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Efficacy and Safety of Rituximab in Connective Tissue Disease-Associated Thrombotic Thrombocytopenic Purpura/Thrombotic Microangiopathy

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ABSTRACT

Introduction: This study examined the efficacy and safety of Rituximab (RTX) treatment in connective tissue disease (CTD)-associated thrombocytopenic purpura (TTP) and thrombotic microangiopathy (TMA), using historical controls as comparators.

Methods: Patients who were admitted to our department from March 1, 2013 to March 31, 2021, and diagnosed with CTD-associated TTP/TMA refractory to plasma exchange were included in the study. A patient with treatment-resistant disease was treated with RTX in addition to high-dose glucocorticoid (GC) therapy (GC + RTX). As historical controls, we selected patients with CTD-associated TTP/TMA who were admitted to our center and treated with GC and immunosuppressants (IS) such as cyclophosphamide. The primary endpoint was the survival rate 52 weeks after the start of treatment.

Results: Fifteen patients were enrolled in the study (GC + RTX). As a control group, 11 patients were enrolled in the same manner (GC + IS). There were no significant differences in age or sex or laboratory tests between the two groups. The primary endpoint of survival rate was significantly higher in the GC + RTX group than in the GC + IS group. In the immunophenotyping analysis before treatment, among all subsets of immune cells, only plasmocytes were significantly elevated in TTP patients compared to healthy controls. Plasmocytes correlated with serum markers, suggesting increased B cell differentiation, which was markedly decreased after RTX treatment.

Conclusion: In CTD-associated TTP/TMA, B cells may affect pathology, and adding RTX to plasma exchange and GC therapy may be worth considering.

1 | Introduction

Thrombotic thrombocytopenic purpura (TTP) was first described by Moschcowitz in 1924 as a condition presenting with five characteristic features, but has since been shown to be associated with a decrease in a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) activity [1, 2]. Systemic lupus erythematosus (SLE) and other connective tissue diseases present with a variety of symptoms, but TTP is known to occur with worsening disease activity in connective tissue diseases [3, 4]. It is also known that, in some cases called thrombotic microangiopathy (TMA), the disease manifests similarly to TTP, even though ADAMTS13 activity is not reduced. Therefore, it is often referred to as connective tissue disease (CTD)-associated TTP/TMA. CTD-associated TTP/TMA is generally considered to

Summary

- It may be worth considering adding RTX to the treatment of patients with CTD-associated TTP/TMA who show little response to conventional treatments.
- Plasmocytes were inversely correlated with platelet counts and LDH levels in CTD-associated TTP/TMA patients.
- RTX may improve prognosis by affecting the pathogenesis of B cells, including plasmocytes.

have a poorer prognosis than typical TTP without underlying diseases [5]. Treatment of CTD-associated TTP/TMA is often combined with glucocorticoids and other immunosuppressive drugs because of the poor response to plasma exchange alone [6, 7]. However, there is no clarity regarding which immunosuppressants (IS) are most effective for CTD-associated TTP/TMA [4].

Rituximab (RTX) is a drug with established efficacy in CTD, including ANCA-associated vasculitis [8]. RTX is a molecularly targeted drug that targets CD20, depletes B cells, and suppresses antibody production, including autoantibody production. RTX has established efficacy in typical TTP [9–11] and has been covered by TTP insurance in Japan since August 2019. However, although there are case reports and single-arm reports reporting the effect of RTX on CTD-associated TTP/TMA [12–30], with some reports of exacerbations [31], there are no studies comparing an RTX-intervention group to a control group. In our department, RTX has been administered for CTD-associated refractory TTP/TMA after obtaining ethics committee approval and written consent. We enrolled patients with autoimmune diseases in a comprehensive immunophenotyping analysis registry (Flow study) to evaluate immune abnormalities in their peripheral blood [32–34]. In the present study, we investigated the safety and efficacy of RTX treatment for CTD-associated refractory TTP/TMA in our department in real-world settings compared with previous cases in which RTX was not used. Simultaneously, we examined the changes in immune abnormalities before and after RTX administration.

2 | Materials and Methods

2.1 | Study Design and Patients

Patients who were admitted to our department between March 1, 2013 and March 31, 2021, with a diagnosis of TTP/TMA associated with an exacerbation of CTD, and who received RTX were enrolled in the GC + RTX group. In this study, as in previous trials, TTP/TMA was diagnosed when ADAMTS13 activity was < 5%; the pentad of Moschcowitz was fulfilled; or microangiopathic hemolytic anemia, thrombocytopenia, elevated LDH (> 1.5 times the reference value), normal coagulation (PT-INR < 1.5, Fib > 100), and no severely elevated blood pressure (sBP < 180, dBP < 120) were observed [10, 30, 35, 36]. According to the current diagnostic criteria, a marked decrease in ADAMTS13 activity is essential for the diagnosis of TTP. However, ADAMTS13 has only recently become measurable in health insurance examinations. As this was a retrospective study, we examined TTP/TMA cases together, including TTP cases that exhibited a marked decrease in ADAMTS13 activity and TMA cases in which a marked decrease in ADAMTS13 activity could not be confirmed but showed clinical findings similar to those of TTP. We excluded cases of scleroderma renal crisis that responded to ACE inhibitors or ARBs and catastrophic antiphospholipid syndrome that responded to anticoagulant therapy. We defined patients who did not improve after five sessions of plasma exchange according to previous reports as refractory cases [10]. We retrospectively observed the outcomes of patients who received induction therapy with RTX in addition to high-dose glucocorticoid (GC) therapy for refractory cases at our hospital and affiliated hospital. RTX was administered at a dose of 500 mg per body once weekly for a total of four doses as the standard regimen. However, based on the patient's condition and blood test results, the dosing interval was extended up to 4 weeks, and the total number of doses was reduced accordingly. Plasma exchange was performed daily for the first three sessions, followed by 3–5 sessions per week thereafter. However, the frequency was reduced on certain occasions according to the patient's condition and blood test results. To optimize the efficacy of RTX, plasma exchange was withheld for 48 h following RTX administration. Historical control was defined as patients admitted to our hospital with a diagnosis of TTP/TMA associated with exacerbation of CTD. The diagnosis was made in the same manner as for the GC + RTX group, and refractory cases were defined in the same manner. Refractory cases during this period were treated with high-dose GC and IS other than RTX, and their outcomes were retrospectively monitored at our hospital and affiliated hospital. Patients who consented underwent additional comprehensive immunophenotyping analysis, which was performed in our department. This study was approved by the Ethics Committee of the Occupational Medical and Welfare University (H27-014, H23-005) and was conducted in accordance with the Declaration of Helsinki and Ethical Guidelines for Medical Research Involving Human Subjects of the Ministry of Health, Labor, and Welfare. Whenever RTX was administered to patients not covered by insurance, a clinical ethics application was submitted to the ethics committee of the hospital of the University of Occupational and Environmental Health, Japan for review and approval (2015-09), and consent was obtained from the patient.

