

Long-term follow-up of idiopathic thrombotic thrombocytopenic purpura treated with rituximab

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Order of Authors: Jens Marcus Chemnitz, PD; Jens Uener; Michael Hallek, Univ. Prof.; Christof Scheid, PD

Abstract: Rituximab may be used to treat patients with thrombotic thrombocytopenic purpura (TTP) refractory to plasma exchange or recurrent disease. While initial response rates are reported to be high, long-term follow-up data of patients treated with rituximab are not available to date however important to estimate the safety and benefit of this treatment. 12 patients with non-familial idiopathic TTP refractory to plasma exchange or with recurrent disease treated with rituximab between 2000 and 2008 were re-examined. The median follow-up was 49.6 months, ranging from 11 to 97 months. All patients achieved initial complete remission after application of rituximab. During follow-up, nine patients remained disease-free and three patients suffered from recurrent disease. All patients with recurrent disease responded to subsequent rituximab therapy. No long-term side effects were noted during the follow-up period. In conclusion, rituximab represents an effective second-line treatment option in relapsing or refractory TTP. Still, patients need to be closely monitored for relapses with extended follow-up.

Response to Reviewers: Reviewer #1

1. Abstract, line 2: I think "acceptable" is not appropriate until larger studies are done. I would suggest changing to: "Rituximab may be used to treat relapsed or refractory TTP"

Changes were made accordingly.

2. Abstract, line 5: While initial response rates are "reported to be" high..

Changes were made accordingly.

3. Abstract, line 32: "Still, patients need to be closely monitored as the relapse rate increases with longer follow-up." Change to ".monitored for relapses with extended follow-up".

Changes were made accordingly.

4. Introduction, line 41: Remove "not otherwise explained"

Changes were made accordingly.

5. Introduction, line 51: Revise: "Acquired idiopathic TTP is differentiated from hereditary TTP (Upshaw- Schulman syndrome)." ie how are they differentiated?

The differentiation between acquired and congenital TTP follows in detail in the next paragraph:

"Large progress was made in recent years in understanding the pathophysiology of TTP. Moake et al. described unusually large von Willebrand factor (VWF) multimers in the plasma of patients with relapsing acquired or congenital TTP causing intravascular platelet aggregation occluding the microvasculature [2]. Tsai et al. [3] and Furlan et al. [4] independently identified a VWF-cleaving protease (ADAMTS13) missing from the plasma of patients with congenital TTP [5] and severely deficient in patients with acquired idiopathic TTP. Mutations in the ADAMTS13 gene were shown to cause congenital TTP [6], whereas IgG autoantibodies inhibit the enzyme in most cases of the acquired form of the disease [7-8]."

To avoid repetitions we left this part of the introduction as it was. If the editor has a distinct opinion to this point, we are more than willing to change it.

6. Introduction, line 12 (2nd page of introduction) and throughout the paper: "plasma exchange against fresh frozen plasma" change to ". WITH fresh frozen plasma."

Changes were made accordingly.

7. Introduction, line 44 (2nd page of introduction): "approximately 95% of reported patients with refractory or relapsing TTP achieved complete remission"- please change to "although only a small number of patients have been reported worldwide, the majority of patients with refractory or relapsing TTP achieved.."

Changes were made accordingly.

8. Results/ Discussion, line 9 (2nd page): "After a median follow up of nearly 50 months, all patients were re-examined. At this time point, all patients presented in good clinical condition without evidence of TTP related symptoms. Three out of the 12 patients (25%) had experienced relapse after rituximab treatment." Change to: "After nearly 50 months for follow up, 3 of 12 patients had relapsed."

Changes were made accordingly.

9. Results/ Discussion, line 24: change "intermittent B cell recovery" to "transient B cell depletion"

Changes were made accordingly.

10. Results/ Discussion, line 30: "therefore a relapse rate of 25% in the present study still represents a successful therapy, since it was exclusively used as second line treatment after failure of the standard therapy with plasma exchange." This leading statement should be omitted.

Changes were made accordingly.

