

Efficiency of Intensive Immune -Suppression Treatment with Rituximab in Acquired Thrombotic Thrombocytopenic Purpura: A Chinese Case study

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Abstract

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disorder characterized by thrombocytopenia, microangiopathic hemolytic anemia (MAHA) and functional failure of the organs due to ADAMTS13 deficiency. ADAMTS13 inhibitor is the main contributor of pathogenesis in acquired TTP. With the help of retrospective analysis the researchers tried to evaluate the effectiveness of rituximab in preventing the acquired TTP in China. Out of 27 patients with acquired TTP that have registered from 2006 to 2015, eleven cases started rituximab infusion before remission, while sixteen others began rituximab infusion after remission. Twenty-three cases got rituximab with standard doses (375 mg/m², weekly for 4 weeks) and four patients were administered reduced doses (100mg, weekly for 4 weeks) after remission. Till date, there was no patient reported with the acquired TTP relapsed during the follow-up sessions, whenever rituximab treatment was initiated (before or after remission), or what dose of rituximab was administrated (standard dose or reduced dose). There were mild controllable side-effects reported in the case of four patients. The study concludes that rituximab can act remarkably in relieving acquired TTP, while preventing the relapse.

Keywords: Thrombotic thrombocytopenic purpura (TTP); ADAMTS13; Inhibitor; Rituximab

Introduction

Thrombotic Thrombocytopenic Purpura (TTP) is a rare and life-threatening thrombotic microangiopathy characterized by a pentad of a consumptive thrombocytopenia, Microangiopathic Hemolytic Anemia (MAHA) and organ dysfunction most commonly affecting the central nervous system, renal and other abdominal organs [1]. It is well known that the pathogenesis of TTP is due to deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) which is related to gene mutations (congenital) or anti-ADAMTS13 autoantibodies (acquired) [2]. If treated irrelevantly, TTP typically runs to neurological deterioration, cardiopulmonary dysfunction, including death [3]. Therapeutic plasma exchange (PEX) and immunosuppression with corticosteroids has been considered as the standard treatment for acute acquired TTP (aTTP) for decades. Approximately 90% of patients with TTP respond effectively [4]. However, there remains a high rate of 10-20% mortality during the acute phase with a 20-50% of relapse rate among TTP patients [5]. Reducing the TTP relapse rate is a major clinical practice. Starting from 2002, rituximab, an anti-CD20 mouse-human chimeric monoclonal antibody, has been used in refractory or relapsing acquired TTP patients, which lies in its ability to destroy autoreactive B cells responsible for producing the ADAMTS13 antibodies. Indeed, used in remission phase, rituximab

has been found effective in reducing relapse rates [6,7]. Recently it has been reported that when rituximab was used in acute phase of TTP in combination with PEX, it could produce better outcome including lower relapse rates with longer time to relapse [8]. Up till now, no study on how and when to start this B cell depletion therapy. The present retrospective study at the registration center reviews the clinical characteristics of TTP and analyzes the efficiency and safety of rituximab in preventing TTP relapse.

Material and Methods

Patients

Twenty-seven patients with acquired TTP have registered at the clinical centre from January 2006 to January 2015. The diagnosis criteria of TTP were according to the guideline [9]. The initial diagnosis of TTP was based on the presence of Microangiopathic Haemolytic Anemia (MAHA) and acute thrombocytopenia, with or without remarkably increased Lactate Dehydrogenase (LDH). Presence of schistocytes, undulatory neurological signs ranging from headache to coma, renal functional damage and fever were also taken into account [10]. TTP was finally identified using ADAMTS13 activity deficiency and presence of ADAMTS13 inhibitor in plasma before PEX. Pregnancy and HIV infection were ruled out through serum tests for the patients.

