

# Efficacy of Rituximab and Concurrent Plasma Exchange in the Treatment of Thrombotic Thrombocytopenic Purpura

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Thrombotic thrombocytopenic purpura (TTP), a rare disorder first described by Moschowitz in 1924,<sup>1</sup> is classically characterized by the symptom pentad of hemolytic anemia, thrombocytopenia, renal failure, fever, and neurological changes. These signs and symptoms arise due to thrombotic microangiopathy with systemic aggregation of platelets leading to organ ischemia, mechanical hemolysis, and profound thrombocytopenia.<sup>2</sup> Although pregnancy, malignancy, autoimmune disease, drugs, infection, and bone marrow transplantation have all been associated with secondary TTP, most acute acquired cases are idiopathic.<sup>3</sup> The etiology of idiopathic TTP has been recently shown to be an acquired autoimmune deficiency of ADAMTS13, a metalloprotease that cleaves unusually large von Willebrand factor (vWf) multimers.<sup>4,5</sup> It is believed that excess unusually large vWf, caused by decreased amounts of ADAMTS13 due to antibody-mediated inhibition, is responsible for the widespread clotting seen in TTP. Untreated TTP is fatal in 90% of cases.<sup>6</sup> The first curative treatment—plasma infusion—was reported in 1977.<sup>7</sup> Subsequently therapeutic plasma exchange (TPE) was found to be superior to plasma infusion for treating TTP, and is the current therapeutic mainstay.<sup>8</sup> Despite marked improvement in overall survival since the inception of daily TPE for treatment of TTP, 10–30% of all cases are refractory and fatal.<sup>3</sup> Immunomodulating agents (eg, corticosteroids, vincristine,<sup>9</sup> cyclophosphamide,<sup>10</sup> azathioprine, cyclosporine, high-dose immunoglobulins), antiplatelet agents, staphylococcal protein A columns,<sup>11</sup> and splenectomy<sup>12</sup> have all been used to treat refractory TTP, with unreliable efficacies.<sup>2</sup> Modification of TPE regimens, such as increased frequency to twice daily<sup>13</sup> plasmaphereses, use of cryosupernatant<sup>14</sup> in place of fresh frozen plasma (FFP), and variation in the number of

plasma volumes per exchange, have also been attempted with inconsistent results.

Presently rituximab therapy is showing some success in the treatment of refractory idiopathic TTP, presumably by eliminating anti-ADAMTS13 IgG antibody-producing CD20(+) B lymphocytes.<sup>10,15-19</sup> Rituximab, a chemotherapeutic agent currently approved only for the treatment of CD20(+) non-Hodgkin B-cell lymphomas, is a chimeric mouse/human antibody that targets the CD20 B-cell antigen. Off-label uses for rituximab are numerous and include reports of efficacy in treating idiopathic autoimmune hemolytic anemia,<sup>20</sup> rheumatoid arthritis,<sup>21</sup> idiopathic thrombocytopenic purpura,<sup>22</sup> cryoglobulinemia,<sup>23</sup> and chronic inflammatory polyneuropathy,<sup>24</sup> among others. The binding of rituximab to CD20 causes complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity, and apoptosis of B cells. It is the decreased production of anti-ADAMTS13 IgG antibodies, and therefore decreased ADAMTS13-inhibitor levels, following destruction of the CD20(+) B cells, that is believed to be the rationale for rituximab therapy in TTP.

This is a case report of a patient with TPE-refractory idiopathic TTP that responded to rituximab therapy. We present flow cytometric data verifying the therapeutic effect of rituximab on CD20(+) B cells and correlate this with the elimination of ADAMTS13 inhibitor and a good clinical response. With an interval of 8–12 hours between a dose of rituximab and the next TPE procedure (to allow for the medication to take effect), rituximab and TPE could be applied as concurrent therapies in the treatment of TTP. Such concurrent treatment may have contributed to the effective treatment of this patient with refractory TTP.