11. Line 48: "36 respectively 40 months". Please revise.

Changes were made to "36 and 40 months"

12. Line 51: "Both patients did not achieve complete remission until cessation of plasma exchange, but only after rituximab treatment." Please revise.

Changes were made to "The individual participation of plasma exchange or rituximab to successful treatment remains open, however both patients did not achieve complete remission after cessation of plasma exchange."

13. Line 39 (last page): change to: "These limitations in mind, it appears that rituximab is an effective second-line treatment option for patients with idiopathic TTP with SOME PATIENTS ACHIEVING sustained responses even after long-term follow-up."

Changes were made accordingly.

14. Line 46 (last page): "will have to answer the question if" change to "are needed to determine whether."

Changes were made accordingly.

15. It is still not clear, as written in the text, that B cell levels and A13 were measured at the end of follow up.

This point has been further clarified in the "Design and Methods" section ("At the end of follow up, documentation of clinical symptoms including any possible side effects related to rituximab therapy was performed after all patients gave their informed consent. Additional to complete medical history, physical examination and standard laboratory testing, ADAMTS13 enzyme activity and the presence of an inhibitor was analyzed..... To evaluate the degree of present B-cell depletion at the end of follow up, CD19 measurement was performed using flow cytometry.") as well as in the "Results and Discussion" section ("In our patient collective, no patient suffered from consequences of long-term B cell depletion, since no treatment related, especially infectious complications were reported. In all but two patients B cell counts had recovered at the end of follow up..." "At the end of follow-up, ADAMTS13 activity and presence of an inhibitor was analyzed...")

Additionally, it is mentioned in Table 1.

16. In the introduction the authors define remission as clinical criteria plus normal A13 levels; yet in their methods they considered TTP patients regardless of A13 levels. Please reconcile.

In the "Introduction" section, we changed the paragraph to "...complete remission with complete clinical and laboratory responses including normal ADAMTS13 level and disappearance of anti-ADAMTS13 antibodies" and referenced to Sadler et al. [Sadler JE. (2008) Blood 112(1):11-8.].

ADAMTS13 is no diagnostic criteria as a condition sine qua non for considering TTP and testing ADAMTS13 during initial presentation is not mandatory to date. However it likely provides useful prognostic information at diagnosis, since severe ADAMTS13 deficiency identifies a specific mechanism of idiopathic TTP that usually responds well to plasma exchange but tends to relapse.

17. Where the authors discuss the 2 patients treated "exclusively" with rituximab, please describe how much plasma they actually received (ie before having the severe allergic reactions) and I would suggest removing word "exclusively" as it implies that no PE was given.

Patient #1 received 22 x plasma exchange treatment, which was disrupted after severe allergic reactions despite high dose steroid treatment. Patient #2 and developed allergic bronchial asthma after 10 x plasma exchange. The reviewer is right to suggest removing the word "exclusively". The paragraph was changed accordingly.

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Abstract

<u>Rituximab may be used to treat patients</u> with thrombotic thrombocytopenic purpura (TTP) refractory to plasma exchange or recurrent disease. <u>While initial response rates are</u> reported to be high, long-term follow-up data of patients treated with rituximab are not available to date however important to estimate the safety and benefit of this treatment. 12 patients with non-familial idiopathic TTP refractory to plasma exchange or with recurrent disease treated with rituximab between 2000 and 2008 were re-examined. The median follow-up was 49.6 months, ranging from 11 to 97 months. All patients achieved initial complete remission after application of rituximab. During follow-up, nine patients remained disease free and three patients suffered from recurrent disease. All patients with recurrent disease responded to subsequent rituximab therapy. No long-term side effects were noted during the follow-up period. In conclusion, rituximab represents an effective second-line treatment option in relapsing or refractory TTP. Still, patients need to be closely <u>monitored for relapses</u> with extended follow-up.

