MEDICAL PRACTICE

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

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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.

Introduction

Most adult patients with acquired thrombotic thrombocytopenic purpura (TTP) have autoantibodies that inhibit the von Willebrand factor–cleaving protease ADAMTS13 in plasma.[1] Plasmapheresis with fresh frozen plasma replacement has significantly improved the survival rates of these patients from less than 10 to 75%.[2] Failure to control the possible underlying autoimmune process leads to relapse after the initial response in up to one third of patients, however.[3] There are also patients with refractory disease that respond poorly to plasmapheresis.[4] With case reports documenting instances where such patients have responded to various immunosuppressive therapies, we need a promising and non-toxic treatment in patients with ADAMTS13 deficiency. We report a patient with TTP who had chronic hepatitis C infection and membranoproliferative glomerulonephritis (MPGN), diminished plasma ADAMTS13 activity and elevated ADAMTS13 antibody titres. She failed to respond to various therapeutic modalities. Treatment with rituximab resulted in the disappearance of her ADAMTS13 antibodies, normalisation of her ADAMTS13 activity, remission of her thrombotic microangiopathy and nephrotic syndrome, and improved renal function. This improvement was followed by a flare-up of her previously quiescent hepatitis C virus (HCV) infection, however.

Key words
Glomerulonephritis, membranous; Hepatitis C; Purpura, thrombocytopenic, idiopathic; Rituximab; Thrombocytopenia

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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 m^2 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

在丙形肝炎感染的情況下，利用rituximab成功治理難治性血栓血小板減少紫斑症及膜增生性腎小球腎炎

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ADAMTS13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膜增生性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。

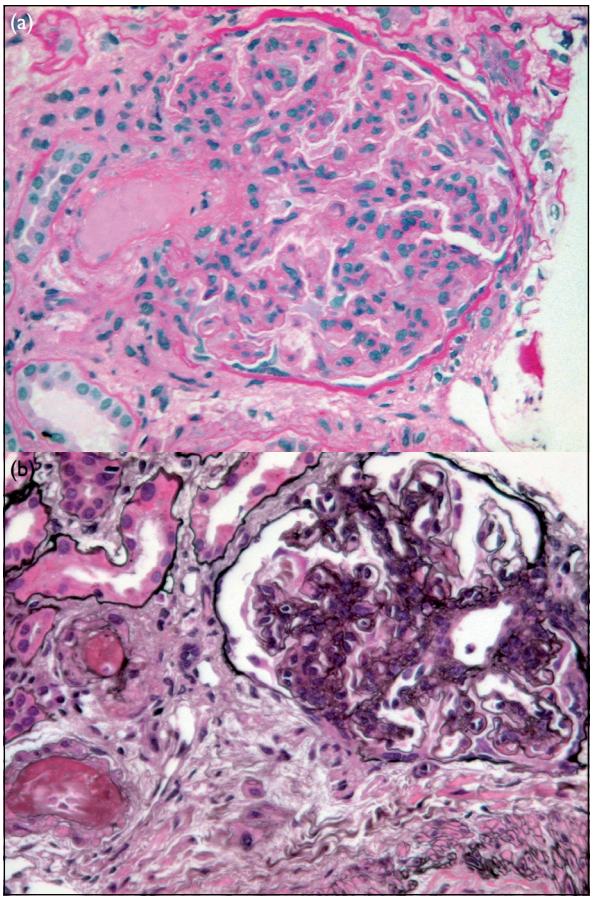

FIG 1. (a) Renal biopsy section revealing lobular accentuation with glomerular hypercellularity. Fibrin thrombus was noted in the glomerular hilum (Periodic acid–Schiff stain, ×400). (b) Glomerulus showing segmental hypercellularity and reduplication of the glomerular basement membrane. Fibrin thrombi were present in the arterioles (Jones silver methenamine stain, ×400)

