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Original Research

Short-term clinical and serological follow-up with conventional and conformational anti-desmoglein antibodies in treatment-naïve and previously treated patients with pemphigus vulgaris after receiving rituximab

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ABSTRACT

Background: Pemphigus vulgaris (PV) is a blistering, life-threatening autoimmune disease. Ethylenediaminetetraacetic acid (EDTA)-treated desmoglein (Dsg) enzyme-linked immunosorbent assay (ELISA) has recently been suggested to detect nonpathogenic antibodies. Rituximab (RTX) is now considered a first-line treatment for PV.
Objective: The primary and secondary aims were to evaluate anti-Dsg and EDTA-treated anti-Dsg ELISA and clinical response before and 3 months after RTX in treatment-naïve and previously treated patients, respectively. In addition, we compared the short-term efficacy of RTX between these groups.
Methods: Seventy-five patients with PV who received RTX (500 mg weekly for 4 weeks or 1000 mg 2 weeks apart) and prednisolone were followed for 3 months. Thirty-seven treatment-naïve newly diagnosed (group A) and 38 relapsed patients (group B) were included. Disease activity was scored with the Pemphigus Disease Area Index (PDAI). Clinical response was also assessed. Serum samples were collected at two points and examined for anti-Dsg1/3 and EDTA-treated anti-Dsg1/3. Conformational anti-Dsg values were calculated by subtracting EDTA-treated from conventional anti-Dsg values.
Results: The correlation of conventional and conformational anti-Dsg values was perfect (correlation coefficient > 0.98; $p < .001$) at every time point for both anti-Dsgs. There was no difference with regard to PDAI and anti-Dsg values between the two groups at baseline. The frequency of responders was significantly higher in group A (100%) than in group B (89%; $p = .006$). Three patients relapsed, and five patients had persistent disease activity in group B. After 3 months, conventional and conformational anti-Dsg values were significantly higher in group B compared with group A (anti-Dsg3: $p = .017$ and .021, respectively; anti-Dsg1: $p = .014$ and .016, respectively). Total and scalp PDAI were significantly lower in group A than in group B ($p = .042$ and .016, respectively).
Conclusion: EDTA-treated anti-Dsg ELISA had no added value. Using RTX as first-line treatment in patients with PV appears to be associated with better clinical response and immunologic profile than delayed treatment in the short term.

Introduction

Pemphigus encompasses a group of autoimmune bullous diseases that can affect the skin and/or mucous membranes. The two major clinical forms of the disease are pemphigus vulgaris (PV) and pemphigus foliaceus. Autoantibodies are players in the immunopathogenesis of pemphigus and are mainly directed against desmoglein (Dsg)1 and Dsg3, leading to acantholysis. The role of non-Dsg autoantibodies has also been reported in some studies (Ahmed et al., 2016; Amber et al., 2018). Circulating anti-Dsg 1/3 auto-antibodies also could be used as a marker to determine disease severity in patients with pemphigus. Anti-Dsg3 and anti-Dsg1 values were generally positively correlated with the severity of oral and skin involvement, respectively, implying the

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critical roles of these autoantibodies in the pathogenesis of pemphigus (Daneshpazhooh et al., 2007; Gandhi et al., 2014; Harman et al., 2001; Kumar et al., 2006; Mortazavi et al., 2009; Sharma et al., 2006). Anti-Dsg1 values were found to be significantly associated with the course of skin lesions (Abasq et al., 2009; Hebert et al., 2019; Patsatsi et al., 2014).

