Title: Mucosal involvement is a risk factor for poor clinical outcomes and relapse in patients with pemphigus treated with rituximab

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ABSTRACT

Many studies have reported the outcome of rituximab use in pemphigus but studies regarding the clinical risk factors for poor clinical outcomes or relapse are lacking. To clarify the risk factors for poor clinical outcomes or relapse in patients with pemphigus treated with rituximab, a retrospective chart analysis was performed on patients with pemphigus who were treated with rituximab in the dermatology clinic of Seoul National University Hospital. Forty patients with pemphigus were treated with rituximab, of which 39 (97.5%) experienced remission and 19 (48.7%) experienced relapse. Patients with mucosal lesions demonstrated poor clinical outcomes. The risk for relapse was 4.626 (confidence interval: 1.126-19.001, P = 0.034) times higher in patients with mucosal lesions than in those without lesions. In patients with pemphigus treated with rituximab, the presence of mucosal lesions resulted in poor clinical outcomes and frequent recurrence.

INTRODUCTION

Pemphigus is a rare, life-threatening, autoimmune blistering disorder (Cho, Jin & Chung, 2014). It is characterized by flaccid blisters and erosion of the skin and mucosa. Pemphigus is divided into two types according to clinical features and causal circulating autoantibody; pemphigus vulgaris (PV) is a more severe blistering disease, with frequent mucosal involvement, and is caused by autoantibodies against desmoglein (Dsg) 1 or 3, while pemphigus foliaceus (PF) is a less severe scaly disease and is affected by autoantibodies against Dsg 1.

Because pemphigus is potentially life-threatening without treatment, long-term immunosuppressive therapy, such as systemic corticosteroid, methotrexate, or intravenous immunoglobulin, has been chosen as first-line treatment for pemphigus (Wang, Liu et al. 2015). Side effects are the main obstacles to the prolonged application of immunosuppressive agents.

Rituximab is a chimeric anti-CD20 monoclonal antibody against B cells. Several reports have demonstrated that rituximab therapy is effective for severe and refractory pemphigus (Joly et al., 2007, Ahmed & Shetty, 2015). Recent articles recommend rituximab for pemphigus, although relapse is common (Murrell et al., 2018). Thus, we investigated the clinical risk factors for poor clinical outcomes and relapse in pemphigus.

METHODS

Study design

This was a retrospective chart review involving pemphigus patients (≥18 years old) recruited from the Seoul National University Hospital between January 2006 and July 2017. The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (IRB No. 1709-073-885).

The retrospective chart review included demographics, diagnosis (PV or PF), disease duration, pemphigus severity score, presence of mucosal lesions (including oral lesions), pretreatment CD19 count, number of rituximab infusions, co-medication at time of rituximab infusion, clinical outcome (remission, relapse), and treatment after relapse. Disease severity was evaluated with the pemphigus severity score (Herbst & Bystryn, 2000; Cho, Jin & Chung, 2014).

Treatment protocol

All patients with pemphigus were treated using the lymphoma protocol, which is defined as four-weekly infusions of rituximab with a dosage of 375 mg/m^2-body surface area. Under the medical chart review, the patients were divided into two groups: patients who received two or fewer infusions and those who received three or more infusions at initial treatment.

Outcome measurement

The outcomes of patients were evaluated by remission status and the presence of relapse. Remission was defined as complete remission on-therapy, complete remission off-therapy,

partial remission on-therapy, and partial remission off-therapy. Complete remission indicated the absence of new or preexisting lesions for at least 2 months, while partial remission implied the presence of transient new lesions which healed within 1 week. On-therapy signified the receiving of minimal therapy (e.g. prednisone less than 10 mg per day), while off-therapy denoted the non-use of systemic therapy. Disease relapse was defined as the appearance of three or more skin lesions lasting longer than 1 month which did not heal spontaneously within 1 week.

Statistical analysis

IBM SPSS version 23.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Binary logistic regression was performed to identify the risk factors for a poor clinical outcome (partial remission) or relapse. All continuous variables are expressed as mean ± standard error. The differences in variables were considered significant if $P < 0.05$.