2.2 | Assessment and Endpoints

The primary endpoint in this study was the survival rate 52 weeks after the initiation of induction therapy. The secondary endpoints included remission rate, plasma exchange independent rates, thrombocyte remission rates, hemodialysis-independent rates, and various blood tests (hemoglobin, platelet count, LDH, and Cre). Remission was defined as platelet normalization (> 150 000), LDH normality (< 1.5 × the upper limit of normal), and no TTP symptoms without plasma exchange [36]. In addition, comprehensive immunophenotyping analysis was performed to evaluate the changes before and after RTX treatment. Adverse events were defined as new events or unexpected worsening of a medical

condition, irrespective of cause, during the observation period as compared to before starting induction therapy. Severity was classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

2.3 | Flow Cytometric Analysis

Flow cytometric analysis was performed as previously described [32–34]. Briefly, peripheral blood mononuclear cells were isolated at the onset of TTP/TMA and approximately 6 months after RTX treatment. Peripheral blood mononuclear cells were resuspended in PBS/3% human IgG (Baxter International Inc., Vienna, Austria) to block Fc receptors and prevent nonspecific antibody binding, and then incubated for 15 min at 4°C in the dark. The cells were then washed with PBS containing 1% bovine serum albumin. Background fluorescence was assessed using the appropriate isotype- and fluorochrome-matched control monoclonal antibodies. After staining with the indicated antibodies, cells were analyzed by multicolor flow cytometry (FACSVerse; BD Biosciences, San Jose, CA, USA) and analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

2.4 | Gating Strategy of Flow Cytometric Analysis

The phenotype of immune cell subsets was defined based on the HIP protocol of comprehensive eight-color flow cytometric analysis proposed by the National Institutes of Health (NIH)/the Federation of Clinical Immunology Societies (FOCIS), with some modifications for detecting Tfh cells [37]. Details of the gating strategy for the flow cytometric analysis are described in Table S1. The clones and names of the antibodies used in this study are listed in Table S2.

2.5 | Statistical Methods

Patient characteristics are expressed as mean (SD), median (interquartile range [IQR]), or number (%) of patients. Survival rates were assessed using the Kaplan–Meier method. Student's *t*-test and Mann–Whitney *U* test were used for between-group comparisons, and Fisher's exact test was used to compare categorical variables. The degree of contribution and contribution ratio were calculated using the bootstrap forest method. All reported *p* values were two-sided and were not adjusted for multiple testing. The level of statistical significance was set at $p < 0.05$. The last observation was used for patients whose laboratory values were not measured. All analyses were performed using JMP version 13.0 (SAS Institute Inc., Cary, NC, USA).

3 | Results

3.1 | Baseline Characteristics

Thirty-five cases were diagnosed with TTP/TMA, seven of which were due to causes other than CTD and 13 did not meet the definition of refractory disease (Figure S1). Of the remaining 15 patients, all received RTX. Finally, 15 patients were enrolled in the study as refractory CTD-associated TTP/TMA (GC + RTX group). Twenty-seven patients were diagnosed with TTP or TMA, five had TTP due to causes other than CTD, and 11 did not meet the definition of refractory disease. None of the patients were treated with RTX, and 11 patients were enrolled (GC + IS group).

Table 1 shows the baseline patient characteristics for both groups. There were no significant differences in age (GC + RTX/GC + IS:52 [40–68]/65 [50–73] years) or sex (percentage of women, GC + RTX/GC + IS:12 (80)/8 (72)) between the two groups. There were no significant differences in background CTD, although SLE accounted for more than 1/3 of the cases in both groups. No difference was observed between the two groups in the proportion of patients with other collagen diseases. In terms of treatment history, only one patient in the GC + RTX group had previously received RTX. All patients had microangiopathic hemolytic anemia and thrombocytopenia, and other Moschcowitz pentad, such as fever (10 (67)/5 (45)), central nervous system abnormalities (10 (67)/7 (64)), and renal dysfunction (13 (87)/7 (64)) did not differ between the two groups. French score (1 [0–1]/1 [1–1]), PLASMIC score (5 [5–6]/5 [5–6]), and severity score (3 [2–4]/2 [2–3]) also did not differ between the two groups [38, 39]. The SLICC Damage Index (1 [0–2]/1 [0–2]) at the time of diagnosis also did not differ between the two groups.