Currently, corticosteroids are widely used as a first-line treatment for patients with pemphigus. Because of their severe and long-term side effects, immunosuppressants such as azathioprine, mycophenolate mofetil, and methotrexate were used to minimize the steroid dosage (Atzmony et al., 2015). However, the steroid sparing effects of these drugs were not considerable. Rituximab (RTX), a revolutionary treatment in various autoimmune diseases such as rheumatoid arthritis, has been shown as a promising therapeutic option for patients with refractory PV (Salopek et al., 2002; Tavakolpour et al., 2017). This drug has been shown to be very effective and safe in newly diagnosed PV as a first-line therapy in a recent robust clinical trial conducted by Joly et al. (2017). RTX is now recommended as a first-line treatment (Murrell et al., 2018) and was recently approved by the U.S. Food and Drug Administration for the treatment of PV. RTX could be associated with relatively long-lasting remission, less cumulative steroid dosage, and probably improved quality of life, although nonresponse and (in extremely rare cases) disease worsening (Mahmoudi et al., 2019) have been reported.

Moreover, anti-Dsg values as measured by a conventional enzyme-linked immunosorbent assay (ELISA) are reported as valuable predictors of clinical relapse (Albers et al., 2017; Daneshpazhooh et al., 2016). However, the presence of some patients with PV in remission with high anti-Dsg 3 values suggested that anti-Dsg antibodies include pathogenic (conformational) and nonpathogenic components. Antibodies against the Ca 2+-dependent conformational epitopes of Dsg have been speculated as the main contributor in the pathogenesis of PV. Ethylenediaminetetraacetic acid (EDTA) added to Dsg-coated plates was used to detect nonpathogenic non-Ca2+-dependent antibodies (Kamiya et al., 2012, 2013). By subtracting EDTA-treated values from conventional values, conformational antibodies were calculated. However, the usefulness of this method in disease monitoring has not been determined.

Herein, we designed an observational study to evaluate the changes of anti-Dsg 1/3 values (both conventional and conformational) as well as Pemphigus Disease Area Index (PDAI) scores 3 months after RTX in patients with treatment-naïve and relapsed PV. Additionally, we attempted to explore the association between anti-Dsg1/3 and clinical response and the difference between the two patient subgroups.

Methods

Patient population

Seventy-five patients with histologically, clinically, and direct immunofluorescence confirmation of PV were included. Patients had been treated with RTX, either as a first-line treatment (group A) or second- or third-line therapy (group B). All patients signed a written informed consent, which was designed in accordance with the Declaration of Helsinki. Patients were planned to be followed for 3 months after the final RTX infusion.

At baseline, patients were subdivided into cutaneous, mucosal, and mucocutaneous types on the basis of the clinical examination. Clinical disease activity was assessed in patients using the PDAI before and 3 months after RTX administration. Additionally, demographic data, duration of disease, corticosteroid dose, and tapering profile were registered for each subgroup during the 3-month follow-up. At the baseline of RTX treatment and 3 months after the last infusion, serum samples were collected and stored at -70°C. Conventional Dsg1 and Dsg3 antibodies and EDTA-Dsg 1 and -Dsg3 antibodies were determined at the end of the study by using ELISA (Euroimmun, Lubeck, Germany). Conformational Dsg ELISA was calculated by subtracting EDTA-Dsg ELISA from conventional Dsg ELISA (Kamiya et al., 2012, 2013). Values $\geq$20 U/ml were considered positive for both anti-Dsg 1 and Dsg3.

Definitions

Complete and partial remission (off therapy and on minimal therapy) and relapse and failure of therapy were defined according to consensus (Murrell et al., 2008). We also added two other outcome measures. Remission on more than minimal therapy was defined as the absence of new or established lesions while the patient was receiving >10 mg prednisolone for at least 2 months. Persistent disease activity was defined as the presence of persistent and/or slow-healing lesions during prednisolone tapering in the absence of new lesions while receiving more than minimal therapy.

Rituximab infusion

All included patients received RTX infusions in accordance with either a modified lymphoma protocol (four weekly infusions of RTX at a dose of 500 mg) or rheumatoid arthritis protocol (two infusions of 1000 mg 2 weeks apart).

