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Rituximab as first-line treatment for acquired thrombotic thrombocytopenic purpura

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Abstract

Objective: To investigate the efficacy and safety of rituximab (RTX) as first-line treatment of acquired thrombotic thrombocytopenic purpura (aTTP).

Methods: Twenty-five patients with acute aTTP and/or severe a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) deficiency were admitted to our centre from April 2009 to March 2015. Fourteen patients received RTX plus standard therapy (plasma exchange and corticosteroids) at acute episodes. Haemoglobin, platelet count, schistocytes, lactate dehydrogenase levels, ADAMTS13 activity and its inhibitors, and the ratio of B lymphocytes in the peripheral blood, were monitored. The number of plasma exchange (PEXs), total plasma volume, remission time, relapse ratio, and adverse effects were recorded.

Results: The median number of PEXs was 5 (2–17) sessions and median total plasma volume was 168.43 ml/kg (62.86–469.52 ml/kg). Patients achieved haematological remission at a median of 15 days (5–22 days), and the median time of immunological remission was 2 weeks (2–8 weeks) with a median follow-up of 13 months (3–61 months). ADAMTS13 activity significantly increased after 2 weeks. The B lymphocyte percentage in peripheral blood was reduced 1 week after the first dose of RTX infusion compared with before treatment ($2.21\% \pm 5.23\%$ vs $18.47\% \pm 7.34\%$, $P = 0.000$ [the result of statistical software]), and began to gradually increase 9 months later. Severe adverse effects and relapsing TTP were not observed during therapy and follow-up. However, one patient who had sustained immunological remission died of severe pneumonia 7 months later.

Conclusion: Although our study was limited by its small sample number and it was a non-controlled, clinical trial, it showed potential benefits of RTX therapy for acute aTTP. RTX may be administered as a first-line therapy for lowering patients' relapse rate in the long term. Randomized, controlled trials of RTX for aTTP are required.

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Introduction

Acquired thrombotic thrombocytopenic purpura (aTTP) is a rare, but life-threatening, haematological emergency. This condition is initially characterized by thrombocytopenia, microangiopathic haemolytic anaemia (MAHA), renal function abnormality, neurological dysfunction, and fever.[1,2] Although only the minority of patients with aTTP (5%) manifest with the classic "pentad", the presence of thrombocytopenia and MAHA are necessary to be consistent with the diagnosis of TTP.[3] Acquired TTP arises from autoantibody-mediated inhibition of a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13). Ultra-large-vWF connects with platelet membrane glycoprotein Ib, further causing intravascular thrombosis, platelet aggregation, and development of TTP.[4,5] TTP has high morbidity and mortality up to 90% if left untreated. Plasma exchange (PEX) plus corticosteroids as standard therapy reduces the mortality of TTP to approximately 10–15%.[3] However, the recurrence rate of TTP is 50–60% by standard treatment.[1] Rituximab (RTX) is a monoclonal antibody that targets the CD20 antigen present on B lymphatic cells. RTX is used in refractory and/or relapsed TTP with a high response. However, there have been few studies on RTX in the treatment of acute aTTP.[6] Because of the high mortality and recurrence rate in patients with aTTP, RTX rapidly depletes circulating B lymphocytes, resulting in a reduction in the antibody of ADAMTS13.[6–8] Therefore, the combination of RTX with standard therapy for aTTP might be useful to 1) reduce the recurrence rate and sustain a long-term response, and 2) reduce the volume of PEX for acute episodes because abundant plasma is unavailable in most medical centres in China.

Patients and methods

Patients

A total of 25 patients with acute aTTP and/or severe ADAMTS13 deficiency were admitted to our centre from April 2009 to March 2015. Fourteen patients with acute aTTP who were newly diagnosed received RTX plus standard therapy as first-line treatment. Five of 14 patients were male and the other patients were female. The median age was 40.5 years (20–70 years). Only two patients had the classic pentad, five had a tetrad (thrombocytopenia, MAHA, neurological deficiency, and fever), and the remaining patients had a triad (thrombocytopenia, MAHA, and neurological deficiency) (Table 1).

