Anti B-cell therapy against refractory thrombocytopenia in SLE and MCTD patients: long term follow up and review of the literature.

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ABSTRACT

The objective of this study was to retrospectively evaluate the clinical and immunological effects of anti-B cell treatment in patients with systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD) with autoimmune thrombocytopenia (AITP) refractory to conventional immunosuppressive treatment. Rituximab (RTX) was added to the ongoing treatment of 16 patients (median age 36 years, range 17 – 84, all female) with treatment resistant autoimmune thrombocytopenia. Thirteen patients had SLE and 3 had MCTD. RTX was given intravenously on four occasions during 4 consecutive weeks at a dose of 375mg/m^2. Clinical and laboratory disease activity variables recorded at every follow up visit were analyzed.

The median disease duration before RTX-treatment was 9 years (range 0.2 – 27) and the median post-treatment follow up time was 28 months (range 3 – 92). 10 patients (63%) were treated repeatedly with RTX during the follow up period. Complete depletion of B cells was achieved in 94% of cases 1 month after RTX treatment. A significant increase ($p = 0.0001$) of platelet counts was seen already after 1 month (median $58x10^9$/ml versus $110x10^9$/ml) whereas within 3 months platelet counts normalized in 10 patients (median $223x10^9$/ml). Three patients did not respond to RTX treatment (median platelet count $69x10^9$/ml). High titers of anti-platelet antibodies were detected in 7 patients before RTX treatment and the autoantibody titers decreased significantly ($p < 0.03$) after RTX treatment in 6 of these patients that also achieved complete remission. A review of literature revealed 24 articles including 18 case reports, 1 retrospective cohort study and 5 prospective studies documenting the outcomes of 65 RTX treated patients with SLE or MCTD related thrombocytopenia with an overall treatment response rate 80%. In conclusion, these findings indicate that RTX is an additional potent therapeutic treatment option for SLE patients with autoimmune thrombocytopenia refractory to conventional immunosuppressive treatment whereas best response may be expected in patients with high titers of anti-platelet antibodies at baseline.

INTRODUCTION

Systemic lupus erythematosus (SLE) and Mixed Connective Tissue Disease (MCTD) are complex multi-systemic autoimmune diseases with heterogeneous presentation of varying severity. SLE is characterized by polyclonal B cell hyper-reactivity that is considered to be the most important pathogenic event. The mechanism behind the abnormal B cell activation is not fully understood although disturbances in several immune regulation mechanisms have been suggested [1]. The pathogenic role of B cells in SLE pathogenesis involves several pathways such as formation of auto-antibodies and immune complexes, activation of dendritic and T cells, cytokine production as well as chemokine-mediated reactions [2]. MCTD, clinically presenting manifestations that overlap with SLE, scleroderma, rheumatoid arthritis or inflammatory myopathies, is immunologically characterized by the presence auto-antibodies and T cells reactive with U1-ribonucleoprotein [3]. The production of characteristic patterns of auto-antibodies, some of which are clearly involved in tissue damage, has been shown to play an important role in both SLE and MCTD.

Autoimmune thrombocytopenia (AITP), a common hematological manifestation of SLE, is found in 20-40 % of patients [4] and results from accelerated platelet destruction mediated by auto-antibodies to platelet glycoproteins [4]. Antibody-coated platelets are removed by the reticuloendothelial system, mostly in the spleen. Severe form of SLE-related immune thrombocytopenia is relatively rare [5], but potentially life-threatening and often unresponsive to standard treatment. Severe thrombocytopenia has also been shown to be an independent predictor of damage accrual and mortality [6-8].

The most common treatments for AITP are corticosteroids and intravenous immunoglobulin [9, 10]. However, in some SLE patients, disease becomes either resistant to this therapy or steroid-dependent requiring the use of second-line agents or splenectomy [11, 12]. A subset of patients, despite therapy with systemic corticosteroids and immunosuppressive agents, still remains refractory or develop unacceptable toxicity. Therefore, new effective and less toxic treatment strategies are needed.

Considering the multi-functional role of B-cells in the pathogenesis of these autoimmune diseases, a depletion of B cells by targeting CD20 using rituximab (RTX) has emerged as a promising treatment option in SLE. Rituximab has been used to successfully treat thrombocytopenia in patients with steroid-resistant disease [13]. To date, there are no

randomized clinical studies and only few published reports of the use of RTX in patients with SLE or MCTD-related autoimmune thrombocytopenia.

The aim of this study was to retrospectively evaluate the long term clinical and immunological outcomes of anti B-cell treatment in SLE and MCTD patients with autoimmune thrombocytopenia and review published reports regarding rituximab treatment in SLE or MCTD-related thrombocytopenia.