Laboratory parameters included Hb (82 [73–89]/82 [75–88] g/L), platelet count (5.8 [2.8–6.4]/2.6 [1.2–6.4] $\times 10^9$/L), LDH (621 [304–1027]/479 [345–778] U/L), Cre (144.1 [118.5–417.2]/172.4 [85.7–263.4] μmol/L), eGFR (24.50 [7.97–32.28]/22.87 [13.12–48.23] mL/min/1.7 m^2), haptoglobin (9 [9–53]/9 [9–66] mg/dL), and ADAMTS13 functional activity (37.2 [26.0–65.2]/27.5 [23.1–40.1]) were not significantly different. Ferritin (986 [368–9293]/3426 [216–5801]), an indicator of macrophage activation, did not differ between the two groups. No differences were observed in complement or autoantibody levels between the two groups.

There was no difference in the initial glucocorticoid dose (56 [50–65] mg/day/50 [40–68] mg/day) or glucocorticoid pulse therapy (10 (67) cases/9 (81) cases) between the two groups. No difference was observed between the two groups in the number of days from TTP/TMA diagnosis to the first plasma exchange. RTX was administered once in one case, twice in seven cases, three times in one case, and four times in six cases. In the GC + IS group, patients were treated with IVCY and AZA in addition to glucocorticoids.

3.2 | Effectiveness and Safety

The primary endpoint of the survival rate was 80.0% (12/15) in the GC + RTX group and 45.5% (5/11) in the GC + IS group after 52 weeks, which was significantly higher in the GC + RTX group than in the GC + IS group (Figure 1A). Deaths in the GC + RTX group included two from sepsis and one from intestinal perforation. In contrast, in the GC + IS group, there were two cases of lower gastrointestinal bleeding, one case of laryngeal hemorrhage, and three deaths due to sepsis (Table S3). The remission rate after 8 weeks did not differ between the two groups, nor did the cumulative remission rate after 52 weeks (Figure 1B,C). There was no difference in the thrombocyte remission rate between the

TABLE 1 | Baseline characteristics of patients with thrombotic microangiopathy in this study.

	GC + RTX $n = 15$	GC + IS; historical control $n = 11$
Age (years)	54.3 ± 16.0	61.3 ± 12.8
Sex (female)	12 (80)	8 (72)
The constitution of CTDs		
RA	0	1
SLE	6	4
IIM	3	2
SSc	2	1
MCTD	1	1
AOSD	1	1
PAN	0	1
MPA	2	0
Disease duration from onset of underlying disease (months)	57 [14–206]	6 [3–141]
Relapsing TTP/TMA	1 (7)	0 (0)
Coexistence of malignant tumors	1 (7)	0 (0)
Organ disorder; Moschcowitz's clinical pentad		
Microangiopathic hemolytic anemia	15 (100)	11 (100)
Thrombocytopenia	15 (100)	11 (100)
Fever	10 (67)	5 (45)
Central nervous system abnormalities	10 (67)	7 (64)
Renal dysfunction	13 (87)	7 (64)
The number of symptom combinations fulfilling the clinical pentad	4 [3–4]	3 [3–4]
French score	1 [0–1]	1 [1–1]
PLASMIC score	5 [5–6]	5 [5–6]
The severity index	3 [2–4]	2 [2–3]
Rose and Eldor score	5 [4–5]	5 [4–5]
Simple prognostic index	4 [2–4]	4 [4–4]
The French TMA Reference Center Score	2 [1–3]	2 [1–3]
Mortality In TTP Score (MITS)	3 [1–4]	3 [1–3]
Damage Index	1 [0–2]	1 [0–2]
Laboratory data		
Hemoglobin (g/L)	81 ± 107	80 ± 127
Platelet count ($\times 10^9$/L)	4.9 ± 2.4	4.0 ± 3.5
Creatinine (μmol/L)	222.2 ± 151.5	183.7 ± 115.6
eGFR (mL/min/1.7 m^2)	24.50 [7.97–32.28]	22.87 [13.12–48.23]
Ferritin (ng/mL)	986 [368–9293]	3426 [216–5801]
LDH (U/L)	621 [304–1027]	479 [345–778]

(Continues)

TABLE 1 | (Continued)

	GC + RTX $n = 15$	GC + IS; historical control $n = 11$
Haptoglobin (mg/dL)	9 [9–59]	9 [9–66]
ADAMTS13 functional activity (%)	37.2 [26.0–65.2]	27.5 [23.1–40.4]
PT-INR	1.07 [1.00–1.20]	1.14 [1.01–1.18]
APTT (s)	37.0 [26.0–44.3]	37.4 [23.7–55.0]
FDP (μg/mL)	10.9 [7.0–67.6]	10.5 [7.3–37.4]
Fibrinogen (mg/dL)	317 [246–410]	211 [135–303]
C3 (mg/dL)	59 [49–88]	61 [38–97]
C4 (mg/dL)	10 [7–19]	22[11–38]
CH50 (U/mL)	36 [27–59]	43 [26–52]
Rheumatoid factor (IU/mL)	10.4 [6.1–68.1]	7.5 [3.2–17.2]
Antinuclear antibody positive (a titer of ≥ 1:80)	10 (67)	7 (64)
Antiphospholipid antibodies positive	3 (20)	2 (18)
Anti-Ro antibodies positive	4 (27)	2 (18)
Anti-La antibodies positive	2 (13)	0 (0)
Anti-dsDNA antibodies positive	4 (27)	3 (27)
Anti-RNP antibodies positive	7 (47)	4 (36)
Anti-Sm antibodies positive	4 (27)	2 (18)
Anti-citrullinated protein antibodies positive	1 (7)	0 (0)
Anti-ARS antibodies positive	2 (13)	1 (9)
Anti-centromere antibodies positive	1 (7)	0 (0)
Anti-RNA polymerase III antibodies positive	0 (0)	1 (9)
Anti-myeloperoxidase-ANCA positive	2 (13)	0 (0)
Treatment		
Number of days from diagnosis to the first plasma exchange (days)	2 [2–4]	2 [1–4]
GC dose (mg/day, PSL equivalent)	56 [50–65]	50 [40–68]
Pulse glucocorticoid therapy	10 (67)	9 (81)
Immunosuppressants	RTX (15)	IVCY 5, Cyclosporine 1, Tacrolimus 1, Azathioprine 4

