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Clinical and Therapeutic Studies of Acquired Thrombotic Thrombocytopenic Purpura in China

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Abstract

Thrombotic thrombocytopenic purpura (TTP) is a rare and severe disorder mediated by autoantibodies against ADAMTS13. In this report, we study the clinical features, laboratory aberration, and treatment effect of 55 patients with acquired TTP in China. The classic pentad occurred in only 33% of TTP patients. Severe ADAMTS13 deficiency was detected in 85% of patients. Advanced age and hyperbilirubinemia might be risk factors for poor prognosis. Early and sufficient plasma exchange is the most important approach. The addition of rituximab to plasma exchange and corticosteroids appears to be effective in inducing and sustaining long-term remission in TTP, and is suitable to be administrated during the first episode. However, more optimal therapeutic regimen warrants further investigation to treat refractory cases and to reduce relapse rate.

Keywords: Thrombotic thrombocytopenic purpura; ADAMTS13; Clinical characteristics; Plasma exchange; Rituximab

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare severe disorder, the incidence of which is estimated to be 3.7 per million [1]. It is characterized by microangiopathic hemolytic anemia, thrombocytopenia and various clinical symptoms, especially in association with neurological and renal damages [2,3]. TTP is mainly associated with a severe deficiency of ADAMTS13 activity, while there are some patients who fulfil the diagnostic criteria for TTP do not have severe ADAMTS13 deficiency. TTP is almost always acquired, congenital TTP caused by a mutation of the ADAMTS13 gene is quite rare. In the acquired TTP, a small proportion of patients are associated with other diseases, while the remaining patients are categorized as idiopathic.

Rapid and widespread advances have been made in clinical and fundamental researches on TTP since identifying a deficiency of ADAMTS13 activity related to TTP [4,5], and confirming the efficacy of plasma exchange (PE), which increases the survival rate of patients with TTP from 10% to about 80% [6,7].

Corticosteroids and other immunosuppressive agents have also been widely used in the treatment of TTP, but there is scanty unanimous evidence documenting their efficacy. In China, most published literature is still only of case reports or small retrospective studies, mainly because measurements of ADAMTS13 activity have not yet become popular. In this report, we comprehensively analyzed the clinical characteristics, treatment strategies and outcomes of 55 patients with TTP.

Patients and Methods

Patients

Fifty-five patients (36 female and 19 male) with a median age of 41 years (range: 13-90) diagnosed between January 2007 and November 2012 were included in this retrospective study. The diagnosis of TTP was established according to Guidelines on the diagnosis and management of the thrombotic microangiopathic haemolytic anaemias by British Society for Haematology [3]. Informed consent to perform the studies was obtained from each patient, and ethical approval was obtained from the ethics committee of our institute according to the Declaration of Helsinki.

The clinical and laboratory features of these patients were variable. Bleeding due to thrombocytopenia included mucocutaneous purpura, epistaxis, gum bleeding, hematuria, melena, and menorrhagia. The manifestations of hemolytic anemia included icterus, hyperbilirubinemia and raised lactate dehydrogenase (LDH) level. Neurological manifestations were headache, altered mental state, confusion, paresthesia, epilepsy, stroke and coma. Renal impairment was revealed as proteinuria, hematuria, raised levels of serum creatinine and urea nitrogen, and renal failure.

Laboratory examinations

Laboratory indexes were regularly observed such as platelet count, LDH level, and striking red cell fragmentation. Platelet recovery time was defined as time from treatment with plasma exchange and corticosteroids to a normal platelet count (>100 × 10^9/L) for at least 3 consecutive days. LDH recovery time was defined as time from effective treatment to a normal LDH level (100-225 U/L) for at least 3 consecutive days. The recovery time of red cell fragmentation was defined when fragmented red cells could not be detected in blood film.

