Rituximab Can Be Combined With Daily Plasma Exchange to Achieve Effective B-Cell Depletion and Clinical Improvement in Acute Autoimmune TTP

Kamran Darabi, MD, Anders H. Berg, MD, PhD
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Kamran Darabi, MD,1 and Anders H. Berg, MD, PhD2

Key Words: Thrombotic thrombocytopenic purpura; Therapeutic apheresis; Rituximab; Autoimmune

Abstract

Much of idiopathic thrombotic thrombocytopenic purpura (TTP) is attributed to the presence of an autoantibody to ADAMTS-13, the metalloprotease that degrades ultralarge von Willebrand protein multimers. Most patients respond to treatment with therapeutic plasma exchange (TPE), which replaces the missing protease and removes the circulating inhibitor. However, a substantial fraction of idiopathic TTP cases (10%-20%) might not respond to TPE alone, and, therefore, interest has been gathering around the use of the novel immunosuppressive anti–B-cell antibody, rituximab. We report 2 cases of refractory TTP in which the combined use of daily plasma exchange and rituximab was associated with clinical resolution of active TTP, and we discuss the benefits and possible timing of combined therapy.

Thrombotic thrombocytopenic purpura (TTP) is attributed to a deficiency of the ultralarge von Willebrand factor (vWF) multimer-degrading serum metalloprotease ADAMTS-13,1 leading to accumulation of vWF multimers and systemic platelet aggregation, microangiopathic thrombosis, hemolytic anemia, and end-organ ischemia. Most cases of acquired idiopathic TTP are believed to be autoimmune in nature, usually attributed to the presence of an autoantibody to ADAMTS-13. TTP usually responds to therapeutic plasma exchange (TPE), which replaces the metalloprotease and removes circulating inhibitors.2 In many cases of acquired TTP, TPE is able to mitigate the microvascular thrombotic process until the disease remits; however, 10% to 30% of all cases are refractory to TPE.2

A growing number of case reports have demonstrated intriguing successes in treating refractory autoimmune TTP with the new immunomodulator rituximab.3-8 Rituximab is a humanized monoclonal antibody raised against CD20 that causes complement- and cell-mediated destruction of CD20-expressing cells. B cells and pre–B cells express leukocyte antigens CD19 and CD20, and then lose their expression of CD20 when they differentiate into antibody-secreting plasma cells. Although rituximab does not remove existing antibody-producing plasma cells, it eliminates the next generation of antibody-producing cells and prevents expansion of the reactive process. Accordingly, the proposed mechanism of this drug in the treatment of autoimmune TTP is believed to be the destruction of the CD20+ precursors of B cells that produce anti-ADAMTS-13.8 However, the issue has been raised whether TPE removes rituximab from the plasma before it can exert its full effect.

We report 2 cases of severe autoimmune TTP refractory to plasma exchange and corticosteroids that exhibited rapid
improvement when rituximab was added to their treatment regimens. In both cases, effective CD20-lymphocyte depletion was demonstrated by flow cytometry despite continuation of TPE after rituximab administration.

Materials and Methods

Plasma exchange was performed on COBE Spectra apheresis units with software version 7 (COBE BCT, Lakewood, CO) at both treatment centers. Isovolemic exchanges were done using either fresh frozen plasma (FFP) or cryosupernatant (cryo-poor plasma) as the replacement fluids. In case 1 Figure 1, 1.0 or 1.5 total plasma volume exchanges were performed daily or twice daily (1.0 + 1.5 total plasma volume, spaced 12 hours apart) depending on the patient’s clinical condition. In case 2 Figure 2, exchanges of 1.0 to 1.5 total plasma volumes were performed daily, except for a 3-day period during an attempt to taper treatment when only 0.7 volume was exchanged. Following evidence of response, exchanges were tapered and stopped. Informed consent for the procedures was obtained from the patients or their next-of-kin when appropriate.

ADAMTS-13 enzyme and inhibitor testing for both cases was performed at the Blood Center of Southeastern Wisconsin, Milwaukee. Enzyme levels were quantitated by using enzyme-linked immunosorbent assay and inhibitor levels by a Bethesda-type mixing study with normal serum looking for quenching of vWF cleavage activity.

