Therapeutic Modality of 11 Patients with TTP in a Single Institution in Miyazaki from 2000 to 2011

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Abstract

Objective Thrombotic thrombocytopenic purpura (TTP) is a life-threatening generalized disease with pathological features that are termed thrombotic microangiopathies. Since the discovery of the von Willebrand factor-cleaving protease [a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13)], it is widely known that approximately two-thirds of TTP patients have a severe deficiency of ADAMTS13 activity due to gene mutations or acquired autoantibodies to this enzyme. However, the remaining one-third of TTP patients have only moderately reduced or almost normal ADAMTS13 activity. To elucidate the clinical characteristics and outcomes of these two types of TTP, we have retrospectively analyzed the cases of acquired TTP patients treated in a single institution from 2000 to 2011.

Methods Our case studies include 11 TTP patients, of which 5 were considered idiopathic and 6 had cases of TTP associated with underlying diseases such as non-Hodgkin lymphoma or connective tissue diseases.

Results These patients were treated with a combination therapy of plasma exchange and steroids and with several adjunctive therapeutic regimens including the on-label use of cyclophosphamide and cyclosporine and the off-label use of high-dose steroid or immunoglobulin with rituximab. Splenectomies were not performed. As a result of these treatments, 6 out of the 7 patients with ADAMTS13 activity deficient TTP achieved a complete remission without relapse, but the remaining 4 patients with non-ADAMTS13 activity deficient TTP all died without complete remission.

Conclusion We present herein the detailed clinical courses of 11 patients with TTP and address our experiences with the efficacy of various therapeutic regimens. This case-oriented study should be helpful to the physicians who directly care for TTP patients, and may provide a future direction for developing a more efficient treatment modality.

Key words: thrombotic thrombocytopenic purpura, ADAMTS13, plasma exchange, adjunctive therapeutic regimen, rituximab

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening generalized disease with pathological features that are termed thrombotic microangiopathies (TMAs). These features are characterized by the triad of microangiopathic hemolytic anemia, destructive thrombocytopenia, and organ (renal) failure due to platelet thrombi (1-5).

In 1996, Amorosi and Ultmann (2) defined the classic “pentad” of clinical features of TTP which included the aforementioned triad plus fluctuating neurological signs and...
fever. Regarding TTP treatment, Rock et al. (6) published a breakthrough report in 1991 showing that plasma exchange (PE) therapy saved the lives of 90% of TTP patients. However, the pathogenesis of TTP was not addressed until the discovery of the von Willebrand factor (VWF)-cleaving protease, now known as ADAMTS13—a disintegrin-like and metalloproteinase with a thrombospondin type 1 motif s (7, 8). Subsequent studies have indicated that TTP is caused by severe deficiency of ADAMTS13 activity in approximately two-thirds of patients. Of these patients, a minor population (circa 5%) have the gene mutations which define Upshaw-Schulman syndrome (9, 10) and a major population (circa 95%) have acquired autoantibodies to this enzyme (11, 12). Therefore, the pathogenesis of TTP is not well defined and the efficacy of PE therapy has been controversial in the remaining one-third of acquired TTP patients who do not show severe deficiency of ADAMTS13 activity. Furthermore, recent studies indicate that some populations of acquired TTP patients with severe deficiency of ADAMTS13 activity do not respond well to PE therapy, and the reasons for this must be explored (13).

We treated 11 patients with acquired TTP from 2000 to 2011 in Miyazaki Prefectural Hospital, a regional referral hospital in Japan. In these patients, the basic PE and steroid therapy was supplemented with several adjunctive therapeutic regimens. These included the on-label use of cyclophosphamide and cyclosporine and the off-label use of high-dose steroid or immunoglobulin and rituximab, but did not include splenectomy. We analyzed the detailed clinical courses of our 11 TTP patients and evaluated the efficacy of the therapeutic regimens. This case-oriented study should be helpful to the physicians who directly care for TTP patients and may provide a future direction for the development of new treatment modalities.

Materials and Methods

Patients: From January 2000 to December 2011, 11 patients with acquired TTP were diagnosed at the Miyazaki Prefectural Hospital (Table 1). The diagnosis of TTP was made by the classic pentad with the following laboratory markers (14): (i) microangiopathic hemolytic anemia (hemoglobin [Hb] ≤12 g/dL), Coombs test negative, non-detectable serum haptoglobin (<10 mg/dL), more than 2 fragmented red cells (schistocytes) in a microscopic field with a magnification of 100, and the concurrent increase of serum lactate dehydrogenase (LDH) above the institutional baseline; (ii) thrombocytopenia (platelet count ≤100x10^9/L); (iii) fever ≥ 37°C; (iv) central nervous system (CNS) involvement ranging from headache to coma and including neurological dysfunction, convulsion, or clouding of consciousness; and (v) renal involvement including abnormal urinalysis in addition to the elevation of the serum creatinine level.