Once the TTP was diagnosed, plasma exchange treatment (PEX) would be initiated for the affected patients to replace it with a fresh frozen plasma at a dosage of 40-60 ml/kg daily along with prednisone 1 mg/kg/day until the platelet counts reach $150 \times 10^9/L$ for 2 consecutive days [11,12]. It has been confirmed that PEX treatment can remove the autoantibodies against ADAMTS13, circulating ultralarge VWF (ULVWF) and supply with ADAMTS13 enzyme. Out of 27 patients with TTP, eleven patients were simultaneously administered with rituximab at a dose of 375 mg/m² weekly for 4 times, before platelet recovering to $150 \times 10^9/L$. The remaining TTP patients were administered with rituximab infusion at the same regimen for 12 patients. A dose of 100 mg weekly for 4 times have been given for 4 patients after platelet count recovery to $150 \times 10^9/L$ to eradicate ADAMTS13 inhibitor to prevent TTP relapse. PEX was withheld for at least 4 hours after the rituximab infusion [13].

Follow-up

Clinical manifestations and signs were recorded regularly. The routine blood test, ADAMTS13 activity and ADAMTS13 inhibitor of the TTP patients were reviewed monthly and the peripheral blood CD19 and CD20 cell counts were also measured once in every 3 months lasting for a period of more than a year.

Clinical definition

Complete response to the treatment is defined as a platelet count of $>150 \times 10^9/L$ for 2 consecutive days and without neurological symptom and renal failure. Refractory is defined as failure to acquire a well-pleasing response with initial treatment of PEX and corticosteroids [14]. Relapse is defined as recurrence of thrombocytopenia ($>150 \times 10^9/L$ for 2 consecutive days) with or without neurological manifestations and renal failure 30 days after recovery from an acute episode [15].

Measurement of ADAMTS13 activity and ADAMTS13 inhibitor

Measurement of ADAMTS13 activity and its inhibitor were mainly based on residual collagen binding assay (R-CBA). Blood samples from the TTP patients before plasma exchange were collected with

3.8% sodium citrate as anticoagulant (at 1:9 ratio). After centrifugation at 2500 r/min for 5 minutes, the plasma was collected for ADAMTS13 activity test and the rest of it was kept in the -30°C for further tests. Plasma sample to be tested was mixed with pooled healthy plasma at the rate of 1:1 and hatched for 2 hours in 37°C to detect ADAMTS13 inhibitor. After having dialyzed in 1.5 mol/L urea solutions overnight, the plasma and mixed plasma were added to ELISA plate wells coated with type III human collagen. HRP-conjugated rabbit-anti-human VWF antibodies (DAKO company, 1:4000 dilution) were used to bind the residual VWF adhered to collagen. ADAMTS13 activity deficiency was defined as ADAMTS13 activity <5% in plasma. ADAMTS13 activity deficiency found in mixed plasma meant the ADAMTS13 inhibitor positive. The ADAMTS13 activity and its inhibitor of TTP patients were measured once a week during the first month. The same was repeated once in 2-3 months after the remission.

Results

27 TTP patients, consisting 8 male (29.6%) and 19 female (70.4%) have enrolled with a median age of 38 years old, ranging from 22 to 70 years. Among these patients, there were four patients having previous episodes of TTP, the remaining being newly diagnosed.

Clinical characteristics

The demographics and clinical characteristics were shown in Table 1. 11.1% of patients had typical pentalogy of TTP, whereas manifestation of Microangiopathic Hemolytic Anemia (MAHA) was found on the entire (100%) population that took part in the study. Thrombocytopenia was also noticed universally on all. While 88.9% of them reported having neurological abnormalities, 66.7% of them complained of fever. Renal abnormalities were noticed among 18.5% of the TTP patients. There were fifteen cases got head imaging tests (CT or MRI) due to neurological abnormalities. Only six cases (40%) presenting positive results, including infarction, encephalomalacia and ventricular system decreases. ADAMTS13 deficiency and ADAMTS13 inhibitor was present among the entire population that participated in the study. Among this group patients, antinuclear antibodies (ANA) were detected in thirteen cases. Three patients had pulmonary infection and one patient was carrier of HBV infection.

