Addition of rituximab to standard therapy improves response rate and progression-free survival in relapsed or refractory thrombotic thrombocytopenic purpura and autoimmune haemolytic anaemia

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Summary
Treatment of relapsed or refractory autoimmune mediated haemolytic syndromes, such as autoimmune haemolytic anaemia (AIHA) and thrombotic thrombocytopenic purpura (TTP), represents a therapeutic challenge. Here we report on our experience with the monoclonal anti-CD20 antibody rituximab (R) compared to standard treatment in these diseases. Patients with non-familial TTP or AIHA and no underlying malignancy were included in our analysis. Safety and efficacy of R-treatment were compared to results obtained in standard treatment approaches. Altogether, 27 patients were analyzed, comprising 15 patients with TTP and 12 patients with AIHA. The patients' average age at the time of diagnosis was 54 years. Eleven patients received antibody treatment (8 TTP, 3 AIHA). No acute or late WHO grade III/IV toxicity associated with rituximab was noted.

Keywords
Rituximab, TTP, AIHA

With standard therapy, the overall response rate (ORR) was 66.7% for AIHA and 65.8% for TTP, respectively. For the R-containing regimens the ORR was 100%. In patients with TTP, median progression free survival (PFS) with R-treatment was 3.8 years, as compared to 0.1 years in the standard-treatment group. In patients with AIHA, median PFS was not reached for R-containing treatment; all patients are in sustained remissions with a median follow up of 12.5 months. In the absence of prospective trials, our data underline the safety and efficacy of rituximab in relapsed and refractory autoimmune anaemias with favourable response rates and promising long-term progression-free survival. Therefore, prospective clinical trials evaluating rituximab as salvage- and first-line-therapy are clearly warranted.

Introduction
Idiopathic autoimmune hemolytic anemia (AIHA) is frequently associated with viral infections and has to be clearly distinguished from tumor-associated hemolytic syndromes, where treatment of the underlying disease often results in disease improvement. Commonly, in AIHA, autoantibodies cross-reacting to circulating red blood cells lead to direct hemolysis. Hence, immunosuppressive drugs like corticosteroids, cyclophosphamide (CPM), and azathioprine (AZA) are the therapeutic mainstay for these conditions (1).

Thrombotic thrombocytopenic purpura (TTP) is characterized by a typical clinical pentad of symptoms such as thrombocytopenia, microangiopathic hemolytic anemia, neurologic complications, fever and renal failure; however, oligosymptomatic courses are present in some patients (e.g. microangiopathic hemolytic anemia and thrombocytopenia alone). In TTP, the production of inhibitory auto-antibodies to von-Willebrand factor cleaving metalloprotease (ADAMTS 13) leads to a critical decrease of its enzyme activity. Eventually, this results in the formation of platelet-rich thrombi in the small vessels, caused by ultralarge von-Willebrand multimer proteins and leading to severe microangiopathic hemolysis. Inhibitory autoantibodies of ADAMTS13 could be recently detected in at least 65% of patients with TTP (2) and with improved detection tools will probably be diagnosed in a higher proportion of patients. Additionally, in some rare cases of inherited TTP, so-called familial TTP (3, 4), gene mutations of ADAMTS 13 have been identified (5).

While initial reports of this syndrome showed a mortality rate of more than 80%, new therapeutic approaches such as plasma exchange, immunoadsorption and plasma infusion in combination with steroids reduced the mortality rate to about 20% (6). However, relapses occur in more than one third of patients and up to one third of cases remain chronic or refractory (7, 8). Relapsed or refractory disease is associated with increased mortality rates of up to 30% (9). Drugs like vincristine (VCR), intra-
venous immunoglobulines (IVIG) or cyclophosphamide (CPM) are used additionally in desperate cases with some success. Splenectomy may be considered as an ultima ratio, but surgical intervention during active or refractory disease, however, revealed a high mortality rate of up to 40% (10–12). Recent studies on administration of high-dose immunoglobulins (13) have failed to prove any beneficial effect in the treatment of recurrent TTP. Furthermore repetitive treatment is highly cost-intensive and associated with reduced quality of life. In AIHA as well as in TTP an expanded clone of affected B-cells eventually differentiates into an antibody-producing plasma cell, thereby defining the cellular equivalent of the pathogenic antibodies. Specifically targeting these B-cells would be an attractive therapeutic objective. Therefore, recent reports on the efficacy of the monoclonal anti-CD20 antibody rituximab for TTP and AIHA treatment have attracted much attention (14–25).

