface hepatitis. The hepatic lobules showed many reactive Kupffer cells in sinusoids. Scattered acidophilic degeneration was noted. There were no fatty changes and lymphoid aggregates. Special stains showed a fine spidery network of increased reticulin, indicating hepatocyte dropout. There was no portal, perivenular fibrosis or fibrin deposit. In summary there were no classical histological features of active hepatitis C.

The liver aminotransferase levels remained elevated. She had no jaundice or hypoalbuminaemia and her clotting profile was normal. We repeated the liver biopsy 9 months later to look for disease progression, when her HCV RNA level had risen to above 5×10^6 IU/mL. She was then on prednisolone 4 mg daily, her TTP was stable and her proteinuria had fallen to 0.28 g/d. The liver architecture was preserved and there was no fibrosis. A few portal tracts had mild lymphocytic infiltration, with one displaying mild focal, piecemeal necrosis. The bile ducts were unremarkable. The liver plates were one to two cells thick and had increased numbers of reticulin fibres between the cell plates, scattered apoptotic bodies and mild lobular inflammation. Haemosiderin pigments were seen in Kupffer cells.
but not in hepatocytes. The features were those of chronic hepatitis with increased lobular activity (Metavir grade 2 and stage 0).

Her liver function improved gradually with the aminotransferase levels returning to normal 4 months after the repeat biopsy. There have been no further liver problems since. We are continuing to reduce her corticosteroids, but because of a failed synacthen stimulation test, she is currently on maintenance hydrocortisone therapy. At the last follow-up her HCV RNA had fallen to $6.3 \times 10^4$ IU/mL (Fig 5). As for her HCV infection, she was sceptical about starting interferon therapy, because she was aware of interferon-α-induced thrombotic microangiopathy or TTP.

**ADAMTS13 assays**

The ADAMTS13 antigen level was measured using the commercial Sandwich Enzyme-linked Immunosorbent Assays (ELISA; American Diagnostica Inc, Stamford, CT, US) chemiluminescence detection method. Citrated plasma samples were double-spun to avoid platelet contamination. Diluted plasma samples were added to microwells coated with a rabbit polyclonal antibody against ADAMTS13. After incubation and washing, a biotinylated rabbit anti-ADAMTS13 polyclonal antibody was added, followed by streptavidin-horseradish peroxidase conjugate and then substrate to create a colour reaction. Absorbance was measured at 450 nm on the Amerlite enhanced luminescence microtitre plate reader (Amersham International), and the result was obtained from a standard curve ranging from 0 to 100 ng/mL. Apart from the internal control samples provided, a plasma sample from a patient with congenital TTP was included as a positive control. The reference range was generated from normal subjects ($n=43$; ADAMTS13 antigen, 1250±517.8 ng/mL; reference range, 519-3152 ng/mL).

The ELISA method was also used to detect the ADAMTS13 auto-antibody. Citrated plasma samples were double-spun. Diluted plasma samples were added to microwells coated with a full-length recombinant ADAMTS13 protein. After incubation and washing, a goat anti-human IgG antibody labelled with horseradish peroxidase was added. The substrate was added following another washing step and a colour reaction developed. Absorbance was measured at 450 nm and the result was obtained from a standard curve ranging from 0 to 60 U/mL. The mean plus two standard deviations of the results from normal subjects was used as the cut-off for positivity ($n=43$; the ADAMTS13 auto-antibody cut-off was taken as the mean+2 standard deviations=25.6 U/mL). One arbitrary unit/mL is equal to 1 μg/mL of affinity purified human anti-ADAMTS13 IgG.

We took the blood tested that was for ADAMTS13 antigen and antibody levels when our patient’s condition was not responding to traditional therapies, in order to confirm the diagnosis and to exclude other possibilities. She had low levels of the ADAMTS13 antigen and a weakly positive ADAMTS13 antibody (Table). Both returned to normal after treatment with rituximab.
Discussion

Thrombotic thrombocytopenic purpura is a life-threatening disease characterised by development of thrombi in the arterioles and capillaries of multiple organs, thrombocytopenia, microscopic haemolytic anaemia, neurological deficits, renal dysfunction and fever. Decreased activity of the protease, ADAMTS13, leads to the accumulation of the unusually large von Willebrand factor multimers (ULVWF). These ULVWF have been observed in patients with chronic relapsing TTP and ADAMTS13 deficiency has been implicated in the pathogenesis of TTP and reported in 60 to 70% of patients. The ULVWF exhibit a higher capacity for supporting platelet aggregation and are believed to bind to platelets where blood flow exerts high shear stress and thus be involved in the formation of microvascular thrombosis. A decrease in synthesis or the synthesis of a functionally abnormal molecule with decreased protease activity has been found in familial TTP. Immunoglobulin G antibodies that inhibit ADAMTS13 activity have been detected in acquired TTP. These antibodies have been reported in 48 to 80% of patients with recurrent TTP, suggesting that their presence may be associated with relapse. The use of ADAMTS13 antigens and antibodies should permit early diagnosis of TTP but the associated costs and need for expert manpower, the difficulties with inhibitor assays caused by the disease biology, and the varying levels among patients with different TTP aetiologies are all issues to consider when using these tests. The question of whether TTP can be defined as a pro-thrombotic state in the microvasculature caused by severe ADAMTS13 deficiency, as proposed by Tsai, remains unanswered. Such a definition allows prompt provision of appropriate therapy but also highlights the importance of developing easy, reliable assays for use in clinical laboratories, without the current wide variation in the assay methods.

