Long-term experience with rituximab therapy for treatment-resistant moderate-to-severe pemphigus

Burc¸in Cansu Bozca, Aslı Bilgic¸ and Soner Uzun

Dermatology and Venereology, Akdeniz University School of Medicine, Antalya, Turkey

ABSTRACT

Background: Rituximab appears to be effective for treating pemphigus, although there are limited long-term data.

Methods: This retrospective single-center study evaluated patients with conventional treatment-resistant pemphigus who received rituximab during September 2010–December 2019. The first rituximab cycle was based on the rheumatoid arthritis protocol in all patients except one patient, and additional single doses (500 mg or 1000 mg) were administered after clinical and/or serological relapse. The consensus definitions were used for complete remission off therapy, complete remission on minimal therapy, and clinical relapse. Serological relapse was defined as a progressive ≥2-fold increase in anti-desmoglein titers (vs. previous the measurement).

Results: The study included 52 patients with pemphigus vulgaris and 1 patient with pemphigus foliaceus. The mean number of infusions was 5 and the average follow-up after the first infusion was 56 months. The average time to clinical and/or serological relapse was 12 months. Complete remission was achieved in 84.9% of patients, including after the first rituximab cycle in 25 patients (47.1%). Two patients died during the follow-up period.

Conclusion: Additional rituximab cycles may help achieve and prolong remission in patients with moderate-to-severe pemphigus resistant to conventional therapies. However, prospective trials are needed to identify the optimal dosing protocol.

Introduction

Pemphigus is a potentially life-threatening autoimmune bullous disease that involves the skin and mucous membranes, which is characterized by flaccid bullae, erosions, and detectable serum concentrations of autoantibodies against desmoglein 1 (dsg1) and desmoglein 3 (dsg3) (1–3). Rituximab (RTX) is a human-mouse chimeric monoclonal antibody that targets CD20 on B lymphocytes and has been used to treat pemphigus since 2002 (4). There is an increasing body of evidence from case reports and case series that RTX has high efficacy in this setting, especially for patients who do not respond to traditional treatments (5–15). A randomized controlled trial also confirmed the potent efficacy of RTX and its superiority over systemic corticosteroids (16), which has led to RTX being included in recent guidelines as a first-line treatment option for pemphigus (17,18). However, there are limited long-term data regarding RTX therapy for pemphigus (19–27). Therefore, the present study aimed to evaluate the long-term safety and efficacy of RTX therapy for patients with moderate-to-severe pemphigus that was resistant to conventional therapies.

Materials and methods

Study design and patients

This single-center retrospective study evaluated patients with pemphigus who were treated between September 2010 and December 2019, with the first cycle of RTX therapy administered before January 2017, at our Bullous Diseases Unit (Dermatology and Venereology Department, Akdeniz University). The diagnoses were confirmed in all patients based on clinical, histopathological, and direct immunofluorescence findings, with or without enzyme-linked immunosorbent assay results. All patients had provided informed consent for the RTX treatment and the retrospective study protocol was approved by the local ethics committee (211/04.03.2020) and the Ministry of Health (16358815-506.01-E.190167).

Treatment procedures

The RTX therapy had been administered to patients with pemphigus if they (i) were unable to achieve remission despite receiving the maximum tolerable dose of methylprednisolone (MP), (ii) had corticosteroid (CS)-dependent disease (i.e. reduction of the CS dose was not possible), or (iii) had serious complications related to CS and/or immunosuppressive therapies. Based on our previous report, we administered premedication (1 g oral paracetamol and IV pheniramine maleate 45.5 mg/2 ml and 100 mg methylprednisolone administered 1 h prior to each infusion) before the RTX infusion and prophylaxis against Pneumocystis jirovecii (Trimethoprim–sulfamethoxazole from the initiation of each RTX cycle and continuing for the next 6 months as a dose of two forte tablets per week) (28). Patients had been considered ineligible for RTX therapy of they (i) were...
pregnant, (ii) were breastfeeding, (iii) had a history of murine protein sensitivity, (iv) had a history of any malignancy, (v) had active and severe infections (including tuberculosis, sepsis, opportunistic infections, and uncontrolled hepatitis), or (vi) had severe uncontrolled cardiac disease.