ankle oedema. There was no bleeding tendency and her blood pressure was 150/90 mm Hg. She had a low fever but the septic workup was negative. Her haemoglobin was 86 g/L and platelet count 123×10^9/L, which continued to drop, reaching 36×10^9/L. A peripheral blood smear showed schizocytes (5% of red cells). She had heavy proteinuria (11.09 g/d), hyaline casts in the urine, and a creatinine clearance of 87 mL/min/1.73 m^2. Her serum urea was 12.1 mmol/L, serum creatinine 148 μmol/L, and albumin 19 g/L. The C3 and C4 complement levels were depressed (0.38 g/L and 0.1 g/L, respectively; reference range of C4, 0.2-0.59 g/L) and she had no serological markers. She also had no hepatitis B surface antigen (HBsAg) and no human immunodeficiency virus antibodies but did have an HCV antibody. Her serum lactate dehydrogenase (LDH) level was 544 U/L and her haptoglobin level was lower than 0.06 g/L. Her clotting profile was normal and she had no anti-cardiolipin antibody, lupus anticoagulant nor cryoglobulins. A stool examination was negative for verotoxin-producing *Escherichia coli*. Her echocardiogram revealed normal pulmonary pressures.

She was diagnosed with TTP, and plasmapheresis (1-1.5 volumes daily, replaced by fresh frozen plasma) was started. Her platelet count increased to 150×10^9/L by day 18, at which point we did a renal biopsy.

Renal biopsy findings

On light microscopy, 13 glomeruli were present. There was no glomerulosclerosis or crescents. The glomeruli showed lobular accentuation with diffuse global glomerular hypercellularity (Fig 1). The endothelial cells were swollen and this was associated with widespread narrowing and obliteration of glomerular capillary lumens. There was fibrin amongst the endothelial cells and fibrin thrombi in arterioles extending to the glomerular roots. Silver staining showed double contours in several capillary loops. There was focal tubular atrophy, as well as interstitial fibrosis. The arterioles showed severe endothelial swelling and disorganisation with abundant fibrin and fragmented red cell depositions within the vessel walls, leading to markedly narrowed lumens. The arteries showed intimal fibrosis with duplication of internal elastic laminae. Immunofluorescence staining showed diffuse, segmental, granular, moderate-to-strong deposition of immunoglobulin (Ig) G, IgA, IgM and C3 along the capillary walls. Under electron microscopy, focal small amounts of subepithelial electron-dense deposits and a moderate level of subendothelial deposits were seen (Fig 2). Focal expansion of the subendothelial zone by electron lucent materials was present. Mesangial interpositioning with inner new basement membrane–like material was noted. The mesangial cells and matrix showed diffuse, global, moderate increases with moderate numbers of mesangial deposits.

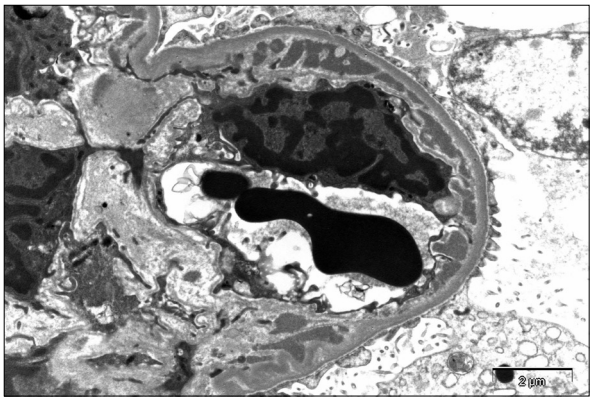

FIG 2. Electron microscopy of a representative renal biopsy section showed glomerular subendothelial electron-dense deposits and 'double' basement membrane–like material in capillary walls (Uranyl acetate and lead citrate, ×8000)