MATERIALS AND METHODS

Patients

Sixteen patients were treated with RTX at the Rheumatology Clinic, Sahlgrenska University Hospital, during the period April 2002-December 2009. Thirteen patients had SLE, diagnosed according to the American College of Rheumatology criteria [14]. Four of these patients had also nephritis and 3 patients with SLE had anti-phospholipid antibody syndrome. Three patients fulfilled MCTD diagnosis according to classification criteria by Kasukawa [15, 16]. Results from 10 patients with thrombocytopenia diagnosed during the period April 2002 – November 2006 have been reported previously [17] and these patients were continuously followed up in the present study.

All patients except one had failed at least one course of methylprednisolone pulse therapy and/or intravenous immunoglobulin (IVIG) and high dose corticosteroids in combination with immunosuppressive drugs. Three patients received concomitantly IVIG and all patients were treated with immunosuppressive agents such as corticosteroids (n = 15), methotrexate (n = 6), azathioprine (n = 5), cyclophosphamide (n = 1), cyclosporine (n = 2) and mycophenolate mofetil (n = 3). One patient had undergone splenectomy without improvement. The patients´ main characteristics are summarized in the Table 1.

Anti-B cells treatment

RTX (Mabthera®) treatment was added to the ongoing immunosuppressive treatment regimen in patients that did not sufficiently responded to conventional therapy. RTX was given as 4 consecutive intravenous infusions once weekly at a dose of 375 mg/m^2 body surface. All patients except 2 were given pre-medication therapy with paracetamol (Panodil®, 1 g) orally, antihistamine klemastin (Tavegyl®, 2 mg) and hydrocortisone (Solu-Cortef®, 100 mg)

according to local treatment guidelines intravenously before RTX infusion. Two patients received methylprednisolone (Solu-Medrol® 125 mg) as a premedication.

Laboratory analyses

The clinical effect of RTX treatment in patients with thrombocytopenia was evaluated by analyses of platelet counts. All evaluations were performed at baseline and after 1, 3, 6, 12, and 24 months following RTX treatment. Levels of antibodies against double-stranded DNA were determined by radioimmunoassay.

Evaluation of anti-thrombocyte antibodies

For detection of anti-thrombocyte antibodies, the blood samples were collected into heparinized tubes, the thrombocyte counts were determined in a cell counter (Sysmex K-4500) and thereafter the samples washed twice with thrombocyte-buffer containing 1.85 g EDTA in 1000 ml phosphate buffered saline (PBS). The samples were incubated with following antibodies: PE-conjugated anti-human CD41a ((BD Bioscience); FITC-labeled rabbit anti-human IgG $(Fab)_2$ and IgM $(Fab)_2$ (Dako, Glostrup, Denmark) for 30 minutes at 4°C. Red blood cells were lysed using lysing solution (BD Bioscience). After washing steps the cells were suspended in 200 µl thrombocyte-buffer and analyzed on a FACScalibur (Beckton-Dickinson). The amounts of bound IgM and IgG antibodies on the surface of thrombocytes were calculated and the results expressed as a percentage of positive platelets. The samples from healthy blood donors were analyzed in parallel as negative controls in every experiment.

Evaluation of circulating B cells

The number of circulating B cells defined as CD19+ and CD20+ lymphocytes in peripheral blood was assessed at baseline and after 1, 3, 6, 12, and 24 months by flow cytometry in the routine analysis laboratory in the Department of Clinical Immunology at the Sahlgrenska University Hospital [17]. CD19+ B cells were chosen for determination of the number of circulating B cells before and after anti-CD20 treatment to avoid any possible interference of RTX with the flow cytometric assay. The minimum of 5000 cells were counted in the lymphocyte gate. The detection level of the method was 1%. Undetectable levels of CD19+ B (levels < 1% of the total lymphocyte population) were considered to indicate B cell depletion from the peripheral blood. Serum levels of immunoglobulin subclasses (IgG, IgM, IgA) were

determined by nephelometry, and the number of circulating immunoglobulin-producing cells was determined by an ELISPOT assay.

Response criteria

Complete response was defined by achievement of a platelet count >100x10^9/ml or by maintaining a platelet count >100x10^9/ml. Partial response was defined if the platelet count was 50–100x10^9/ml. The treatment failure was determined when no improvement occurred with regard to platelet counts [18]. Time to response was defined as the time from the first RTX administration to the achievement of any degree of response. Remission was defined as stable platelet count > 150x10^9/ml at least 3 months without or while tapering out corticosteroid treatment. Relapse or progression was defined as loss of response/remission criteria.

