Long-Term Response to Rituximab in Patients with Relapsing Thrombotic Thrombocytopenic Purpura

Gideon Y. Stein MD\(^1\), Dorit Blickstein MD\(^2\), Jerome Orlin MD\(^3\), Galit Sarig MD\(^4\) and Aida Inbal MD\(^2\)

\(^1\)Department of Internal Medicine B, \(^2\)Thrombosis and Hemostasis Unit and Hematology Institute, and \(^3\)Department of Transfusion Medicine, Rabin Medical Center (Beilinson Campus), Petah Tikva, affiliated with Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

\(^4\)Hematology Laboratory, Thrombosis and Hemostasis Unit, Rambam Health Care Campus, Haifa, Israel

**ABSTRACT:** Acquired thrombotic thrombocytopenic purpura (TTP) is an uncommon disease in adults, characterized by fever, neurological manifestations, microangiopathic hemolytic anemia, thrombocytopenia, renal dysfunction, and the presence of antibodies against the enzyme ADAMTS13. Treatment with plasmapheresis has increased the survival from 10% to more than 90%. Still, there is a subset of patients with resistant TTP who fail to respond to plasmapheresis or remain dependent on this procedure. There is mounting evidence that rituximab may play an important role in remission induction of resistant/relapsing TTP, but the extent of the remission is unknown. We present here four patients with chronic-relapsing TTP who responded favorably to rituximab. All four patients achieved prolonged remission of 23 to 82 months after the treatment. One patient relapsed 6 years after the initial treatment with rituximab and re-entered remission following retreatment.

**KEY WORDS:** thrombotic thrombocytopenic purpura, ADAMTS13, rituximab

**TTP = thrombotic thrombocytopenic purpura**

**vWF = von Willebrand factor**

**Plasmapheresis using fresh frozen plasma has become the standard treatment for TTP. Most patients respond to this treatment and achieve remission. Some patients, however, experience multiple relapses, developing a chronic debilitating form of the disease that can be life threatening, or they become plasmapheresis-dependent [5,6]. Recently, a number of reports have suggested an important role for rituximab in the treatment of resistant/relapsing acquired TTP, especially in patients with anti-ADAMTS13 antibodies [7,8]. It is assumed that by eliminating B cells, rituximab also eliminates autoantibody-secreting B cells, which are associated with TTP and other autoantibody-related diseases. In the absence of anti-ADAMTS-13 antibodies, high molecular weight vWF multimers are cleaved, while the remaining multimers no longer aggregate platelets to form arterial thrombi. Although the role of rituximab in remission induction of relapsing TTP has been recognized, the length of the remission is unknown. We report on four patients with chronic-relapsing TTP treated with rituximab. All patients achieved prolonged remissions following treatment.**

**PATIENTS AND METHODS**

The diagnosis of TTP was established by the detection of microangiopathic anemia on blood film, thrombocytopenia and the presence of anti-ADAMTS13 antibodies (in some patients). All other reasons for thrombocytopenia or hemolytic anemia were ruled out. Laboratory and clinical data are summarized in Table 1.

**PATIENT 1**

A 55 year old woman presented in 2007 with a non-traumatic epidural hematoma and seizures. Treatment with corticosteroids and plasmapheresis resulted in a complete remission. During the ensuing 3 months two asymptomatic relapses were diagnosed during routine follow-up. Both were successfully treated with plasmapheresis and corticosteroids. Due to high operative risk, splenectomy was not performed. Because of her history of...
only short-lived remissions, she was treated with rituximab and achieved complete remission lasting more than 44 months.

**PATIENT 2**

A 37 year old man presented in 2008 with abdominal pain and nephritic syndrome. Plasmapheresis resulted in a complete remission. A relapse 9 months later was treated with plasmapheresis, however without response. Administration of rituximab resulted in complete remission, lasting over 23 months. It is worth mentioning that following rituximab treatment the nephritic syndrome also resolved.

**PATIENT 3**

A 41 year old man presented in 1993 with fever, hemiparesis, purpura and hematuria. Treatment with plasmapheresis resulted in complete remission. After five relapses, which were successfully treated with plasmapheresis, a sixth relapse, 6 years after the initial presentation, failed to respond to plasmapheresis. Remission was achieved only after splenectomy and treatment with high-dose steroids, intravenous immunoglobulin and vincristine. In the following years the disease entered a chronic-relapsing phase and was treated with therapeutic/prophylactic plasmapheresis every few weeks. In 2007, laboratory testing revealed a high titer of anti-ADAMTS13 antibodies and low activity of ADAMTS13. Rituximab was administered in 2008, and a complete remission, lasting more than 42 months, was achieved. Interestingly, although the patient was asymptomatic, ADAMTS13 activity has remained persistently low even after treatment with rituximab.