Clinical outcomes

The outcomes were judged based on the consensus definitions of pemphigus, endpoints, and therapeutic response (29). The consensus definitions were used for complete remission (CR) off therapy (CRoffT), minimal therapy (MT), and clinical relapse/flare. However, CR on minimal therapy (CRonMT) was defined as the absence of new or established lesions while the patient received MT. In addition, CR on therapy (CRonT) was defined as the absence of new or established lesions while the patient received treatment that exceeded MT. Partial remission (PR) was evaluated based on the consensus definition (29), although it was not a priority in the present study. We defined serological relapse as a ≥2-fold increase in anti-dsg1 and/or anti-dsg3 titers, relative to the previous measurement.

Statistical analysis

All analyses were performed using SPSS software (version 20; IBM Corp., Armonk, NY, USA) and differences were considered statistically significant at p-values of <.05. Data were presented as mean ± standard deviation (range), median (interquartile range), or number (percentage). The distributions of continuous data were evaluated using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Non-normally distributed continuous data were analyzed using the Mann-Whitney U test to compare the CRoffT and no CRoffT groups (CRonMT, CRonT, PR, and no response) and to compare groups that received additional RTX cycles at doses of 500 mg and 1000 mg. The related-samples Wilcoxon signed-rank sum test was used to analyze variables before and after RTX therapy. Cochran’s Q test was used to compare relapse frequencies at different time intervals.

Results

Patients

The study included 53 patients (33 women and 20 men) who had a mean age of 51.1 ± 11.9 years (range: 24–79 years). One patient had pemphigus foliaceus (PF) and 52 patients had pemphigus vulgaris (PV) (34 mucocutaneous cases and 18 mucosal cases). The most common comorbidities were hypertension, chronic inactive hepatitis B infection, thyroid disease, and asthma. The median age at disease onset was 42.0 ± 12.1 years (range: 37.0–49.0 years) (Table 1).

Treatment using RTX and outcomes

The first RTX cycle was administered to most patients using the rheumatoid arthritis protocol (1000 mg intravenously, 2 weeks apart), although one patient started the lymphoma protocol (375 mg/m² intravenously each week for 4 consecutive weeks). During subsequent cycles, the RTX was administered as a single dose of 500 mg or 1000 mg. The median duration of conventional treatment before the first RTX infusion was 4.3 ± 4.5 years (range: 0–20 years). The median follow-up time after the first RTX cycle was 56 months (range: 1–114 months) (Supplemental Table S1).

After the first RTX cycle, 25 patients (47.1%) achieved CRoffT. All patients who achieved remission relapsed. The median interval to relapse was 12 months (range: 4–72 months). Patients

| Characteristic                          | Patients (n = 53) |
|----------------------------------------|-------------------|
| Previous CS treatment, n (%)           | 53 (100.0)        |
| Previous CS sparing treatment, n (%)   | 43 (81.1)         |
| Azathioprine                           | 30 (56.6)         |
| Methotrexate                           | 4 (7.5)           |
| Mycophenolate mofetil                  | 3 (5.7)           |
| IVIG                                    | 2 (3.8)           |
| Plasmapheresis                         | 2 (3.8)           |
| Dapsone                                 | 1 (1.9)           |
| Doxycycline                            | 1 (1.9)           |
| Duration of disease before rituximab (years), median (IQR) | 4.0 (1.0–6.0)    |
| Adverse effects of treatments before rituximab, n (%) | 48 (90.6)        |
| Osteoporosis                           | 42 (79.2)         |
| Striae                                 | 12 (22.6)         |
| Cushing syndrome                       | 11 (20.8)         |
| Diabetes mellitus                      | 10 (18.9)         |
| Depression                             | 6 (11.3)          |
| Avascular necrosis                     | 6 (11.3)          |
| Cataract                               | 5 (9.4)           |
| Glaucoma                               | 4 (7.5)           |
| Hypertension                           | 3 (5.7)           |
| Myopathy                               | 3 (5.7)           |
| Compression fracture                   | 3 (5.7)           |
| Herpetic infection                     | 2 (3.8)           |
| Psychosis                              | 1 (1.9)           |
| Bone marrow suppression                | 1 (1.9)           |
| Anti-dsg1 level before rituximab, median (IQR) | 26.0 (0.0–140.0) |
| Anti-dsg3 level before rituximab, median (IQR) | 200.0 (122.0–200.0) |
| Number of rituximab infusion, median (range) | 5.0 (1.0–9.0) |