SL.No	Sex	Age	CNS symptoms	Temp (°C)	Renal damage	HB (g/L)	Plt ($\times 10^9/L$)	schistocytes (%)	LDH	ADAMTS13 %	ADAMTS13 inhibitor	Previous episodes (N)	TTP	ANA	hospital stay (days)
1	F	38	Yes	38.8	No	53	4	18	947	<5%	Yes	No		+	21
2	F	37	Yes	38.5	No	60	9	7	825	<5%	Yes	No		+	32
3	F	60	Yes	38	No	65	8	10	610	<5%	Yes	No		-	28
4	M	60	Yes	37.9	No	109	9	5	758	<5%	Yes	No		+	37
5	M	70	Yes	36.5	No	78	12	13	1242	<5%	Yes	No		-	39
6	F	48	Yes	36.4	No	71	8	11	1089	<5%	Yes	No		+	17
7	F	25	Yes	37.5	No	87	7	8	1672	<5%	Yes	No		+	26
8	M	45	Yes	36.8	Yes	108	4	1	2031	<5%	Yes	No		+	27
9	M	38	No	39	No	62	5	21	900	<5%	Yes	No		-	10
10	F	35	Yes	36.1	Yes	64	19	2	934	<5%	Yes	No		+	11

11	F	33	Yes	39.5	No	69	15	3	1368	<5%	Yes	No	-	33
12	F	32	Yes	39.7	No	35	2	3	2524	<5%	Yes	No	-	37
13	F	47	Yes	38.6	No	78	18	2	1146	<5%	Yes	No	-	21
14	M	70	Yes	38.3	No	95	3	6	560	<5%	Yes	Yes (3)	-	34
15	M	23	Yes	37.8	Yes	90	10	1	450	<5%	Yes	Yes (2)	+	42
16	F	47	No	37.2	No	84	37	6	1035	<5%	Yes	No	+	34
17	F	51	Yes	36.9	No	80	10	2	796	<5%	Yes	No	-	38
18	F	28	Yes	38.5	No	65	10	2	531	<5%	Yes	No	+	27
19	F	53	Yes	37.2	Yes	89	10	7	735	<5%	Yes	No	+	30
20	F	36	Yes	36.4	No	72	15	5	398	<5%	Yes	No	-	25
21	F	40	No	40	No	110	5	2	534	<5%	Yes	No	-	17
22	M	45	Yes	36.7	No	78	8	3	379	<5%	Yes	Yes(1)	-	35
23	F	22	Yes	38.6	No	43	7	2	893	<5%	Yes	No	-	28
24	F	23	Yes	37	No	104	12	6	1554	<5%	Yes	No	-	23
25	M	39	Yes	41	Yes	97	51	7	1193	<5%	Yes	No	+	39
26	F	34	Yes	36.8	No	54	6	3	621	<5%	Yes	Yes(2)	+	26
27	F	24	Yes	36.9	No	102	13	3	1737	<5%	Yes	No	-	29

CNS: Central Nervous System; HB: Hemoglobin; Plt: Platelet; LDH: Lactate Dehydrogenase; ANA: Antinuclear Antibodies.

Table1: Demographics and clinical characteristics of aTTP patients who received rituximab.

Treatment outcomes and follow-up

The outcomes of treatment and follow-up of TTP patients was depicted in Table 2. In addition to the PEX and glucocorticoids, there were twenty-three cases that had been administered with the standard doses of rituximab infusion for 4 weeks (a refractory patient received rituximab infusion 6 times). Four of them have been given doses of 100 mg rituximab weekly for 4 weeks. The median time of hospital stay was 28 days (10-42 days) and the median times of plasma

exchange was 7, ranging from 2 to 30 times. And the median time of ADAMTS13 recovery was 25 days. Out of 27 TTP patients, eleven of them got rituximab injected before remission and the remaining 16 of them got rituximab infusion after the recovery of platelets. No significant change in the time taken for the recovery of platelets (15.5 days vs.16 days) and ADAMTS13 (24 days vs. 25 days) was noticed on either of these methods in the combination of rituximab, PEX and glucocorticoids in our cohort.

	Initiated rituximab before remission	Initiated rituximab after remission
The number of patients	11	16
The median age of the patients (years)	38 (25-70)	37.5 (22-70)
The median hemoglobin (g/L)	87 (53-110)	75 (35-109)
The median platelet ($\times 10^9/L$)	7 (3-37)	11 (2-51)
The median LDH	825 (450-2031)	1011.5 (379-2524)
The median time of platelets recovery	15.5 (7-33)	16 (3-35)
The median time of ADAMTS13 recovery	24 (14-78)	25 (13-78)
The median time of hospital stay	27 (10-42)	28.5 (11-39)
Number of patients who relapsed	0	0
Adverse effects	1 (9.0%)	3 (18.8%)

Table 2: Outcomes of aTTP patients who initiated rituximab before or after remission.