Introduction

Thrombotic thrombocytopenic pupura (TTP) is a life-threatening disease <u>characterized</u> <u>by microangiopathic hemolytic anemia</u>, consumptive thrombocytopenia and various organ dysfunctions such as neurological symptoms, renal damage and fever. Idiopathic TTP is distinguished from secondary TTP following predisposing conditions like cancer, eclampsia, drug toxicity or hematopoetic stem cell transplantation [1]. Acquired idiopathic TTP is differentiated from hereditary TTP (Upshaw-Schulman syndrome). Large progress was made in recent years in understanding the pathophysiology of TTP. Moake et al. described unusually large von Willebrand factor (VWF) multimers in the plasma of patients with relapsing acquired or congenital TTP causing intravascular platelet aggregation occluding the microvasculature [2]. Tsai et al. [3] and Furlan et al. [4] independently identified a VWF- cleaving protease (ADAMTS13) missing from the plasma of patients with congenital TTP [5] and severely deficient in patients with acquired idiopathic TTP. Mutations in the ADAMTS13 gene were shown to cause congenital TTP [6], whereas IgG autoantibodies inhibit the enzyme in most cases of the acquired form of the disease [7-8]. The standard treatment of acquired idiopathic TTP consists of plasma exchange with fresh frozen plasma [9-10], thereby removing large VWF multimers as well as autoantibodies to ADAMTS13 and replacing the enzyme [11-12]. As a result of this therapy the overall survival could be improved from 10% to more than 80% [13-14]. However, up to one third of patients relapse after successful treatment of an acute episode, and especially patients with initial severe ADAMTS13 deficiency have a significantly increased risk of relapsing TTP [11, 15]. In case of plasmarefractory TTP, which is defined as persistent thrombocytopenia or lactate dehydrogenase elevation after a total of seven daily plasma exchange procedures, current guidelines advise to intensify plasma exchange and to add corticosteroids in patients not receiving immunosuppressive drugs [12]. Due to the autoimmune nature of acquired idiopathic TTP rituximab has been used as a second-line immunosuppressive intervention in TTP cases refractory to plasma exchange as well as in frequently relapsing patients [16-19]. According to treatment schedules in Non-Hodgkin's lymphoma, rituximab is mainly administered at doses of 375 mg/m^2 weekly for an average of 4 doses. Using this regimen, although only a small number of patients have been reported worldwide, the majority of patients with refractory or relapsing TTP achieved complete remission with complete clinical and laboratory responses including normal ADAMTS13 level and disappearance of anti-ADAMTS13 antibodies [11]. In the reported cases, only 10% - 13% of patients relapsed after rituximab treatment after a median follow-up of 11 months [17-18]. Especially when considering B-cell recovery nine to twelve months after rituximab application, data for longterm follow-up is required.

Design and Methods

Diagnosis of TTP was based on clinical and laboratory findings - including consumptive thrombocytopenia, hemolytic anemia and elevated schistocytes in the peripheral blood smear - irrespective of ADAMTS13 activity. All patients were initially treated with standard treatment including daily <u>plasma exchange with fresh frozen plasma</u> (1.5 x plasma volume) and corticosteroids. Decision of rituximab treatment initiation was made in case of non-response to daily plasma exchange, severe allergic reactions to plasma exchange leading to treatment discontinuation or relapse after initial successful therapy. During 2000 and 2008, 30 patients with an acute episode of non-familial idiopathic TTP were treated at the University Hospital of Cologne, 12 patients treated with rituximab were identified and re-examined. Due to different pathophysiology, patients with TTP secondary to bone marrow transplantation, rheumatologic diseases or active cancer were excluded.

In most cases, treatment schedule consisted of rituximab at a dose of 375 mg/m² per week for 4 consecutive weeks administered in parallel to daily plasma exchange, except for two patients with intolerance to plasma exchange after a total of 22 and 10 exchange procedures leading to disruption of plasma exchange therapy. After application of rituximab, plasma exchange was interrupted for 24 hours. In general, plasma exchange was continued until clinical remission and platelet count above 150 x $10^9/1$ for longer than 48 hours.