## Case

The patient is a 39-year-old white woman with a past medical history significant only for depression and congenital

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absence of her right kidney, who presented with acute mental status changes in the setting of direct antiglobulin-negative microangiopathic hemolytic anemia. Her peripheral blood showed a marked schistocytosis, hemoglobin of 5.8 (normal, 12–16 g/dL), hematocrit of 17% (normal, 36–46%), platelet count of 10,000/ $\mu$ L (normal, 150,000–350,000/ $\mu$ L), lactate dehydrogenase (LDH) of 1,606 U/L (normal, 110–210 U/L), and a normal white blood cell count. Her liver transaminases were mildly elevated with a direct bilirubin level of 1.2 mg/dL (normal, 0–0.4 mg/dL) and indirect bilirubin of 2.4 mg/dL (normal, 0–0.6 mg/dL). The patient was not pregnant and did not have any pathogens recovered on microbiology samples of stool (including *Escherichia coli* 0157:H7 and *Shigella* spp) or blood. She did have an asymptomatic *E. coli* urinary tract infection that was treated successfully with levofloxacin. Her basic chemistries and renal function tests were within normal limits. Head computed tomography showed no appreciable abnormalities.

Initially, she received daily one-plasma-volume TPE and corticosteroids and showed laboratory and clinical improvement for the first week, including LDH falling to 485 U/L and platelets increasing to 35,000/ $\mu$ L (Figure 1). On day 7, the patient became agitated and confused, with worsening hemolysis, and was given vincristine 1 mg/m<sup>2</sup> after her daily TPE. By day 8, the patient was exhibiting expressive and receptive aphasia with marked agitation, and she had LDH and platelet levels trending back to her values at admission. Between days 8 and 17, TPE was usually performed twice daily, with the sum of the exchanges ranging from 1.5 to 2.5 total plasma volumes per day. Cryosupernatant (cryoprecipitate-reduced plasma) was used as exchange fluid in place of FFP on days 9–11; however, the patient developed epistaxis, hematemesis, and hemochezia, with little improvement in laboratory values or mental status. Consequently FFP exchanges were restarted and the mucosal bleeding stopped. On day 11, the first of four doses of weekly rituximab 375 mg/m<sup>2</sup> was administered after a single TPE procedure that day, in order to increase the drug's length of time in circulation. The patient received a second dose of vincristine 1 mg/m<sup>2</sup> on day 12 and twice-daily TPE was resumed. Between days 11 and 14, the patient developed profound neurological dysfunction that culminated in a near-comatose state. Her laboratory values indicated severe hemolysis with LDH 2,780 U/L, total bilirubin 21.4 mg/dL (direct bilirubin 11.8 mg/dL), and platelets 7,000/ $\mu$ L. On day 14 the patient showed a glimpse of returning neurological function as she was seen to visually track her husband's face for a few seconds. On day 15, her LDH level fell to 742 U/L and, although still verbally perseverating and experiencing frank hallucinations (possibly due to steroid psychosis), she started to regain mental function. Her platelet count