Among the 11 patients with TTP, 5 patients had no underlying disease and were classified as having acquired idiopathic TTP (ai-TTP) and 5 patients had TTP associated with connective tissue diseases. In this patient group, 2 had systemic lupus erythematosus (SLE), 2 had systemic sclerosis (SSc), and 1 had overlapping diseases (OS: SLE + SSc). The remaining patient had TTP associated with non-Hodgkin lymphoma (NHL) (Table 1). This retrospective study was conducted in compliance with good clinical practices and the ethical principles of the Declaration of Helsinki.

ADAMTS13 assays: Through March 2005, the ADAMTS13 activity was measured at Nara Medical University by the classic von Willebrand factor multimer (VWFM) assay with a detection limit of 3% of the normal control (9). Thereafter, the ADAMTS13 activity was determined by a chromogenic ADAMTS13-act-ELISA (Chr-act-ELISA) with a detection limit of 0.5% of the normal (15). For consistency, all the samples tested prior to 2005 were re-evaluated by Chr-act-ELISA using the plasmas that had been stored at -80°C.

The plasma ADAMTS13 inhibitor titers were analyzed using patient plasmas that had been heat-inactivated at 56°C for 30 minutes (15) according to the original method established for the measurement of factor VIII inhibitor (16). The results were expressed in Bethesda units (BU) where one unit was defined as the amount necessary to reduce the ADAMTS13 activity to 50% of the control levels. The ADAMTS13 inhibitor titers were considered negative for values of less than 0.5 BU/mL, marginal for values between 0.5 and 1 BU/mL, and positive for values greater than 1 BU/mL (14).

Plasma exchange therapy: We performed plasma exchange (PE) therapy with fresh frozen plasma (FFP) at 60 mL/kg body weight until we observed the recovery of the following variables: increased platelet count (>150x10^9/L), decreased lactate dehydrogenase (LDH) levels, and decreased neurological abnormalities (4, 8). The PE therapy was performed for 3 consecutive days from the diagnosis of TTP and then the frequency was gradually tapered off. A diagnosis of complete remission (CR) was considered when a normalization of both the physical and the laboratory findings were achieved as previously described (4). The PE therapy was usually accompanied by high-dose methylprednisolone (mPSL) pulse therapy with an intravenous drip infusion rate of 1 g mPSL/day for 3 consecutive days.

Case series: The clinical and laboratory findings at admission for 11 patients with acquired TTP are shown in Table 1 and their treatment regimens and therapeutic outcomes are summarized in Table 2.

Case 1: In 2003, a 56-year-old man developed petechiae and fever, then fell into a coma prior to his admission to our hospital. Based on the clinical diagnosis of TTP, PE therapy was immediately initiated with high-dose mPSL pulse therapy under sedation and with intubation. The patient was extubated on hospital day 13 but the thrombocytopenia remained, and, thus, a second course of mPSL pulse therapy was initiated on hospital day 14. On hospital day 16, the laboratory results showed that a low plasma level of ADAMTS13 activity (<3%) and positivity for an ADAMTS
Table 1. Clinical and Laboratory Findings on Admission of Eleven Patients with Acquired TTP

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Age (yo)</th>
<th>Body Weight (kg)</th>
<th>Year</th>
<th>Initial clinical signs</th>
<th>Etiology</th>
<th>Blood chemistry</th>
<th>Hemostatic test</th>
<th>Peripheral blood</th>
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<tr>
<td>1</td>
<td>M</td>
<td>56</td>
<td>48</td>
<td>2003</td>
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<td>Idiopathic</td>
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<td>2</td>
<td>M</td>
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<td>70</td>
<td>2003</td>
<td>Fever, Anemia, Purpura</td>
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<td>3</td>
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<td>4</td>
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<td>50</td>
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<td>65</td>
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<td>6</td>
<td>M</td>
<td>48</td>
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<td>2003</td>
<td>Fever, Anemia, Purpura</td>
<td>Idiopathic</td>
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<td>7</td>
<td>F</td>
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<td>59</td>
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<td>SSc</td>
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<td>45</td>
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<td>58</td>
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Among the 11 patients with TTP, 8 had no underlying diseases and were termed acquired idiopathic TTP (a-TTP) and 5 had TTP that could be associated with connective tissue diseases. Of the second group, 2 patients had systemic lupus erythematosus (SLE), 2 patients had systemic sclerosis (SS), and 1 patient had overlapping disease (OS: SLE + SS). The remaining non-idiopathic patient had non-Hodgkin lymphoma (NHL).
13 inhibitor (1.8 BU/mL) were present on admission, confirming the diagnosis of ai-TTP with a severe deficiency of ADAMTS13 activity. Because thrombocytopenia persisted (10×10^9/L), 4 cycles of vincristine at a dose of 1.0 mg/m² per week were initiated starting on hospital day 21. Moreover, an intravenous infusion of gamma globulin (IVIG) was administered at a dose of 400 mg/kg for 5 consecutive days starting on hospital day 49 because of persistent thrombocytopenia (25×10^9/L). After these treatments, combined with 30 rounds of PE therapy, a CR was achieved on hospital day 63. To date, the patient maintains disease-free survival (DFS) with an oral intake of 5 mg PSL/day.