Statistical analyses

Nonparametric methods were used for statistical evaluation of data in most cases due to small sample size and uneven distribution. Clinical measures and all laboratory data are presented as medians and 25^{th} -75^{th} percentiles (IQR). Responses to RTX treatment at 1, 3, 6, and 12 months were compared with baseline values. Wilcoxon signed rank test for paired samples was used for comparison of different variables at baseline and follow up. P value < 0.05 was considered as statistically significant. All analyses were performed using StatView Software version 5.0.1 (SAS Institute Inc., NY, USA).

RESULTS

Patients' characteristics and demographics at baseline

All patients were females and the median age was 34 years (range 17-84). The majority of patients were Caucasian (n = 13) and 3 were Asian in origin. The median duration of the rheumatic disease before RTX-treatment was 9 years (range 0.2 – 27) and post-treatment follow up time 28 months (range 3 – 92). 10 patients (63%) were treated repeatedly with RTX either 2 (6 patients), 3 (2 patients) or 4 times (2 patients) during the follow up period. The indication for re-treatment was in 7 patients thrombocytopenia flare, in 2 patients APS and 1 patient received RTX 3 times due to nephritis flare. The baseline characteristics of patients are shown in table 1.

The clinical and immunological effects of RTX treatment

Complete depletion of B cells was achieved in 94% of cases 1 month after RTX treatment. In all but one patient there were no detectable CD19+ B cells in the circulation after first RTX cycle. A significant increase ($p = 0.0001$) of platelet counts was seen already after 1 month (median $58x10^9$/ml vs. $110x10^9$/ml) (Fig. 1). Within 3 months after first RTX cycle the platelet counts normalized in 10 patients (median $223x10^9$/ml) and in seven of them (44%) complete remission was achieved and maintained during the median follow up period of 18 (range 3 – 86) months. However, in 3 of these patients RTX treatment was repeated due to other SLE indication than thrombocytopenia. In 4 out of 16 patients the treatment response following first RTX was transient with relapses in thrombocytopenia occurring within median time of 5 (range 3-8) months. After the second RTX cycle 3 patients achieved complete remission that was maintained during the follow up period of respective 6, 11 and 30 months. In one patient with partial response to second treatment the third RTX cycle was needed to induce 31 months remission. Three out of seven patients who received the second RTX cycle due to thrombocytopenia flare had measurable levels of CD19+ cells (5%, 3% and 6% in respective patients) in the peripheral blood, four patients displayed undetectable levels of B cells.

Three patients (18%) did not respond to RTX treatment and their baseline median thrombocyte counts remained basically unchanged following 1, 3 and 6 months RTX treatment ($60x10^9$/ml versus $75x10^9$/ml, $69x10^9$/ml and $65x10^9$/ml, respectively).

Antibodies of IgM and IgG isotypes were measured in 12 out of 16 patients before treatment and 7 patients had follow up data. High levels (>10% of platelets with auto-antibodies bound to their surface) were detected in 7 patients before RTX treatment (IgG; median 15%, range 5-67%; IgM; median 11%, range 5-46%). The autoantibody levels decreased significantly following RTX treatment (Fig. 2) and 6 patients achieved complete remission after treatment. Of note, these patients had also high titers of anti-thrombocyte antibodies. Nine patients had anti–dsDNA antibodies prior RTX treatment and decrease was observed in 8 patients within 6 months following RTX treatment.

At RTX treatment initiation 88% of patients received oral corticosteroids. The median oral daily prednisolone dose decreased significantly ($p < 0.05$) already 1 month after treatment as compared to baseline. The baseline prednisolone dose (median 22.5 (interquartile range 11-36) mg) was tapered to 10 (5-15) mg and 6.2 (5-10) mg within 3 and 6 months ($p = 0.0056$ and $p = 0.0033$), respectively.

Safety aspects

RTX treatment was generally well tolerated. Twelve patients (75%) did not develop any severe allergic reactions or adverse effects after RTX infusions. Four patients discontinued treatment due to side effects. One patient developed osteitis/inflammation in the jaw probably originating from deep tooth infection present before RTX treatment and RTX treatment was stopped after the first infusion. The serum sickness symptoms with fever and rash developed in another patient after the second infusion during the first treatment cycle and the treatment was discontinued. However, 6.5 years later the patient received the second RTX cycle (4 weekly infusions) without complications. During the third and fourth RTX cycle only 1 weekly infusion was given. The treatment was also discontinued after two infusions during the second RTX cycle in the third patient due to the development of fever, rash and suspected allergic reaction. The development of leucopenia was seen in the fourth patient after the third infusion during the second RTX cycle. Three patients were lost for follow up: two patients moved 3 and 18 months, respectively; the third patient deceased due to dilated cardiomyopathy 18 months after RTX treatment.