For case 1, the laboratory references ranges were as follows: hemoglobin, 12 to 16 g/dL (120-160 g/L); hematocrit, 36% to 46% (0.36-0.46); platelet count, 150 to 350 × 10^9/L; serum lactate dehydrogenase (LDH), 110 to 210 U/L; serum direct bilirubin, 0.0 to 0.4 mg/dL (0.0-6.8 µmol/L); and serum indirect bilirubin, 0.0 to 0.6 mg/dL. Schistocytosis of 3+ corresponds to approximately 5 to 9 schistocytes per high-power field.

For case 2, laboratory references ranges were as follows: hemoglobin, 11.5 to 16.4 g/dL (115-164 g/L); hematocrit, 26% to 48% (0.26-0.48); platelet count, 150 to 350 × 10^9/L; LDH, 107 to 231 U/L; total bilirubin, 0.2 to 1.2 mg/dL (3.4-20.5 µmol/L); cardiac troponin I, less than 0.1 ng/mL; and creatinine kinase-MB fraction, 0.0 to 5.0 ng/mL (0.0-5.0 µg/L). Schistocytosis of 2+ corresponds to approximately 3 to 5 schistocytes per high-power field.

Flow cytometry was performed according to standard operating procedures based on techniques described elsewhere. Flow cytometry was performed using a Becton Dickinson FACSCalibur flow cytometer, and data were analyzed using CellQuest software, version 3.3. All equipment, reagents, and software were purchased from Becton Dickinson, Franklin Lakes, NJ. In brief, EDTA-anticoagulated
Peripheral blood was hemolyzed and resuspended in Isoton II solution (Beckman Coulter, Fullerton, CA). Mononuclear cells were stained with mouse monoclonal antibodies to CD10, CD20, and CD19 conjugated with fluorescein isothiocyanate, R-phycoerythrin, and peridinin chlorophyll protein, respectively. The population of B cells was isolated and quantitated by sequentially gating on lymphocytes by forward and side scatter, and then identifying B cells by CD19 and CD20 staining.

Case 1

A 39-year-old woman with a history of depression was transferred from an outside hospital where she had been given a diagnosis of TTP. She had received FFP infusions en route to the referral hospital and appeared alert and oriented on arrival. She responded to questions appropriately, and physical examination findings were remarkable only for widespread petechiae. Her initial laboratory results (Figure 1) showed a Coombs-negative microangiopathic hemolytic anemia with 3+ schistocytosis and a hemoglobin level of 5.8 g/dL (58 g/L), a hematocrit of 17% (0.17), a platelet count of 10 × 10^3/µL (10 × 10^9/L), an LDH level of 1,606 U/L, and a normal WBC count. Her liver transaminase values were mildly elevated, and she had elevated serum bilirubin (direct bilirubin level, 1.2 mg/dL [20.5 µmol/L] and indirect bilirubin, 2.4 mg/dL). Qualitative β-human chorionic gonadotropin was negative, and stool cultures were negative for Escherichia coli O157:H7 and Shigella species. Her basic chemistry and renal function test results were within reference ranges.

The patient was treated initially with daily 1.0 plasma volume TPE with FFP for replacement and methylprednisolone (Solu-Medrol, 60 mg, intravenously [IV] once daily), which improved her laboratory values for the first week, with the LDH level falling to 485 U/L and the platelet count increasing to 35 × 10^9/L (35 × 10^9/L). However, on day 7, the patient became agitated and confused with worsening signs of hemolysis and, therefore, received intravenous vincristine, 1 mg/m², after that day’s plasma exchange, and 125 mg of IV methylprednisolone was given each day thereafter.

As her clinical condition continued to worsen, TPE was performed twice daily for 4 days, with treatments spaced 10 to 12 hours apart and the sum of the exchanges ranging from 1.5 to 2.5 total plasma volumes per day. Cryosupernatant was used as exchange fluid in place of FFP temporarily on days 9 through 11, with little improvement in laboratory values or clinical status.