Note: The severity index was determined by the number of the following criteria: ADAMTS13 inhibitor 2 BU/mL or higher, renal dysfunction, neuropsychiatric disorder, cardiac disorder, intestinal disorder, deep bleeding or deep thrombus, failure to respond to GC treatment, and recurrent cases, as defined by the Ministry of Health, Labor and Welfare. Data are shown as mean ± standard deviation, median [quartile] or *n* (%). *p* values were determined using Student's *t*-test, the Wilcoxon rank-sum test or Fisher's exact probability test.

Abbreviations: ADAMTS13, a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13; ANCA, anti-neutrophil cytoplasmic antibody; APTT, activated partial thromboplastin time; ARS, aminoacyl-tRNA synthetase; CH50, 50% hemolytic unit of complement; dsDNA, double-stranded deoxyribonucleic acid; FDP, fibrin/fibrinogen degradation products; GC, glucocorticoid; IVCY, intravenous cyclophosphamide pulse therapy; LDH, lactate dehydrogenase; PSL, prednisolone; PT-INR, prothrombin time-international normalized ratio; RNA, ribonucleic acid; RNP, ribonucleoprotein; Sm, Smith.

two groups during the observation period, nor was there any difference in the number of patients weaned off plasma exchange (Figure 1D,E). Seven patients in the GC + RTX group and six patients in the GC + IS group were started on hemodialysis at the onset of the disease, and five patients (71.4%) in the GC + RTX group and one patient (16.7%) in the GC + IS group were weaned off hemodialysis, with no difference between the two groups (Figure 1F). The platelet count improved in both groups; however, it improved significantly in the GC + RTX group after Week 8 and continued to improve at Week 52 (Figure 2A). Hgb was also significantly improved in the GC + RTX group after Week 8, and LDH was significantly improved in the GC + RTX group at Week 52; however, there was no significant difference in Cre between the two groups throughout the entire period (Figure 2B–D).

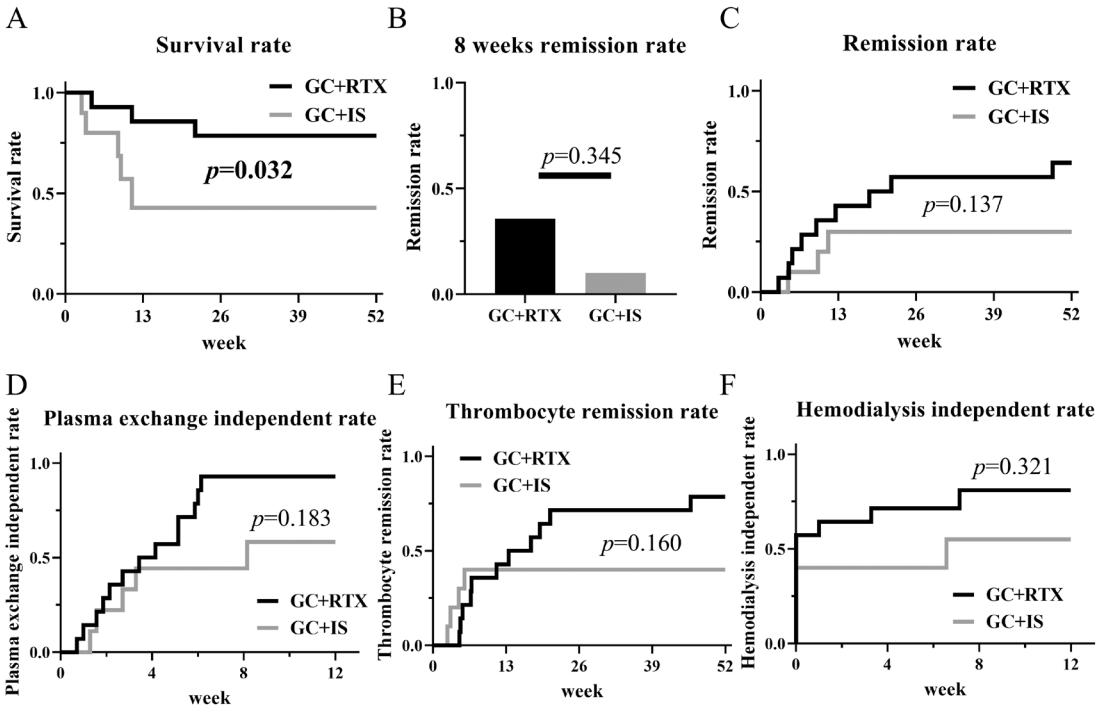

FIGURE 1 | (a) Survival rate up to 52weeks. (b) Remission rate of patients treated with GC + RTX or patients treated with GC + IS at 8weeks. (c) The remission rates up to 52weeks after the introduction therapy. (d) Plasma exchange independent rates up to 12weeks after the introduction therapy. (e) Thrombocyte remission rates up to 52weeks after the introduction therapy. (f) Hemodialysis independent rates up to 12weeks after the introduction therapy.