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 was 82×10^9/L, serum creatinine level had decreased to 188 µmol/L, LDH was 505 U/L, and schizocytes were present in her peripheral blood, she was still receiving alternate-day plasma exchange (PE). At this point weekly doses of intravenous rituximab (375 mg/m^2, MabThera, F. Hoffmann-La Roche Ltd, Basel) were commenced and continued for 6 weeks. Her platelet count crept up to 150×10^9/L within 7 days and it was possible to decrease the plasmapheresis frequency to every 3 to 4 days. These were discontinued altogether by day 97. Cyclosporine A was added on day 106 with a view to it serving as a maintenance therapy, but her platelet count dropped from 262 to 131×10^9/L after 12 days of treatment so the drug was stopped. On day 122, the 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/1.73m^2 (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

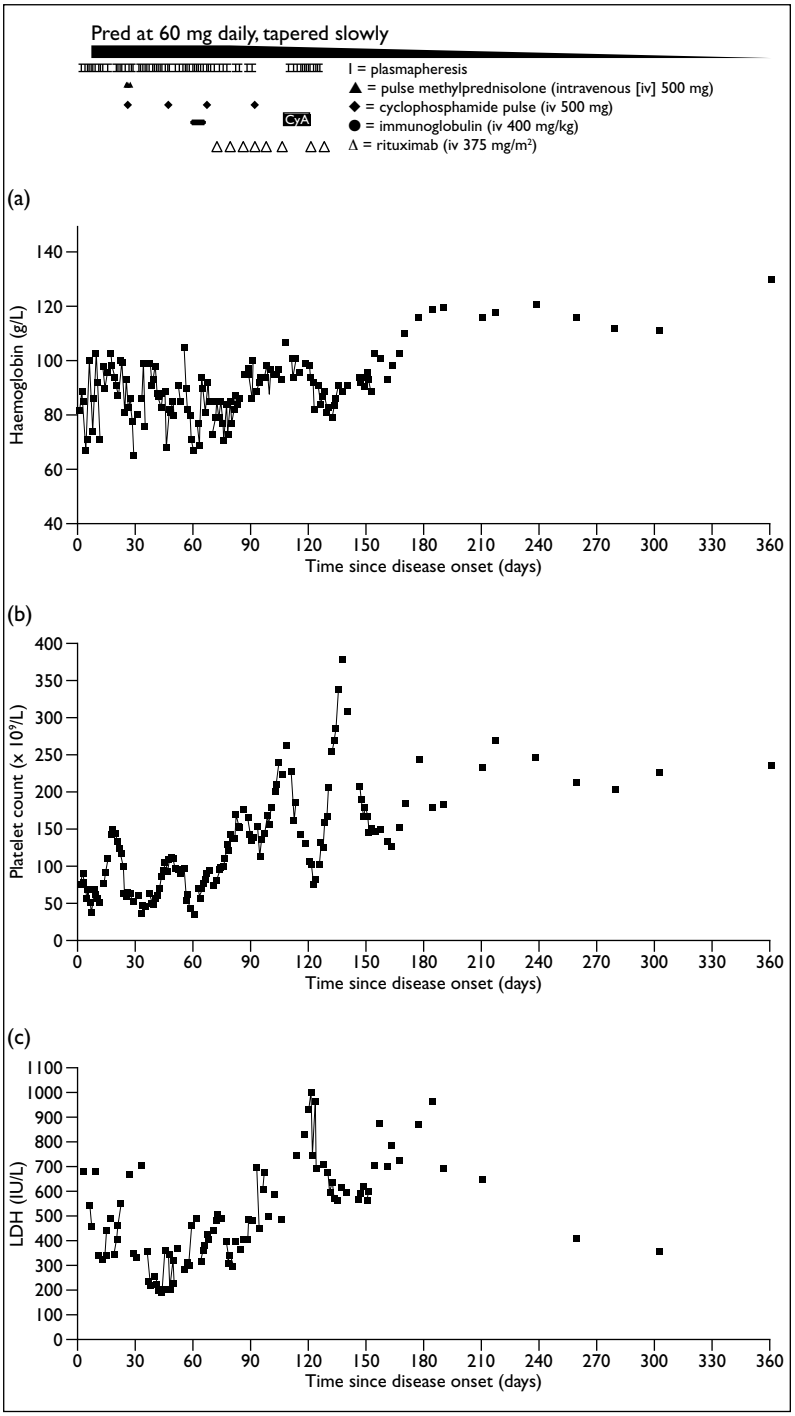

FIG 3. Time course for haemoglobin, platelet count and serum lactate dehydrogenase (LDH) levels up to 1 year from disease onset, in relation to various therapies
Pred denotes prednisolone; and CyA cyclosporine