Plasma exchange with fresh frozen plasma replacement is the only proven treatment modality for TTP and many patients have self-limited disease that remits after 1 to several weeks' treatment. Plasma exchange alone has only a small, transient effect on ADAMTS13 antibody levels, however, an effect seen in other autoimmune diseases. Additional immunosuppression should thus be considered if the syndrome persists despite PE, particularly when a persistent inhibitor can be demonstrated. Approximately 36 to 65% of patients who survive the acute episodes experience relapses and up to 14% have no response to plasmapheresis therapy. The majority of relapses occur within 1 month. Various treatment modalities have been tried with varying success in such patients, including corticosteroids, vincristine, cyclophosphamide, azathioprine, cyclosporine, Ig, staphylococcal protein A columns and splenectomy.
Rituximab, a murine/human chimeric monoclonal antibody, binds to CD20+ B cells resulting in a rapid and sustained depletion of these cells in both the circulation and lymphoid tissues via antibody-dependent cellular cytotoxicity, inducing apoptosis, and complement-mediated lysis. The total lymphocyte count does not change during treatment. B cell recovery is usually apparent 6 to 12 months after treatment, but the level can remain lower than normal for a period of up to 750 days. Patients with TTP successfully treated with rituximab, have an increased level of ADAMTS13 and decreased antibodies. The partial B cell recovery has been associated with a decline in ADAMTS13 activity and the reappearance of the antibodies. Persistently low ADAMTS13 activity has been reported following successful treatment with rituximab, suggesting either that triggering factors are essential to precipitate a relapse, or low levels of ADAMTS13 activity (at 5-10%) are sufficient for protection from disease recurrence. Case reports suggest that alleviation of disease activity and a decreased need for PE is observed within 2 to 5 weeks of the first dose of rituximab.

On presentation, our patient had a deficiency of the von Willebrand factor–cleaving protease associated with IgG antibodies. We believe that her modestly low ADAMTS13 antigen level and weakly positive ADAMTS13 antibody levels were related to the timing of the tests, which were first done when she had already received different therapies. The changes in her ADAMTS13 Ag and auto-antibody titres were associated with a rapid and durable improvement in her other parameters. A complete remission lasting 29 months and still ongoing was achieved only after treatment with rituximab. Whether the drug can serve as an ideal second-line therapy that helps retard or even prevent relapses remains unknown. It has been noted that patients with high ADAMTS13 antibody titres tend not to respond to PE alone, but the level defining these high-risk patients has yet to be established.

Rituximab is often administered as weekly doses for 4 to 8 weeks in patients with TTP. When given concurrently with PE, its efficacy is believed to be unchanged due to the fact that most of the antibodies bind rapidly to CD20+ cells and very few stay in the circulation 24 hours after the infusion. Any potential removal of rituximab by PE can be minimised by giving the drug after the daily PE session. As most of the reported patients with TTP who were given rituximab had ADAMTS13 antibodies, it is unknown whether the agent should be tried in patients without these antibodies, or whether, indeed, fresh frozen plasma infusion alone should be enough.

Viral infections, including cytomegalovirus infection, parvovirus B19, acute hepatitis B (with loss of the HBs antibody) and hepatitis C, have all been reported with the use of rituximab in patients with lymphoproliferative disorders. On the other hand, rituximab has been used successfully in HCV-mixed cryoglobulinaemia vasculitis.

Along with the typical features of TTP, our patient’s renal biopsy showed immune complexes and the features of MPGN. Serum cryoglobulins were not detected on repeated testing and her renal biopsy lacked typical cryoglobulinaemia features, particularly the electron-dense fibrils seen on electron microscopy. Patients who are HCV-positive may have MPGN with no associated cryoglobulinaemia, but cases of this are rare compared with those associated with type II cryoglobulinaemia. There is no reported link between hepatitis C infection and TTP, except in cases associated with antiphospholipid antibodies. Immunosuppressive agents and plasmapheresis are often used to control the acute phase of MPGN associated with cryoglobulinaemia in HCV-positive patients, but this therapy is often poorly tolerated. The rise in HCV RNA concentration observed during immunosuppressive therapy may be harmful for HCV-related liver disease, as in our patient. Rituximab has been used successfully in patients with HCV-associated mixed cryoglobulinaemic syndrome. This is echoed by findings of a stable viral load by other authors.

Further controlled studies are required to explore the usefulness of rituximab and the optimal dosage in patients with unresponsive TTP and ADAMTS13 antibodies. The concept of a pathogenetic association between the hepatitis C infection and TTP in our patient remains intriguing, in view of the
simultaneous onset of both HCV-related MPGN and TTP, and their parallel response to treatment.

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Declaration

The authors declared no conflicts of interest.

References