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Clinical and serological predictors of relapse in pemphigus: a study of 143 patients

Running head: predictors of relapse in pemphigus

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What is already known about this topic?

- Clinical and immunopathological factors predictive of relapse and earliness of relapse in pemphigus have not yet been uniquely identified

- Although anti-desmoglein (Dsg)1 and anti-Dsg3 titres have been directly correlated to disease activity, the role of serological monitoring through serial Enzyme Linked Immunosorbent Assays in pemphigus patients' follow-up is controversial

What does this study add?

- A Body Surface Area (BSA) score of 3 is a significant predictor of relapse compared with a BSA score lower than 3
- Negativity of the autoantibody titer at clinical remission in patients stratified on the basis of their anti-Dsg1/anti-Dsg3 profile at diagnosis may be a reliable tool in predicting relapse.

ABSTRACT

Background: Pemphigus is an autoimmune bullous disease mediated by autoantibodies targeting epithelial cell–cell adhesion molecules. Predictors of relapse have not yet been uniquely identified.

Objectives: To identify factors at diagnosis and during follow-up which could be predictors of relapse.

Methods: Clinical and immunopathological data at diagnosis, clinical remission, and first relapse from patients with pemphigus vulgaris or foliaceus and at least a 36-month follow-up were retrospectively collected. Based on autoantibody profile at diagnosis, three serological patients' subsets were devised: anti-desmoglein (Dsg)1-positive/anti-Dsg3-negative; anti-Dsg1-negative/anti-Dsg3-positive; anti-Dsg1/anti-Dsg3-positive.

Results: Data from 143 patients were collected. No significant differences were found between relapsers (n=90) and non-relapsers (n=53) in terms of time to remission and anti-Dsg1/anti-Dsg3 titers at diagnosis and remission. Considering all patients, a Body Surface Area (BSA) of 3 compared to BSA<3 (OR=3.30, 95%CI:1.17-9.28; p=0.0240) and a positive titer in either anti-Dsg1 or anti-Dsg3 autoantibodies at remission compared to having both negative (OR=2.42, 95%CI 1.21-4.85, p=0.0130) predicted a higher risk of relapse. In patients with anti-Dsg3-positive/anti-Dsg1-negative at diagnosis, failure to achieve anti-Dsg3 negativity at clinical remission was a significant relapse predictor (OR=7.89, 95%CI:2.06-30.21; p=0.0026). Conversely, failure to achieve anti-Dsg1 negativity at clinical remission was a significant relapse predictor in patients with both anti-Dsg1 and anti-Dsg3 positivity at diagnosis (OR=5.74, 95%CI:1.15-28.61; p=0.0331), but not in those with positive anti-Dsg1-positive/anti-Dsg3-negative at diagnosis (OR=1.08, 95%CI:0.27-4.30; p=0.9093).

Conclusion: Regardless of pemphigus subtype, autoantibody titer negativity at clinical remission in patients classified based on their anti-Dsg1/anti-Dsg3 profile at diagnosis and BSA were useful tools in predicting relapse.

INTRODUCTION

Pemphigus is a group of mucocutaneous autoimmune bullous diseases mediated by circulating autoantibodies targeting epithelial cell–cell adhesion molecules of the cadherin family, particularly desmoglein (Dsg)3 and Dsg1.[1] Most of these autoantibodies may be detected and quantified by means of enzyme-linked immunosorbent assays (ELISA).[1] The most frequent variant of pemphigus is pemphigus vulgaris (PV), which typically exhibits a chronic-relapsing course and is characterized by most relapses occurring within two years from the diagnosis[2]; a less frequent variant is pemphigus foliaceus (PF), which is generally regarded as more responsive to the treatment and rarely characterized by a chronic course.[3]

Although many studies explored the role of different clinical and immunopathological factors in predicting relapse, markers predictive of relapse have not yet been uniquely identified.