Statistical analysis

Pearson's correlation coefficient was used to evaluate the correlations between two variables. Paired and independent *t* tests were used to compare anti-Dsg1/3 values in the same patients or to compare the antibody values in each group, respectively. For evaluation of the significance of the differences between the categorical variables, the χ^2 or Fisher exact test was used. Statistical significance was defined as < .05, two-tailed. The statistical analysis was performed with IBM SPSS Statistics, version 19.0 (IBM Corp.; IBM SPSS Statistics for Windows, Version 22.0; Armonk, NY).

Results

Patients demographics

A total of 37 (men: 11; women: 26) and 38 (men: 15; women: 23) patients with PV were treated with RTX as first-line therapy (group A) or second- or third-line treatment (group B), respectively. All patients completed a 3-month follow-up. The number of included women was higher in both groups, but there was no significant difference in sex distribution (p = .38). The mean age of patients in groups A and B was 38.95 ± 9.70 years (range, 20-59 years) and 44.03 ± 11.44 years (range, 20-66 years), respectively (p= .042). Descriptive statistics of the patients are presented in Table 1.

Baseline serology and clinical status

Baseline values of total or conventional Dsg1 and Dsg3 antibodies were not significantly different between the two groups (P-values: anti-Dsg1 = .088; anti-Dsg3 = .700). This was also true for the pathogenic or conformational values of these two antibodies (P-value: anti-Dsg1 = .104; anti-Dsg3 = .717) (Table 2).

The mean initial prednisolone dosage was 46.83 ± 19.44 mg in group A and 20.13 ± 17.53 mg in group B; the difference was sig-

Table 1
Baseline characteristics of pemphigus vulgaris patients receiving rituximab: Newly diagnosed (group A) and relapsed (group B) patients

Variable		Group A (n = 37)	Group B (n = 38)	*p*-value
Sex				
	Male (%)	11 (29.7)	15 (29.5)	.382
	Female (%)	26 (70.3)	23 (60.5)	
Disease phenotype (%)				**.047**
	Mucosal	12 (32.4)	4 (10.5)	
	Cutaneous	1 (2.7)	2 (5.3)	
	Mucocutaneous	24 (64.9)	32 (84.2)	
Age at treatment ± standard deviation (range)		38.95 ± 9.70 (20-59)	44.03 ± 11.44 (20-66)	**.042**

Table 2
Response rate to rituximab in patients with pemphigus vulgaris in groups A and B

			Group A	Group B	*p*-value
Response (%)			37 (100)	30 (79.0)	**.006**
	Complete remission (%)		22 (59.4)	15 (39.5)	.093
		Off therapy (%)	3 (8.1)	1 (2.6)	.295
		Minimal therapy (%)	19 (51.4)	14 (36.8)	.211
	Partial remission (%)		15 (40.5)	15 (39.4)	.923
		Off therapy (%)	0 (0)	2 (5.3)	.164
		Minimal therapy (%)	6 (16.2)	10 (26.3)	.293
		More than minimal therapy (%)	9 (24.3)	3 (7.9)	.060
No response (%)			0 (0)	8 (21.0)	**.006**
	Relapse (%)		0 (0)	3 (7.9)	.090
	Persistent disease activity (%)		0 (0)	5 (13.2)	**.028**

nificant ($p < .001$). With regard to disease severity, no significant difference was observed among baseline mucosal PDAI ($p = .726$), cutaneous PDAI ($p = .750$), and total PDAI (P = .636) scores between the two groups.

Clinical and serological outcome

In general, evaluation of disease severity before and 3 months after RTX revealed that patients in group A had a better outcome than patients in the relapsed group. The number of responders to RTX among patients who received RTX as first-line therapy was significantly higher than in group B (37 patients [100%] vs. 30 patients [79%]; $p = .006$). Moreover, no patients in group A experienced disease relapse or persistent disease activity during the 3-month follow-up, but eight patients (21%) were categorized as nonresponders in group B (relapse = 3; treatment failure = 5; $p = .028$). More details of clinical response in both groups are summarized. Patients in group A achieved a significantly lower scalp ($p = .016$) and total ($p = .042$) PDAI scores compared with patients in group B after 3 months. However, there was no significant difference in skin ($p = .083$) and mucosal ($p = .867$) PDAI scores between the two groups (Table 2).