**PATIENT 4**

A 23 year old woman presented in 1992 with weakness, low back pain and confusion. Plasmapheresis resulted in a complete remission. A year later, a relapse was treated by splenectomy, resulting in a disease-free interval of 5 years. In 1998 she experienced a severe relapse, manifested by hemolytic anemia, thrombocytopenia, renal failure and neurological complications including confusion, aphasia and coma with respiratory arrest. She was treated with plasmapheresis, corticosteroids, IVIG, vincristine and supportive care. A complete clinical and laboratory remission was achieved again, as published in a previous report [9]. In 2003 the patient experienced another severe relapse, manifested by epistaxis, hemoptysis and macrohematuria. She again responded to treatment with plasmapheresis and corticosteroids; however, all attempts to taper plasmapheresis resulted in relapse. After 2 months of intensive treatment that was complicated by sepsis and seizures, the patient received a single dose of cyclophosphamide, 1.2 g, and four weekly courses of rituximab [Table 1]. For the next 6 years, the patient remained in remission, as reported previously by some of us [10]. In November 2009, another relapse occurred that manifested as generalized weakness, epistaxis, hematuria and severe thrombocytopenia. High levels of anti-ADAMTS13 antibodies were detected in the patient’s plasma, accompanied by undetectable levels of ADAMTS13 activity. The patient had been previously exposed to hepatitis B virus, with positive anti-HBc and anti-HBs antibodies, negative HBsAg levels and undetectable HBV viral load. Since treatment with plasmapheresis failed to induce remission, the patient was again treated with four weekly courses of rituximab. Due to several reports of HBV reactivation following rituximab therapy [11-13], prophylactic treatment with lamivudin was initiated. The patient did not need plasmapheresis after the second rituximab course and is now in complete remission for more than 19 months, with normal levels of liver enzymes.

**METHODS**

Levels of ADAMTS-13 activity were measured in plasma samples by chromogenic enzyme-linked immunosorbent

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**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Gender</th>
<th>Age at presentation (yrs)</th>
<th>Plt (10⁹/L)</th>
<th>Hb (g/dL)</th>
<th>LDH (units/L)</th>
<th>MAHA</th>
<th>ADAMTS13 activity (%)*</th>
<th>Anti-ADAMTS13 Ab (U/ml)**</th>
<th>TTP episodes</th>
<th>Treatment modalities</th>
<th>Rituximab</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>55</td>
<td>20</td>
<td>7.0</td>
<td>1873</td>
<td>+</td>
<td>N/A</td>
<td>N/A</td>
<td>3</td>
<td>Steroids, plasmapheresis</td>
<td>600 mg/wk/4 wks</td>
<td>Complete remission (3 yrs, 8 mos)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>37</td>
<td>13</td>
<td>15.4</td>
<td>2574</td>
<td>+</td>
<td>N/A</td>
<td>N/A</td>
<td>3</td>
<td>Steroids, plasmapheresis</td>
<td>700 mg/wk/4 wks</td>
<td>Complete remission (1 yr, 11 mos)</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>41</td>
<td>7</td>
<td>8.7</td>
<td>3700</td>
<td>+</td>
<td>15</td>
<td>42</td>
<td>6</td>
<td>Steroids, plasmapheresis, splenectomy</td>
<td>670 mg/wk/4 wks</td>
<td>Complete remission (3 yrs, 6 mos)</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>26</td>
<td>13</td>
<td>7.1</td>
<td>4259</td>
<td>+</td>
<td>&lt; 1.0</td>
<td>100</td>
<td>4</td>
<td>Steroids, plasmapheresis, splenectomy, vincristine</td>
<td>500 mg/wk/4 wks</td>
<td>Relapse after 6 years, complete remission after retreatment, 19 mos remission</td>
</tr>
</tbody>
</table>

*Normal range 65–120%  
**Normal range 0–15 U/ml  
Plt = platelets, LDH = lactate dehydrogenase, TTP = thrombotic thrombocytopenic purpura, MAHA = microangiopathic hemolytic anemia, N/A = not available
assay using TECHNOZYM® ADAMTS-13 Activity ELISA kit, Technoclone GmbH, Vienna, Austria. Antibodies against ADAMTS-13 were tested by TECHNOZYM® ADAMTS-13 INH ELISA kit (Technoclone GmbH, Vienna, Austria). Briefly, the detection wells were coated with recombinant ADAMTS-13. Antibodies against ADAMTS-13 from the plasma sample were recognized by conjugated anti-human IgG. The peroxidase level was determined by a chromogenic reaction that is proportional to the anti ADAMTS-13 antibody level.

**DISCUSSION**

Plasmapheresis, the standard treatment for TTP, improves prognosis and induces remission in more than 90% of patients. However, relapses are common [6]. There are no standard guidelines for the treatment and prevention of recurrent TTP. The options include plasmapheresis, corticosteroids alone or in combination with other immunosuppressive drugs (azathioprine, vincristine or cyclophosphamide), splenectomy or high-dose intravenous immune globulins [14-18]. However, an effective prophylactic regimen to prevent recurrent relapses is lacking.