The presence of mucosal involvement at pemphigus onset[4,5] and the positivity of direct immunofluorescence in PV patients in clinical remission[6] have been found to be associated with a higher risk of relapse. Ujiie *et al*. showed also that in patients with mucocutaneous PV, initial doses of systemic corticosteroids were significantly lower in relapsing than in non-relapsing cases.[7]

Changes in titres of circulating autoantibodies evaluated by means of Enzyme Linked Immunosorbent Assay (ELISA) may help the clinician undertake therapeutic decisions in the remission phase.[8] A correlation between anti-Dsg1/anti-Dsg3 titres and disease activity has been widely demonstrated,[9-16] even though serial ELISAs cannot be considered absolute indicators of disease activity. Indeed, elevated autoantibody titres may persist in phases of clinical remission,[17,18] conceivably due to a high percentage of non-pathogenetic autoantibodies (e.g., IgG1-type autoantibodies or autoantibodies directed against non-disease-associated epitopes) in these patients. The correlation of anti-Dsg1 antibodies to skin relapses would seem to be more significant than that of anti-Dsg3 antibodies to mucosal relapses.[9,14,19] On the other hand, Daneshpazooh *et al*.[6] demonstrated that positive (> 20 U/mL) anti-Dsg3 titres were associated with an earlier relapse in PV patients in remission. In a prospective study on pemphigus relapses in rituximab-treated patients, lower Dsg-1 levels were associated with a longer time to relapse.[20] Finally, another study on biomarkers predictive of relapse in rituximab-treated pemphigus patients showed that the relapse was associated with positivity for either anti-Dsg1 or anti-Dsg3 antibodies in serial ELISA tests after rituximab treatment.[21]

The primary endpoints of our single-center study were: i) to compare the demographic, clinical and immunopathological features at diagnosis and during follow-up between relapsing and non-relapsing patients; ii) to identify factors at diagnosis and during follow-up which could be predictive of relapse.

MATERIALS AND METHODS

Patients

Clinical data of all patients with pemphigus seen in the 2007-2019 period at the Dermatology Unit of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan were retrospectively analysed.

Eligibility criteria were as follows: i) diagnosis of PV or PF based on typical clinical (mucosal and/or cutaneous blisters and/or erosions), histopathological (acantholysis) and immunopathological (IgG and/or C3 intercellular deposits on direct and/or indirect immunofluorescence microscopy) criteria; ii) positivity at onset of at least one test between anti-Dsg1 and Dsg3; iii) availability of clinical data and anti-Dsg1/Dsg3 ELISAs at least at diagnosis, clinical remission and first relapse; iv) follow-up period of at least 36 months from the diagnosis. Patients who did not achieve clinical remission during the follow-up period, patients with pemphigus subtypes other than PV or PF and patients treated with rituximab before the first relapse were ruled out. Four clinical phenotypes[22] were distinguished: cutaneous PV [cPV] (suprabasal acantholysis, presence of anti-Dsg3 autoantibodies with or without anti-Dsg1 antibodies and exclusively cutaneous lesions)[23], mucosal PV [mPV] (suprabasal acantholysis, presence of anti-Dsg3 antibodies with or without anti-Dsg1 antibodies and exclusively mucosal lesions) and mucocutaneous PV [mcPV] (suprabasal acantholysis, presence of anti-Dsg3 antibodies with or without anti-Dsg1 antibodies and lesions of the skin and mucous membranes) and PF (superficial acantholysis, presence of anti-Dsg1 autoantibodies without anti-Dsg3 autoantibodies and no mucosal involvement).

This study was conducted in accordance with the Declaration of Helsinki and all patients provided written informed consent for study participation.

Clinical and serological assessment: definitions

Based on criteria adapted from the consensus-based definitions proposed by Murrell *et al*.,[24] clinical remission was defined as the absence of old or new lesions for at least two months in a patient with minimal (prednisone [or equivalent] at a dose of less than 10 mg/day and/or minimal adjuvant therapy) or no therapy, while relapse was defined as the onset of more than three new lesions (blisters, erosions) per month that do not heal within 1 week, or the extension of established lesions in a patient who had achieved clinical remission.