CS: Corticosteroid; IVIG: Intravenous immunoglobulin; IQR: Interquartile range; PDAI: Pemphigus Disease Area Index; Anti-dsg1: Antidesmoglein 1; Anti-dsg3: Antidesmoglein 3.
who experienced clinical and/or serological relapse received additional RTX cycles. A second RTX cycle was provided to 44 patients (500 mg: 26 patients, 1000 mg: 18 patients) and CRoffT was achieved by 32 patients (72.2%). The median interval to relapse after the second cycle was 12 months (range: 2–84 months). A third RTX cycle was provided to 35 patients (500 mg: 14 patients, 1000 mg: 21 patients) and CRoffT was achieved by 23 patients (65.7%). Twenty-eight patients experienced a third relapse after a median interval of 13 months (range: 4–28 months). A fourth RTX cycle was provided to 27 patients (500 mg: 7 patients, 1000 mg: 20 patients) and CRoffT was achieved by 20 patients (74.1%). Among 23 patients who achieved CR (on or off treatment), 22 patients experienced a fourth relapse after a median interval of 12 months (range: 7–39 months). A fifth RTX cycle was provided to 13 patients (500 mg: 3 patients, 1000 mg: 10 patients) and CRoffT was achieved by 10 patients (76.9%). The median times to relapse were 12 months after the fifth cycle (range: 7–52 months), 13 months after the sixth cycle (range: 6–20 months), and 13 months after the seventh cycle (range: 6–21 months). Only 2 patients received an eighth RTX cycle and both patients achieved CR (1 patient with CRonMT and 1 patient with CRoffT), although 1 patient experienced relapse after 11 months. Thus, among the 53 patients, the overall treatment responses were CRoffT in 42 patients, CRonMT in 3 patients. RTX effect could not be evaluated due to death in one patient (Table 2 and Supplemental Table S1). Comparisons of the groups with and without CRoffT revealed no significant differences after the first RTX cycle or throughout the follow-up period in terms of disease type, sex, age, and disease duration before RTX initiation (Supplemental Table S2).

Clinical relapse frequencies were examined at 3 months, 6 months, and 12 months after starting RTX therapy, which revealed some significant differences (Table 3). The post-hoc comparison analysis revealed that this was related to the difference in clinical relapse frequencies at 6 months and 12 months. Furthermore, the frequency of clinical relapse decreased between the second and fifth cycles, although the difference was not statistically significant during the follow-up period (Table 3). Serological relapse frequencies were also evaluated at 3 months, 6 months, and 12 months. The only significant difference in the serological relapse rates was observed during the first cycle (Table 4). The post-hoc comparison analysis revealed that the 12-month serological relapse rate was significantly higher than the 3-month and 6-month relapse rates.

The changes in anti-dsg1 and anti-dsg3 titers between the pretreatment evaluation, 3 months, and 6 months after starting therapy are shown in Figure 1. Significant differences were observed in the anti-dsg1 and anti-dsg3 titers at the pretreatment evaluation, 3 months, 6 months, and 12 months after the first RTX cycle (p < .001). The groups with and without CRoffT had similar anti-dsg1 and anti-dsg3 titers before the first RTX cycle, after the first RTX cycle, and at all points during follow-up. However, a titer of ≤100 U/mL after the first RTX cycle was significantly associated with CRoffT (p = .03).

During the second to fourth RTX cycles, the median time to relapse was longer in the 1000 mg group than in the 500 mg group, although these differences were not statistically significant. However, the 1000 mg group had significantly higher rates of CR, CRoffT, and CRonMT during the third RTX cycle. The other clinical responses were generally similar and there were no time points where the anti-dsg1 and anti-dsg3 titers were significantly different between the 500 mg and 1000 mg groups.