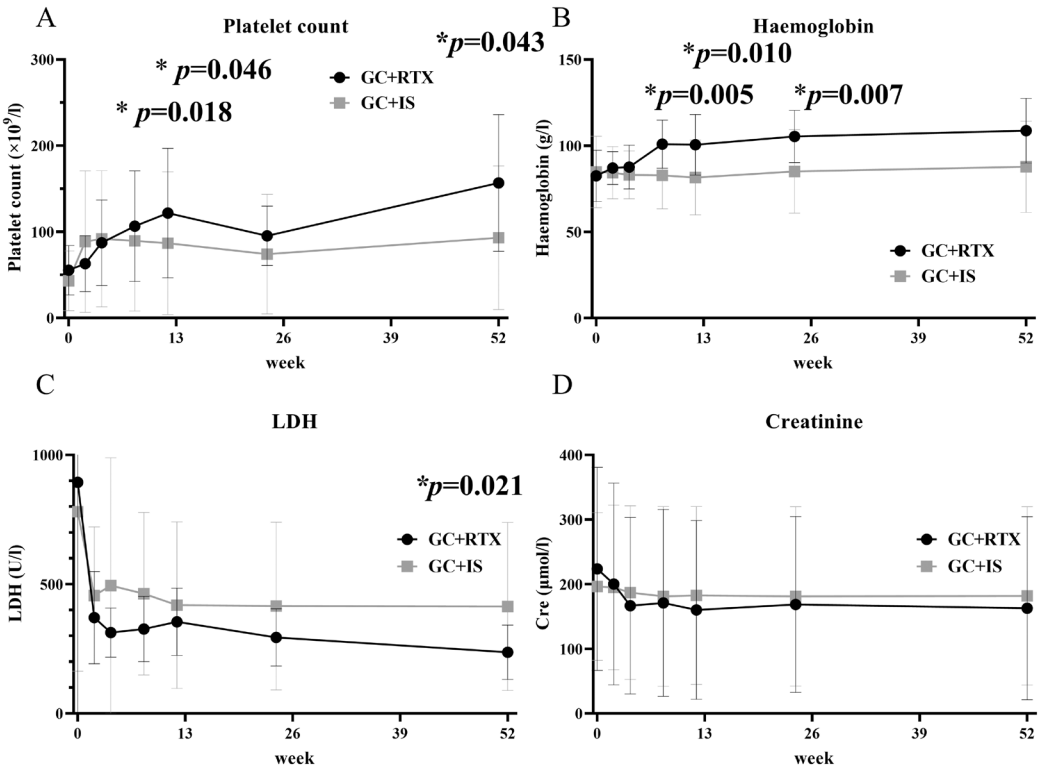

FIGURE 2 | (A) Change in platelet count(10^9/L) in TTP patients. (B) Change in hemoglobin (g/L) in TTP patients. (C) Change in lactate dehydrogenase (U/L) in TTP patients. (D) Change in Cre (μmol/L) in TTP patients. The points denote the mean value and the bars indicate the standard deviation. Cre; creatine; LDH; lactate dehydrogenase.

3.3 | Adverse Events

Adverse events occurred in all patients in the GC + RTX group during the observation period, but there was no difference compared to the GC + IS group. There was also no difference in serious adverse events (Table 2). In terms of CTCAE grade ≥ 3 adverse events, the all-cause mortality rate tended to be higher in the GC + IS group. Otherwise, there were no

TABLE 2 | Adverse events within 52 weeks after treatment.

	GC + RTX $n = 15$	GC + IS; historical control $n = 11$	p
All adverse events	15 (100)	11 (100)	1.000
Serious adverse events	14 (93)	11 (100)	1.000
Infection events	12 (80)	10 (91)	0.614
Serious infection events	11 (73)	8 (73)	1.000
Death (CTCAE grade 5 adverse events)	3 (20)	6 (63)	0.103
CTCAE grade 4 adverse events	2 (13)	3 (27)	0.617
CTCAE grade 3/4 adverse events	14 (93)	11 (100)	1.000
Adverse event of special interest			
Sepsis	3 (20)	3 (27)	0.674
Lung infection	8 (53)	6 (55)	1.000
Hepatitis B reactivation	0 (0)	0 (0)	1.000
Details of all adverse events			
Infection			
Sepsis	Grade 4; 1 Grade 5: 2	Grade 5: 3	
Lung infection	Grade 3; 7 Grade 4: 1	Grade 3; 3 Grade 4: 3	
Biliary tract infection	Grade 4; 1		
CMV infection reactivation	Grade 3; 5	Grade 3; 5	
Febrile neutropenia	Grade 3; 1	Grade 3; 1	
Urinary tract infection	Grade 3; 1		
Enterocolitis infectious	Grade 3; 1		
Shingles		Grade 3; 1	
Adverse drug reactions			
Colonic perforation	Grade 5; 1		
Lower gastrointestinal hemorrhage	Grade 4; 1	Grade 5; 2	
Laryngeal hemorrhage		Grade 5; 1	
Seizure	Grade 3; 1		
Uveitis	Grade 3; 1		
Thromboembolic event		Grade 3; 1	
Duodenal perforation		Grade 3; 1	
Malabsorption		Grade 3; 1	
Laboratory test abnormality			
ALT increased	Grade 3; 1		
ALP increased		Grade 3; 1	

Note: p values were determined by Fisher's exact probability test.

significant differences in the number of adverse events between the GC + RTX and GC + IS groups.