In order to avoid potential misclassification, a patient was considered as negative for anti-Dsg1 and/or anti-Dsg3 autoantibodies at clinical remission only if no subsequent positivity was recorded in the following 6 months. Similarly, a patient was considered as positive for anti-Dsg1 and/or anti-Dsg3 autoantibodies at clinical remission only if no subsequent negativity was recorded in the following 6 months. Further serological changes occurring more than 6 months after the achievement of clinical remission were not taken into consideration in those who did not relapse during the available follow-up period.

Similarly to what reported by Ujiie *et al*.,[7] skin severity at diagnosis was graded according to body surface area (BSA) involved in 0 (no lesions), 1 (up to 5% of BSA involved), 2 (5-15% of BSA involved), 3 (> 15% of BSA involved) and oral severity was graded according to oral cavity surface area (OSA) in 0 (no lesions), 1 (up to 5% of OSA involved), 2 (5-30% of OSA involved), 3 (>30% of OSA involved).[7] BSA and OSA

scores had been documented at each visit always by the same investigators (G.G. and A.V.M.) and were available in their written reports. In all cases, total body clinical photographs were also available and were employed for confirmation.

The following data were collected: sex; age at onset; pemphigus subtype; mucosal and/or cutaneous involvement; involvement of oral, nasal, laryngeal, conjunctival, anogenital mucosal subsites; BSA; OSA; anti-Dsg1 and anti-Dsg3 autoantibody titer at diagnosis, remission and first relapse; time from diagnosis to clinical remission, time from diagnosis to first relapse; treatment at diagnosis and at first relapse; follow-up duration time.

Based on anti-Dsg1 and anti-Dsg3 autoantibody profile at diagnosis, three serological subsets were created: anti-Dsg1-positive/anti-Dsg3-negative patients; anti-Dsg1-negative/anti-Dsg3-positive patients; anti-Dsg1 /anti-Dsg3-positive patients.

ELISA test

To identify anti-Dsg1 and anti-Dsg3 autoantibodies in patients' serum, an ELISA test (MESACUP Desmoglein-1 and MESACUP Desmoglein-3 respectively, MBL, Nagoya, Japan) was used. The assay was conducted according to the manufacturers' instructions. A cut-off value > 20 U/ml was considered as positive either for anti-Dsg1 and anti-Dsg3 autoantibodies.

Statistical analysis

Categorical data are reported as counts (percentages) whereas continuous variables are reported as median (interquartile range, IQR). Comparisons of clinical and serological features between relapsing and non-relapsing patients were performed using the Fisher exact test (for categorical variables) or the Mann-Whitney non-parametric test (for continuous variables). Correlation between quantitative variables was assessed with Spearman's rank correlation coefficient (r). Considering only relapsing patients, variation of anti-Dsg1 and anti-Dsg3 from diagnosis to first relapse was calculated for each patient (within-patient analysis). Then the non-parametric sign test for paired data was used to investigate whether anti-Dsg1 and anti-Dsg3 values changed from diagnosis to relapse. Subgroup analyses were also conducted considering pemphigus subtypes. Finally, univariate and multivariate logistic regression analyses were performed to assess the effect of some predefined factors on the risk of relapse. The following factors were considered as potential predictors of relapse in univariate models: age, sex, pemphigus subtype, mucosal/cutaneous involvement, BSA, OSA, nasal/laryngeal involvement, anogenital involvement, ocular involvement, anti-Dsg1 and anti-Dsg3 positivity at diagnosis, anti-Dsg1 and anti-Dsg3 negativity at remission (at least one of the two or both). Only those factors that showed a statistically significant association at univariate stage were considered in multivariate model. The logistic regression analyses were carried out on the whole sample and on the three subgroups identified according to anti-Dsg1 and anti-Dsg3 autoantibody profile at diagnosis, as described above (anti-Dsg1-positive/anti-Dsg3-negative; anti-Dsg1-negative/anti-Dsg3-positive; anti-Dsg1/anti-Dsg3-positive). Odds ratios with their 95% confidence intervals (CI) were obtained from logistic models. P values lower than 0.05, two sided, were considered statistically significant. All the

statistical analyses were conducted with the statistical software SAS (release 9.4, SAS Institute, Inc., Cary, North Carolina).