### Table 2. Clinical outcomes in patients with pemphigus who received RTX therapy (Summarized).

| Treatment cycle | Clinical relapse | Time intervals |
|-----------------|------------------|----------------|
| 3rd month       | 6th month        | 12th month     |
| Present, n (%)  | 2 (3.8)          | 6 (11.5)       | 27 (51.9) |
| Absent, n (%)   | 3 (5.7)          | 3 (5.7)        | 6 (11.5)  |
| Present, n (%)  | 3 (5.7)          | 7 (13.5)       | 13 (24.5) |
| Absent, n (%)   | 3 (5.7)          | 4 (7.7)        | 10 (18.8) |
| Present, n (%)  | 0 (0.0)          | 0 (0.0)        | 2 (3.8)   |
| Absent, n (%)   | 0 (0.0)          | 0 (0.0)        | 27 (51.9) |
| Present, n (%)  | 1 (2.9)          | 2 (3.8)        | 8 (15.1)  |
| Absent, n (%)   | 1 (2.9)          | 2 (3.8)        | 10 (18.8) |
| Present, n (%)  | 3 (5.7)          | 6 (11.5)       | 13 (24.5) |
| Absent, n (%)   | 3 (5.7)          | 4 (7.7)        | 10 (18.8) |
| Present, n (%)  | 0 (0.0)          | 0 (0.0)        | 2 (3.8)   |
| Absent, n (%)   | 0 (0.0)          | 0 (0.0)        | 27 (51.9) |
| Present, n (%)  | 2 (3.8)          | 4 (7.7)        | 10 (18.8) |
| Absent, n (%)   | 2 (3.8)          | 4 (7.7)        | 10 (18.8) |
| Present, n (%)  | 0 (0.0)          | 0 (0.0)        | 2 (3.8)   |
| Absent, n (%)   | 0 (0.0)          | 0 (0.0)        | 27 (51.9) |

### Table 3. Comparison of clinical relapse frequency among different time intervals and treatment cycles.

| Treatment cycle | Clinical relapse | Time intervals |
|-----------------|------------------|----------------|
| 3rd month       | 6th month        | 12th month     |
| CRoffT          | Absent, n (%)    | 6 (11.5)       | 13 (24.5) |
| CRonMT          | Present, n (%)   | 2 (3.8)        | 6 (11.5)  |
| CRonT           | Absent, n (%)    | 0 (0.0)        | 0 (0.0)   |
| NR              | Present, n (%)   | 1 (2.9)        | 2 (3.8)   |
| Death           | Present, n (%)   | 0 (0.0)        | 0 (0.0)   |
| LFU             | Present, n (%)   | 0 (0.0)        | 0 (0.0)   |

### Table 4. Comparison of serological relapse frequency among different time intervals and treatment cycles.

| Treatment cycle | Serological relapse | Time intervals |
|-----------------|---------------------|----------------|
| 3rd month       | 6th month           | 12th month     |
| Present, n (%)  | 0 (0.0)             | 3 (6.5)        | 10 (26.3) |
| Absent, n (%)   | 46 (100.0)          | 43 (93.5)      | 25 (73.7) |
| Present, n (%)  | 1 (2.9)             | 2 (3.8)        | 3 (18.8)  |
| Absent, n (%)   | 33 (97.1)           | 30 (96.5)      | 13 (81.2) |
| Present, n (%)  | 2 (6.9)             | 3 (10.3)       | 2 (10.5)  |
| Absent, n (%)   | 27 (93.1)           | 26 (89.7)      | 17 (89.5) |
| Present, n (%)  | 1 (4.8)             | 2 (6.9)        | 2 (11.1)  |
| Absent, n (%)   | 21 (95.2)           | 19 (90.5)      | 16 (88.9) |
| Present, n (%)  | 1 (2.9)             | 2 (6.9)        | 2 (11.1)  |
| Absent, n (%)   | 12 (100.0)          | 11 (100.0)     | 8 (88.9)  |
| Present, n (%)  | 0 (0.0)             | 0 (0.0)        | 0 (0.0)   |
| Absent, n (%)   | 6 (100.0)           | 6 (100.0)      | 4 (100.0) |

*Related Samples Cochran’s Q Test was used. Bold p values indicate statistical significance.

### Adverse events

Adverse events during RTX infusions were identified for 12 patients (22.64%). The most common adverse event was hypertension (7.55%), which was followed by headache (5.66%), facial...

**Table 2. Clinical outcomes in patients with pemphigus who received RTX therapy (Summarized).**

| Treatment cycle | Clinical relapse | Time intervals |
|-----------------|------------------|----------------|
| 3rd month       | 6th month        | 12th month     |
| CRoffT          | Absent, n (%)    | 6 (11.5)       | 13 (24.5) |
| CRonMT          | Present, n (%)   | 2 (3.8)        | 6 (11.5)  |
| CRonT           | Absent, n (%)    | 0 (0.0)        | 0 (0.0)   |
| NR              | Present, n (%)   | 1 (2.9)        | 2 (3.8)   |
| Death           | Present, n (%)   | 0 (0.0)        | 0 (0.0)   |
| LFU             | Present, n (%)   | 0 (0.0)        | 0 (0.0)   |

*Related Samples Cochran’s Q Test was used. Bold p values indicate statistical significance.*
erythema (5.66%), and transient hyperglycemia (3.77%). None of these cases involved severe infusion-related adverse event.