Measuring the different subtypes of anti-Dsg1 3 months after RTX administration revealed that the values of conventional ($p = .014$) and conformational ($p = .016$) anti-Dsg1 were significantly lower in group A compared with group B. With regard to anti-Dsg3 values, conventional ($p = .017$) and conformational ($p = .021$) anti-Dsg3 values were also found to be lower in group A than in group B (Table 3). The number of patients with negative and positive anti-Dsg1 and Dsg3 was not significantly different at baseline between the two groups. Three months after RTX, the number of patients with negative anti-Dsg1, but not anti-Dsg3, was significantly higher among new cases compared with relapsed patients ($p = .008$). Table 4 demonstrates data related to the number of patients with positive/negative anti-Dsg1/3 in each group. At the end of the 3-month follow-up, the percentage of patients with negative anti-Dsg1 was significantly higher than the percentage of patients with negative anti-Dsg3 among patients who went into complete remission ($p < .001$). This significant difference was also true for patients who achieved partial remission ($p = .002$). In contrast, no association between the number of nonresponders to RTX and anti-Dsg1/3 positivity has been ascertained 3 months after RTX (Table 5).

Furthermore, despite the significantly higher daily prescribed steroid dosage in group A ($p < .001$), no significant difference was observed after 3 months in the two groups ($p = .23$) (Table 2). This shows that the pace of steroid tapering in new cases treated with RTX was higher.

Correlation between markers and clinical response

The mean values of both anti-Dsg1 and anti-Dsg3 antibodies were inversely correlated with clinically desirable outcomes. Indeed, the values increased when moving from responders toward the nonresponding group (Table 6). The values of conventional and conformational Dsg1 and Dsg3 antibodies were found to be positively correlated with cutaneous (skin and scalp) (conventional anti-Dsg1: correlation coefficient = 0.621; $p < .001$; conformational anti-Dsg1: correlation coefficient = 0.624; $p < .001$) and mucosal (conventional Dsg3: correlation coefficient = 0.288; $p < .012$; conformational Dsg3: correlation coefficient = 0.296; $p < .01$) PDAI scores, respectively. Moreover, the total PDAI score was significantly correlated with both anti-Dsg1 and anti-Dsg3 (conventional anti-Dsg1: correlation coefficient = 0.459; $p = .001$; conformational anti-Dsg1: correlation coefficient = 0.464; $p = .001$; conventional anti-Dsg3: correlation coefficient = 0.332; $p = .004$; conformational Dsg3: correlation coefficient = 0.347; $p = .002$).

A perfect correlation was observed between conventional and conformational anti-Dsg1 and Dsg3 before and after treatment (conventional and conformational anti-Dsg1, before treatment: correlation coefficient = 0.986; $p < .001$; conventional and conformational anti-Dsg1, after 3 months of follow-up: correlation coefficient = 0.999; $p < .001$; conventional and conformational anti-Dsg3, before treatment: correlation coefficient = 0.982; $p < .001$; conventional and conformational anti-Dsg3, after 3 months of follow-up: correlation coefficient = 0.998; $p < .001$).