RESULTS

Demographic, clinical and immunopathological features

As shown in Table 1, data from 143 patients with pemphigus were collected, including 83 (58.0%) females and 60 (42.0%) males. Median age at onset was 55 (IQR: 43-67) years. Twenty-nine (20.3%) patients with PF, 15 (10.5%) with cPV, 27 (18.9%) with mPV and 72 (50.4%) with mcPV were identified (Table S1). Ninety out of 143 (62.9%) patients developed at least one relapse, while 53/143 (37.1%) never developed relapses. Median follow-up time was 74 (IQR: 58-98) months. Skin involvement was observed in 117 (81.8%) patients (BSA=1 in 27 [18.9%], BSA=2 in 62 [43.4%] and BSA=3 in 28 [19.6%] patients) while oral involvement was observed in 95 (66.4%) patients (OSA=1 in 17 [11.9%], OSA=2 in 58 [40.6%] and OSA=3 in 20 [14.0%] patients). Median titer of anti-Dsg1 antibodies was 56.8 (IQR: 10.6-121.1) U/ml, while median titer of anti-Dsg3 antibodies was 139.6 (IQR: 14.4-180.1) U/ml. Thirty-seven (25.9%) and 47 (32.9%) patients had exclusive anti-Dsg1 or anti-Dsg3 autoantibody positivity at diagnosis, respectively. Fifty-nine (41.3%) patients had both anti-Dsg1 and anti-Dsg3 autoantibodies at diagnosis. Median time to remission was not significantly different between relapsing and non-relapsing patients (5 [IQR: 3-8] vs 5 [IQR: 3-7] months; p=0.5224). At diagnosis, median anti-Dsg1 antibody values were 62.9 U/ml in relapsing and 30.3 U/ml in non-relapsing patients (p=0.2015), while median anti-Dsg3 antibody values were 142.8 U/ml in relapsing and 137.8 U/ml in non-relapsing patients (p=0.5580), respectively. At diagnosis, considering all 143 patients, moderate yet statistically significant correlations between anti-Dsg1 and BSA (Spearman r=0.446, p<0.0001) and between anti-Dsg3 and OSA (r=0.533, p<0.0001) were found. At remission, median anti-Dsg1 antibody values were 8.7 U/ml in relapsing and 8.1 U/ml in non-relapsing patients (p=0.4568), while median anti-Dsg3 antibody values were 7.3 U/ml in relapsing and 5.8 U/ml in non-relapsing patients (p=0.4876), respectively.

No subsequent serological negativity/positivity was recorded in those with positive/negative anti-Dsg1 and/or anti-Dsg3 antibody titer at remission in the 6 months following the achievement of remission.

Induction therapy at diagnosis with either systemic corticosteroid alone (n=69; 48.3%) or combined with immunosuppressive adjuvant drugs (n=74; 51.7%) was documented. Maintenance therapy at first relapse consisted mainly of systemic corticosteroid monotherapy with (n=36; 40.0%) or without (n=42; 46.7%) an immunosuppressive adjuvant drug. Mean systemic corticosteroid dosage at the time of relapse was 0.12 mg/kg/day of prednisone. Only one patient was taking a non-steroidal immunosuppressive drug. Eleven patients (12.2%) were not taking any medication.

After stratification according to pemphigus subtype at diagnosis, no statistically significant differences between relapsing and non-relapsing patients were found in terms of time to remission and anti-Dsg1/anti-Dsg3 antibody titers at diagnosis and remission.

Clinical and immunopathological features of relapsing patients

In relapsing patients, median time to remission was 5 (IQR 3-8) months and median time to relapse was 29 (IQR 18-44) months with a median disease-free interval of 22 (IQR 12-36) months. Median value of anti-Dsg1 antibodies was 62.9 U/ml at diagnosis and decreased to 8.7 U/ml at remission, subsequently rising at

19.1 U/ml at first relapse. The median value of within-patient anti-Dsg1 titers' reduction at relapse compared to diagnosis (i.e., the median of the difference between titer at diagnosis and titer at relapse for each patients) was 8.3 U/ml (p=0.001). Median value of anti-Dsg3 antibodies was 142.8 U/ml at diagnosis and decreased to 7.3 U/ml at remission, subsequently rising to 83.9 U/ml at first relapse. The median value of within-patient anti-Dsg3 titers' reduction at relapse compared to diagnosis was 2.7 U/ml (p=0.0558). At relapse, significant correlations between anti-Dsg1 and BSA (r=0.450, p<0.0001) and between anti-Dsg3 and OSA (r=0.407, p=0.0001) were noted.