DISCUSSION AND LITERATURE REVIEW

Approximately 7-30 % of patients with SLE will develop thrombocytopenia and in up to 16 % of patients it can predate the SLE diagnosis and be the initial disease manifestation [9, 19]. In a recent retrospective study by Ziakas et al [8] the prevalence of thrombocytopenia at the time of lupus diagnosis was found particularly high and was present in 58% of cases. Recent studies indicate that B cells participate in the pathogenesis of lupus and MCTD in several ways by producing polyclonal antibodies, cytokines and supporting the activation of dendritic and T cells [1]. Increased peripheral destruction of thrombocytes, associated with the presence of anti-thrombocyte antibodies on the surface of platelets, is the most likely pathogenetic mechanism [20]. The central role for B lymphocytes in the pathogenesis of these autoimmune diseases provides a rationale for the use of B-cell depletion with RTX, a chimeric anti-CD20 monoclonal antibody. Rituximab attacks B cells by several mechanisms including antibody-dependent cellular cytotoxicity, complement-dependent destruction of B cells as well as apoptosis induction [21, 22].

To date, RTX has been used in SLE patients as an off-label rescue medication in cases of different severe disease manifestations, including autoimmune thrombocytopenia refractory to conventional treatment with corticosteroids, IVIG and DMARDs [23, 24]. There is great evidence regarding the outcome of RTX treatment of SLE previously reviewed and published [24-26]. Also, two randomized clinical trials investigating B cell depletion in non-renal [27] and renal [28] SLE have been conducted but generated conflicting results and failed to meet their primary endpoints probably attributable to study design and the heterogeneity of the SLE cohorts. In these trials and reviews, however, the outcome of RTX specifically in SLE-related thrombocytopenias has not been addressed. In this study, we have evaluated the outcome of RTX treated SLE and MCTD patients with thrombocytopenia and reviewed the available published literature in this regard.

We performed a comprehensive PubMed search to identify all English language articles through December 2011 that documented RTX in the treatment of SLE and MCTD-related autoimmune thrombocytopenia (AITP) and thrombotic thrombocytopenia (TTP). The following combinations of MeSH terms were used: MCTD and RTX, SLE and RTX and thrombocytopenia; SLE and RTX. Articles describing the effect of RTX treatment in children with SLE-related thrombocytopenia, cases with primary idiopathic (not SLE/MCTD-related) ITP/TTP as well as primary antiphospholipid antibody syndrome have been reviewed elsewhere [26, 29-31] and were not included. All case falls were identified and patients with thrombocytopenia from larger SLE cohorts treated with RTX were extracted for analysis, if possible. References and review articles were searched for unique cases.

The first successful case of RTX therapy in the treatment of SLE and autoimmune thrombocytopenia was described in 2002 by Kneitz et al [32]. During the last decade published evidence on off-label RTX treatment in SLE-associated thrombocytopenia has increased and suggests a favorable effect. Our search yielded 24 articles including 18 case reports, 1 retrospective cohort study and 5 prospective studies documenting 65 patients [18, 32-55]. Fifty five received RTX due to immune thrombocytopenia and in 10 patients thrombotic thrombocytopenia was diagnosed. The results are summarized in Table 2. Only few cases have been published regarding use of RTX in the treatment of MCTD-related thrombocytopenia [17, 39, 49]. Fifty one patients out of 65 (78%) reviewed here (Table 2) achieved complete and 6 patients (9%) partial response within median time to response 4 (range 1-24) weeks and median follow up time 12.5 (2 – 60) months. There was no improvement regarding thrombocytopenia in 3 cases (4.6%) and 3 patients (4.6%) died. Relapses occurred in 5 out of 38 (13 %) patients within median time of 9 (range 9-24) months.

In corroboration with these results, in our study cohort the overall response rate to RTX was 81 % of which 11 patients (69 %) displayed complete and 2 patients (12.5 %) partial treatment response regarding thrombocytopenia. In addition, 7 patients (44 %) achieved complete long term remission regarding thrombocytopenia after first RTX cycle; 6 of 16 patients (37.5%) had relapse within period of 3 to 9 months and positively responded to one (n = 3) or two (n = 2) re-treatments with RTX. In one patient, only partial response was achieved and 3 patients (18.5 %) did not respond, whereas 1 patient (6%) died. Our study showed that relapses occurred during first nine months, and one to two re-treatments were sufficient to introduce patients in long term remission.

In addition to reviewed cases, Ten et al. recently reported analyzing data from randomized, double-blind, placebo-controlled EXPLORER trial that platelet levels normalized in a subgroup of 16 RTX-treated patients who entered the study with low baseline counts [56]. Unfortunately, these patients were not further analyzed in detail. Also, two prospective studies reported by Terrier[51] and Garcia-Carrasco[53] with 13 and 8 patients out of 136 and 52, respectively, showed that thrombocytopenia responded to RTX in 85% of cases. These results diminish the publication bias that otherwise would be obvious while analyzing data from case falls.