26 TTP patients reported complete response and only one patient died on the 21th day of admission. The mean follow-up time (in months) was 12.5 months, ranging from 3 to 112 months and no person relapsed during this time. 15.4% of TTP patients have experienced adverse effects in our cohort, two of them had pulmonary infections within 6 months after rituximab infusion and responsive to antibiotic therapy. Two of them have experienced transient hypotension. Seven patients took the measurement of CD19+CD20+ cells count in peripheral blood during and after the infusion of rituximab. All of them had a decreased CD19+CD20+ cell counts to 0% after 2 times of rituximab infusion, the CD19+CD20+ cell absence lasted at least 6 months.

Discussion

Exact diagnosis and plasma exchange were essential for TTP patients to remission. Except for life-threatening hemorrhage, platelets infusion is contradicted among TTP patients prior to the introduction of PEX, which exacerbate the severity of microvascular thrombi formation, worsen the patient's condition seriously [16]. Although PEX was used 7 times and rituximab infusion given twice, a patient died in our cohort because of platelet infusion before the diagnosis. It is important for the doctor to exclude TTP before platelet infusion in thrombocytopenic patients. TTP should be diagnosed ahead of MAHA and thrombocytopenia for the patients with neurological abnormalities. ADAMTS13 activity and inhibitor should be tested and PEX should be started immediately.

Recent times have witnessed the increasing number of TTP cases yet, have promptly received PEX therapy, dramatically decreasing the mortality due to this disease. Relapse of this disease however still remains as a challenge that needs to be addressed among the patients affected with TTP. Eradication of ADAMTS13 inhibitor would be an ideal strategy to prevent relapse.

Rituximab, an anti-CD20 monoclonal antibody has been successfully used in autoimmune disorders, such as Immune Thrombocytopenia (ITP), Autoimmune Hemolytic Anemia (AIHA) to deplete B lymphocytes. The effect of depletion usually lasts for 6 to 9 months on B-cell and return to the normal count after 12 months. The side effects of rituximab are mild, mainly with cytokines related reactions during the first infusion [17].

Rituximab was used among TTP affected patients during the second phase of the study and the researchers came to a conclusion that early use of rituximab (≤ 3 days from admission) was better than later administration with fewer PEX, allowing short period of time to remission [18].

In our cohort of TTP patients, we found that the platelets recovery time and ADAMTS13 recovery time were almost same whether the patients got rituximab administered before or after platelet count recovery. This indicates the fact that there was no specific benefit of early infusion of rituximab in the recovery of platelets.

According to 12-year follow-up data comparing the relapse rates with or without rituximab after remission, Hie et al. [19] reported that rituximab administration after remission can decrease the relapse rate from 0.57 episodes/year to 0 episode/year for TTP patients along with a longer relapse-free survival time. We also found that there was no relapse occurred during the follow-up time for the entire TTP patients infused with rituximab before or after remission, which further

approved that rituximab administration can effectively prevent TTP patients from relapses and improves the quality of life.

Although the standard protocol of rituximab in 375 mg/m² in a weekly dose was given for a month, low doses of rituximab (100 mg per week, for 4 weeks) has also shown the same benefit for other autoimmune diseases. There were 4 TTP patients receiving the low doses rituximab after remission in this study and there was no relapse during follow-up in these cases. It was suggested that rituximab infusion at short intervals would be more valid as pharmacokinetics and pharmacodynamics of rituximab might influence plasma exchange therapy [20,21]. So the study concludes that rituximab should be administrated during the 1st, 3rd, 7th and 14th days in succession [22].

In conclusion, intensive immunosuppression treatment with rituximab administration weekly combined with PEX and steroids is safe, effective, and well tolerated by the TTP patients both during the acute and remission phase and shortens the time taken for the remission by preventing the relapse. The low doses rituximab regimen (100 mg per week, for 4 weeks) might also work. As a retrospective study, our results support the fact that rituximab infusion prevents relapse of TTP. The study recommends the need for a randomized, prospective and multi-dimensional studies to determine the timing of infusions, appropriate dosing, the maintenance therapy, and long-term side effects.

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