RESULTS

Demographic and clinical characteristics of the study population

Forty patients (20 male, 20 female) who had pemphigus treated with rituximab were admitted at Seoul National University Hospital. Their mean age was 52.6±2.5 years. Twenty-eight (70%) were diagnosed with PV, while 12 (30%) were diagnosed with PF. Most of the PV patients had mucosal lesions (22/28, 78.6%), unlike the PF group (1/12, 8.3%). The most common site of mucosal lesions was the oral cavity (16/23, 69.6%), followed by the lip

(11/23, 47.8%), eye (2/23, 8.7%) and posterior pharynx (2/23, 8.7%). All patients had at least an oral or lip lesion. Twenty-seven (67.5%) patients had ≤ 2 infusions of rituximab, while 13 (32.5%) had ≥ 3 infusions. All patients underwent immunosuppressive treatment such as prednisolone, cyclosporine, azathioprine, or intravenous immunoglobulin before rituximab treatment. During treatment, all except one patient (97.5%) received co-medication as follows: systemic corticosteroid (n=38, 95.0%), azathioprine (n=9, 22.5%), cyclosporine (n=8, 20.0%), and dapsone (n=1, 2.5%). One patient died during rituximab treatment due to pneumonia. After treatment, all patients except the one that died experienced remission; complete remission (on-therapy: 16, off-therapy: 8) or partial remission (on-therapy: 15). The mean time to disease control was 4.37±0.35 weeks. The mean follow-up period after rituximab treatment was 57.4±7.7 months. Nineteen (48.7%) patients experienced relapse: 2 in complete remission off-therapy, 5 in complete remission on-therapy, and 12 in partial remission on-therapy. The mean period before relapse was 19.2±2.6 months (Fig. 1).

Clinical risk factors for a poor clinical outcome after rituximab treatment in pemphigus

Several clinical factors were investigated to elucidate the risk factors for a poor outcome. Of these, only the presence of mucosal lesions demonstrated a significantly increased risk for a poor outcome. (Odds ratio 4.626, confidence interval: 1.126-19.001, P = 0.034) The outcome did not change even after adjustment for age and sex (Table 1). The mean cumulative dosage of systemic corticosteroid required to obtain remission was 1950.73±257.61 mg in patients with complete remission and 1442.63±207.98 mg in patients without relapse (P = 0.173). Other co-medications also did not influence the outcome (data not shown).

Clinical risk factors for pemphigus relapse after rituximab treatment

As mentioned above, 19 patients experienced relapse 19.2±2.6 months after the last rituximab infusion. Those with mucosal lesions showed a significantly higher risk of relapse than those without such lesions. Other clinical factors did not demonstrate any significant risk (Table 2). The mean cumulative dosage of systemic corticosteroid required to obtain remission was 1713.11±244.05 mg in patients with relapse and 1795.40±269.81 mg in patients without relapse (P = 0.823). The mean maintenance dose of systemic corticosteroid was 8.16±1.86 mg/day in patients with relapse and 5.33±1.15 mg/day in patients without relapse (P = 0.199). No other co-medication affected relapse (data not shown).

DISCUSSION

Rituximab has been successfully applied to autoimmune bullous diseases (Schmidt, Hunzelmann, Zillikens, Brocker & Goebeler, 2006). Before its application to pemphigus, rituximab had been approved for non-Hodgkin's lymphoma and rheumatoid arthritis, and both have different protocols. Both the lymphoma and rheumatoid arthritis protocols have been applied to the treatment of pemphigus (Ahmed & Shetty, 2015; Amber & Hertl, 2015; Cho, Jin & Chung, 2014; Kanwar et al., 2014; Kim, Kim, Kim & Kim, 2011; Wang, Liu, Li & Huang, 2015).