We observed that patients with high detectable levels of anti-platelet antibodies responded better to RTX therapy than patients without antibodies. This is in line with several others, although few, studies in which the levels of anti-platelet antibodies were analyzed [32, 34, 38, 42, 43, 50] (Table 2). In 6 SLE patients anti-platelet antibodies were identified and 5 of them (83 %) had no flare whereas one relapsed 12 months. Thrombocytopenia as a manifestation of APS in patients with SLE is frequently related to the presence of antiphospholipid antibodies and also in these patients RTX has shown beneficial effect [26, 57]. This finding is consistent with favorable effect of RTX treatment with patients with primary ITP [29, 30] and supports a pathogenetic role for auto-antibodies in platelet destruction.

In most published studies RTX has been given intravenously once weekly during 4 consecutive weeks at the dosage of 375mg/m^2 and in some studies rheumatoid arthritis schedule with 1000 mg twice biweekly has been administered. However, in a recent prospective study by Chen et al. [18] RTX at a low dose of 100 mg once weekly for 4 weeks was shown to induce complete response in 60% of SLE patients with severe refractory thrombocytopenia at week 12.

In conclusion, current evidence indicates that rituximab therapy is efficacious in approximately 80 % of patients with AITP secondary to SLE/MCTD whose conditions had

failed previous immunosuppressive therapies. Best response may be expected in patients with high titers of anti-platelet antibodies and RTX can induce long-term complete remission in these patients. We believe that these results warrant further prospective trials to confirm the safety and efficacy of RTX in severe ITP secondary to SLE and/ or MCTD.

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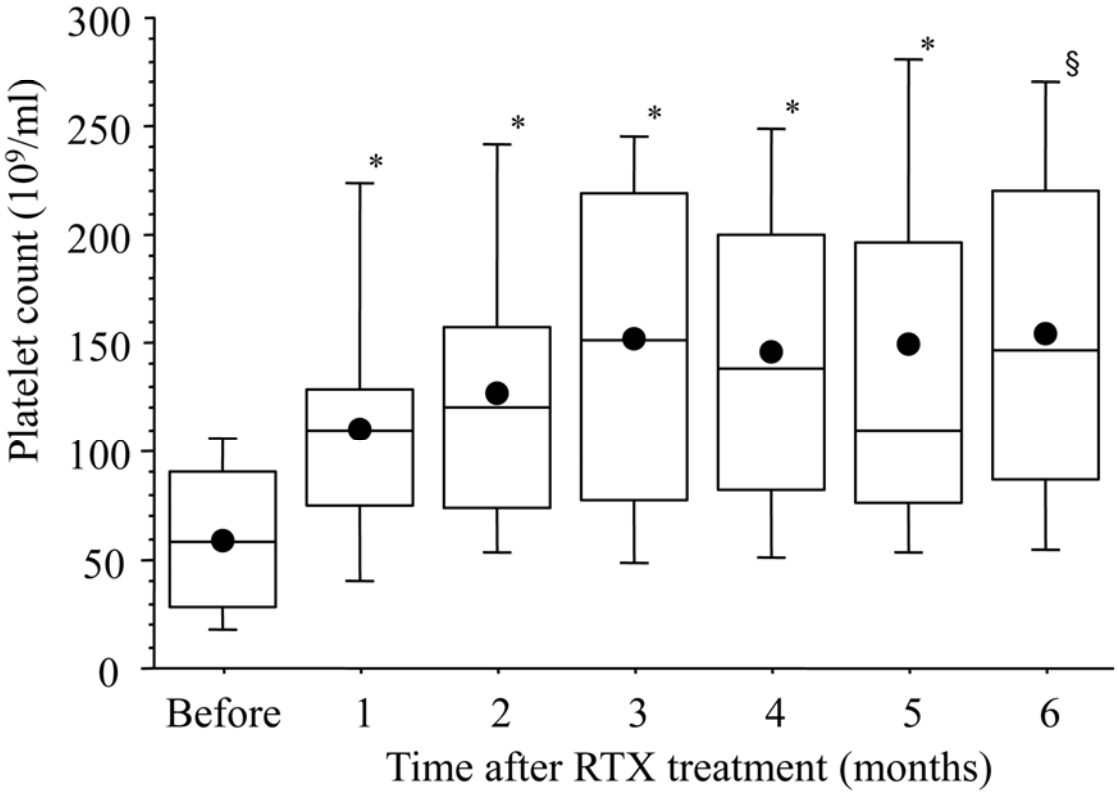
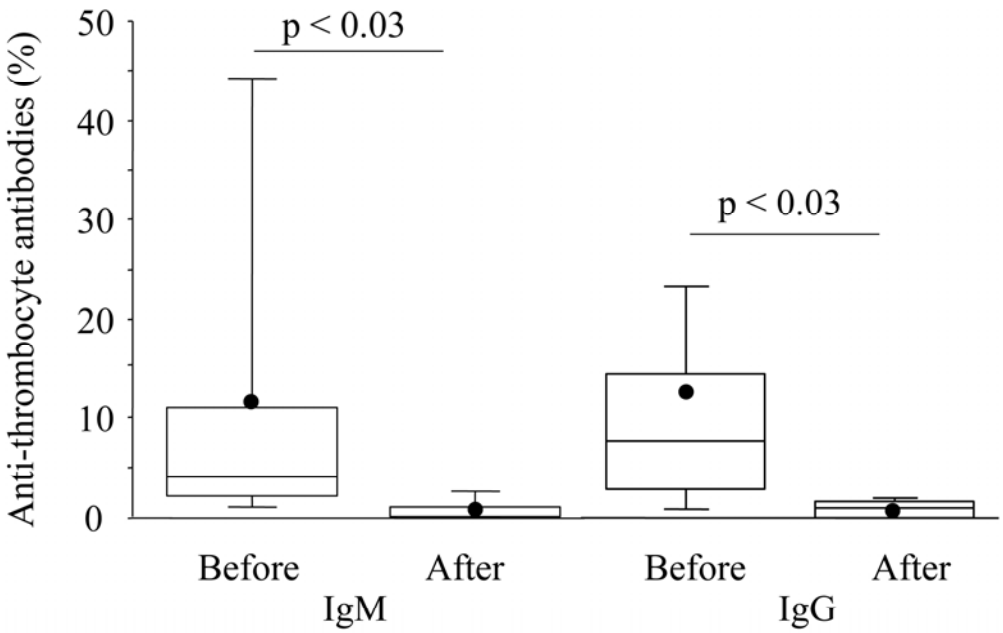
Figure 1.