3.4 | Flow Cytometry Analysis

Figure 3 shows a heat map of cell counts by subset of various immune cells in the patients' peripheral blood compared to healthy controls. First, for $CD4^+$ T cells, most subsets were reduced or unchanged compared to healthy controls. As for $CD8^+$ T cells, although activated $CD8^+$ T cells and activated $CXCR3^+CCR6^-CD8^+$ T cells were also increased, these changes were not significant, and cell numbers decreased in most of the subsets. However, in B cells, plasmocytes were significantly elevated in TTP patients (23.5/μL) compared to healthy controls (3.1/μL) (Table S4). Plasmocytes were the

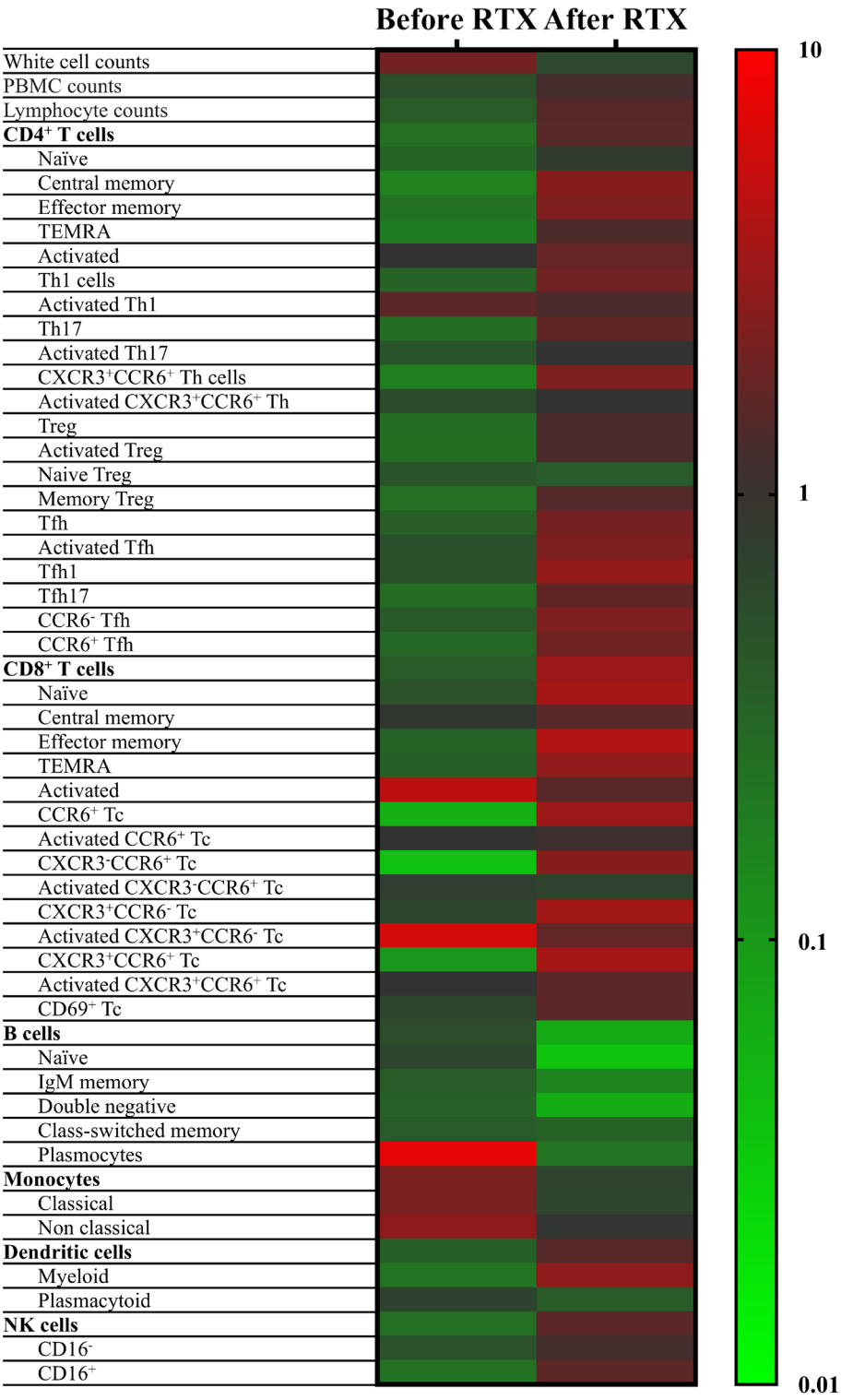

FIGURE 3 | The heat map shows the number of cells per various immune cell subsets in the peripheral blood compared to healthy controls. The left column shows the numbers before RTX treatment, and the right column shows the numbers after RTX treatment.