Non-response to plasma exchange was defined as either persistent thrombocytopenia or elevated lactate dehydrogenase (LDH) after one week of ongoing daily <u>plasma exchange with</u> <u>fresh frozen plasma</u>. Relapse was defined as recurrence of at least one further acute TTP episode after initial successful standard therapy with plasma exchange. Complete response

was defined as a sustained normal platelet count, absence of clinical symptoms of TTP and cessation of plasma exchange therapy.

At the end of follow up, documentation of clinical symptoms including any possible side effects related to rituximab therapy was performed after all patients gave their informed consent. Additional to complete medical history, physical examination and standard laboratory testing, ADAMTS13 enzyme activity and the presence of an inhibitor was analyzed. ADAMTS13 activity was analyzed using the TECHNOZYM ADAMTS13 activity ELISA according to manufacturer's instructions. The presence of an inhibitor was analyzed using TECHNOZYM ADAMTS13 INH ELISA (both Technoclone Deutschland, Heidelberg, Germany). To evaluate the degree of present B-cell depletion at the end of follow up, CD19 measurement was performed using flow cytometry. Prior to the collection of data, the study was examined by the local ethics committee.

Results and Discussion

At the time of initial TTP diagnosis, median age of the 12 patients was 42.5 years. 75% of patients were female. The main clinical manifestations during acute TTP episode consisted of neurological symptoms (n = 7), renal impairment (n = 2) or abdominal pain (n = 4). At presentation, the mean platelet count was decreased to 19.8 x 10^9 /l and mean LDH was elevated to 1018 U/l (reference < 250 U/l). All patients had <u>plasma exchange with fresh frozen plasma</u> and steroid treatment prior to rituximab, 4 patients were additionally treated with vincristine (2 mg i.v.). 7 patients were treated during the first episode of acute TTP, among those 5 received rituximab because of insufficient standard therapy with plasma exchange; <u>2 patients suffered from severe allergic reaction leading to disruption of plasma exchange after 22 and 10 exchange procedures</u>. 5 patients received rituximab because of relapse after at least one prior episode of acute TTP initially successfully treated with standard therapy. Baseline characteristics of all patients are shown in **Table 1**. Due to assay

availability, unfortunately only in a minority of patients ADAMTS13 activity was analyzed before initiation of rituximab therapy. After application of rituximab no severe acute side effects were noted and all patients responded with complete remission. This is in line with previous data reporting acute response rates of more than 90% [11, 20].

After nearly 50 months follow up, 3 of 12 patients had relapsed. Previous reports estimate the relapse rate after rituximab at ca. 10% [11, 17 - 18]. However, this existing data is based on few patients with short follow-up, thus objective statements to frequency of relapse after rituximab can not be made to this time point. The higher relapse rate in our collective is probably reflecting the longer follow-up period reported in the present study with transient B cell depletion and subsequent formation of new antibodies directed against ADAMTS13. Notably, 5 patients suffered from partly frequent relapses before initiation of rituximab treatment, 3 of these patients remained disease free even after long-term follow-up. Only one patient relapsed after rituximab treatment during the initial TTP episode. All relapsing patients responded to subsequent rituximab therapy. Interestingly, the two patients treated with rituximab during initial TTP episode due to severe allergic reaction after initial standard therapy with plasma exchange had a sustained response without recurrence of TTP after a follow up of 36 and 40 months. The individual participation of plasma exchange or rituximab to successful treatment remains open, however both patients did not achieve complete remission after cessation of plasma exchange. In two patients a maintenance therapy with rituximab was initiated consisting of 375 mg/m^2 every four weeks. One patient remains disease free with ongoing maintenance therapy, the other is in sustained complete remission despite abortion of maintenance therapy after three months.