**Case 2:** In 2005, a 50-year-old woman developed petechiae and a fever, and thereafter fell into a coma prior to her admission to our hospital. Based on the clinical diagnosis of TTP, PE therapy was immediately initiated with high-dose mPSL pulse therapy under sedation and with intubation. The patient was extubated on hospital day 8. On hospital day 11, laboratory findings revealed that a low plasma level of ADAMTS13 activity (<3%) and positivity for an ADAMTS13 inhibitor (1.3 BU/mL) were present on admission, confirming the diagnosis of ai-TTP with a severe deficiency of ADAMTS13 activity. Because the clinical and laboratory findings were exacerbated when the PE therapy was tapered off, a second course of high-dose mPSL pulse therapy was initiated starting on hospital day 11. In addition, a high-dose IVIG treatment was administered for 5 consecutive days starting on hospital day 15, which remarkably improved the neurological and hematological findings. A CR was achieved on hospital day 42 after 12 rounds of PE therapy. Relapse has not been observed to date with an oral intake of 5 mg PSL/day. However, the patient did develop osteonecrosis of the right femur requiring an anterior rotation osteotomy in April 2011 which may have been associated with an adverse reaction to the two courses of high-dose mPSL pulse therapy.

**Case 3:** In 2007, a 48-year-old man developed a fever with subsequent eye deviation to the right and right hemiplegia prior to his admission to our hospital. Based on the clinical diagnosis of TTP, PE therapy was immediately initiated with high-dose mPSL pulse therapy under sedation and with intubation. The patient was extubated on hospital day 4. On hospital day 10, laboratory findings revealed that a low plasma level of ADAMTS13 activity (<3%) and positivity for an ADAMTS13 inhibitor (1.6 BU/mL) were present at admission, confirming the diagnosis of ai-TTP with a severe deficiency of ADAMTS13 activity. Because clinical aggravations were observed while tapering off the administration of PE and PSL, a high-dose IVIG treatment was administered for 5 consecutive days starting on hospital day 21. A CR was achieved on hospital day 40 after 19 rounds of PE therapy. To date, the patient maintains DFS with an oral intake of 5 mg PSL/day.

**Case 4:** In 2007, a 56-year-old man developed petechiae without a fever and subsequently developed anemia and thrombocytopenia prior to his admission to our hospital. The clinical and laboratory findings upon admission suggested a diagnosis of TTP, but this patient also had a history of non-Hodgkin lymphoma (NHL) with CS IIIA at the age of 54. He achieved a CR from the NHL after 8 courses of treatment with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) therapy, but relapsed at the age of 55. However, he achieved a second CR after being treated in December 2006 with salvage therapy and...
of anti-nuclear antibodies (ANA), the anti-ds DNA antibody, complement, the presence of a skin rash, and the presence and thrombocytopenia. Based on her low serum levels of hospital after her family doctor found evidence of anemia general fatigue, and dermatitis. She was admitted to our receiving PE therapy 6 times.

Staphylococcus aureus (MRSA) on hospital day 76 after re-

condition. He died of sepsis due to methicillin-resistant

started on hospital day 29, but did not improve his clinical

were initiated starting on hospital day 15, but the patient

Platelet count did not increase. For this reason, 4 cycles of vincristine administered at a dose of 1.0 mg/m² in 2007, but his condition soon worsened and he fell into a coma on hospital day 3. Under a clinical diagnosis of PBSCT-associated TTP, he received PE therapy with high-dose mPSL therapy starting on hospital day 7. On that day, the laboratory findings revealed that a slightly decreased plasma level of ADAMTS13 activity (39%) and negativity for an ADAMTS13 inhibitor were present on admission. The exacerbation of the neurological symptoms and thrombocytopenia were observed while tapering off the administration of PE and PSL. Thus, cyclophosphamide (500 mg/m²) was administered starting on hospital day 15 which resulted in a transient rise in her platelet count. A partial remission (PR) was achieved on hospital day 44 after 14 rounds of PE therapy. However, the patient died of an uncontrolled progression of aspergillus pneumonia on hospital day 102 despite the administration of liposomal amphotericin B. During the autopsy, platelet thrombi stained with the anti-VWF polyclonal antibody were detected in her kidney, heart, brain, and intestine (Figure). Since platelet thrombi in the microvascular-structures that are rich in VWF have been hallmarks of ADAMTS13 activity deficient TTP (17), it was interesting