Clinical, demographic and serological predictors of relapse

Considering all patients, a Body Surface Area (BSA) of 3, compared to BSA<3, (OR=3.30, 95%CI:1.17-9.28; p=0.0240) and a positive titer in either anti-Dsg1 or anti-Dsg3 autoantibodies at remission compared to having both negative (OR=2.42, 95% CI 1.21-4.85, p=0.0130) predicted a higher risk of relapse.

Considering anti-Dsg1 and anti-Dsg3 autoantibody positivity separately, both at diagnosis and at remission, neither was found to be a statistically significant predictor of relapse. However, a higher, yet not statistically significant, risk of relapse (OR=2.16, 95% CI: 0.90-5.23, p=0.0864) was indeed recorded in patients with positive anti-Dsg1 autoantibodies at remission.

Concerning induction therapy at diagnosis across our cohort, the employment of systemic corticosteroids plus immunosuppressive adjuvant drugs versus systemic corticosteroid monotherapy predicted a higher, yet not statistically significant, risk of relapse (OR=1.51, 95% CI: 0.76-2.99, p=0.2361).

Considering patients with sole anti-Dsg3 autoantibody positivity (i.e., those with positive anti-Dsg3 and negative anti-Dsg1 autoantibodies) at diagnosis (n=47), failure to achieve anti-Dsg3 titer negativity at clinical remission was a statistically significant predictor of relapse (OR=7.89, 95% CI: 2.06-30.21, p=0.0026). Conversely, in patients with sole anti-Dsg1 autoantibody positivity (i.e., those with positive anti-Dsg1 and negative anti-Dsg3 autoantibodies) at diagnosis (n=37), failure to achieve anti-Dsg1 titer negativity at clinical remission was not a reliable predictor of relapse (OR=1.08, 95% CI: 0.27-4.29; p=0.9093). Finally, failure to achieve anti-Dsg1 negativity at clinical remission in patients with both anti-Dsg1 and anti-Dsg3 autoantibody positivity at diagnosis (n=59) was a statistically significant predictor of relapse (OR=5.74, 95% CI: 1.15-28.61; p=0.0331) (Table 2).

DISCUSSION

In the last decades, several studies investigated possible clinical and/or laboratory predictors of relapse in pemphigus, focusing mainly on the relationship between disease activity and autoantibody titres evaluated by means of ELISA. Considering that pemphigus relapses usually occur in the first two years from disease onset, only patients with at least a three-year follow-up were included in this study. In line with the work by Kyriakis *et al*.,[2] median time from diagnosis to first relapse was 29 months, with a median disease-free interval of about 22 months.

Among the collected clinical variables, only a BSA score of 3 at diagnosis, corresponding to 15% or more body surface involvement, was shown to be a significant predictor of relapse, when compared with less extensive cutaneous disease scored as BSA < 3. In contrast with previous works[4,5,24] indicating the possible role of mucosal involvement at pemphigus onset as a predictor of relapse, in our cohort neither mucosal involvement at onset nor any of the other clinical variables was found to be a significant predictive factor of relapse.