On day 11, the first of 4 doses of intravenous rituximab (375 mg/m² once per week) was administered after TPE. A second TPE was not performed that day to increase the length of time the drug would remain in the circulation. The twice-daily TPE schedule was resumed 24 hours after rituximab.

![Figure 2](Case 2) Longitudinal graph of ongoing treatments and laboratory parameters. **A**, Timeline linear graph of platelet counts (solid line) and hematocrit values (dotted line), coinciding plasma exchange regimen (daily therapeutic plasma exchange [TPE], upper bar), corticosteroids (lower bar), RBC transfusions (small arrows), and rituximab infusions (large arrows). **B**, Timeline linear graph of lactate dehydrogenase (LDH; solid line) and total bilirubin (dotted line) values coinciding with values and treatment conditions shown in Figure 2A. The x-axis is numbered by day of admission; the longer vertical line connects the ADAMTS-13 assay value with the corresponding day of admission on which it was drawn. ADAMTS-13 enzyme activity (percentage of normal control) and ADAMTS-13 inhibitor (in Bethesda U/mL) were measured as described in the “Materials and Methods” section. Values are given in conventional units; conversions to Système International (SI) units are as follows: platelet count (× 10^9/L), multiply by 1; hematocrit (proportion of 1.0), multiply by 0.001; and bilirubin (µmol/L), multiply by 17.1. (For LDH, conventional and SI units are the same.)
administration. A second dose of vincristine (1 mg/m²) was administered on day 12 after the evening TPE, resulting in a transient rise in the platelet count to 21 × 10⁹/µL (21 × 10⁹/L). On day 13, the platelet count decreased again to 7 × 10⁹/µL (7 × 10⁹/L). Hemolysis peaked at that time, with the LDH level reaching 2,780 U/L and the total bilirubin reaching 21.4 mg/dL (365.9 µmol/L).

On day 15, the patient suddenly became lucid, and although she was experiencing perseveration, she could answer basic questions and follow simple commands. Her condition continued to improve dramatically throughout the following days. A blood specimen taken that day showed complete CD19+/CD20+ cell depletion by flow cytometry (quantitative data no longer available). A specimen obtained on day 16 showed the first evidence of improvement in her circulating ADAMTS-13 levels and disappearance of the inhibitor. On day 16, she received a third dose of vincristine and second and third doses of rituximab on days 18 and 25. She was discharged from our facility on day 28.

She spent 1 evening at home but was rehospitalized at another facility near her home to resume daily TPE because her platelet count fell to just below 100 × 10³/µL (100 × 10⁹/L) with no significant rise in LDH. After another 2 weeks of TPE and the final (fourth) dose of rituximab, her platelet count returned to greater than 150 × 10³/µL (>150 × 10⁹/L), and she was discharged home with careful plans for follow-up.

Case 2

A 62-year-old woman with a medical history of systemic lupus erythematosus was transferred from an outside hospital because of right shoulder paresthesias, clumsiness in both hands, slurred speech, an elevated LDH level, a decreased hematocrit value, and significantly elevated troponin I levels. On transfer, her complaints were limited to headache and fatigue. Review of systems was otherwise unremarkable. She had been given a diagnosis of lupus associated with arthritis 12 years earlier but had not taken any immunomodulatory therapies for many years. The physical examination findings were unremarkable and showed no petechiae and no focal neurologic deficits or muscle weakness. She was alert and oriented to person, place, and time.

Admission laboratory results demonstrated a Coombs-negative microangiopathic anemia with 2+ schistocytosis, decreased hemoglobin (9.1 mg/dL [91 g/L]) and hematocrit (26.5% [0.27]) values, a severely decreased platelet count (18 × 10⁹/µL [18 × 10⁹/L]), an elevated LDH level (908 U/L), a mildly elevated total bilirubin level (1.5 mg/dL [25.7 µmol/L]), and normal liver transaminase levels, direct bilirubin level, and WBC count. The results of routine coagulation assays and fibrinogen levels were within reference ranges.