More than 10 years ago an autoimmune process was implicated in the pathophysiology of some forms of TTP [2]. Arterial thrombi, the hallmark of TTP, consist of unusually large multimers of von Willebrand factor. These multimers are cleaved by a serum metalloprotease (ADAMTS-13) [1]. In patients with non-familial TTP, the presence of an autoantibody results in decreased levels of this metalloprotease [2]. Thus, targeting the activity of B lymphocytes which produce these autoantibodies may be beneficial in treating TTP. Rituximab, a chimeric antibody against the CD20 antigen presented on B lymphocytes, is used for the treatment of B cell lymphoma, idiopathic thrombocytopenic purpura, and other autoantibody-mediated autoimmune diseases [19-21]. Recent reports have described a beneficial response to rituximab with or without cyclophosphamide [22,23] in chronic-relapsing TTP. Rituximab attaches to the CD20 antigen in B lymphocytes, apparently triggering an immunological response, which eliminates B cells, including the clone that produces the anti-ADAMTS-13 antibodies.

All patients who fulfill the diagnosis of TTP are treated with plasmapheresis. According to the largest TTP-HUS registry from Oklahoma, the estimated risk for relapse is 41% at 7.5 years [24]. Most of the relapses occur during the first year after recovery. This group of relapsed TTP patients is a candidate for rituximab treatment, which in recent years has gained popularity.

This article describes four patients with recurrent or refractory TTP who achieved long-term remissions (13–72 months) following treatment with rituximab. Our experience with these patients is consistent with previously published data and substantiates a role for rituximab treatment in this potentially fatal disease, after relapses.

Patient 4 in our series had been previously exposed to hepatitis B virus. Reactivation of HBV leading to fulminant hepatic failure has been observed in these patients following treatment with rituximab [25]. In one series, 4.2% of 95 HBsAg-negative rituximab-treated lymphoma patients developed HBV reactivation [13]. Yeo et al. [11] reported that in HBsAg negative/anti-HBc positive lymphoma patients being treated with either CHOP or R-CHOP chemotherapy protocols, 5 of 21 patients treated with rituximab (R-CHOP) developed HBV reactivation (including one who died from hepatic failure), as compared to none of 25 patients receiving only CHOP [11]. Several reports have suggested that prophylactic treatment with lamivudin may reduce the rates of HBV reactivation [11,13]. In our patient 4, lamivudin was initiated at the time of the first rituximab cycle and continued for 6 months. HBV reactivation did not occur.

In conclusion, prolonged remissions of TTP-relapsing patients, following treatment with rituximab, as reported in this work, warrant further substantiation in future studies.

**Corresponding author:**

Dr. A. Inbal

Thrombosis and Hemostasis Unit, Rabin Medical Center (Beilinson Campus), Petah Tikva 49101, Israel

Phone: (972-3) 937-7912

Fax: (972-3) 924-0145

email: aidainbal@hotmail.com

**References**


Suppression of lung adenocarcinoma progression by Nkx2-1

Despite the high prevalence and poor outcome of patients with metastatic lung cancer, the mechanisms of tumor progression and metastasis remain largely uncharacterized. Winslow et al. modeled human lung adenocarcinoma, which frequently harbors activating point mutations in KRAS and inactivation of the p53 pathway, using conditional alleles in vivo. Interrogation of Nkx2-1 in the lung epithelial cells of KrasLSL-G12D; p53flox/flox mice initiates lung adenocarcinoma development. Although tumors are initiated synchronously by defined genetic alterations, only a subset becomes malignant, indicating that disease progression requires additional alterations. Identification of the lentiviral integration sites allowed the authors to distinguish metastatic from non-metastatic tumors and determine the gene expression alterations that distinguish these tumor types. Cross-species analysis identified the Nk2-related homeobox transcription factor Nkx2-1 (also called Ttf-1 or Titf1) as a candidate suppressor of malignant progression.

In this mouse model, Nkx2-1 negativity is pathognomonic of high-grade poorly differentiated tumors. Gain- and loss-of-function experiments in cells derived from metastatic and non-metastatic tumors demonstrated that Nkx2-1 controls tumor differentiation and limits metastatic potential in vivo. Interrogation of Nkx2-1-regulated genes, analysis of tumors at defined developmental stages, and functional complementation experiments indicate that Nkx2-1 constrains tumors in part by repressing the embryonically restricted chromatin regulator Hmga2. Whereas focal amplification of Nkx2-1 in a fraction of human lung adenocarcinomas has focused attention on its oncogenic function, their data specifically link Nkx2-1 downregulation to loss of differentiation, enhanced tumor seeding ability and increased metastatic proclivity. Thus, the oncogenic and suppressive functions of Nkx2-1 in the same tumor type substantiate its role as a dual function lineage factor.

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Eitan Israeli

“Eat food. Not too much. Mostly plants”
Michael Pollan (b. 1955), American author, journalist, activist, and professor of journalism at the University of California, Berkeley. A 2006 New York Times books review describes him as a “liberal foodie intellectual”

“In all affairs it’s a healthy thing now and then to hang a question mark on the things you have long taken for granted”
Bertrand Russell (1872-1970), British philosopher, mathematician, author, Nobel laureate