Platelet count before and following RTX treatment

Box plots show the 25th and 75th percentiles. Horizontal black solid lines within boxes indicate medians, filled circles indicate means. Vertical bars indicate the 5th and 95th percentiles. * $p < 0.0005$; § $p < 0.005$ as compared to levels before

Figure 2.



Changes in anti-platelet antibodies following RTX treatment

Box plots show the 25th and 75th percentiles. Horizontal black solid lines within boxes indicate medians, filled circles indicate means. Vertical bars indicate the 5th and 95th percentiles

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Table 1

Characteristics of rituximab treated SLE and MCTD patients with immune thrombocytopenia.

Pat No.	Age	DD, years	Follow up, months	Previous treatment	Diagnosis and reason for RTX treatment	Con-comitant treatment	RTX repeated, times, (interval between treatments months)	Response and time to response, months	Time to ITP flare, months
1	33	8	92	CS, AZA, IVIG, CYA, splenectomy	SLE, Thrombocytopenia	CS, AZA or MTX, IVIG	4 (78, 7, 6)	1) No response 2) PR – 4 m 3) PR – 2 m 4) PR – 1 m	5 m 3 m 6 m
2	17	10	3 [a]	CS,IVIG,CYA	SLE, Thrombocytopenia	CS, IVIG	1	CR – 1 m	No flare 3 months
3	52	21	86	CS, CYC, AZA, MTX (splenectomy) [b]	SLE, Thrombocytopenia Nephritis, APS	CS, MTX	2 [c] (65)	CR – 3 m	No flare / Remission 86 months
4	34	10	18	CS, IVIG, AZA, MTX, CYC, CYA, MMF	SLE, Thrombocytopenia	CS, IVIG	1	No response	ǂ
5	21	3	46	CYC, MMF, RTX	SLE, Thrombocytopenia, Nephritis	CYA, MMF	4 [c] (6, 6, 27)	CR – 1 m	No flare / Remission 46 months

6	37	2	47	CS, CYC, CYA	SLE, Thrombocytopenia, APS	CS, MMF	3 (8, 8)	1) CR – 2 m 2) PR – 1 m 3) CR – 1 m	4 m 2 m No flare / Remission 31 months
7	30	10	46	CS, AZA,HCQ	SLE, Thrombocytopenia	CS, AZA	2 (40)	CR – 1 m CR – 1 m	6 m No flare / Remission 6 months
8	84	12	17 [a]	CS, MTX	MCTD, Thrombocytopenia	CS, MTX	2 (6)	CR – 1 m CR – 6 m	3 m No flare / Remission 11 months
9	49	27	38	CS, CYA, AZA, IVIG	SLE, Thrombocytopenia	CS, MTX	2 (23)	No response	
10	30	13	38	CS, AZA	MCTD, Thrombocytopenia	CS, AZA, CYA	2 (8)	CR – 2 m CR – 3 m	8 m No flare / Remission 30 months
11	37	0.25	15	no DMARDs	SLE, Thrombocytopenia	CS, AZA	1	CR – 1 m	No flare / Remission 15 months
12	60	1	28	CS, MTX	MCTD, Thrombocytopenia	CS, MTX	3 (10, 11)	PR – 2 m CR – 3 m CR – 1 m	6 m 9 m No flare / Remission 7 months
13	57	17	27	CS,MMF,AZA	SLE, Thrombocytopenia	CS, MTX	1	No response	