Dsg 1 and 3, the main targets of autoantibodies in pemphigus, show different distributions in the skin and mucous membranes. Dsg 1 occurs in the superficial epidermis and mucosa, while Dsg 3 is found in the deep epidermis and entire mucosa (Pan, Liu & Zheng, 2011). The Dsg compensation theory explains why mucous membrane involvement frequently occurs in

PV but not in PF. The severity of oral lesions in pemphigus is correlated to anti-Dsg 3 antibody levels. (Harman et al., 2001). Furthermore, mucosal involvement in PV indicates future poor clinical outcomes (Mimouni, Bar, Gdalevich, Katzenelson & David, 2010; Seidenbaum, David & Sandbank 1988). In this study, we found that patients who had pemphigus with mucosal involvement had poor clinical outcomes and frequent relapse after rituximab treatment. In contrast, Saleh (Saleh, 2018) reported that patients with early relapsing pemphigus after rituximab treatment tended to have higher anti-Dsg 1 antibody indices than those with late relapsing pemphigus. Larger prospective studies and/or meta-analyses are required to clarify the clinical risk factors for poor clinical outcomes and relapse in patients with pemphigus undergoing rituximab treatment.

This study had some limitations. It was retrospective, observational, and single-center with a relatively small number of patients with pemphigus. Moreover, the severity of pemphigus was assessed using the pemphigus severity score, which is difficult to directly compare with other common severity score indices such as the Pemphigus Disease Area Index (Daniel et al., 2012). Antibody indices such as anti-Dsg 1 or 3 antibody were not measured in majority of patients and were excluded in the analysis.

CONCLUSIONS

In conclusion, this study showed that clinical factors like mucosal lesions are needed to be considered a high-risk factor for relapsing pemphigus after rituximab dose. In patients with mucosal lesions, close follow-up after rituximab treatment seems necessary for proper maintenance.

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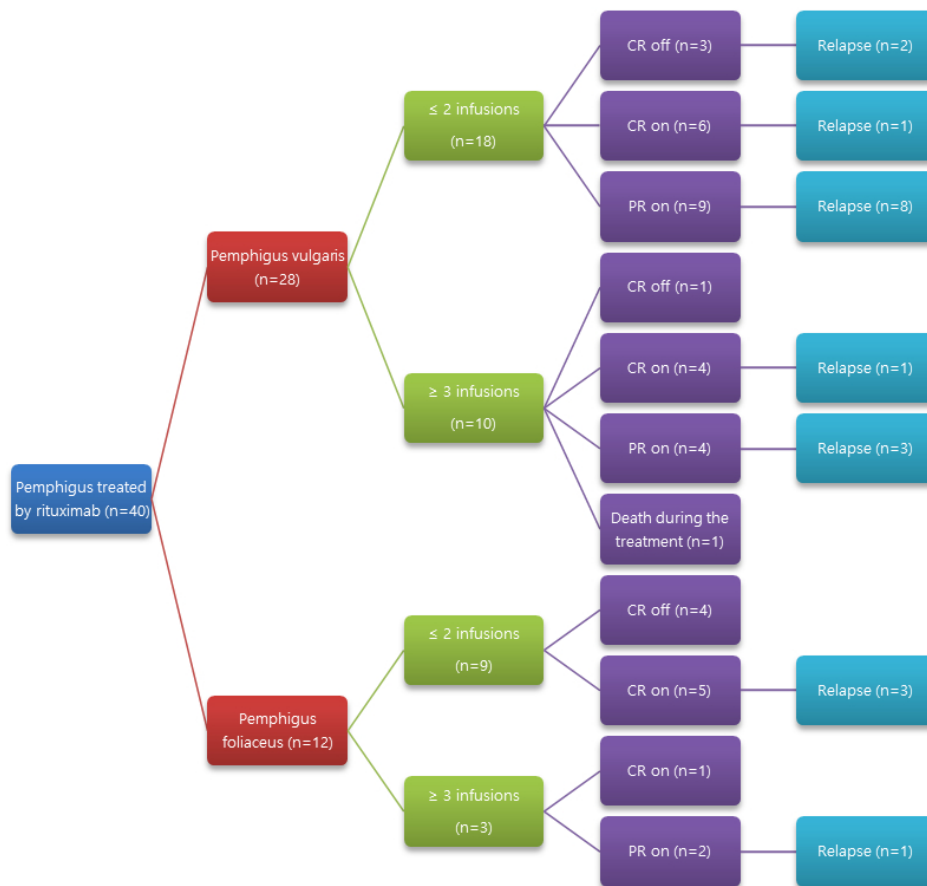
Figure 1. Summary of clinical outcomes and relapse in patients with pemphigus treated with rituximab infusion (CR: complete remission, PR: partial remission)