high-dose chemotherapy followed by an autologous periph-

eral blood stem cell transplantation (PBSCT). Because of his clinical background, he was initially treated with oral PSL (1 mg/kg) in 2007, but his condition soon worsened and he fell into a coma on hospital day 3. Under a clinical diagnosis of PBSCT-associated TTP, he received PE therapy with high-dose mPSL therapy starting on hospital day 7. On that day, the laboratory findings revealed that a slightly decreased plasma level of ADAMTS13 activity (39%) and negativity for an ADAMTS13 inhibitor were present on admission. Despite treatments with PE and mPSL therapy, his platelet count did not increase. For this reason, 4 cycles of vincristine administered at a dose of 1.0 mg/m² per week were initiated starting on hospital day 29, but did not improve his clinical condition. He died of sepsis due to methicillin-resistant Staphylococcus aureus (MRSA) on hospital day 76 after receiving PE therapy 6 times.

Case 5: In 2007, a 50-year-old woman developed a fever, general fatigue, and dermatitis. She was admitted to our hospital after her family doctor found evidence of anemia and thrombocytopenia. Based on her low serum levels of complement, the presence of a skin rash, and the presence of anti-nuclear antibodies (ANA), the anti-ds DNA antibody, the anti-Sm antibody, and the anti-Scl 70 antibody, she was diagnosed with OS (SLE and SSc) and was treated with oral PSL (1 mg/kg). However, she developed the neurological symptoms of coma and tonic-clonic convulsions on hospital day 3. Taken together, these findings indicated a diagnosis of OS-associated TTP. PE therapy was initiated with high-dose mPSL therapy in parallel with continuous hemodialysis under sedation and with intubation from hospital day 3. The patient was extubated on hospital day 10. On that same day, laboratory findings revealed that a slightly decreased plasma level of ADAMTS13 activity (49%) and negativity for an ADAMTS13 inhibitor were present on admission. The exacerbation of the neurological symptoms and thrombocytopenia were observed while tapering off the administration of PE and PSL. Thus, cyclophosphamide (500 mg/m²) was administered starting on hospital day 15 which resulted in a transient rise in her platelet count. A partial remission (PR) was achieved on hospital day 44 after 14 rounds of PE therapy. However, the patient died of an uncontrolled progression of aspergillus pneumonia on hospital day 102 despite the administration of liposomal amphotericin B. During the autopsy, platelet thrombi stained with the anti-VWF polyclonal antibody were detected in her kidney, heart, brain, and intestine (Figure). Since platelet thrombi in the microvascular-structures that are rich in VWF have been hallmarks of ADAMTS13 activity deficient TTP (17), it was interesting

Figure. A post-mortem histological examination of a renal specimen from a patient with non-AD-

AMTS13 activity deficient TTP (See Case 5). Both Figures A and B show Hematoxylin and Eosin staining, and the arrows indicate platelet thrombi. Figure C is the immuno-staining with the anti-fi-
brininogen polyclonal antibody (DAKO, Glostrup, Denmark), and Figure D is the immune-staining with the anti-von Willebrand factor (VWF) polyclonal antibody (DAKO, Glostrup, Denmark). Note that the VWF-rich platelet thrombi in this patient show a sharp contrast to those in patients with disseminated intravascular coagulation (DIC) (17).

The high-dose chemotherapy followed by an autologous peripheral blood stem cell transplantation (PBSCT). Because of his clinical background, he was initially treated with oral PSL (1 mg/kg) in 2007, but his condition soon worsened and he fell into a coma on hospital day 3. Under a clinical diagnosis of PBSCT-associated TTP, he received PE therapy with high-dose mPSL therapy starting on hospital day 7. On that day, the laboratory findings revealed that a slightly decreased plasma level of ADAMTS13 activity (39%) and negativity for an ADAMTS13 inhibitor were present on admission. Despite treatments with PE and mPSL therapy, his platelet count did not increase. For this reason, 4 cycles of vincristine administered at a dose of 1.0 mg/m² per week were initiated starting on hospital day 29, but did not improve his clinical condition. He died of sepsis due to methicillin-resistant Staphylococcus aureus (MRSA) on hospital day 76 after receiving PE therapy 6 times.