Criteria of diagnosis of aTTP and efficacy of treatment

The diagnosis of aTTP met the criterion of the British Society for Haematology (BCSH), and inherited TTP and other types of thrombotic microangiopathy were excluded. Evaluation of efficacy was determined by referring to the BCSH and the standards proposed by other authors,[1] and was categorized into: 1) haematological remission (HR): recovery of haematological and biochemical parameters to normal after ceasing PEX, and disappearance of clinical symptoms and signs; 2) immunological remission (IR), based on HR, an increase in ADAMTS13 activity to $> 20\%$, and its inhibitor becomes negative; 3) cure, with sustained HR or IR for at least 18 months;[9] and 4) relapse, where patients with HR

Table 1. Clinical characteristics and laboratory parameters at diagnosis.

Female/male	9/5
Initial therapy (*n*)	14
Median age (years)	40.5 (20–70)
Clinical symptoms, *n* (%)	
Triad	100% (14/14)
Tetrad	35.71% (5/14)
Pentad	14.29% (2/14)
ADAMTS13	
Activity, %, M (range)	0 (0–2)
Inhibitor	Positive
Platelet count, $\times 10^9$/L, M (range)	15 (4–31)
Haemoglobin, g/l, M (range)	70 (47–90)
LDH, U/L, M (range)	1065 (522–2963)
Schistocytes, %, M (range)	8 (6–26)
Reticulocytes, M (range)	0.078 (0.012–0.098)
Creatinine, μmol/L, M (range)	52.6 (37.8–159)
B lymphocytes, %, M (range)*	20.9 (8.4–50.3)
HBsAg*/HBV DNA load	Negative/normal

*Measured in peripheral blood. ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; LDH, lactate dehydrogenase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

present with TTP-related laboratory abnormalities and/or clinical manifestations and signs again.

Therapeutic methods

Standard treatment: PEX plus steroids is the standard therapy. PEX (20–40 ml/kg) was provided daily and increased to twice a day for patients with severe nervous system damage, until clinical symptoms and laboratory parameters were improved (PLT $> 50 \times 10^9$/L). When PEX was insufficient, patients also received plasma infusion, or plasma combined with albumin diluted to saline, instead of PEX. The initial dosage of prednisone was 1–2 mg/kg/day, or equivalent methylprednisolone and dexamethasone, with a progressively reduced dosage after clinical remission.

RTX treatment: Once the diagnosis of aTTP was confirmed, RTX was administered immediately after the PEX session. Eleven patients received weekly RTX (375 mg/m^2) for 4 consecutive weeks with the addition of PEX and steroids. Three patients were administered RTX 375 mg/m^2 weekly for the first 2 weeks, and then a fixed dose of 100 mg weekly was provided for the next 2 weeks. This was performed because one patient died of severe pneumonia owing to severe immune deficiency. Steroids, calcium gluconate, and antihistamine were provided before RTX infusion. Hepatitis B serology or/and virus (if necessary) were screened for in all of the patients before starting RTX for all indications.

Detection of ADAMTS13 activity and its inhibitor

Peripheral blood was collected from patients with TTP and healthy controls before PEX. The blood was centrifuged for 5 min at 3000 rpm/min (1600 g). The plasma was aliquoted and frozen at −30°C. The plasma of patients and healthy volunteers was mixed at a ratio of 1:9 and incubated for 2 h at 37°C. Plasma ADAMTS13 activity was determined by the residual collagen binding assay. ADAMTS13 inhibitor was defined as positive if the value was $< 10\%$.

Detection of peripheral blood B lymphocytes

Peripheral blood was collected into EDTA vacutainers. The cell number was adjusted to half (one million per ml) within 6 h. A volume of 100 μl of cell suspension was collected and labelled by immunofluorescence. Lymphocytes were gated using forward scatter versus side scatter plots by flow cytometric analysis. The relative count of B lymphocytes was determined as the percentage of CD3+/CD19+ cells in lymphocytes that were selected in the CD45/SSC dot plots.

Monoclonal antibodies, including CD3, CD19, CD20, and CD45, and the corresponding negative controls were purchased from Immunotech (Marseilles, France). Data were analysed using Expo32 ADC software.

Definition of follow-up and events

The duration of follow-up was calculated from the day of diagnosis of aTTP to the day of death or to March 2015. A total of 14 patients were followed up at the outpatient department or via telephone. ADAMTS13 activity and its inhibitors, and peripheral blood B lymphocytes were assayed weekly after the first dose of RTX in the first month and then every 2 to 3 months after this time. Adverse events consisted of any cause of death, infection, hospitalization, or relapse in TTP.