Tables

Table 1. Results of the logistic regression analysis of the clinical outcome (complete or partial remission) after rituximab treatment before and after adjustment for age and sex

	CR/PR (Number or mean ± SE)	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Age (years)					
≤ 64	20/10	Reference		Reference	
≥ 65	4/5	2.500 (0.548-11.410)	0.237	2.567 (0.556-11.845)	0.227
Sex					
Male	13/7	Reference		Reference	
Female	11/8	1.351 (0.370-4.925)	0.649	1.423 (0.378-5.347)	0.602
Disease duration					
Duration (months)	42.2±7.3 /38.3±9.8	0.997 (0.978-1.015)	0.739	1.000 (0.980-1.019)	0.976
Diagnosis					
Pemphigus foliaceus	10/2	Reference		Reference	
Pemphigus vulgaris	14/13	4.643 (0.852-25.301)	0.076	5.082 (0.872-29.628)	0.071

Mucosal lesion

No	14/3	Reference		Reference	
Yes	10/12	5.600 (1.246-25.174)	0.025	6.090 (1.256-29.531)	0.025

Disease severity

Severity score	5.5±0.5 /7.1±0.7	1.314 (0.989-1.745)	0.059	1.340 (0.999-1.799)	0.051

Pre-treatment CD19

Count	212.1±39.3 /225.6±58.8	1.000 (0.996-1.004)	0.838	1.000 (0.996-1.005)	0.917

Treatment protocol

≤ 2 infusions	18/9	Reference		Reference	
≥ 3 infusions	6/6	2.000 (0.500-7.997)	0.327	2.210 (0.520-9.397)	0.283

(CI: Confidence interval, CR: Complete remission, OR: Odds ratio, PR: Partial remission, SE: Standard error)

Table 2. Results of the logistic regression analysis of pemphigus relapse after rituximab treatment

before and after adjustment for age and sex

	Relapse: Yes/No (Number or mean ± SE)	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Age (years)					
≤ 64	16/14	Reference		Reference	
≥ 65	3/6	0.438 (0.092-2.083)	0.299	0.434 (0.091-2.072)	0.295
Sex					
Male	10/10	Reference		Reference	
Female	9/10	0.900 (0.256-3.162)	0.869	0.868 (0.242-3.114)	0.828
Disease duration					
Duration (months)	35.7±7.9 /45.4±8.6	0.992 (0.974-1.011)	0.405	0.989 (0.970-1.008)	0.259
Diagnosis					

Pemphigus foliaceus	4/8	Reference		Reference	
Pemphigus vulgaris	15/12	2.500 (0.604-10.344)	0.206	2.571 (0.603-10.957)	0.202
Mucosal lesion					
No	5/12	Reference		Reference	
Yes	14/8	4.200 (1.081-16.324)	0.038	4.626 (1.126-19.001)	0.034
Disease severity					
Severity score	6.4±0.7 /6.0±0.5	1.072 (0.829-1.387)	0.596	1.070 (0.823-1.393)	0.613
Pre-treatment CD19					
Count	204.9±47.1 /230.6±46.3	0.999 (0.995-1.003)	0.690	0.998 (0.993-1.003)	0.361
Treatment protocol					
≤ 2 infusions	14/13	Reference		Reference	
≥ 3 infusions	5/7	0.663 (0.168-2.620)	0.558	0.611 (0.148-2.515)	0.495

(CI: Confidence interval, OR: Odds ratio, SE: Standard error)