Table 3
Clinical and serological changes 3 months after rituximab therapy

	Group A			Group B			Compare group A with group B	*p*-value† of month 3 values
	Baseline	After 3 months	*p*-value*	Baseline	After 3 months	*p*-value*	*p*-value† of baseline values	
Prednisolone dose (mg)	46.83 ± 19.44 (0-80)	9.73 ± 5.95 (0-30)	**< .001**	20.13 ± 17.53 (0-80)	8.16 ± 5.30 (0-25)	**< .001**	<0.001	.23
Mucosal PDAI	13.99 ± 14.47	1.40 ± 3.27	**< .001**	12.79±15.05	1.526 ± 2.97	**< .001**	0.726	.867
Cutaneous PDAI	10.28 ± 13.86	0.14 ± 0.54	**< .001**	9.40±9.43	0.77 ± 2.14	**< .001**	0.750	.083
Skin PDAI	8.33 ± 11.76	0.135 ± 0.53	**< .001**	6.29±7.65	0.771 ± 2.14	**< .001**	0.377	.083
Scalp PDAI	1.95 ± 3.14	0.027 ± 0.16	**.001**	3.11±3.88	1.43 ± 3.41	**.003**	0.158	**.016**
Total PDAI	24.25 ± 19.88	1.57 ± 3.25	**< .001**	22.171±17.98	3.72 ± 5.4	**< .001**	0.636	**.042**
Total anti-Dsg1 Ab (U/ml)	62.80 ± 80.98	3.20 ± 4.42	**< .001**	100.65±106.24	27.28 ± 57.33	**< .001**	0.88	**.014**
Pathogenic anti-Dsg1 Ab (U/ml)	58.46 ± 81.00	2.12 ± 4.23	**< .001**	94.37±105.84	25.24 ± 56.34	**< .001**	0.104	**.016**
Non-pathogenic anti-Dsg1 Ab (U/ml)	4.34 ± 11.16	1.08 ± 0.28	**< .001**	4.26±8.67	2.03 ± 3.03	**< .001**	0.974	.062
Total anti-Dsg3 Ab (U/ml)	180.39 ± 100.81	45.99 ± 65.35	**< .001**	171.89±89.10	88.23 ± 83.45	**< .001**	0.700	**.017**
Pathogenic anti-Dsg3 Ab (U/ml)	164.45 ± 91.41	43.71 ± 63.93	**< .001**	157.24±80.07	82.54 ± 77.87	**< .001**	0.717	**.021**
Non-pathogenic anti-Dsg3 Ab (U/ml)	15.94 ± 19.99	2.28 ± 2.24	**< .001**	14.65±19.18	5.69 ± 8.37	**.001**	0.777	**.020**

Ab, antibody; Dsg, desmoglein; PDAI, Pemphigus Disease Area Index.
* Paired t test.
† Independent t test.

Table 4
Differences in total anti-Dsg1/3 values before and after the treatment in each group

	Group A		Group B		*p*-value
	Negative (<20)	Positive (≥20)	Negative (<20)	Positive (≥20)	
Baseline total anti-Dsg1 Ab (%)	21 (56.8)	16 (43.2)	14 (36.8)	24 (63.2)	.084
T3 total anti-Dsg1 Ab (%)	36 (97.3)	1 (2.7)	29 (76.3)	9 (23.7)	**.008**
Baseline total anti-Dsg3 Ab (%)	2 (5.4)	35 (94.6)	6 (15.8)	32 (84.2)	.145
T3 total anti-Dsg3 Ab (%)	19 (51.4)	18 (48.6)	13 (34.2)	25 (65.8)	.133

Ab, antibody; Dsg, desmoglein

Table 5
Frequency of positive and negative anti-Dsg1/3 Ab in patients with pemphigus vulgaris who achieved CR, PR, and NR after rituximab

	Anti-Dsg1		Anti-Dsg3		*p*-value
	Negative	Positive	Negative	Positive	
CR (%)	35 (94.6)	2 (5.4)	17 (45.9)	20 (54.1)	**< .001**
PR (%)	26 (86.7)	4 (13.3)	14 (46.7)	16 (53.3)	**.002**
NR (%)	4 (50.0)	4 (50.0)	1 (12.5)	7 (87.5)	.282

Ab, antibody; CR, complete remission; Dsg, desmoglein; PR, partial remission; NR, nonresponding

Table 6
Means± SD of anti-Dsg1/3 values in patients with pemphigus vulgaris 3 months after rituximab therapy (based on clinical response)