Case 5: In 2007, a 50-year-old woman developed a fever, general fatigue, and dermatitis. She was admitted to our hospital after her family doctor found evidence of anemia and thrombocytopenia. Based on her low serum levels of complement, the presence of a skin rash, and the presence of anti-nuclear antibodies (ANA), the anti-ds DNA antibody, the anti-Sm antibody, and the anti-Scl 70 antibody, she was diagnosed with OS (SLE and SSc) and was treated with oral PSL (1 mg/kg). However, she developed the neurological symptoms of coma and tonic-clonic convulsions on hospital day 3. Taken together, these findings indicated a diagnosis of OS-associated TTP. PE therapy was initiated with high-dose mPSL therapy in parallel with continuous hemodialysis under sedation and with intubation from hospital day 3. The patient was extubated on hospital day 10. On that same day, laboratory findings revealed that a slightly decreased plasma level of ADAMTS13 activity (49%) and negativity for an ADAMTS13 inhibitor were present on admission. The exacerbation of the neurological symptoms and thrombocytopenia were observed while tapering off the administration of PE and PSL. Thus, cyclophosphamide (500 mg/m²) was administered starting on hospital day 15 which resulted in a transient rise in her platelet count. A partial remission (PR) was achieved on hospital day 44 after 14 rounds of PE therapy. However, the patient died of an uncontrolled progression of aspergillus pneumonia on hospital day 102 despite the administration of liposomal amphotericin B. During the autopsy, platelet thrombi stained with the anti-VWF polyclonal antibody were detected in her kidney, heart, brain, and intestine (Figure). Since platelet thrombi in the microvascular-structures that are rich in VWF have been hallmarks of ADAMTS13 activity deficient TTP (17), it was interesting
to note that the same pathological change was observed in this instance of non-ADAMTS13 activity deficient TTP. This finding indicates that both the severe deficiency of ADAMTS13 activity and a moderate deficiency with an extremely low ratio of ADAMTS13:Unusually Large VWF Multimers (UL-VWFM) lead to the same pathological results.

Case 6: In 2008, a 17-year-old female patient developed a fever, general fatigue, headache, vomiting, and arthralgia. She was admitted to our hospital after her family doctor found evidence of anemia and thrombocytopenia. She also had Raynaud’s phenomenon, low serum levels of complement, and was positive for ANA, the anti-ds DNA antibody, and the anti-Sm antibody. These findings indicated a diagnosis of SLE and she was treated with PSL (1 mg/kg). She developed the neurological symptom of tonic-clonic convulsions on hospital day 3. By this point, she had been diagnosed with SLE-associated TTP, and PE therapy with high-dose mPSL pulse therapy under sedation and with intubation was instituted starting on hospital day 3. The patient was extubated on hospital day 5 after her platelet count increased (70x10^9/L). On hospital day 7, the laboratory findings revealed that a low plasma level of ADAMTS13 activity (<0.5%) and positivity for an ADAMTS13 inhibitor (1.4 BU/mL) were present on admission, confirming a diagnosis of SLE-associated acquired TTP with a severe deficiency of ADAMTS13 activity. Her neurological symptoms worsened on hospital day 12 despite intensive PE and mPSL therapy. As a result, an off-label treatment with rituximab was initiated starting on hospital day 13 which remarkably improved the abnormal clinical and laboratory findings. A CR was achieved on hospital day 45 after 6 rounds of PE therapy. To date, the patient maintains DFS with an oral intake of 5 mg PSL/day.

Case 7: In 2008, a 71-year-old woman developed a fever and chest pain and soon fell into a coma prior to being transferred to our hospital. The findings of abnormal electrocardiogram, elevated cardiac enzymes, and the asynnergy of the antero-septal wall by ultrasound cardiomogram confirmed a cardiac failure due to acute myocardial infarction (AMI). Due to her cardiac condition, PE therapy with a reduced amount of FFP (30 mL/kg) was initiated with high-dose mPSL therapy under sedation and with intubation starting on hospital day 1 in parallel with continuous hemodialysis. The laboratory findings on hospital day 9 revealed that a low plasma level of ADAMTS13 activity (<0.5%) and positivity for an ADAMTS13 inhibitor (1.1 BU/mL) were present on admission, thus confirming a diagnosis of ai-TTP with a severe deficiency of ADAMTS13 activity. The neurological symptoms and pulmonary hemorrhage transiently improved the clinical signs and a PR was achieved on hospital day 42 after 8 rounds of PE therapy. However, the progression of a nosocomial aspergillus pneumonia could not be controlled despite the use of amphotericin B, and the patient died on hospital day 56.