Cardiac enzyme assays revealed a markedly elevated cardiac troponin I level (14.67 ng/mL) and creatinine kinase-MB fraction (9.1 ng/mL [9.1 µg/L]); however, an electrocardiogram was indicative of concentric left ventricular hypertrophy only with no other abnormalities. A chest radiograph showed signs of mild pulmonary edema in all lung fields.

ADAMTS-13 activity and inhibitor assays were ordered on admission, but the initial specimen was lost; another specimen was sent on day 14, after 2 weeks of plasma exchange; although no inhibitor could be detected, enzyme levels were below the limits of detection (<4% of control). She was given a presumptive diagnosis of autoimmune TTP. The elevated cardiac markers indicated microthrombi-induced myocardial ischemia. Treatment was initiated with daily exchange of 1.0 total body plasma volumes with cryo-poor plasma replacement and no corticosteroids or other immunosuppressive agents.

Initially, the patient responded well. The platelet count at the outset of therapy was 18 × 10⁹/µL (18 × 10⁹/L) and increased to 162 × 10⁹/µL (162 × 10⁹/L) after 7 days of plasma exchange. The LDH level decreased from 908 U/L to 259 U/L; the hematocrit value went from 26.5% (0.27) to 39.2% (0.39) after only 1 unit of RBCs transfused (Figure 2). The patient had no other changes in clinical status through this week. Daily exchange was continued until laboratory values had been within normal limits for 3 consecutive days, at which time it was decided to taper plasma exchange, which then was omitted for 1 day.

During the 1-day hiatus, dysarthria and visual hallucinations developed, and laboratory values all worsened precipitously; between days 10 and 12 the patient’s platelet count dropped from 221 × 10⁹/µL (221 × 10⁹/L) to 21 × 10⁹/µL (21 × 10⁹/L), the LDH level rose from 206 to 452 U/L, and the hematocrit value dropped from 36.1% (0.36) to 29.7% (0.30). Daily TPE was resumed, increasing to 1.5 total plasma volumes exchanged. When the patient’s clinical status and laboratory values failed to respond, treatment was intensified with prednisone, 60 mg twice daily.

When there was no evidence of improvement after 42 days of combined plasma exchange and corticosteroids, it was decided to add weekly rituximab to the patient’s regimen while maintaining her schedule of TPE. On day 42 of hospitalization, a dose of rituximab (375 mg/m²) was administered, and TPE was performed 36 hours later. Another 12 hours after this, a peripheral blood sample was obtained for quantitation of circulating B cells by flow cytometry. Pre-rituximab flow cytometric results demonstrated that 0.66% of all lymphocytes were CD19+/CD20+, compared with 0.00% of lymphocytes in the postrituximab sample (0 of 2,331 lymphocytes). This demonstrated complete disappearance of circulating CD19+/CD20+ B cells.

During the week following the first dose of rituximab, the patient’s platelet counts began to recover slowly. As recovery...
proceeded, rituximab dosing and daily plasma exchange were continued on a near-weekly basis (days 48, 53, 63). Because of worsening congestive heart failure, severe edema with status changes, and the adverse effects of high-dose corticosteroids, the patient’s prednisone was tapered before tapering plasma exchange, but once it was demonstrated that the platelet and RBC counts could be maintained without corticosteroids, plasma exchange also was tapered and stopped. At this point, the patient’s platelet counts recovered completely, and the patient was transferred to a skilled nursing facility for rehabilitation.

**Discussion**

The number of published case reports describing the use of rituximab to treat patients with refractory and relapsed TTP is growing, and, although the studies are not randomized or controlled, they are suggestive of a potentially impressive therapeutic efficacy. A recent search of the PubMed database returned 15 reports containing 36 cases with almost all cases responding rapidly and with relatively long relapse-free periods. For our 2 patients, evidence of any causal effects by rituximab was confounded by multiple ongoing therapies; however, it is fair to add these cases to the list of successful outcomes after rituximab administration. In case 1, in addition to frequent TPE treatments and daily corticosteroids, the patient had received vincristine on days 7, 12, 18, and 25 and rituximab on days 11, 18, and 25. Although the patient’s condition improved soon thereafter, it was impossible to credit only one therapy for the patient’s improvement. Case 2 was more suggestive of an effect by rituximab treatment, however, because clinical improvement was achieved during a 4-week course of rituximab therapy initiated after 42 days of unsuccessful TPE and corticosteroid therapy. The more significant observation made from these cases was the interaction between rituximab and uninterrupted daily TPE.