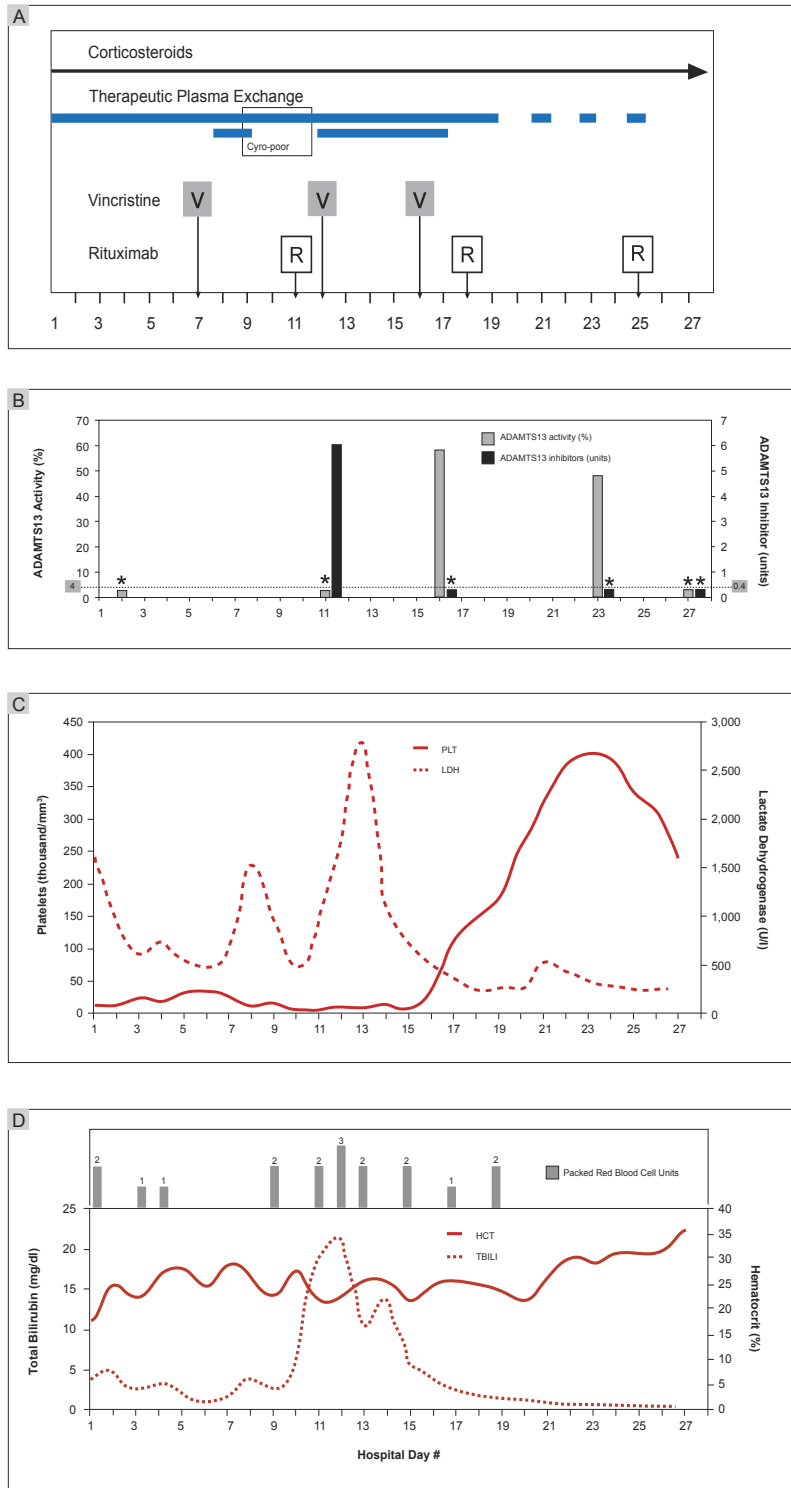
and LDH levels stabilized, and her mental status normalized. She received a third dose of vincristine on day 16 and second and third doses of rituximab on days 18 and 25. She was discharged from our facility on day 28. She spent an evening at home, but was rehospitalized at a different healthcare facility when her platelets were observed to fall below 100,000/ $\mu$ L (with no significant rise in her LDH or schistocytes on smear); there she was restarted on daily TPE. The decreased platelet count was believed to be drug-induced rather than a relapse of her TTP as there was no evidence of hemolysis and her mental status remained normal. After another 2 weeks of TPE at the out-of-state facility and a final dose of rituximab, her platelet count surpassed 150,000/ $\mu$ L and she was discharged.