Two patients died during the follow-up period. The first patient was a 62-year-old man with PV who died because of thromboembolism at 1 week after the first RTX infusion. Although the potential adverse cardiac effects of RTX could not be excluded, this patient also had long-term immobilization because of various comorbidities and prolonged high-dose CS

Figure 1. (a) Anti-dsg1 and (b) Anti-dsg3 responses to RTX therapy in patients with pemphigus. Related Samples Wilcoxon Signed Rank Sum Test was used for comparing levels before and after RTX therapy. Anti-dsg1: Anti-desmoglein 1; Anti-dsg3: Anti-desmoglein 3; RTX: Rituximab.
usage. The second patient was a 37-year-old woman with PV, non-Hodgkin lymphoma, and severe myasthenia gravis who died because of bacterial pneumonia and sepsis at 17 months after the second RTX administration. The patient’s infection was likely related to her neurological diseases and increased risk of related complications, such as lower respiratory tract infection.

Discussion

This retrospective study evaluated our accumulated experience over an approximately 10-year period using RTX to treat pemphigus. The results indicate that RTX therapy maintained CR in 84.9% of patients with moderate-to-severe pemphigus during the follow-up period, and also permitted complete withdrawal of systemic CS in 79.2% of the patients. Moreover, among all patients with complete follow-up data, the first RTX cycle permitted complete discontinuation of the adjuvant immunosuppressive agents.

Our experience indicates that the clinical effects of RTX treatment in this setting emerge during the first cycle, with agrees with previously reported CR rates of 42–100% after the first RTX cycle (19–25,30,31). To the best of our knowledge, our CR (both CRoffT, CRonMT and CRonT) rate of 88.5% after the first cycle is the second highest rate, after that reported by Vinay et al. (22). A meta-analysis conducted by Wang et al. (32) identified a CR rate of 77.5%, based on the pemphigus consensus definitions (29). These differences may be related to the proportion of patients who received lower RTX doses during in the first cycle (vs. our study) and the heterogeneity related to the large number of patients who were included in the meta-analysis.

Previous studies (19,25,30,31) and our results indicate that CR rates increased during follow-up. Furthermore, even after relapse, use of additional RTX cycles can allow patients to re-achieve CR (19,25,26,30,31). Thus, a CR rate of 80–90% seems reasonable if patients can continue RTX treatment with regular follow-up. Furthermore, remission can be maintained while reducing or eliminating the need for systemic CS and adjuvant immunosuppressive treatment (16,32–34).

Relapses are not uncommon following RTX therapy (32), which agrees with our results. We also observed that clinical relapse began to emerge at 6 months after starting RTX therapy and began to increase significantly at 12 months, which agrees with previous reports that relapse tends to develop at 6–24 months after starting treatment (27,35). Clinical relapse reflects the immunological modification of B lymphocytes after RTX treatment, which significantly depletes the B lymphocyte population for ≥6 months and up to 1–2 years in some patients (36). We did not detect significant differences in the clinical relapse rates at 3 months, 6 months, and 12 months during the different RTX cycles, although it is possible this was related to the relatively small sample size. Nevertheless, we observed a decrease in the rates of clinical relapse at 3 months and 6 months after the second to fifth RTX cycles, which may be a clinically relevant finding. Given the possibility that clinical relapse might become less common at higher numbers of cycles, further studies are needed to examine whether repeated RTX cycles have cumulative effects on B lymphocyte depletion and repopulation.

Interestingly, in contrast with the trend for clinical relapse, we only observed a substantial decrease in serological relapse after the first RTX cycle. However, this comparison may be limited, as anti-dsg1 and anti-dsg3 titers of >200 U/L were not measured quantitatively. For example, some patients with pretreatment titers of >200 U/L had their titers decrease to <200 U/L after the first RTX cycle, while other patients had antibody titers remain at ≥200 U/L regardless of their clinical response. Thus, it is possible that an unmeasured temporal change occurred in patients with titers of >200 U/mL, although it remains unclear whether the response in anti-dsg titers is related to clinical response. Nevertheless, we observed that pretreatment anti-dsg titers of ≤100 U/mL were associated with better CRoffT rates. Similarly, Shimanovich et al. (25) reported that anti-dsg antibody titers of ≤250 U/L were associated with a higher CRoffT rate after the initial RTX cycle. In contrast, other studies have indicated that baseline anti-dsg1 titers of >100 U/mL (vs. ≤100 U/mL) predicted a greater reduction in CS dose at 6 months after RTX treatment (23,37,38). Therefore, additional studies are needed to clarify whether antibody titers are related to RTX response in patients.