Measurement of plasma ADAMTS13 activity

Peripheral blood samples of both TTP patients before the initiation of first plasma exchange and healthy donors were collected with one-tenth volume of 3.2% trisodium citrate. The samples were centrifuged at g for 10 min to prepare platelet-poor plasma, which was then kept at -30 until analysis. Plasma ADAMTS13 activity was measured by residual-collagen binding assay according to the method of Rick [8]. Less than 5% of ADAMTS13 activity was considered as severe deficiency, and the presence of ADAMTS13 inhibitor was assessed in all samples with its severe deficiency.

B lymphocytes detection using flow cytometry

The flow cytometry measurement of B lymphocytes was performed using EDTA-anticoagulant peripheral blood of the patients treated with retuximab. The white blood cells were adjusted to (0.5-1) × 10^9 cells/ml. Samples of 100 µL were incubated for 15 min in the dark with saturating amounts of fluorescent MoAbs anti-CD3, CD19, CD20 and CD45 (Immunotech, France), and then washed with hemolytic agent to lysis red blood cells. Cells were analyzed by a EpicsXL flow cytometry (Beckman Coulter, USA). Total events of 10000 were gated based on forward (FSC) and side-scatter (SSC) characteristics and dot plots for B lymphocytes were gated.

Response criteria

Response criteria to treatment was defined according to that described by Zhan [9] and Gurkan [10]: 1. complete response: a platelet count >100 × 10^9/L and no new clinical events; 2. partial response: a platelet count ≥50 × 10^9/L or more than doubled the baseline count, and no new clinical events; 3. no response: a platelet count <20 × 10^9/L or less than doubled the baseline count, and deterioration of the patient's clinical status; and 4. relapse: recurrence of clinical manifestations and laboratory abnormalities after a complete remission for 30 days or longer.

Statistical analysis

Data were expressed as the median and the range. Comparisons between response group and death group were made using Student's t-test. A two-tailed p-value <0.05 was considered statistically significant. Relevant statistical analyses were performed by GraphPad Prism 5 software.

Results

Clinical features

Among the 55 patients in our cohort with TTP, 10 (18%) had autoimmune disorders, 6 (11%) had infection, and pregnancy was associated with 4 patients (7%). The remaining 35 patients (64%) had no obvious underlying causes and were considered to have idiopathic TTP.

Symptoms of the patients with TTP came on abruptly and progressed quickly. The clinical features at the onset included fever, fatigue, thrombocytopenia, hemolytic anemia, and neurological abnormalities, which could occur either simultaneously or subsequently, and exacerbate until initiation of plasma exchange. Clinical variants appeared upon admission. All patients had thrombocytopenia with 74% showing bleeding phenomena, 51(91) of patients had microangiopathic hemolytic anemia, 45 (82%) had neurologic abnormalities, 37 (72%) had fever, and 36 (65%) had renal abnormalities. Among our cohort, 18 patients (33%) exhibited the typical pentad of clinical features (Table 1).

Clinical manifestations	n (%)
Bleeding	41 (74%)
skin ecchymosis	24 (47%)
hematuria	5 (8%)
epistaxis	5 (8%)
GI bleeding	3 (6%)
Menorrhagia	2 (4%)
Gum bleeding	1 (2%)
Microangiopathic hemolytic anemia	51 (91%)
Neurological manifestations	45 (82%)
Confusion, coma	28 (51%)
Altered mental state	10 (18%)
Headache, vomiting	6 (10%)
Delirium, convulsion	5 (8%)
Paresthesia	5 (8%)
Delusion	1 (2%)
Fever	37 (67%)
Renal impairment	36 (65%)
GI: Gastrointestinal	

Table 1: Clinical manifestations of TTP patients.

Laboratory findings

At presentation, the median platelet count was 11 × 10^9/L (range, 5 × 10^9/L-72 × 10^9/L), the median hemoglobin level was 71 g/L (range, 50 g/L-156 g/L) with 77% of patients having moderate to severe anemia. Red cell fragmentation was detected in 86% of patients with median percentage of 5.0% (range, 1-15%).