## Methods

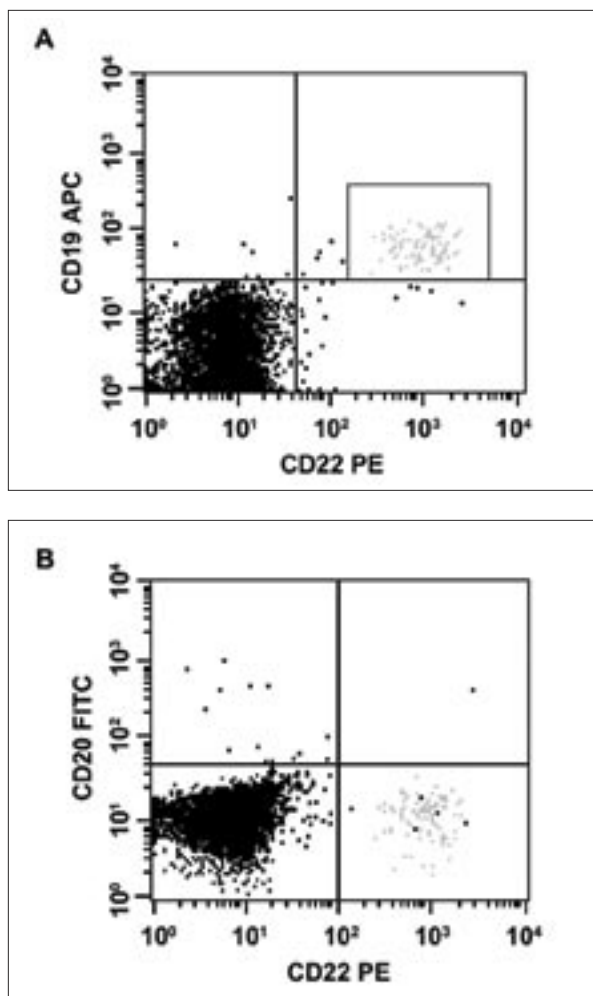
ADAMTS13 activity and inhibitor levels were assayed on days 2, 11, 16, 23, and 27 of the hospital stay by the Blood Center of Southeastern Wisconsin, Inc. in Milwaukee, using previously published methodology.<sup>25</sup> Post-therapeutic flow cytometric assessment of CD20(+) B cells was performed on day 16, five days after the first dose of rituximab was administered. Platelet, hematocrit, serum LDH, and total serum bilirubin levels were obtained daily.

## Results

Initially this patient had no detectable ADAMTS13 activity (normal >67%) and a detectable ADAMTS13 inhibitor of 1.5 units (normal <0.4 units) (Figure 1B). By day 11, after a total of 14 TPE procedures, and 4 days after administering the first dose of vincristine, the patient still showed no detectable ADAMTS13 activity but exhibited a four-fold increase in her inhibitor level, to 6.0 units. Her clinical and laboratory signs were consistent with worsening disease (Figures 1C, 1D). Later on day 11, the patient received her first dose of rituximab, followed by a 24-hour TPE hiatus in order to allow for the maximum drug effect. The following day she received another dose of vincristine. Days 11–14 corresponded with her most profound laboratory and clinical manifestations of TTP. By day 16, the patient demonstrated remarkable improvement. Five days after receiving the first dose of rituximab, testing revealed nearly normal ADAMTS13 activity at 58% and no detectable inhibitor. Concurrent flow cytometric testing for rituximab efficacy demonstrated no detectable CD20 on CD19(+)/CD22(+) B cells, suggesting that rituximab had bound her B lymphocytes to saturation (Figure 2). The patient's platelet count peaked at day 24 and then began trending downward again. However, this decrease was not associ-



**Figure 1.** The patient’s entire hospitalization (hospital days 1–27) for thrombotic thrombocytopenia is represented by these graphs. On day 16, flow cytometry for rituximab efficacy demonstrated no detectable CD20 B cells. (A) Daily treatment regimen. Corticosteroids were given throughout entire hospitalization. For therapeutic plasma exchange the number of bars corresponds with the number of plasmaphereses the patient received each day. The “cryo-poor” box indicates when the patient received cryosupernatant as an exchange fluid. Vincristine and rituximab were administered on the indicated days. (B) ADAMTS13 activity and inhibitor levels. ADAMTS13 activity levels are indicated by black bars. Normal activity levels are  $\geq 67\%$ . ADAMTS13 inhibitor levels are indicated by gray bars. Normal inhibitor levels are  $\leq 0.4$  units. Levels marked with an asterisk (\*) below the dotted horizontal line (activity  $< 4\%$ ; inhibitor  $< 0.4$  units) are considered undetectable. (C) Platelet and serum lactate dehydrogenase (LDH) levels. The solid line indicates the number of platelets (normal, 150,000–350,000/ $\mu\text{L}$ ) and the dotted line indicates serum LDH levels (normal, 110–210 U/L). The patient’s mental status corresponded roughly to the serum LDH levels with the best mental function seen when LDH levels were low and the worst mental status seen when LDH levels were high. (D) Top: Packed red blood cell units. Each bar represents the number of transfused units of packed red blood cells. Bottom: Total serum bilirubin level and hematocrit. The dotted line indicates the patient’s total serum bilirubin levels (normal, 0–1.0 mg/dL) and solid line indicates hematocrit (normal, 36–46%).