Until now, only small series reporting on the efficacy of rituximab in TTP have been published, the largest on 11 patients treated for relapsed or refractory disease (26). For AIHA, several small series have been published for rituximab treatment in children as well as in adults with successful outcome even in refractory disease or related syndromes (27–29). Besides these ‘salvage’ therapies applied additionally to immunosuppressive treatment, several case reports on monotherapy have shown positive results with long-term remissions even upon re-therapy in relapse after rituximab. However, despite the favourable effects.

### Table 1: Baseline characteristics of 27 patients included into this analysis.

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<th>Patient #</th>
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<th>Treatments</th>
<th>Rituximab</th>
<th>Number of applications</th>
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<td>Yes‡</td>
<td>2 + 4</td>
<td>&lt; 6.25</td>
<td>+</td>
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* p = plasma exchange, S = steroids, C = cytotoxic agents, I = immunoglobulines, A = azathioprine, ULM = unusually large multimers of von Willebrand’s factor; ADAMTS13 = von Willebrand’s factor cleaving protease; n.e. = not evaluable; n.a. = not applicable. § documented since 2001, former relapses were not evaluable. * One patient with TTP received no plasma exchange due to death before initiation of PE therapy. † Treatments given before first administration of rituximab. ‡ Patients experiencing relapse after rituximab treatment. †† Material obtained after first plasma exchange. × Normal range: >50%.
reported in both diseases, no comparative analyses of rituximab-containing treatment approaches versus standard treatment are available.

Here we report on our experience with the addition of rituximab to standard therapy versus standard therapy alone in a patient population diagnosed with TTP or AIHA who were seen at two university centres within a six-year period.

Materials and methods

Patients
From two institutional databases (University Hospital Mainz, Department of Hematology; University Hospital Frankfurt a. M., Department of Hemostaseology) we identified patients >18 years of age who required treatment for TTP or AIHA between 2000 and 2005. All patients with an underlying malignant disease or drug-induced TTP (e.g. cyclosporine, mitomycin, clopidogrel) were excluded from this analysis. Non-familial TTP was considered if there was no positive family history and if available, results of ADAMTS-13 testing in relatives gave normal results. ‘Conventional’ treatment lines (plasma exchange, immunosuppressives) of all eligible patients were considered as control group. All treatment lines including the antibody rituximab (R) were evaluated in terms of efficacy in comparison to ‘conventional’ treatment options as indicated above. Patients were asked for their informed consent for the evaluation of their disease specific data. This study was performed in accordance with the declaration of Helsinki.

Overall, 27 patients with idiopathic AIHA or TTP were included in our analysis (18 female, 9 male). Fifteen patients had TTP and 12 patients presented with AIHA. Median age was 54 years for all patients (range 21–77 years). Prior treatment lines are depicted in Table 1.

For AIHA, 11/12 patients received corticosteroids, 3/12 received intravenous immunoglobulins, 1/12 was treated with AZA, and 2/12 were treated with cytotoxic agents (VCR/CPM).

Of 15 patients diagnosed with TTP, 14 (93.3%) received plasma exchange. One patient died before plasma exchange could be initiated. All 15 patients received corticosteroids, 1/12 was treated with immunoglobulins, and six patients out of 15 received cytotoxic agents.

Altogether, 11 patients received Rituximab-treatment: eight with the diagnosis of TTP and three with AIHA. Median number of rituximab doses was eight for AIHA (all 8), and four for TTP (range: 1–8), respectively.

Diagnostic criteria
TTP was diagnosed by the concomitance of thrombocytopenia, hemolytic anemia (by determination of lactatehydrogenase [LDH] and haptoglobin levels), schistocytes in the blood smear, negative Coombs test and onset of clinical symptoms (including neurologic symptoms and bleeding complications). ADAMTS13 activity was determined as previously described (30).

Patients were diagnosed with AIHA by the presence of hemolytic anemia (indicated above) and determination of accompanying antibodies. Patients with IgG-warm antibodies and cold-antibodies were included in this analysis.

Criteria for clinical response and remission
For the purpose of this study, clinical remissions were defined as follows:

AIHA: synopsis of hemoglobin level >10 g/dl (normal range: 12–16 g/dl), LDH <1.5 x upper limit of normal (ULN) (normal range: 130–260 U/l), and haptoglobin >0.3 g/l (normal range=0.3–2.6 g/l).

TTP: synopsis of platelet count >100,000/µl, hemoglobin level >10 g/dl, LDH <1.5 x ULN and haptoglobin >0.3 g/l. In addition, the absence of clinical symptoms was mandatory.

Time to response
Time to response was defined as time from receiving adequate therapy to the time remission was achieved according to the response criteria stated above.