Because rituximab might be removed by TPE, it may seem counterproductive to continue a daily TPE schedule after this medication has been administered. Therefore, some authors have advocated postponing plasma exchange as long as clinically possible after rituximab administration. However, discontinuation of TPE might lead to adverse clinical outcomes. The drug is given in weekly doses, and according to the manufacturer, the mean half-life of the first dose is 76.3 hours. Based on this information alone, it is reasonable to assume that resumption of daily exchange shortly after rituximab administration might remove a substantial amount of it from the circulation and impede its clinical effect.

The standard dosing schedule and circulating half-life are not necessarily indicative of the time required for significant B-cell removal, however. Like most antibodies, rituximab would be expected to bind its target and start cytolysis immediately. A study by Maloney et al demonstrated that efficient B-cell depletion, as measured by flow cytometry, occurred by day 4 after rituximab administration; however, none of the subjects underwent TPE. A study in macaques (that express the same CD20 antigen on B cells) showed that human-equivalent doses of rituximab effectively depleted peripheral blood circulating CD19+/CD20+ cells (which represent pre-B-cell and mature B-cell populations) within 24 hours of administration. Furthermore, this 1 dose was sufficient to remove peripheral B cells for the entire month. As another testament of the rapid efficacy of rituximab, a study in human patients with lymphomas and chronic lymphocytic leukemias showed that rituximab administration depleted more than 50% of the circulating CD19+/CD20+ lymphocytes during and immediately after administration. Considering the large numbers of CD20+ cells present in these patients, this observation is a testament to the rapidity and capacity for B-cell killing by this agent.

Based on the time course that can be inferred from these studies, we expected that a standard dose of rituximab would have significantly rapid effects and that therapeutic efficacy could be achieved despite quick resumption of plasma exchange. Indeed, when plasma exchange was resumed 24 and 36 hours after rituximab administration and blood specimens were obtained for flow cytometry 72 and 12 hours after TPE (cases 1 and 2, respectively), no peripheral circulating CD19+/CD20+ cells were found, suggesting that these regularly scheduled plasma exchanges did not interfere with the immunosuppressive effects of rituximab.

The clinical improvements in the condition of both patients further intimated that the potential clinical efficacy of rituximab, whatever it may be, might not necessarily be compromised by uninterrupted daily plasma exchanges. Note that the absence of CD19+/CD20+ cells demonstrated effective removal of all peripheral B cells, not just CD20 antigen saturation. It also should be noted that although peripheral clearance of B cells after 1 dose of rituximab implies that our TPE schedule might not interfere with the immunosuppressive efficacy of rituximab, it does not imply that 1 dose can always effect total body ablation of B cells. Recrudescence after 1 dose of rituximab is typical in the treatment of lymphoma, which is why multiple doses usually are required. Additional trials are required to determine the effective dosing regimen in cases of TTP.

Although further studies on the coordination of these therapies is indicated to corroborate these observations, we believe these 2 cases provide good preliminary evidence that delays in plasma exchange during concomitant rituximab therapy are unnecessary.

From the 1Joint Program in Transfusion Medicine of Harvard Medical School at Massachusetts General Hospital, Brigham and
Women’s Hospital, Dana-Farber Cancer Institute, Beth Israel Deaconess Medical Center and Children’s Hospital; and 2Department of Pathology, Brigham and Women’s Hospital, Boston.

Address reprint requests to Dr Darabi: Blood Transfusion Service, GRJ-148, Massachusetts General Hospital, 55 Fruit St, Boston, MA 02114. kamrandarubi@yahoo.com.

References