14	45	0.1	12	CS, AZA	SLE, Thrombocytopenia APS	CS, AZA	2 [c] (8)	CR – 1 m	No flare / Remission 12 months
15	29	2	18	CS,AZA, CYA, MMF	SLE, Thrombocytopenia, Nephritis	CS, MMF	1	CR – 1 m	No flare / Remission 18 months
16	33	8	20	CS, IVIG, AZA, MMF	SLE, Thrombocytopenia, Nephritis	CS, CYC→ MMF	1	CR – 1 m	No flare / Remission 20 months

[a] – Patients moved and were lost for follow up

[b] – The patient underwent splenectomy due to traumatic bleeding 3 months after the second RTX cycle.

[c] – RTX re-treatment was repeated due to other indication than thrombocytopenia;

ǂ – Patient died;

DD – disease duration; ITP – immune thrombocytopenia; DMARDs – disease modifying anti-rheumatic drugs; AZA – azathioprine; IVIG – intravenous gamma globulin; MMF – mycophenolate mofetil; CYA – cyclosporine; MTX – methotrexate; HCQ – hydroxychloroquine; CYC – cyclophosphamide; CS – corticosteroids; APS – antiphospholipid syndrome; CR – complete response, defined as platelet count >100x10^9/ml; PR – partial response, defined as platelet count 50-100x10^9/ml. Remission was defined as stable platelet count >150x10^9/ml at least 3 months while decreasing corticosteroid dose.

Table 2 Previous reports on the use of rituximab in thrombocytopenia related to SLE and/or connective tissue diseases.

Author	Year	Platelet-associated anti-bodies	Study	Indication (Diagnosis)	Pat No	Previous treatment that failed	RTX Dose	Response regarding ITP	Time to response/ remission (weeks)	Follow up (month)	Relapses during follow up
Kneitz [28]	2002	Positive → no change	Case report	1– AITP (SLE) 2 –A ITP, nephritis	2*	1) CS, splenectomy 2) CS, CYC, AZA, MMF,	375 mg/m^2/week x 4 infusions	1 – CR 2 – PR	12 w -----	30 m ------	No -----
Tomietto [30]	2004	Positive →negative	Case report	AITP, AIHA, CNS (SLE)	1	CS, CYA, IVIG, MMF, AZA	375 mg/m^2/week x 4 infusions	1 – CR	2 w	16 m	12 m
Van den Bergh [31]	2005		Case report	AITP, AIHA, nephritis, pneumonitis, (SLE/SS overlap)	1	CS, CYC, IVIG, plasmaferesis	375 mg/m^2/week x 4 infusions	CR	4 w	12 m	No
Ahn [32]	2005		Case report	AITP , (SLE, severe APS)	1	CYC, plasmaferesis	375 mg/m^2/week x 4 infusions	CR	7 w	15 m	No
Paran [33]	2006		Case report	AITP, nephritis, (SLE)	1	CS, CYC, IVIG	375 mg/m^2/week x 4 infusions	CR	rapid		No
Anandacoo-marasamy [34]	2006	Positive → NA	Case report	AITP, (SLE, severe APS)	1	CS, CYC, IVIG, CYA, VCR, DPS	375 mg/m^2/week x 4 infusions	CR	ca 6 w	30 m	No
Niewold [35]	2006		Case report	1 – TTP (SLE) 2 – TTP (overlap CTD)	2	1) CS , plasma exchange 2) CS , plasma exchange, hemo-dialysis	375 mg/m^2/week x 2 and 4 infusions, respectively	CR	1 w	2 m	No
Ng [36]	2007		Open prospective study	2 – AITP (SLE) 1 – AITP,	3	1) CS, HCQ 2) CS, HCQ,AZA 3) CS,HCQ,CYC,	1000 mg / 2 weeks x 2 infusions	CR		9, 47, 60 m	No