There are limited data regarding the strategy for managing relapse after the first RTX cycle, with most practice based on data from a prospective randomized controlled study conducted by Joly et al. (16). In that study, patients received a single 500-mg RTX dose at 12 month and 18 months. The primary endpoint was the proportion of patients who achieved CRoffT at week 24, which was almost 3-fold higher in the RTX group than in the control group that received systemic CS alone. In a recent study, patients who received 500 mg additional RTX infusions at 6 months and 12 months after the first RTX cycle had lower relapse rates in the first three years after the first infusion than those who did not receive additional infusions (39). However, to the best of our knowledge, no studies have compared the clinical and/or serological responses between patients who received addition RTX doses of 500 mg or 1000 mg. The present study failed to identify a significant difference in the clinical response between the two doses, with the exception of after the third cycle, and no significant difference in the serological response was observed during any cycle. Caution is warranted when interpreting this result, as patients did not always receive the same RTX dose in subsequent cycles (e.g. the same patient could receive 500 mg in one cycle and 1000 mg in a later cycle), although we speculate that higher doses at later cycles may provide better clinical and serological responses (vs. lower doses or no dose). Studies in the literature evaluate the effectiveness of different doses of RTX administered in the induction cycle. Several studies (40–42) have found that 500 mg RTX administered at two-week intervals in the first cycle is effective and safe. Another study (43) found that 1000 mg RTX administered two weeks apart was clinically and immunologically superior to 500 mg RTX administered. Russo et al. (44) also recently investigated the effectiveness of ultra-low-dose RTX in 8 patients with PV, and reported that a single 200-mg infusion provided positive clinical effects (CR in 5 patients and PR in 3 patients), with 1 patient experiencing relapse after 58 weeks. Russo et al. noted that their strategy was based on a previous study (45), which indicated that a low RTX dose (1 mg/m²) provided effective CD20+ B lymphocyte depletion in healthy subjects, and suggested that relatively low RTX doses might provide sufficient efficacy in autoimmune diseases such as pemphigus (45,46).

There are conflicting data regarding whether an increasing number of RTX infusions can prolong the remission interval between cycles. One study revealed that the remission duration increased at higher numbers of RTX infusions (20), while our results and other studies (25,26,30) failed to identify such an
effect. We also failed to detect a significantly prolonged remission duration when a dose of 1000 mg was used (vs. 500 mg). Nevertheless, our results and previous studies (19,20,25,30,31) indicate that CR rates increased with prolonged follow-up and that use of addition RTX cycles after relapse can help patients to re-achieve CR (19,20,25,26,30,31). In addition, additional RTX cycles after a patient achieves PR can increase the likelihood of that patient achieving CR (25,26). Nevertheless, there is some heterogeneity in this population, as some patients can achieve CR or PR after subsequent cycles even if they do not achieve remission during the first RTX cycle (30,38), while other patients may continue to be unresponsive (21,24,25). There are also reports regarding patients who do not respond at all to RTX (12,14,47,48). Schmidt et al. (49) have detected anti-RTX antibodies in 2 of 11 patients with pemphigus who had never received RTX infusions. Those patients with anti-RTX antibodies only achieved PR after RTX treatment and increases in their anti-RTX titers appeared to parallel their clinical deterioration (49). However, the patients with anti-RTX antibodies only received a single cycle of RTX therapy and it is unclear whether additional cycles might have improved their treatment response. We did not encounter any patients who failed to respond to RTX therapy, except for one patient whose RTX response could not be evaluated due to death, and additional RTX cycles provided CR even in patients who exhibited no response after earlier cycles. Therefore, clinicians should consider personalized assessments of whether to administer additional RTX cycles in patients with PR or no response after the first RTX cycle. The generally accepted approach is currently to not use prophylactic RTX cycles (17,18,50), and many markers have been suggested for predicting clinical relapse. The main starting point involves identifying B-cell re-population (35,51,52), although relapse can be detected before B-cell re-population (53), which highlights the need for robust biomarkers to predict relapse. Circulating anti-dsg titers are reportedly useful for predicting relapse in clinical practice (54), and Albers et al. (35) have reported that anti-dsg3 titers might predict relapse in all patients with PV, while anti-dsg1 titers might be more useful in patients with PV and mucocutaneous disease. In contrast, other studies have indicated that anti-dsg1 titers may be more reliable than anti-dsg3 titers for predicting relapse, and that anti-dsg1 titers are the most effective marker in patients with skin involvement (53,55). The recently updated S2K guidelines for managing pemphigus (18) recommend slowly reducing the CS dose after starting RTX therapy in this setting, as the risk of cutaneous relapse may be high when anti-dsg1 titers are >50 U/L. Furthermore, the persistence of high anti-dsg1 titers is useful for predicting skin relapse, while anti-dsg3 titers are less effective for predicting mucosal relapse unless they are >130 U/L. In the present study, additional RTX cycles were administered at or before clinical relapse to patients with progressive increases in antibody titers (>2-fold vs. the previous measurement). This strategy may seem overprotective. However, RTX treatment for pemphigus is subject to an off-label application process in Turkey and the time needed for approval might be relatively long when clinical relapse occurs. Moreover, a few weeks may be needed to receive approval even in cases of clinical relapse, and systemic CS treatment during this period may not be tolerated by fragile patients who have experienced previous CS-related complications (e.g. ophthalmologic complications, avascular necrosis, and unregulated diabetes).