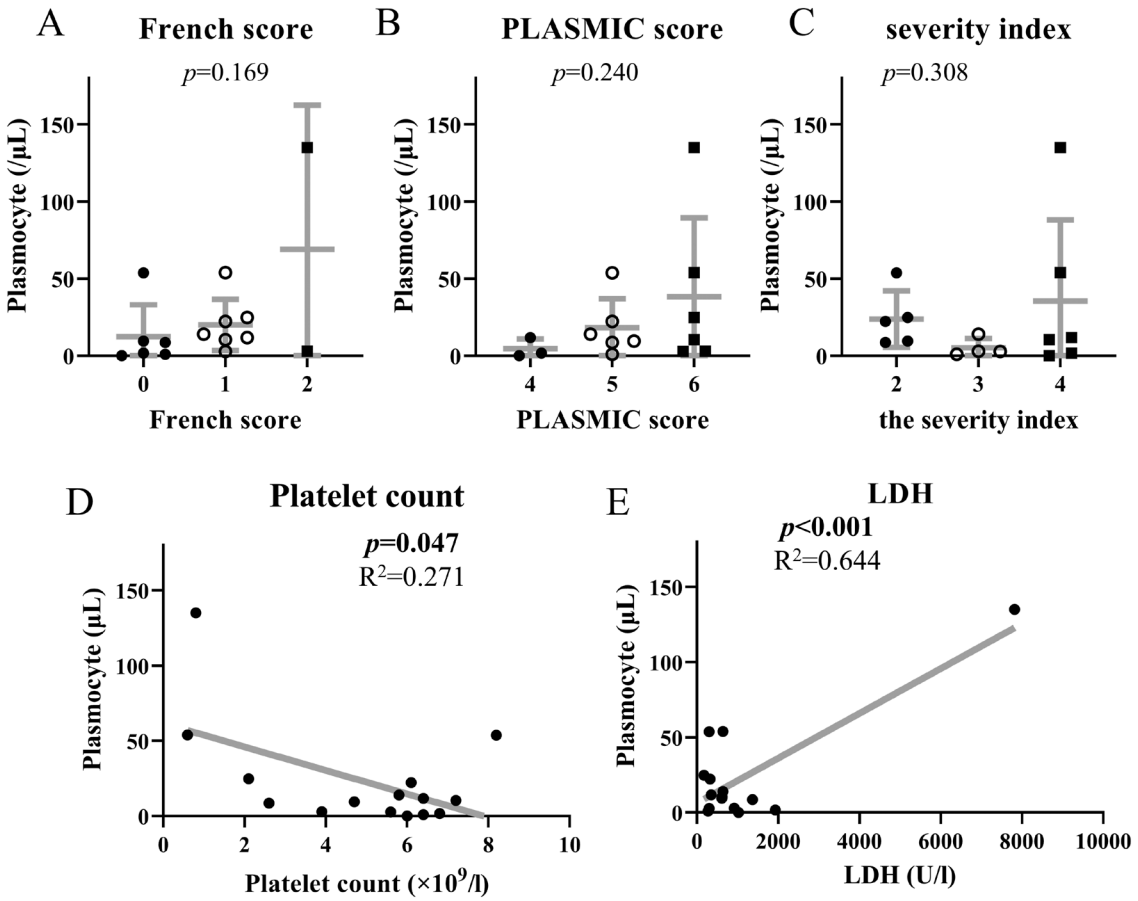

FIGURE 4 | (A) Comparison of plasmocyte with the severity index. (B) Comparison of plasmocyte with PLASMIC score. (C) Comparison of plasmocyte with French score. (D) Comparison of plasmocyte with platelet count. (E) Comparison of plasmocyte with LDH.

only subset that was also significantly increased in all cell subsets. Plasmocytes were not correlated with the severity score, PLASMIC score, or French score(Figure 4a–c), but were significantly inversely correlated with platelet count and significantly correlated with LDH (Figure 4d,e). This suggests that plasmocytes may be involved in the pathogenesis of TTP. In monocytes, all subsets were slightly increased, but not significantly different, and in DCs, all subsets were significantly lower in TTP than in healthy controls. Among NK cells, $CD16^+$ NK cells significantly decreased. These findings revealed that both the acquired and innate immune systems were abnormal in CTD-associated TTP/TMA. In particular, the acquired immune system has been found to exhibit abnormalities in plasmocyte differentiation.

RTX treatment decreased cell counts in all B cells, including plasmocytes (Figure 3; Table S5). With regard to T cells, both $CD4^+$ and $CD8^+$ T-cell counts recovered, with some subsets showing higher counts than in healthy controls. In the individual subset analysis, among $CD4^+$ T cells, effector memory $CD4^+$ T cells significantly recovered, and among $CD8^+$ T cells, $CXCR3^+CCR6^-$ $CD8^+$ T cells significantly recovered; however, no specific subset was biased toward recovery (Table S5). In the innate immune system, myeloid DCs, which were low before treatment, were significantly higher after treatment, although there were no significant changes in monocytes or NK cells.

4 | Discussion

This study examined the efficacy of adding RTX to plasma exchange and high-dose glucocorticoid therapy in refractory CTD-associated TTP/TMA in real-world settings.

CTD-associated TTP/TMA has been implicated in some autoimmune mechanisms, since the incidence of TMA in patients with CTD differs greatly from the incidence of TMA in the general population [40–42]. As mildly decreased ADAMTS13 activity is frequently observed in CTD-associated TTP/TMA, one hypothesis includes increased clearance by non-neutralizing antibodies. Other possible pathogeneses include vascular endothelial damage and complement hyperactivation due to abnormalities in complement regulatory factors, but the details are unspecified [43–45]. Although the treatment of CTD-associated TTP/TMA with glucocorticoids, cyclophosphamide, and other agents has been reported in a case report [6, 46, 47], many reports have used RTX, partly because RTX is effective in typical TTP [12–30].