Several investigations validated the correlation between disease activity and anti-Dsg1 and/or anti-Dsg3 autoantibody titers in pemphigus.[9-16] In agreement with them, a significant correlation was noted in our cohort both at diagnosis and at relapse between anti-Dsg1 autoantibody titers and BSA scores, and between anti-Dsg3 autoantibody titers and OSA scores. The role of autoantibody titers as immunologic predictors of relapse is more nuanced, with available evidence being controversial.[9,14,19]

In our study, three serological subsets were devised to assess the utility of each autoantibody titer within highly coherent subgroups. When considering our cohort in its entirety, neither anti-Dsg1 nor anti-Dsg3 autoantibody positivity, at diagnosis or at remission, was found to be a significant predictor of relapse. However, focusing separately on each serological subset, we found that failure to achieve anti-Dsg1 autoantibody titer negativity predicted relapse in anti-Dsg1/anti-Dsg3 positive patients as well as failure to achieve anti-Dsg3 titer negativity at clinical remission was a valuable predictor of relapse in patients with sole anti-Dsg3 autoantibody positivity at diagnosis. In contrast, anti-Dsg1 autoantibody titer did not provide adequate immunological guidance in predicting relapse in anti-Dsg1 positive/anti-Dsg3 negative patients. It can be speculated that the above-mentioned serological subsets at diagnosis faithfully identify groups of patients with homogeneous immunopathological profiles and it is conceivable that monitoring such groups may allow a better prediction of relapse than considering anti-Dsg1 or anti-Dsg3 separately. Failure of anti-Dsg1 antibodies in predicting relapse in the subset with exclusive anti-Dgs1 positivity might be due to the high proportion of patients with elevated non-pathogenetic anti-Dsg1 at remission who did not relapse, as suggested also by Kamiya *et al.*[26]

Our study has some limitations. Due to its retrospective nature, assessment of disease severity was not performed by means of a validated scoring system, such as Pemphigus Disease Area Index (PDAI) or Autoimmune Bullous Skin Disorder Intensity Score (ABSIS). Moreover, even if a minimum of 36 months of follow-up was required for inclusion, follow-up duration was heterogeneous. However, considering that most pemphigus relapses occur within the first two years since onset, it is likely that patients classified as "non-relapsing" in our cohort did not relapse after follow-up termination. Further, to simplify our analyses,

we focused on clinical and serological data at first relapse only. Concerning induction therapy at diagnosis, the use of an immunosuppressive adjuvant drug alongside systemic corticosteroids did not predict a lower relapse risk relative to systemic corticosteroid monotherapy, rather the opposite was true, albeit not reaching statistical significance. This finding possibly reflects the choice of immunosuppressive adjuvants in patients with a more severe clinical picture at diagnosis. It also shows the limited impact of induction therapy at diagnosis as a confounder in the rest of our analyses. It was not feasible to retrospectively assess the predictive role of treatment during follow-up, as this would have required an arbitrary timepoint selection for the comparison of non-relapsers versus relapsers (i.e., treatment at an arbitrary timepoint during remission versus treatment at first relapse, respectively). However, it must be underscored that most patients were receiving systemic corticosteroids and/or adjuvant treatments at the moment of relapse and only a minority of patients was untreated. Finally, the small sample sizes of individual subgroups stratified by clinical subtype lacked the statistical power to demonstrate an effect in relapse prediction.

In conclusion, patients' classification based on anti-Dsg1 and anti-Dsg3 autoantibody positivity at diagnosis, regardless of pemphigus subtype, and subsequent assessment of antibody titers at clinical remission may be of use in relapse prediction. More specifically, failure to achieve anti-Dsg3 titer negativity at clinical remission appears to be a significant predictor of relapse in those who had isolated anti-Dsg3 autoantibody positivity at diagnosis; conversely, failure to achieve anti-Dsg1 titer negativity at remission seems to predict relapse only in patients with both anti-Dsg1 and anti-Dsg3 autoantibody positivity, but not in those who had isolated anti-Dsg1 autoantibody positivity at diagnosis.

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TABLES

Table 1. Clinical and serological features of relapsing and non-relapsing pemphigus patients included in the study.