Treatment decision making in case of further relapse after rituximab is difficult and not evidence based to this time point. Rituximab has been used as pre-emptive treatment in patients with anti-ADAMTS13 autoantibodies [21], thus rituximab maintenance maybe an option, however possibly related to long term side effects like progressive multifocal leucoencephalopathy [22]. An alternative treatment option would be a splenectomy, which is supported by data provided by Kappers-Klunne et al. 2005 [23]. 33 patients with TTP were retrospectively reported who were splectomised due to refractory or relapsing disease. After median follow up of 109 months the 10-year relapse-free survival was 70%. As these data were generated without rituximab being a treatment alternative, one has to balance between risks of the operation procedure, postoperative complications as well as infectious complications post-splenectomy against consequences of long-term B cell depletion during rituximab maintenance therapy.

In our patient collective, no patient suffered from consequences of long-term B cell depletion, since no treatment related, especially infectious complications were reported. <u>In all but two patients B cell counts had recovered at the end of follow up</u>, one of the two patients is currently treated with rituximab maintenance therapy. Thus, the safety profile of rituximab used in this hematological autoimmune disorder seems to be justifiable, however, the number of patients presented here is too small to conclude rituximab as being a safe treatment option in refractory or relapsing TTP.

At the end of follow-up, ADAMTS13 activity and presence of an inhibitor was analyzed. Testing ADAMTS13 activity during remission is recommended, since idiopathic TTP caused by ADAMTS13 deficiency relapses more frequently, and monitoring might identify patients presenting without TTP related symptoms however with imminent relapse [11]. In our patient collective ADAMTS13 activity was reduced in 25%. No patient had an enzyme activity less than 5% which would imply the need of subsequent rituximab therapy, although patient #12 presenting with ADAMTS13 activity of 7% and high inhibitor concentrations (64 U/ml) is closely monitored. Inhibitors against ADAMTS13 at time of reevaluation were detected in four patients.

The favor of the reported data is a long follow up. A median follow-up of nearly 50 months is reflecting the longest follow-up period after rituximab treatment reported to date. In

the existing literature patients treated with plasma exchange and rituximab have been followed for up to three years [17, 18], however median follow-up is reported less than 11 months [12]. Longer follow-up is required for addressing possible long-term side effects of rituximab and the safety of this treatment procedure [24]. Moreover, reflecting the initial high response rates after rituximab treatment, longer follow-up periods are required to assess the exact benefit of this therapy, since late relapses have to be taken into account. The limitation of the present study is the retrospective design possibly leading to incomplete reporting of adverse events during the long follow up period. However, most patients were seen on a regular basis in our outpatient clinic and medical history as well as laboratory testing was routinely performed. Another limitation is the small patient number making it difficult to draw final conclusions especially regarding the safety of rituximab treatment in TTP. These limitations in mind, it appears that rituximab is an effective second-line treatment option for patients with idiopathic TTP with some patients achieving sustained responses even after long-term follow-up. Prospective multicenter studies are needed to determine whether rituximab can be safely used just as salvage therapy or even initially in addition to plasma exchange to decrease the relapse rate after first line treatment [25].

Authorship and Disclosures

JMC recruited the patients and wrote the paper, JU recruited the patients, MH discussed the results. CS wrote the paper and takes the main responsibility for the paper. The authors reported no potential conflicts of interest.

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Table 1.Follow-up of reported patients

Case #	Age (years)	Sex	Prior TTP episodes	Reason for rituximab initiation	# of Rituximab applications 375 mg/m2	Follow- up (months)	Relapse after rituximab	Maintenance therapy	ADAMTS13 baseline (%) (a)	ADAMTS13 end of follow up (%) (a)	Inhibitor end of follow up (U/ml) (b)	CD19 end of follow up (%) (c)
1	57	Male	-	Allergy to PE	4	36	-	-	n.a.	77	22	n.a.
2	34	Female	-	Allergy to PE	4	40	-	-	< 3	77	11	11
3	36	Female	-	Refractory to PE	4	41	-	-	n.a.	57	n.a.	7
4	46	Female	3	Relapse	4	52	-	-	< 3	15	7	14
5	26	Female	1	Relapse	4	67	4	+	< 3	102	n.a.	0
6	22	Male	1	Relapse	4	11	1	+	90	103	13	12
7	45	Male	3	Relapse	4	46	-	-	< 3	29	n.a.	19
8	41	Female	-	Refractory to PE	4	46	-	-	n.a.	72	38	17
9	72	Female	-	Refractory to PE	7	22	-	-	76	95	14	3
10	51	Female	-	Refractory to PE	4	67	-	-	n.a.	53	48	10
11	44	Female	1	Relapse	4	70	-	-	n.a.	76	11	n.a.
12 (d)	36	Female	-	Refractory to PE	2	97	1	-	7	7	64	8