Case 8: In 2008, a 68-year-old woman with dermatitis and chronic heart failure was diagnosed with anemia and thrombocytopenia by her family doctor and admitted to our hospital. Due to the presence of a skin rash, a positive ANA result, and the presence of the anti-Scl 70 antibody, she was diagnosed with SSc and was treated with PSL (1 mg/kg). During the treatment period, she went into a coma and experienced a pulmonary hemorrhage on hospital day 7. She was subsequently diagnosed with SSc-associated TTP. PE therapy at a reduced volume of FFP (30 mL/kg) with high-dose mPSL therapy was initiated in parallel with continuous hemodialysis with intubation starting on hospital day 7. The patient was extubated on hospital day 13. On that same day, the laboratory findings revealed that a slightly decreased plasma level of ADAMTS13 activity (47%) and negativity for an ADAMTS13 inhibitor were present on admission. The neurological symptoms and pulmonary hemorrhage were exacerbated when the PE and PSL administration were tapered off. As a result, we added cyclophosphamide (500 mg/m^2) pulse therapy on hospital day 14, but the patient failed to improve. We subsequently initiated off-label therapy with rituximab starting on hospital day 21. This treatment transiently improved the clinical signs and a PR was achieved on hospital day 42 after 8 rounds of PE therapy. However, the progression of a nosocomial aspergillus pneumonia could not be controlled despite the use of amphotericin B, and the patient died on hospital day 56.

Case 9: In 2008, a 56-year-old woman visited a local physician who identified dermatitis of Gottron’s sign, arthritis, and the presence of ANA and the anti-SCL70 antibody. She was diagnosed with SSc and treated with PSL (1 mg/kg). Soon after, she developed anemia and thrombocytopenia and fell into a coma with cerebral hemorrhage. Simultaneously, she had lung lesions indicative of interstitial pneumonia with pulmonary hemorrhage. Due to these serious clinical conditions, she received a platelet transfusion with a dose of 10 units (2x10^11 platelets) after which her clinical signs worsened and necessitated her transfer to our hospital. Clinical and laboratory findings upon admission indicated a clinical diagnosis of SSc-related TTP. The patient immediately received PE therapy for 2 consecutive days concurrently with high-dose mPSL pulse therapy. However, she died from the progression of a cerebral hemorrhage on hospital day 3 without appreciable clinical improvements. After her death, laboratory findings revealed that a normal plasma level of ADAMTS13 activity (69%) and negativity for an ADAMTS13 inhibitor were present on admission.

Case 10: In 2009, a 59-year-old man developed a fever, diarrhea, and gastrointestinal hemorrhage. The patient subsequently fell into a coma with left hemiplegia and was admitted to our hospital. PE therapy was initiated with high-dose mPSL pulse therapy after sedation and with intubation on hospital day 1. The patient was extubated on hospital day 10 because his clinical signs had improved. On hospital day 10, laboratory findings revealed that a deficient plasma ADAMTS13 activity (<0.5%) with positivity for an
ADAMTS13 inhibitor (2.0 BU/mL) were present upon admission, thus confirming a diagnosis of ai-TTP with severe deficiency of ADAMTS13 activity. However, exacerbation of the thrombocytopenia was observed when PE and PSL administrations were tapered off. Thus, cyclosporine (5 mg/kg) was administered starting on hospital day 20 which resulted in an increased platelet count. A CR was achieved on hospital day 35 after 13 rounds of PE therapy. To date, the patient maintains DFS with an oral intake of PSL 5 mg/day.

Case 11: In 2009, a 51-year-old woman developed a fever and sore throat and subsequently fell into a coma prior to her transfer to our hospital. In addition, she had a skin rash, low serum levels of complement, and was positive for ANA, the anti-ds DNA antibody, and the anti-Sm antibody. On the basis of these additional findings, she was diagnosed with SLE-associated TTP. PE therapy was initiated immediately with high-dose mPSL therapy under sedation and with intubation starting on hospital day 1. These treatments rapidly improved her laboratory and clinical findings, and she was extubated on hospital day 7. On hospital day 9, the laboratory findings revealed a low plasma level of ADAMTS13 activity (<0.5%) and positivity for an ADAMTS13 inhibitor (2.2 BU/mL), confirming the diagnosis of SLE-associated TTP with a severe ADAMTS13 deficiency. The neurological symptoms and thrombocytopenia were exacerbated when the PE and PSL administrations were tapered off. Thus, cyclophosphamide (500 mg/m²) pulse therapy was added starting on hospital day 20, which gradually improved thrombocytopenia. A CR was achieved on hospital day 32 after 14 rounds of PE therapy. The patient maintains DFS with an oral intake of 5 mg PSL/day.