The median total bilirubin level of plasma was elevated to 49.9 µmol/L (range, 7.7 µmol/L to 153.1 µmol/L), and the median LDH concentration was 1166.4 U/L (range, 109 U/L-2953 U/L), with 29 patients (53%) having LDH >1000 U/L. median serum creatinine concentration of 40 patients with renal impairement or renal failure was 167.4 µmol/L (range, 34.8 µmol/L-735.0 µmol/L), and median urea nitrogen concentration was 13.1 mmol/L (range, 4.1 mmol/L-49.1 mmol/L). The results of coagulation tests were generally normal, only three patients showed mild prolongation of activated partial thromboplastin time or thrombin time.

Forty-one patients had ADAMTSl3 activity measured on their admission. In 35 (85%) patients, ADAMTS13 activity was confirmed to be <5%, of whom 25 (61%) were antibody-positive; the remaining 6 patients (15%) showed normal ADAMTS13 activity including one who had received PE.

Treatment

Three of 55 patients died during the first acute episode before sufficient PE therapy. One patient died who was treated only with corticosteroid. Other 51 received plasma exchange with or without corticosteroid combined with such immunosuppressive agents as vincristine, cyclophosphamide, and cyclosporine. Among them, 9 patients were also treated with additional rituximab (375 mg/m^2, once weekly for 4 weeks) (Table 2).

In most of 40 survivors (73%), platelet count was increased after second or third daily PE and then reached normal range (>100×10^9/L), followed by decrease of the high LDH level. In our cohort, 15 patients (27%) died during the disease course. The death rate in patients receiving only PE was 55%, that in patients treated with plasma exchange in combination with corticosteroids and immunosuppressants was 18%. Nine patients treated with additional rituximab are all surviving (Table 2). In analyzing prognostic risk factors, we found that the mean age of the death group was older than the remission group, and total bilirubin level was higher in the death group, while other characteristics on admission in the two groups were similar such as body temperature, platelet count, white cell count, hemoglobin level, creatinine and LDH (Table 3).

Therapeutic regimens	n	remission (%)	death (%)
No treatment	1	0	1 (100%)
Steroids	3	0	3 (100%)
PE	9	4 (45%)	5 (55%)
PE+steroids+IP	33	27 (82%)	6 (18%)
PE+steroids+IP+rituximab	9	9 (100%)	0 (0%)
PE: Plasma Exchange; IP: Immunosuppressive Agents (vincristine, cyclophosphamide or cyclosporine)			

Table 2: Effect of different therapeutic regimens in TTP patients.

Clinical and lab index	Remission (n=40)	Death (n=15)	P value
Age (y)	37.5 ±14.5	50.1 ± 18.9	0.008
Temperature (°C)	38.0 ±1.0	37.9 ± 0.8	0.957
WBC (×10^9/L)	11.2 ± 6.4	13.3 ± 9.7	0.485
Hemoglobin (g/L)	71.4 ± 15.4	85.7 ± 38.2	0.086
Platelet (×10^9/L)	15.8 ± 13.9	14.4 ± 10.32	0.630
Total bilirubin (μ mol/L)	43.3 ± 23.5	63.7 ± 37.7	0.036
Creatinine (μ mol/L)	145.8 ±124.9	152.8 ±131.5	0.999
Urea nitrogen (μ mol/L	11.3 ± 6.2	13.8 ±10.9	0.444
LDH (U/L)	1091.9 ± 585.0	1328.9 ± 659.3	0.098
LDH = lactate dehydrogenase			

Table 3: Influence of clinical and laboratory indexes on prognosis.

Alteration of B lymphocyte percentage during rituximab treatment

The B lymphocyte percentage in the peripheral blood before treatment was 18.39 ± 7.15%, which was decreased to 2.19 ± 5.11% , 0.53 ± 1.16% and 0.14 ± 0.40% after the first, second and fourth administration of rituximab, respectively (P values <0.001).