			(32 pat)	nephritis, AIHA, (SLE)		AZA, MMF, IVIG					
Limal [37]	2007		Case report	TTP (SLE,APS)	1	CS, AZA, HCQ, plasma exchange	375 mg/m^2/week x 4 infusions	CR	1 w	15 m	No
Fukushima [38]	2008	IgG decrease	Case report	AMT, CNS, serositis, nephritis, (SLE)	1	CS, CYA	375 mg/m^2/week x 2 infusions	CR	2.5 m	8 m	No
Kittaka [39]	2008	IgG decrease	Case report	AIHA, AITP, (SLE)	1	CS, CYA, IVIG, splenectomy	375 mg/m^2/week x 2 infusions	CR	4 w	6 m	No
Hundae [29]	2008		Case report	TTP, nephritis, (SLE)	1	CS, HCQ, plasma exchange	2 courses	CR			No
Nadri [40]	2009		Case report	AITP, ESRD (SLE)	1	CS, CYC, IVIG, MMF, CYA, dialysis, plasma exchange	375 mg/m^2/week x 4 infusions	CR	4 w		No
Reis [41]	2009		Case report	AITP (SLE)	2*	1) CS, AZA 2) CS, AZA, IVIG, DAZ	375 mg/m^2/week x 4 infusions	CR CR		17 m 9 m	No 9 m
Mardjuardi [42]	2009		Case report	AITP, nephritis, (SLE)	1	CS, MMF	500 mg / 2 weeks x 3 infusions	CR	2 w	7 m	No
Letchumanan [43]	2009		Cohort study	TTP (SLE)	3*	plasmaferesis, CS (3), MMF (3) , CYC (1)	375 mg/m^2/week x 1, 4 and 11 infusions	2 – died 1 – CR	3 w		
Lateef [44]	2010		Retrospective cohort **(10 pat)**	AITP (SLE)	3*	CS (3), MMF (3), CYC(2), HCQ (3), CYA(3), AZA (2), IVIG (1)	375 mg/m^2/ 2 weeks	2 – CR 1 –PR		median 18 m	2 – no 1 – 24 m
Zheng [45]	2010		Case report	AITP (3 SLE, 1 CTD)	4*	CS (4), CYC(3), IVIG (3), VCR (3),	375 mg/m^2/week x 3-4 infusions	3 – CR 1 – Death	1-4 w	6, 13, 35 m	No

						CYA (2), splenectomy (2)					
Lee [46]	2010	Positive → NA	Case report	TTP (SLE)	1	CS, plasmaferesis	375 mg/m^2/week x 3 infusions	CR	2 w	10 m	No
Terrier [47]	2010		Prospective multi-center registry analysis **(136 pat)**	AITP (SLE)	13 *	Various [a]	1000 mg / 2 weeks x 2 infusions or 375 mg/m^2/week x 4 infusions	CR – 10 PR – 2 NR - 1	mean 6 ± 3 m		
Niaz [48]	2010		Case report	TTP (SLE)	1	CS, plasma exchange	375 mg/m^2/week x 3 infusions	CR	2 w	8 m	No
Garcia-Carrasco [49]	2010		Prospective single centre **(52 pat)**	AITP (SLE)	8*	Various [a]	1000 mg / 2 weeks x 2 infusions	1 – AE 6/7 - CR	mean 12 w	6 m	6/7 - No
Chen [12]	2011		Prospective pilot study **(10 pat)**	AITP (SLE)	10	CS, CYC, IVIG, CSA, VCR; splenectomy (1)	100 mg /week x 4 infusions	6 – CR 2 – PR 2 – No	2 – 4 w 4 – 12 w 2 – 24 w	8.5 m	4 – No 2 – w 36
Gupta [50]	2011		Case report	AITP, PRCA (SLE)	1	CS, IVIG, CYC	1000 mg /10 days x 2 infusions + 1 dose to reach 375 mg/m^2/ 4weeks	CR	4 w	7 m	No
Pinto [51]	2011		Prospective observational cohort **(42 pat)**	AITP (SLE)	1*		100 0 mg / 2weeks x 2 infusions	CR	12 w	21 m	No

VCR – vincristine; DAZ – danazol; CYC – cyclophosphamide; CS – corticosteroids; AZA – azathioprine: IVIG – intravenous immunoglobulin; CSA – cyclosporine; MMF – mycophenolate mofetil; DPS – dapsone; SLE – systemic lupus erythematosus; SS – systemic sclerosis; CTD – connective tissue disease; TTP – thrombotic thrombocytopenic purpura; AMT – amegacaryocytic thrombocytopenia; A ITP – autoimmune thrombocytopenia; AIHA – autoimmune hemolytic anemia; PRCA – pure red cell aplasia; NA – not analyzed

CR – complete remission; PR – partial remission; NR – no remission; AE – adverse effects

[*] Only SLE patients with thrombocytopenia were included in the table from larger study cohorts. [a] Larger SLE patient cohorts analyzed and specific treatment was not indicated for AITP patients