Case series summary: Of our 11 patients, 7 had ADAMTS13 activity-deficient TTP and 4 had non-ADAMTS13 activity-deficient TTP. Although idiopathic TTP develops with a fever in the absence of any known etiology, TTP can also be associated with various underlying diseases. Of the 11 patients included in our study, 5 were idiopathic and 6 had cases that were associated with underlying causes such as non-Hodgkin lymphoma or connective tissue diseases. Regarding the clinical pentad of TTP, all 11 of the patients had a fever, hemolytic anemia, thrombocytopenia, and neurological signs upon admission, but 5 lacked renal dysfunction (Table 1). Although the platelet counts and serum LDH levels were highly variable between patients, their serum haptoglobin levels were uniformly very low (<10 mg/dL). Of these 11 patients, 7 had ADAMTS13 activity deficient TTP with low-titer ADAMTS13 inhibitors (1.1-2.2 BU/mL), whereas the remaining 4 had a slightly reduced or almost normal ADAMTS13 activity levels without detectable inhibitors.

Of the 11 patients treated for TTP, 6 are alive with a mean survival time of 1,014.6±1,101.0 days (mean ± SD). The survival rates of ADAMTS13 activity-deficient TTP patients and non-ADAMTS13 activity-deficient TTP patients were 85.7% and 0%, respectively, and the respective mean survival times were 1,816.0±853.3 days and 53.0±38.9 days.

The causes of death identified during this study included cardiogenic shock due to heart failure (patient 5), aspergillus pneumonia (patients 8 and 10), MRSA sepsis (patient 9) and cerebral hemorrhage (patient 11). Patients with idiopathic, ADAMTS13 activity-deficient TTP did not develop any other diseases such as collagen diseases during the follow-up periods.

Discussion

The detailed clinical and laboratory findings were well preserved in this study because all 11 patients with TTP were treated in a single institution at Miyazaki during the past 12 years. Of these 11 patients, 5 had idiopathic TTP and 6 had cases of TTP that were associated with underlying issues such as non-Hodgkin lymphoma or connective tissue diseases. Regarding the clinical pentad of TTP, all 11 of the patients had a fever, hemolytic anemia, thrombocytopenia, and neurological signs upon admission, but 5 lacked renal dysfunction (Table 1). Although the platelet counts and serum LDH levels were highly variable between patients, their serum haptoglobin levels were uniformly very low (<10 mg/dL). Of these 11 patients, 7 had ADAMTS13 activity deficient TTP with low-titer ADAMTS13 inhibitors (1.1-2.2 BU/mL), whereas the remaining 4 had a slightly reduced or almost normal ADAMTS13 activity without any detectable inhibitors. The non-ADAMTS13 activity deficient TTP patients tended to display high LDH values and low bilirubin values. Notably, the plasma levels of fibrin degradation products (FDP)-P and D-dimer were slightly elevated in 3 out of the 4 non-ADAMTS13 activity-deficient TTP patients. These findings suggested that non-ADAMTS13 activity deficient TTP patients who suffer from an underlying disease are prone to suffer from a fibrinogen consumption commonly known as disseminated intravascular coagulation (DIC). Habe et al. (18) recently reported that ADAMTS13/VWF profiles may have important roles in the pathogenesis of DIC, and that the plasma levels of both ADAMTS13 and VWF propeptide are useful indicators for the diagnosis and prognosis of DIC. In contrast, the marker for immune thrombocytopenia (ITP), platelet-associated (PA) IgG, was present in significantly increased levels in all of our TTP patients, thereby indicating that PAIgG is not useful for differentiating between ITP and TTP.

Of our 11 patients with TTP, 6 are still alive. The survival rates of ADAMTS13 activity-deficient TTP patients and non-ADAMTS13 activity-deficient TTP patients were 85.7% and 0%, respectively, and the respective mean survival times were 1,816.0±853.3 days and 53.0±38.9 days. Statistically significant differences between these two groups were observed in the incidence of renal dysfunction (p=0.02) and in
the total bilirubin counts (p=0.002).