Remission rate
Remission rate was defined as percentage of all patients meeting the response criteria mentioned above and receiving standard therapy or rituximab, respectively.

Standard treatments
For AIHA standard treatment normally included the use of steroids (1 mg/kg prednisolone) as first-line therapy. For further treatment lines steroid and/or other immunosuppressive drugs such as CPM or AZA were usually given in refractory or relapsed disease.

First-line treatment for TTP consisted of plasma exchange in combination with corticosteroids (usually 1 mg/kg prednisolone). Treatment was continued until two days after reaching the remission criteria mentioned above. Plasma exchange was performed using the Cobe-Spectra device (Gambro BTC, Lakewood, CO, USA) against fresh frozen plasma (FFP). In severe cases (coma, severe bleeding events) plasma exchange was performed twice daily. For second line treatment besides repeated plasma exchange and corticosteroid treatment, multiple agents were used (VCR, CPM, IVIG, steroid escalation) (Table 1).

Rituximab treatment
Besides refractoriness or relapse of TTP or AIHA, eligibility and time-point of rituximab therapy was dependent on multiple factors such as disease course or availability of alternative treatment options. Due to the experience of R-treatment in Non-Hodgkin’s-Lymphoma the established dose of 375 mg/m² weekly for a maximum of eight weeks was chosen.

Thus, patients treated with rituximab received weekly infusions (1 to 8 courses) at a dose of 375 mg/m² administered in addition to conventional therapy (corticosteroids, plasma exchange). Plasma exchange was performed before infusion of rituximab and discontinued for at least 24 hours (h), if ever possible. Plasma exchange was discontinued 48 h after reaching remission criteria given above. Corticosteroids were given in all patients during plasma exchange therapy and antibody treatment. Tapering of steroids started after reaching remission criteria given above.

Progression-free survival (PFS)
PFS was defined as the time from treatment initiation until relapse, disease progression (i.e. progressive and/or persisting...
symptoms upon therapy) and change of therapeutic schedule or death, whichever came first. Due to the retrospective nature of our analysis with varying time points of rituximab initiation, which usually occurred after failure of 2–3 weeks of conventional treatment, we selected the time point of the rituximab initiation for the calculation of PFS.

**Statistical analysis**
An explorative and descriptive analysis of the data was performed in accordance with recommendations from the Institute for Biomedicine and Epidemiology (Mainz, Germany). Curve comparison was performed using the logrank test. A significant difference was considered with p < 0.05. For statistical analysis GraphPad PRISM™ Version 4.0 (2003, GraphPad Software Inc., San Diego, CA, USA) was used.

**Results**

**Safety and toxicity of rituximab therapy**
Regarding the application of the monoclonal antibody rituximab, no severe toxicities were observed. Only mild forms of immune-mediated reactions (e.g., skin rash, fever, and chills) to antibody treatment were occasionally seen despite standard prophylaxis with antihistaminic drugs and paracetamol. Importantly, no clinical complications of B-cell impairment or immunodeficiency like severe infections after rituximab infusion were documented within the period of this analysis. One patient experienced CMV reactivation after long-term steroid treatment in addition to R-therapy.

**Response evaluation**

**Disease course upon standard treatment**
In patients with TTP remission rate was 65.8% (25 out of 38 therapies induced at least short clinical remissions) in response to standard treatment lines (n=38), and median PFS was 0.9 months (range 0–85.7 months). Median time to response including first-line therapies was 5.5 days (range: 2–181 days).

For patients with AIHA the remission rate was 66.7% (14 out of 21 therapies induced a remission, one patient had missing data). Median PFS was 8.7 months (range: 0–43.5 months), and median time to response was 44 days (range: 7–217) (Table 2, Fig. 1A, B).

**Disease course upon treatment lines including rituximab**

**Patients with TTP:** Eight patients received a total of 10 lines of rituximab containing treatment (8 lines after failure of standard treatment, and 2 lines for relapse after prior R-treatment). The overall response rate was 100% in evaluable patients with a median time to response of 22 days (range: 3–82 days). Data of one patient were not available. Median progression free survival upon rituximab was 45.8 months (range 2.4–50.9 months) (Table 2, Fig. 1B). Altogether, we observed a total of four relapses in three patients upon R-containing therapy, as indicated in Table 1. Patient number 22 (Table 1) suffered from two relapses of which the second was fatal. Interestingly, these patients had received a maximum of four cycles of rituximab, suggesting a dose-dependency for this treatment.