Infections are perhaps the most worrisome side effect of RTX treatment. However, studies regarding long-term biologic treatment for rheumatoid arthritis (56) and accumulating reports regarding RTX treatment for pemphigus (57) suggest that there is only a low risk of serious infections, such as tuberculosis and opportunistic infections. For example, a review of 136 patients, including patients with paraneoplastic pemphigus, identified 17 serious adverse events (including 2 deaths) in 14 of 103 patients (13.5%) with PV and 1 serious adverse event (death) in 20 patients with PF (5%) (33). The other events were bacterial sepsis (n = 4), bacterial pneumonia (n = 3), Pneumocystis jiroveci pneumonia (n = 2), pulmonary embolism (n = 2), and neutropenia (n = 2). Deep vein thrombosis, which we identified in one case, has been reported as a possible adverse event during RTX treatment (33,58). Nevertheless, the most common side effects are mild-to-moderate infusion reactions, which are usually observed at the first infusion, and severe infusion reactions are infrequent (<1%), which include anaphylaxis, anaphylactoid reactions, and angioedema (59). We did not identify any severe infusion reactions and only 2 of the 53 patients died. One of the deaths involved a cardiac complication, and we cannot rule out the potential contribution of RTX, although the other death was caused by sepsis and did not appear to be directly related to RTX. Thus, caution is warranted regarding immune dysregulation that can be caused by pemphigus, as well as the risk of infection that is related to immunosuppressive treatment. However, we do not believe that these side effects should discourage dermatologists from using RTX to effectively treat patients with pemphigus, as their disease itself can be fatal.

The present study has several limitations. First, small retrospective studies are prone to various sources of bias. Second, we did not have an appropriate control group to evaluate the outcomes. Third, we were not able to extract data regarding other immunological markers or quantitative data regarding antibody titers that were >200 U/L. Fourth, the groups of patients who received 500 mg and 1,000 mg RTX dose in additional cycles were heterogeneous. Finally, we did not perform survival analysis because of the small number of patients.

In conclusion, the present study revealed that RTX therapy was effective for patients with moderate-to-severe pemphigus, had a good safety profile, and could prolong remission via additional infusions without the need for systemic CS and/or immunosuppressive treatment. However, prospective randomized controlled studies are needed to investigate the optimal dose and frequency of additional RTX infusions. Furthermore, and perhaps most importantly, it would be useful to develop a way to customize RTX treatment based on immunological markers, rather than applying a standard treatment strategy to all patients.

Disclosure statement
No potential conflict of interest was reported by the author(s).

ORCID
Burçin Cansu Bozca http://orcid.org/0000-0001-7907-5037
Aslı Bilgiç http://orcid.org/0000-0001-7910-7908
Soner Uzun http://orcid.org/0000-0001-7059-5474
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