The results suggest that adding RTX to GC therapy or performing plasma exchange for CTD-associated TTP/TMA may be worthwhile. In particular, focusing on the cause of death, there were no deaths due to hemorrhage associated with the progression of TTP/TMA in the GC + RTX group (Table S3). There was also a concern that RTX would increase deaths

from infections, but there was no difference in infection-related deaths between the two groups. Although the small sample size did not result in significant differences, there was a trend toward higher remission, thrombocyte remission, and weaning rates from plasma exchange in the GC + RTX group. Serum data also showed significant improvements in platelets, hemoglobin, and LDH from 8 to 52 weeks, suggesting the efficacy of RTX. In terms of renal function, there was no difference in Cre between the two groups, and the percentage of patients weaned from hemodialysis was not significantly different; however, more patients were weaned from hemodialysis with RTX. Regarding adverse events, because all patients were under immunosuppression, lung infections and CMV infection reactivation were more common, but there was no increase in infections or other adverse events in the GC + RTX group compared to the GC + IS group.

In the present study, we evaluated immunological abnormalities in CTD-associated TTP/TMA by performing a comprehensive immunophenotyping analysis of the peripheral blood prior to RTX. This is the first study to comprehensively evaluate immunophenotyping of the peripheral blood of patients with CTD-associated TTP/TMA. Although a decreased total lymphocyte count has been associated with poor prognosis of TTP in SLE [48], in the present case, lymphocytes were decreased in all patients, regardless of the underlying disease. This study suggests that immune abnormalities in patients with CTD-associated TTP/TMA are located in the acquired immune system. Among the acquired immune systems, only plasmocytes were significantly increased among all cell subsets. The increase in plasmocytes despite the decrease in PBMC cell counts revealed immunological features of increased B cell differentiation and consequent plasmocyte dominance, particularly in CTD-associated TTP/TMA.

Before treatment, plasmocytes were correlated with platelets and LDH, and RTX depleted the B cells and consequently reduced plasmocytes, which may have improved the pathogenesis of connective tissue pathological TTP/TMA. In contrast, the T cells recovered in cell number as a whole, suggesting that the T cells that were mobilized to peripheral tissues may have returned to the peripheral blood in response to treatment. The analysis of each subset did not recover heavily biased toward any particular subset, and it was not possible to determine which T cells were involved in CTD-associated TTP/TMA in this study. In the innate immune system, although the decrease in mDC was restored after treatment, as in the T-cell system, no judgment could be made regarding its significance.

This study has some limitations. First, this was a retrospective, observational study. TMA has recently been classified according to the underlying disease. However, in this study, the cases were examined retrospectively; therefore, the classification range differs from the current diagnostic criteria for TTP and the narrow definition of secondary TMA. In particular, some cases of secondary TMA overlap with the pathology of aHUS [49]; however, in this study, we could not completely rule out the involvement of aHUS pathology. However, none of the patients included in this study showed characteristic findings in C3 or C4. Second, the study involved an extremely rare condition, CTD-associated TTP/TMA, which forced us to analyze a limited number of cases: 15 in the GC + RTX group and 11 in the GC + IS group. Patients with CTD-associated TTP/TMA have a wide range of backgrounds, and to investigate the efficacy of RTX, a prospective study with a larger number of cases and consistent conditions is needed. Third, one patient with a history of RTX treatment was included in the GC + RTX group, which could introduce potential bias. However, this patient had received RTX treatment more than 2 years prior to the study. Furthermore, concurrent flow cytometry demonstrated sufficient detection of CD20-positive B cells, suggesting that the prior RTX treatment had minimal impact on the patient's clinical condition. Fourth, comprehensive immunophenotyping analysis was performed on the peripheral blood of patients, and immune abnormalities in the tissues were not analyzed. Despite the limitations, we report these results here because we found that plasmocytes correlate with platelet counts and LDH in patients with CTD-associated TTP/TMA, and because we believe the results suggest the clinical efficacy and safety of RTX acting on these progenitor cells.

5 | Conclusion

In CTD-associated TTP/TMA, B cells may influence pathology. Therefore, the addition of RTX to plasma exchange and GC therapies should be considered.

Author Contributions

All authors were involved in the drafting and critical revision of the manuscript. All authors approved the final version of the manuscript. N.O., S.N., and Y.T. designed the research study. S.F., Y.I., H.T., and Y.T. analyzed the data. N.O., S.N., and Y.T. wrote the manuscript. Y.T. supervised the research, created the research concept, and supervised the study.

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Conflicts of Interest

Y.M. has received consulting fees, speaking fees, and/or honoraria from Eli Lilly and has received research grants from GlaxoSmithKline. S.N. has received consulting fees, lecture fees, and/or honoraria from Bristol-Myers, AstraZeneca, Pfizer, GlaxoSmithKline, AbbVie, Astellas, Asahi-kasei, Sanofi, Chugai, Eisai, Gilead Sciences, Boehringer Ingelheim and has received research grants from Mitsubishi-Tanabe. Y.T. has received speaking fees and/or honoraria from Gilead, AbbVie, Boehringer-Ingelheim, Eli Lilly, Mitsubishi-Tanabe, Chugai, Amgen, YL Biologics, Eisai, Astellas, Bristol-Myers, and AstraZeneca; received research grants from Asahi-Kasei, AbbVie, Chugai, Mitsubishi-Tanabe, Eisai, Takeda, Corrona, Daiichi-Sankyo, Kowa, and Boehringer-Ingelheim; and received consultant fees from Eli Lilly, Daiichi-Sankyo, Taisho, Ayumi, Sanofi, GSK, and AbbVie. All other authors declare no conflicts of interest.

Data Availability Statement

Data cannot be shared for ethical/privacy reasons.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.