Informed consent

The study was approved by the 100th Hospital of the People's Liberation Army institutional review board. Appropriate consent was obtained from all patients or their guardians.

Statistical analysis

SPSS 13.0 statistical software was used for data processing. Data that were normally distributed are shown by $\bar{x} \pm SD$, and data that had a skewed distribution are shown as the median. The t-test was used to compare before and after treatment of measurement data. $P < 0.05$ was considered statistically significant.

Results

Efficacy

A total of 14 patients received a mean of 5 (2–17) PEXs during acute episodes, and the mean total plasma volume was 168.43 ml/kg (62.86–469.52 ml/kg).The median time of infusion of the first dose of RTX was 6.5 days (3–14 days) after the diagnosis of aTTP. After the second RTX infusion, the platelet count increased to 50×10^9/L and LDH levels were less than 450 U/L. After three RTX infusions, each patient achieved an HR. The median time of achieving remission was 15 days (5–22 days) and the median time of IR was 2 weeks (2–8 weeks), with a median of 13 months (3–61 months) of follow-up. ADAMTS13 activity ranged from 87% to 100%, and the ADAMTS13 inhibitor became negative. ADAMTS13 activity was maintained and no patient relapsed during the follow-up (Table 2).

Event-free survival analysis

Event-free survival was analysed in patients with aTTP after use of RTX. The event-free survival rate with a median of 13 months of follow-up in the whole cohort was 92.86%.

Analysis of peripheral blood B lymphocytes

The percentage of B lymphocytes in peripheral blood was $22.05\% \pm 10.68\%$ before

Table 2. Therapeutic outcomes after treatment.

Times of PEX, M (range)	5 (2–17)
Mean total plasma volume, ml/kg (range)	168.43 ± 27.13 (62.86–469.52)
Median time to PLT $> 50 \times 10^9$/L, days (range)	13 (3–18)
Timing of initial rituximab infusion, days, M (range)	6.5 (3–14)
Median time to haematological remission (days)	15 (5–22)
Sustained haematological remission rate (%)	100 (14/14)
Median time to immunological remission (weeks)	2 (2–8)
Duration of immunological remission (months)	13 (3–61)
Sustained immunological remission rate (%)	100 (14/14)
Relapse rate (%)	0 (0/14)

RTX infusion. The percentage of B lymphocytes was significantly decreased to $2.53\% \pm 4.71\%$ after one time of RTX infusion, $0.64\% \pm 1.10\%$ after two times of RTX infusion, and $0.15\% \pm 0.37\%$ after four times of RTX infusion. This percentage was subsequently maintained at the level of $< 1\%$ (Figure 1) and gradually recovered within 9 months. The B lymphocyte ratio in two patients was still less than 0.2% 12 months after treatment.

Adverse events

No rash, fever, palpitation or other adverse events occurred during a total of 64 RTX infusions. However, a 45-year-old male patient who had IR died of severe pulmonary infection and respiratory failure 7 months later because of delayed treatment.

Discussion

The standard treatment of aTTP is PEX combined with steroids.[10] Effective PEX treatment decreases the mortality rate from 90% to approximately 10–20%, usually based on a 1.5 times plasma volume daily.[11,12] Patients who experience first-line treatment failure or relapse can be provided intensive treatment via PEX, a greater dosage of methylprednisolone, or combined with other immunosuppressants, such as vincristine and cyclophosphamide. A total of 10–42% of patients show a poor response to PEX and corticosteroids [3]and 20–50% relapse, [1,13] thus requiring additional therapy in each case. RTX, recombinant ADAMTS13, vWF inhibitor, and platelet inhibitors are among novel treatments for aTTP.[14–16] In patients with an episode of refractory TTP, addition of RTX to standard therapy increases the platelet count in approximately 80% of patients and can reduce the time required to achieve a platelet count response.[6] This is because aTTP is a rare disease, there are no high-quality publications on RTX as the initial therapy in acute aTTP based on randomized, controlled trials.[6] In a phase-II study of RTX in acute aTTP, 40 patients were treated with RTX ($375\,mg/m^2 \times 4$ dosages) in conjunction with standard therapy (PEX and steroids). In the RTX group, there was a reduction in the number of PEXs, hospitalization rate in the ICU, and relapse rate among patients who received RTX compared with the control group. Therefore, among patients who