Discussion

The diagnosis of TTP is mainly based on the presence of thrombocytopenia and microangiopathic hemolytic anemia, which are caused by pathogenetic formation of platelet microvascular thrombi [3]. Although the classic pentad of TTP diagnosis has been recognized since it was first described by Moschowitz in 1924, recent data confirmed that most patients present the triad of TTP, including hemolytic anemia, thrombocytopenia and neurologic abnormalities, while renal impairment and fever occur only in small proportion of TTP patients [2,11]. In this study, we found that only 35% of patients had the classical pentad of TTP. All these results emphasize that the "classic pentad" of clinical features is not relevant to current practice.

The pathogenic mechanism of TTP has been associated with ADAMTS13 deficiency [12,13], ADAMTS13 deficiency has a high sensitivity and specificity for TTP diagnosis. However, there have been some patients in almost all reports who had normal ADAMTS13 activity. In our cohort, 17% patients fulfilled diagnostic criteria for TTP, but did not have severe ADAMTS13 deficiency. Documentation of a severe deficiency of plasma ADAMTS13 activity is important, but not essential for the diagnosis of TTP. Therefore, its diagnosis should be based on the presence of clinical symptoms, laboratory aberrations consistent with MAHA (MicroAngiopathic Hemolytic Anemia), decreased ADAMTS13 activity, and possibly presence of anti-ADAMTS13 autoantibodies [14].

PE has been the mainstay of treatment of acute TTP and decreased the mortality rates from 90% to 10-20%. Meanwhile corticosteroids have widely been used as an adjunctive treatment combined with plasma exchange. On the other hand, other immunosuppressive agents are often used in the treatment of refractory or recurrent TTP [3]. In this study, the mortality rate in patients who were solely treated with plasma exchange was as high as 55%, certainly because PE in China is still logistically difficult that leads limited plasma supplement and an unavoidable delay. However, we found that, in this case, PE in combination with corticosteroid and immunosuppressive agents could significantly increase the remission rate to 81%. Other 9 patients treated with rituximab in addition to PE, corticosteroid and immunosuppressive agents were all in remission, and no relapses occurred during the follow-up period of 5-61 months. Rituximab binds to the CD20 antigen on B lymphocytes and results in Fcγ-mediated B-cell lysis. In this study, rituximab significantly eradicated B lymphocytes which are involved in the production of the ADAMTS13 auto-antibody. Our results suggest that a role for the early administration of immunosuppressants, especially rituximab, would be advocated. Recent data have also demonstrated the benefit of the addition of rituximab to standard therapy in sustaining long-term remission in TTP [15,16].

There have been several studies about prognostic factors of TTP. Patients with higher titers of anti-ADAMTS13 auto-antibodies and severe thrombocytopenia might be at greater risk for complications and death [17-19]. Dervenoulas et al. [20] reported advanced age and severe renal impairment as the only parameters associated with

treatment failure and poor outcome. In this study, we found that beside advanced age, hyperbilirubilemia is also a prognostic risk factor. This finding needs to be further investigated by randomized prospective clinical trials conducted with larger series of patients.

Although approximately 80% of patients can survive in the era of plasma exchange, the survival rate has not changed since then, and many patients have refractory TTP. In addition, risk for relapse is as high as 35% after remission [2]. More effective as well as safer treatment such as recombinant ADAMTS13 [14] and bortezomib [21] would improve the prognosis of TTP.

Our study has some limitations. First, due to its retrospective nature, the follow-up is not adequate to evaluate a long-term outcome such as disease relapse. Second, the method used for measuring ADAMTS13 was residual-collagen binding assay, which is less sensitive. A new and highly sensitive enzyme immunoassay of ADAMTS13 activity has just been established in our institute for further prospective investigation.

Declaration of Conflicting Interest

The authors declare that there are no conflicts of interest.

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