	Total anti-Dsg1 (U/ml)	Pathogenic anti-Dsg1 (U/ml)	Nonpathogenic anti-Dsg1 (U/ml)	Total anti-Dsg3 (U/ml)	Pathogenic anti-Dsg3 (U/ml)	Nonpathogenic anti-Dsg3 (U/ml)
CR (n = 37)	7.67 ± 22.41	6.07 ± 22.29	1.60 ± 2.84	50.80 ± 69.00	46.92 ± 63.61	3.88 ± 7.63
PR (n = 30)	11.97 ± 34.01	10.58 ± 32.80	1.39 ± 1.23	71.83 ± 82.45	68.21 ± 79.58	3.62 ± 4.56
NR (n = 8)	64.03 ± 92.64	61.99 ± 90.97	2.04 ± 1.79	127.49 ± 72.38	121.41 ± 68.43	6.08 ± 6.03
p-value*	**.002**	**.002**	.756	**.035**	**.028**	.621

CR, complete remission; Dsg, desmoglein; PR, partial remission; NR, nonresponding; SD, standard deviation
* One-way analysis of variance test.

Discussion

RTX has found its place as a first-line treatment for pemphigus. In this study we aimed to compare anti-Dsg values (both conventional and conformational), PDAI scores, and clinical response after RTX in two groups of patients with PV (newly diagnosed and relapsed) after 3 months. We observed that anti-Dsg values and PDAI scores decreased significantly with RTX in both groups. Nev-

ertheless, anti-Dsg 1/3 values and total PDAI score were significantly lower after RTX in treatment-naïve patients compared with relapsed patients. Considering there was no significant difference between total PDAI score in groups A and B at baseline, the significantly lower total PDAI score after RTX in group A compared with group B could indicate that first-line treatment with RTX was associated with a more favorable outcome. In addition, responders were significantly more frequent in newly diagnosed patients.

With regard to serologic status, anti-Dsg 1 and Dsg 3 values were significantly higher in nonresponders. In addition, anti-Dsg 1 values were also significantly higher in relapsed patients after 3 months of receiving RTX. Moreover, negative anti-Dsg 1 was significantly more frequent than negative anti-Dsg 3 in responders.

Another important finding is related to the significant correlation between the conventional and conformational anti-Dsg1/3 ELISA before and after treatment. The utility of conformational anti-Dsg value in monitoring disease activity has been accompanied by promising results in previous studies (Kamiya et al., 2012, 2013; Li et al., 2015). According to our study, the strong correlation between total and pathogenic anti-Dsg1/3 did not support the use of this method, which is inconsistent with Kamiya et al.'s reports (Kamiya et al., 2012, 2013). In other words, although conventional anti-Dsg1/3 antibodies are not optimal, EDTA-treated ELISA does not add any benefit for monitoring patients, as we have shown previously in patients in clinical remission (Daneshpazhooh et al., 2018).

A notable finding in our study is the better outcomes observed in the new patient group in the short term. In other words, all treatment-naïve patients responded to RTX, but only 89% of patients in the relapsed group responded after 3 months despite being matched by severity. One may speculate that earlier RTX treatment may be associated with a better outcome; however, this theory should be verified by controlled prospective long-term studies. The anti-Dsg1/3 status also supported the observed outcomes statistically. Recently, we showed that early RTX treatment of patients with PV could lead to a higher rate and longer duration of remission (Balighi et al., 2019). As Colliou et al. (2013) reported, an increase in naïve B-cells/ memory B-cells correlated with longer remission after RTX therapy. Higher efficacy of RTX in new cases might also be explained by the lower number of long-lived memory cells in patients with shorter disease duration. Additionally, the role of T cells or even CD20(-) B cells could become more important as disease duration increases.