Since 1991, PE therapy with or without an adjunctive regular or high-dose (pulse) steroid therapy has been used as the first-line treatment of TTP (6, 19-25). The discovery of ADAMTS13 allowed the efficacy of PE therapy in TTP patients to be quantifiably analyzed. Normal hemostasis can now be achieved by the replenishment of ADAMTS13 and regular-sized VWFM in addition to the removal of hazardous materials such as UL-VWFM, anti-ADAMTS13 autoantibodies, and the elevated inflammatory cytokines that up-regulate UL-VWFM release from vascular endothelia cells. All 11 of our patients were treated with the combination regimen of PE therapy with high-dose mPSL pulse therapy because ADAMTS13 data were not readily available upon admission. The patient group received PE therapy between 6 and 30 times, and none of the patients showed a rebound or increase in their inhibitor titers during their PE therapy and subsequent clinical courses. The high-dose mPSL pulse therapy administered in tandem aimed to sedate the severe, fluctuating neurological signs associated with TTP as well as to suppress the anti-ADAMTS13 antibody production in patients with ADAMTS13 activity-deficient TTP. Ito-Habe et al. (26) report that a high-dose mPSL pulse therapy (1 g/day) is now administered in the majority of Japanese institutions rather than standard PSL therapy (1 mg/kg). According to their response to the combination regimen of PE therapy and mPSL pulse therapy, some of our patients were treated with the following adjunctive therapies: high-dose IVIG, cyclosporine, vincristine, cyclophosphamide, or rituximab. A few of these adjunctive drugs now have well-documented efficacies based on pharmacokinetics. The adjunctive therapies were administered based on the underlying disease, the unrecovered clinical symptoms, the unresolved laboratory data, and the degree of organ dysfunction. We tended to administer rituximab for the refractory setting of TTP after the drug became available for off-label use in 2007. Due to our therapeutic regime, 6 out of the 7 patients with ADAMTS13 activity-deficient TTP accomplished CR with the exception from this group being the case that died from a complication of her AMI. In contrast, all 4 of the non-ADAMTS13 activity deficient TTP patients died.

While a few studies documented the efficacy of high-dose IVIG therapy in TTP patients prior to the discovery of ADAMTS13, subsequent studies could not confirm this finding (27, 28). Between 2003 and 2007, we used high-dose IVIG therapy at a dosage of 400 mg/kg IVIG for 5 consecutive days in 3 patients with ADAMTS13 activity-deficient TTP (cases 1-3) as an adjunctive therapy when the clinical aggravation of thrombocytopenia and persistent thrombocytopenia were observed during the tapering off period of the PE and PSL therapies. Interestingly, all the patients showed a good response to high-dose IVIG therapy. However, as described below, rituximab is now preferred over high-dose IVIG therapy for the treatment of TTP patients with relapsing and intractable clinical courses. If the efficacy of high-dose IVIG is proven on a scientific basis in the future, this therapy might be revived.

Among the adjunctive therapies, rituximab treatment may be most reasonable and powerful therapeutic modality for depleting the B cells that produce the autoantibodies for ADAMTS13. Several reports in the literature have clearly shown that rituximab, an anti-CD20 chimeric monoclonal antibody, is highly efficient as an adjunctive therapy for TTP patients who do not respond adequately to a standard combination therapy of PE and steroids (29-31). Globally, the on-label therapeutic use of rituximab is for CD20-positive malignant B-cell lymphoma. Because the B-lymphocyte is an IgG antibody-producing cell, a broad spectrum of autoimmune diseases and associated symptoms can be targeted by rituximab, including the ADAMTS13 deficiency in TTP patients due to the presence of autoantibodies. The most recent study indicates that rituximab administration at a dosage of 375 mg/m² weekly for 4 cycles almost completely depletes the circulating B-lymphocytes from 16 hospital days to 3 months (32). Three of our TTP patients (cases 4, 6, and 8) were treated with rituximab between 2007 and 2008. The patient with ADAMTS13 activity deficient TTP (Case 6) due to a low titer of ADAMTS13 inhibitor (1.4 BU/mL), responded well to this treatment and has maintained DFS to date. However, the two patients with non-ADAMTS13 activity deficient TTP (Cases 4 and 8) died on hospital days 76 and 56, respectively. We therefore did not find any benefit in rituximab therapy for patients with non-ADAMTS13 activity-deficient TTP. However, of our 7 patients with ADAMTS13 activity-deficient TTP due to its inhibitors (Cases 1, 2, 3, 6, 7, 10, and 11), Case 6 alone was treated with rituximab and had a favorable outcome. During her course of treatment, Case 6 received less PE therapy than the other cases (6 rounds in Case 6 versus 9 to 30 times in our other cases), suggesting that the adjunctive use of rituximab has a plasma-sparing effect that must be confirmed in future studies. Scully et al. (32) reported in a Phase 2 study that rituximab treatment with PE was a safe and effective treatment for ai-TTP patients, thus validating our observations. Kameda et al. (33) have recently reported that rituximab treatment may be effective for non-ADAMTS13 activity deficient TTP patients, but we did not confirm this to be true for our study. Their results may be explained by the reduction of excessive cytokine production through the rituximab-driven B cell depletion in non-ADAMTS13 activity deficient TTP patients, but that hypothesis needs to be carefully evaluated in future studies. We are presently unable to discuss the efficacies of the additional adjunct therapies, including including vincristine, high-dose cyclophosphamide, and cyclosporine, due to their low administration frequency to patients in this study.

In conclusion, we believe that this case-oriented study will be highly useful to the physicians who directly care for TTP patients.

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