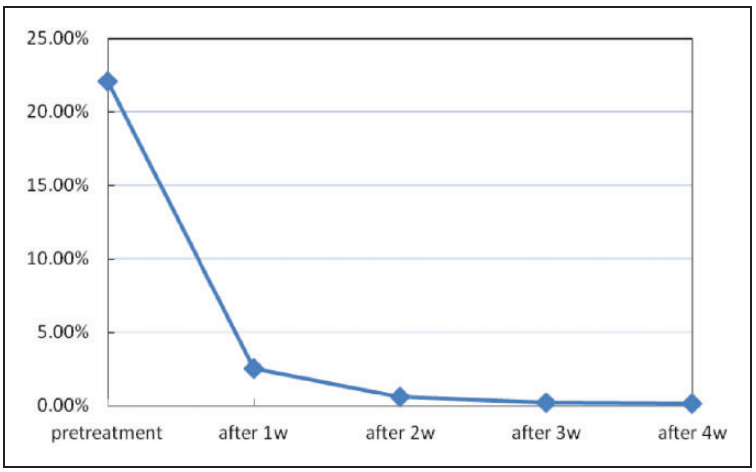

Figure 1. Change in the percentage of peripheral blood B lymphocytes before and after treatment.

receive RTX as soon as possible, the prognosis improves if RTX is administered within 3 days.[9] However, the same conclusion could not be made in another study because of numerous potential confounders.[17]

In the current study, the median follow-up was 13 months and 13 patients had IR at the end of the follow-up. ADAMTS13 activity in all of the patients was less than 5% and the inhibitor was positive when aTTP was diagnosed, thus being consistent with severe ADAMTS13 deficiency. After 1–2 weeks of use of RTX, ADAMTS13 activity dramatically increased up to 50% and was then maintained at normal activity. The mean number of PEXs was only 5 and the mean total plasma volume was only 168.43 ml/kg in our patients. These values were significantly less than those previously reported.[1,6,18] The reasons for this discrepancy between studies may be as follows. (1) In studies from Western countries, PEX was continued until the PLT count was $> 150 \times 10^9$/L,[1,8,9] with a longer duration of PEX treatment and greater plasma volume consumption, while plasma resources are in short supply in China. Therefore, in our study, PEX was ceased when the clinical symptoms improved and laboratory parameters had recovered to a certain degree. (2) In acute episodes, the use of PEX and steroids cleared and reduced ADAMTS13 inhibitor and supplied exogenous ADAMTS13. Therefore, the pathological process of patients was terminated, suggesting that the early haematological response mainly lies in adequate and effective PEX. 3) RTX depletes B lymphocytes in 2 to 4 weeks and maintains them at a low level, thus encouraging further recovery of ADAMTS13 activity.[6] Patients can achieve early IR or even maintain long-term remission without recurrence. Ultimately, RTX can reduce PEX over a long course of treatment.

There are few severe adverse effects of RTX in treatment of the early stage of aTTP,[19] which was confirmed in our study. Humoral immune function due to depletion of B lymphocytes and consequent probable infection should be carefully examined in the long term after use of a standard dose of RTX. Our study and other studies have shown that the risk of severe infection in RTX-treated patients is an inevitable challenge in clinical practice.[6] In our study, after the second dose of RTX, patients had low levels of B lymphocytes in peripheral blood ($< 1\%$). One patient died of severe pulmonary infection due to respiratory failure with sustained IR. Additionally, low-dose RTX is promising in immune thrombocytopenia and TTP treatment.[20–23] Therefore, we decreased the dose of RTX to a fixed dose of 100 mg at the 3rd/4th week. Fortunately, three patients who received the lower dose of RTX had a sustained IR. We would continue to administer this regimen for patients with aTTP to reduce the high cost of RTX and the rate of risk of infection. However, the benefits and efficacy of long-term treatment should be validated in the future.

Although we studied a small number of patients and our study was not a controlled, clinical trial, the current study showed potential benefits of addition of RTX for patients with acute aTTP. We conclude that RTX may be administered to lower the relapse rate in the long term. Nevertheless, a series with more patients is required to validate more precisely the role of RTX as front-line therapy and the optimal dose of RTX in aTTP.

Declaration of conflicting interest

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