(a) Reference: 50 – 110 %

(b) Reference: < 16 U/ml

(c) Reference: 7 - 23 %

(d) This patient had been formally reported (16)

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9. Results/ Discussion, line 24: change "intermittent B cell recovery" to "transient B cell depletion"

Changes were made accordingly.

10. Results/ Discussion, line 30: "therefore a relapse rate of 25% in the present study still represents a successful therapy, since it was exclusively used as second line treatment after failure of the standard therapy with plasma exchange." This leading statement should be omitted.

Changes were made accordingly.

11. Line 48: "36 respectively 40 months". Please revise.

Changes were made to "36 and 40 months"

12. Line 51: "Both patients did not achieve complete remission until cessation of plasma exchange, but only after rituximab treatment." Please revise.

Changes were made to "The individual participation of plasma exchange or rituximab to successful treatment remains open, however both patients did not achieve complete remission after cessation of plasma exchange."

13. Line 39 (last page): change to: "These limitations in mind, it appears that rituximab is an effective second-line treatment option for patients with idiopathic TTP with SOME PATIENTS ACHIEVING sustained responses even after long-term follow-up."

Changes were made accordingly.

14. Line 46 (last page): "will have to answer the question if" change to "are needed to determine whether."

Changes were made accordingly.

15. It is still not clear, as written in the text, that B cell levels and A13 were measured at the end of follow up.

This point has been further clarified in the "Design and Methods" section ("At the end of follow up, documentation of clinical symptoms including any possible side effects related to rituximab therapy was performed after all patients gave their informed consent. Additional to complete medical history, physical examination and standard laboratory testing, ADAMTS13 enzyme activity and the presence of an inhibitor was analyzed..... <u>To evaluate the degree of present B-cell depletion at the end of follow up</u>, CD19 measurement was performed using flow cytometry.") as well as in the "Results and Discussion" section ("In our patient collective, no patient suffered from consequences of long-term B cell depletion, since no treatment related, especially infectious complications were reported. <u>In all but two patients B cell counts had</u> <u>recovered at the end of follow up...</u>" "At the end of follow-up, ADAMTS13 activity and presence of an inhibitor was analyzed...") Additionally, it is mentioned in Table 1.

16. In the introduction the authors define remission as clinical criteria plus normal A13 levels; yet in their methods they considered TTP patients regardless of A13 levels. Please reconcile.

In the "Introduction" section, we changed the paragraph to "...complete remission <u>with</u> complete clinical and laboratory responses including normal ADAMTS13 level and disappearance of anti-ADAMTS13 antibodies" and referenced to Sadler et al. [Sadler JE. (2008) Blood 112(1):11-8.].

ADAMTS13 is no diagnostic criteria as a *condition sine qua non* for considering TTP and testing ADAMTS13 during initial presentation is not mandatory to date. However it likely provides useful prognostic information at diagnosis, since severe ADAMTS13 deficiency identifies a specific mechanism of idiopathic TTP that usually responds well to plasma exchange but tends to relapse.

17. Where the authors discuss the 2 patients treated "exclusively" with rituximab, please describe how much plasma they actually received (ie before having the severe allergic reactions) and I would suggest removing word "exclusively" as it implies that no PE was given.

Patient #1 received 22 x plasma exchange treatment, which was disrupted after severe allergic reactions despite high dose steroid treatment. Patient #2 and developed allergic bronchial asthma after 10 x plasma exchange. The reviewer is right to suggest removing the word "exclusively". The paragraph was changed accordingly.

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