**Figure 2.** Flow cytometry of the patient's blood was performed on hospital day 16, five days after receiving rituximab for the treatment of therapeutic plasma exchange (TPE)-refractory thrombotic thrombocytopenic purpura. (A) Detectable population of CD19(+) and CD22(+) mature B lymphocytes (in box and colored gray). (B) Same CD19(+) and CD22(+) mature B lymphocytes (gray) with no detectable CD20 positivity indicating complete target saturation by anti-CD20 monoclonal antibody (rituximab) despite concomitant daily TPE. The patient did not undergo TPE for a 24-hour period following rituximab administration.

APC = allophycocyanin; FITC = fluorescein isothiocyanate; PE = phycoerythrin.

ated with hemolysis (as evidenced by a normal hematocrit and bilirubin), neurologic dysfunction, or detectable ADAMTS13 inhibitor, although it was associated with decreased ADAMTS13 activity (<4%).

## Discussion

This case of a 39-year-old woman with refractory TTP delineates and exemplifies a number of points relevant to the treatment of TTP with rituximab. We show that rituximab and TPE can quite effectively be applied as concurrent therapies in the treatment of this disease. An interval of 24 hours between administration of rituximab and the next TPE procedure appeared adequate. Of note, in subsequent applications we have allowed an interval of only 8 hours between administration of rituximab and plasmapheresis with apparently similar efficacy.

The National Heart, Lung, and Blood Institute (NHLBI) Clinical Trials Network in Transfusion Medicine and Hemostasis<sup>26</sup> is planning to conduct a multicenter prospective trial that will randomize patients with TTP either to receive steroids plus plasmapheresis plus up-front rituximab or to receive steroids plus plasmapheresis plus placebo. The results of this trial, which is currently in its planning stage, will take a number of years to be reported. Until the results of such a rigorous study are reported, case reports may provide modest insights into improving the design of such trials and current treatment regimens.

In this case, we used flow cytometry to assess the impact of rituximab therapy on metalloprotease antibody inhibitor production by B lymphocytes. Rituximab binds the CD20 receptor on B lymphocytes. We were able to show that saturation of the CD20 receptor sites by rituximab correlated with a decline in laboratory levels of ADAMTS13 inhibitor and with a marked improvement in the patient's clinical condition (and normalization of her other laboratory values). Flow cytometry may thus be a useful adjunct in clarifying the efficacy of rituximab therapy, although not essential if ADAMTS13 inhibitor levels are seen to be concomitantly decreasing.

The efficacies of the other treatments that this patient received are unclear. Vincristine, although historically a component of the arsenal against TTP, was not associated with a decline in ADAMTS13 inhibitor levels and its administration was followed by large increases in LDH the first two (of three) times it was given. Its use was not associated with any clear therapeutic benefit, and the patient appeared to suffer several unwanted side effects including peripheral neuropathy and alopecia. The efficacy of steroids was likewise difficult to assess and may also have contributed to these side effects. The use of cryosupernatant plasma as an exchange fluid for TPE was not associated with any clinical (or laboratory

value) improvement in this patient. The efficacy of twice-daily TPE is difficult to assess. It is possible that the 63% plasma exchange achieved with a one-volume TPE procedure performed twice daily provided an effective rate of overriding clonal antibody inhibitor production (via swift removal and replacement with fresh metalloprotease) during the most clinically critical phase of her disease. Again, the relative efficacies of TPE and rituximab in this regard remain unknown, but it is not inconceivable that the effects of TPE and rituximab may, at least to a degree, be synergistic.

In summary, refractory TTP can be effectively treated (at least in the short term) with concurrent rituximab and TPE. Unknown presently is whether rituximab, alone or in combination with other therapies such as TPE, is best suited for particular presentations of TTP. Considering its blocking effect on B lymphocytes and antibody inhibitor production, rituximab's therapeutic benefit may be greatest in patients with high levels of ADAMTS13 inhibitor. Whether rituximab will ultimately be used concurrently with TPE (as in our case report) or be limited to cases refractory to current forms of treatment (TPE, steroids) is another question the NHLBI Clinical Trials Network in Transfusion Medicine and Hemostasis may eventually answer. Although the long-term outcome for our patient remains to be seen, the remarkable improvement in her refractory clinical condition following administration of rituximab provides support for pursuing a rigorous controlled trial examining this drug's efficacy in the treatment of TTP.

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