Another interesting clinical finding is related to scalp involvement. Scalp disease activity remained significantly higher in the relapsed group after treatment with RTX. This is in accordance with our clinical experience and case reports on recalcitrant localized persistent lesions or vegetating lesions on the scalp in patients with chronic pemphigus (Daneshpazhooh and Chams-Davatchi, 2015; Rackett et al., 1995). Recently, Sar-Pomian et al. (2018) showed that the time required to achieve complete remission was significantly higher in patients with pemphigus with scalp involvement than in those with a disease-free scalp. We also found in a previous study that anagen hair loss was a predictor of anti-Dsg1 values and skin and scalp PDAI scores (Fard et al., 2017). These findings may be partly explained by the high density of pemphigus vulgaris antigens in the scalp and buccal mucosa (Ioannides et al., 1991). Interestingly, these important locations have different levels of expression of Dsg1, with the scalp showing high and the oral mucosa low levels.

The mean age of relapsed patients was higher than that of newly diagnosed patients, which may be simply attributed to the fact that previously treated patients are older. It should be kept in mind that RTX therapy may be associated with better response in younger patients with other conditions, such as systemic lupus erythematosus and idiopathic thrombocytopenic purpura (Marangon et al., 2017; Md Yusof et al., 2017). Further large-scale studies with longer follow-up may clarify the role of age on response to RTX in pemphigus in the future.

Despite the significantly higher initial daily dosage of prednisolone among new cases compared with relapsed patients, steroid dosage did not differ significantly 3 months after RTX, which suggests the advantage of first-line RTX therapy in tapering steroid faster until reaching a minimal dose (Joly et al., 2017). Additionally, the possible presence of more resistant cases in the relapsed group and a higher initial dosage of prednisolone may skew results toward more desirable outcomes in treatment-naïve cases.

Another finding that deserves attention is the percentage of patients with mucocutaneous PV, which was higher in the relapsed group. This may be due to the conversion of milder phenotypes to the mucocutaneous phenotype during the disease course or possibly higher relapse rates in mucocutaneous patients.

When evaluating the serologic evolution after treatment, anti-Dsg 1 disappeared sooner than anti-Dsg3 in both groups, as shown in previous studies (Abasq et al., 2009; Abidi et al., 2017; Naseer et al., 2015), especially among responders. On the other hand, although anti-Dsg 3 values remained positive in a remarkable proportion of patients of both groups after treatment with RTX, the values were significantly higher in the relapsed group. Undetectable anti-Dsg1 in the presence of high values of anti-Dsg3 in patients in remission is consistent with the results of previous studies (Abidi et al., 2017; Daneshpazhooh et al., 2014; Naseer et al., 2015; Reguiai et al., 2012; Sinistro et al., 2015). Lastly, only anti-Dsg1 quantitative status (positive or negative) was significantly different between the two groups. Therefore, our study confirms that anti-Dsg1 values paralleled better the clinical response. There seems to be a paradox regarding why anti-Dsg1 values are so important in PV when Dsg3 is the main target in this disease and Dsg1 has a low expression in the oral mucosa (Shirakata et al., 1998).

One of our aims was to determine whether EDTA-Dsg ELISA would be beneficial for monitoring disease activity in patients receiving RTX. In the study by Li et al. (2015) on 29 patients with pemphigus, both conventional and conformational anti-Dsg1 correlated with total and cutaneous PDAI scores. For anti-Dsg3, both conventional and conformational values correlated with mucosal scores (better seen for conformational); however, only conformational anti-Dsg3 showed a correlation with cutaneous PDAI scores (Li et al., 2015). Our study did not confirm these findings. In other words, EDTA-Dsg ELISA was not found to be beneficial in this setting.

Conclusion

EDTA Dsg ELISA did not add more information than conventional assay. Clinically, patients with newly diagnosed PV appeared to benefit more than relapsing patients from treatment with RTX as first-line therapy in the short term. Early treatment with RTX also minimized the risk of no response. However, these findings could be explained by other aspects. First, the significantly higher dosage of steroid at baseline and higher cumulative dosage in the follow-up period may have influenced the results. Second, relapsing patients may have recalcitrant disease. Serologically, anti-Dsg1 values are much more representative of clinical response. Further large-scale studies with a longer follow-up are encouraged to elucidate the obscure points of PV treatment with RTX.

Conflict of Interest

None.

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Study Approval

The authors confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies.

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