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 6.3×10^4 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.[5]

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,[6] and ADAMTS13 deficiency has been implicated in the pathogenesis of TTP[1,7] and reported in 60 to 70% of patients.[1,8] The ULVWF exhibit a higher capacity for supporting platelet aggregation,[1] and are believed to bind to platelets where blood flow exerts high shear stress[9] 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.[10] Immunoglobulin G antibodies that inhibit ADAMTS13 activity have been detected in acquired TTP.[1] These antibodies have been reported in 48 to 80% of patients with recurrent TTP,[1,11] 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,[1] the difficulties with inhibitor assays caused by the disease biology, and the varying levels among patients with different TTP aetiologies,[1,12] 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,[1] 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,[2,13] 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.[11,14] 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.[15,16] Furthermore, around one third of patients have a chronic relapsing course[3,15,16] and up to 14% have no response to plasmapheresis therapy.[4] The majority of relapses occur within 1 month.[15] 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.[17-19]

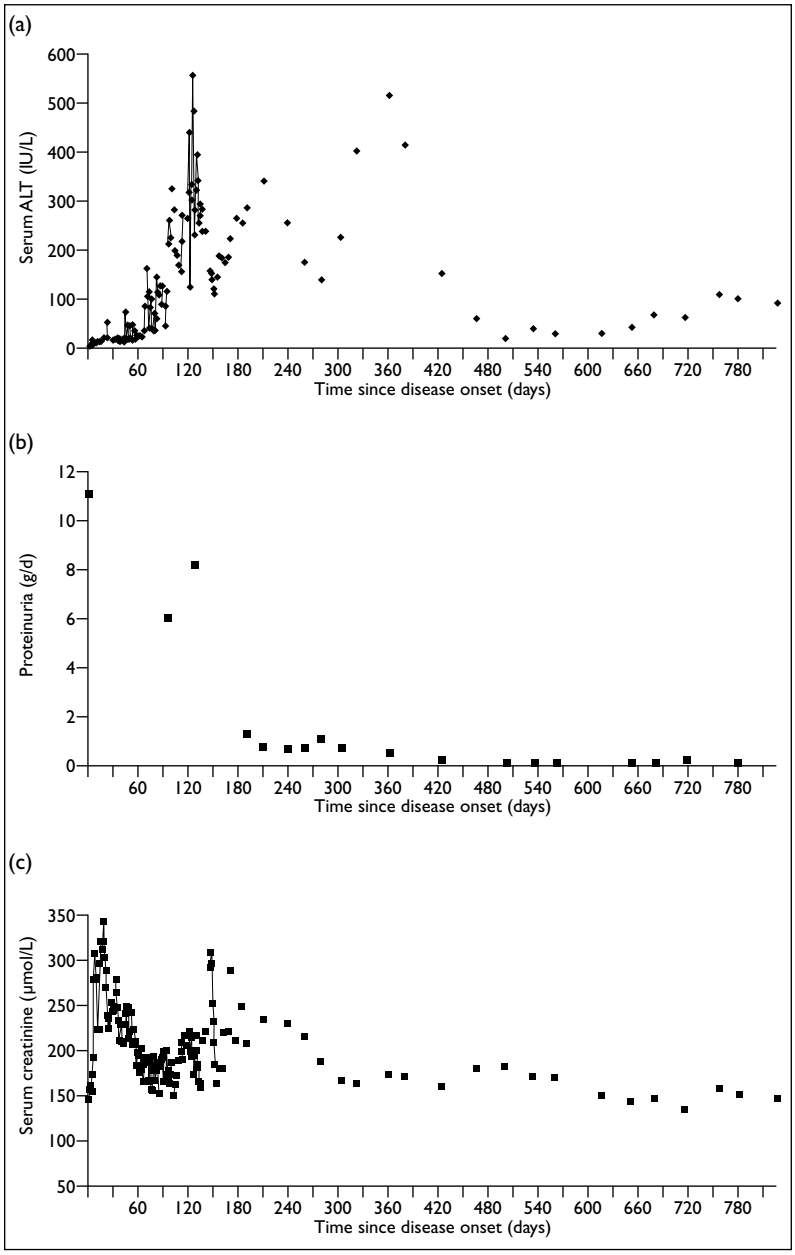

FIG 4. Time course for serum alanine aminotransferase (ALT), proteinuria and serum creatinine levels up to 2 years from disease onset

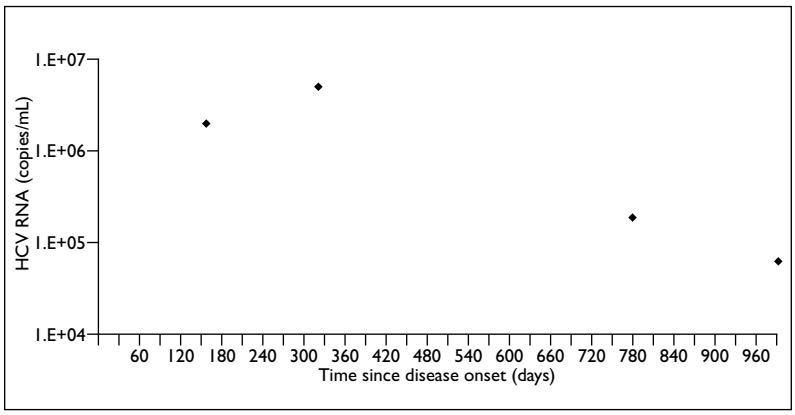

FIG 5. Time course for serum hepatitis C virus (HCV) RNA level up to last follow-up

TABLE. Serial ADAMTS13 antigen (Ag) and auto-antibody (Ab) titres

Time from disease onset (days)	ADAMTS13 Ag (ng/mL)		ADAMTS13 Ab (U/mL)	
74	347	Low	26.08	Weak positive
81	835	Normal	16.72	Negative
88	1658	Normal	10.53	Negative
95	1163	Normal	11.87	Negative
111	1829	Normal	12.31	Negative