Using a Whitney-Man t-test to compare results of standard therapy to R-containing treatments, a statistically significant difference between these groups was demonstrated (p=0.0025; hazard ratio: 4.14; 95% CI 1.46–5.86). Focussing on the results of the last line of treatment without rituximab (which allows a more precise description of disease status, in comparison to the results of the subsequent antibody containing regimens) this difference appeared even to be increased (data not shown).

**Patients with AIHA:** Overall three patients received each eight cycles of rituximab for relapsed or refractory disease. Response rate was 100% and the median time to response was 80 days (range: 55–110 days). Until now, for these patients, no median PFS has been reached as all patients are in continued complete remission with a follow up of 8.7, 12.5 and 24.6 months (median 12.5 months +), respectively (Table 2, Fig. 1A).

**Discussion**

Rapid differentiation of committed B-cells into auto-antibody-producing plasma cells is probably one of the key mechanisms of disease perpetuation in both, TTP and AIHA. Thus, selective B-cell elimination is an attractive therapeutic possibility.

In our analysis, the probability of progression-free survival (PFS) for TTP patients with standard therapy regimens was poor: Median PFS was only about one month. In contrast, for patients refractory to standard treatment or at relapse who received R-treatment, median PFS was 45.8 months (Fig. 1B). Only three patients experienced a total of four relapses after R-therapy. However, in these patients PFS was 23.2, 45.8 and 13.3 months as compared to 0.4, 0.8 and 3.2 months obtained with their individual last line of treatment, respectively. Additionally, one of these patients had received only a single dose of rituximab, which may partly explain its limited therapeutic efficacy.

After relapse, one patient died 73 days after having received one additional dose of rituximab due to relapse. Another patient...
achieved a second ongoing remission upon R-containing treatment. A third patient was lost for follow-up.

Although this has not been a prospective evaluation, we performed a statistical analysis comparing the sum of conventional therapy approaches to addition of antibody treatment. For the TTP patient cohort we observed a tendency for a longer PFS after R-treatment compared to standard treatment \[p=0.0025 (**)\], hazard ratio 4.14; CI 95% 1.46–5.86\]

Analyzing the group of AIHA patients, median PFS for standard therapy was 261 days (8.7 months) and has not been reached for R-treatment, respectively. All three patients who received rituximab were in ongoing remission at the time of this analysis. Even with this limited number of patients statistical approximation strongly supported a significant difference. Our group of patients includes cases with warm- and cold-reacting antibodies. Differences in response rate to rituximab may exist between the two forms of AIHA. However, the use of rituximab is intended to eventually eliminate progenitors of antibody-producing cells, which are essentially identical in all types of AIHA, which would argue against differences between both groups. Furthermore, our series is too small to separate those different subgroups and to allow comparison of differences.

Our data are in line with earlier observations, especially the series of 11 patients reported by Fakhouri et al. (26). However, to the best of our knowledge, until now no competitive report has demonstrated an identical benefit.

Besides higher efficacy (PFS) and a good safety profile, potential economic benefits were conceivable. Without a formal calculation cost effectiveness is strongly supported by shortened hospitalization, decreased number of plasma exchanges and earlier termination of transfusion dependency. Further prospective clinical trials should also address this issue in order to generate additional evidence for using rituximab early in relapsed or refractory AIHA and TTP.

Some questions arise from this analysis. First, the frequency and total number of rituximab infusions that are needed to induce stable remissions remain unclear up to date. Unfortunately, pharmacokinetic data on rituximab are scarce, which makes it difficult to determine the optimal dosing and treatment intervals in each individual. Moreover, the efficacy of rituximab as a single agent could not be clearly determined, as previous and concomitant medication (cytotoxic drugs, corticosteroids) might have contributed to better clinical responses in the group treated with rituximab. However, early responses, as frequently observed in TTP after rituximab administration, may be explained by the combination of B-cell elimination and concurrent elimination of inhibitory antibodies following plasma exchange. Without using this double strategy, observed time to response in AIHA was
80 days in median. As plasma exchange itself is suspected to cause stimulation of B-cells and therefore may partly perpetuate the generation of inhibitory antibodies (rebound effect), rituximab might even directly block this mechanism (31–33). Long-term remissions may be explained by elimination of the oligoclonally expanded B-cells, which results in a terminally abolished production of inhibitory antibodies.

The prognostic and therapeutic significance of repeated inhibitor level- and ADAMTS-13-activity testing during and after treatment cannot be estimated from our or previously published data.

References


Taken together we feel that the use of rituximab as a salvage therapy is justified in relapsing or refractory TTP or AIHA. Prospective multicenter international phase II/III trials evaluating rituximab in refractory or relapsed disease are clearly warranted.

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