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[10,20] 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.[14] Patients with TTP successfully treated with rituximab, have an increased level of ADAMTS13 and decreased antibodies.[21] The partial B cell recovery has been associated with a decline in ADAMTS13 activity and the reappearance of the antibodies.[14,21] Persistently low ADAMTS13 activity has been reported following successful treatment with rituximab,[22,23] 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.[24] 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.[25]

Rituximab is often administered as weekly doses for 4 to 8 weeks in patients with TTP.[26] 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.[27] 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.[1]

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.[28-31] On the other hand, rituximab has been used successfully in HCV-mixed cryoglobulinaemia vasculitis.[32]

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.[33] There is no reported link between hepatitis C infection and TTP, except in cases associated with anticardiolipin antibodies.[34] 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,[35] as in our patient. Rituximab has been used successfully in patients with HCV-associated mixed cryoglobulinaemic syndrome.[36-38] This is echoed by findings of a stable viral load by other authors.[39,40] Sansonno et al,[37] like us, witnessed a doubling of serum HCV RNA in responders to rituximab when it was used in 20 patients with mixed cryoglobulinaemia and HCV-positive chronic active liver disease. This enhanced HCV-viraemia may reflect a decline in IgG anti-HCV antibodies, allowing HCV to avoid immune pressure and thus favouring its replication. The HCV RNA load has decreased in our patient, coinciding with a reduction in her immunosuppressive therapies. It is likely that both her MPGN remission and the resurgence of her hepatitis C infection were not solely related to rituximab, but to the concerted effects of the immunosuppressive therapies she had received.

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.

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