		Relapsing (n=90)	Non-relapsing (n=53)	All patients (n=143)
Males, n (%)		35 (38.9)	25 (47.2)	60 (42.0)
Median age at onset, years (IQR)		54 (42-67)	56 (46-66)	55 (43-67)
Pemphigus subtype at diagnosis, n (%)	Foliaceus	20 (22.2)	9 (17.0)	29 (20.3)
	Vulgaris (cutaneous)	10 (11.1)	5 (9.4)	15 (10.5)
	Vulgaris (mucosal)	19 (21.1)	8 (15.01)	27 (18.9)
	Vulgaris (mucocutaneous)	41 (45.6)	31 (58.5)	72 (50.3)
Involved mucosal sites, n (%)	Oral mucosa	58 (64.4)	37 (69.8)	95 (66.4)
	Nasal/laryngeal mucosa	12 (13.3)	7 (13.2)	19 (13.3)
	Anogenital mucosa	12 (13.3)	7 (13.2)	19 (13.3)
	Conjunctiva	6 (6.7)	3 (5.7)	9 (6.3)
BSA, n (%)	0	18 (20.0)	8 (15.1)	26 (18.2)
	1	14 (15.6)	13 (24.5)	27 (18.9)
	2	35 (38.9)	27 (50.9)	62 (43.4)
	3	23 (25.6)	5 (9.4)	28 (19.6)
OSA, n (%)	0	32 (35.6)	16 (30.2)	48 (33.6)
	1	12 (13.3)	5 (9.4)	17 (11.9)
	2	32 (35.6)	26 (49.1)	58 (40.6)
	3	14 (15.6)	6 (11.3)	20 (14.0)
Therapy at diagnosis (%)	Systemic corticosteroid plus immunosuppressive adjuvant therapy	50 (55.5)	24 (45.3)	74 (51.7)
	Systemic corticosteroid monotherapy	40 (44.4)	29 (54.7)	69 (48.3)
	Immunosuppressive monotherapy	0	0	0
	Mean prednisone equivalent dose (mg/kg/day) (SD)	1.21 (0.80)	1.12 (0.77)	1.17 (0.79)
Therapy at relapse (%)	Systemic corticosteroid plus immunosuppressive adjuvant therapy	36 (40.0)	-	-
	Systemic corticosteroid monotherapy	42 (46.7)	-	-
	Immunosuppressive monotherapy	1 (1.1)	-	-
	None	11 (12.2)	-	-
	Mean prednisone equivalent dose (mg/kg/day)	0.12	-	-
Time between diagnosis and complete remission, months, median (IQR)		5 (3-8)	5 (3-7)	5 (3-7)
Time between diagnosis and first relapse, months, median (IQR)		29 (18-44)	-	-
Disease free time, months, median (IQR)		22 (12-36)	-	-
Follow-up time, months, median (IQR)		78 (60-103.3)	70 (50-91.5)	74 (58-98)
ELISA at diagnosis, median, U/ml (IQR)	anti-Dsg1[§]	62.9 (10.6-151.0)	30.3 (11.1-109.3)	56.8 (10.6-121.1)
	anti-Dsg3[§]	142.8 (10.7-176.7)	137.8 (29.5-180.1)	139.6 (14.4-180.1)

ELISA at remission, median, U/ml (IQR)	anti-Dsg1[§§]	8.7 (5.8-26.6)	8.1 (6.2-10.6)	8.4 (5.8-12.5)
	anti-Dsg3[§§]	7.3 (4.3-94.7)	5.8 (4.2-69.6)	6.8 (4.2-93.2)
ELISA at relapse, median, U/ml (IQR)	anti-Dsg1[§§§]	19.1 (8.0-102.1)	-	
	anti-Dsg3[§§§]	83.9 (4.4-156.1)	-	
Serological subtypes, n (%)	anti-Dsg1-positive and anti-Dsg3-positive	35 (38.9)	24 (45.3)	59 (41.3)
	anti-Dsg1-positive and anti-Dsg3-negative	25 (27.8)	12 (22.6)	37 (25.9)
	anti-Dsg1-negative and anti-Dsg3-positive	30 (33.3)	17 (32.1)	47 (32.9)

BSA=body surface area; Dsg=desmoglein; ELISA=enzyme linked immunosorbent assay; OSA=oral surface area; SD=standard deviation.

§ Among relapsing patients, 60 had a positive anti-Dsg1 autoantibody titer, and 65 had a positive anti-Dsg3 autoantibody titer at diagnosis, respectively. Among non-relapsing patients, 36 had a positive anti-Dsg1 autoantibody titer, and 41 had a positive anti-Dsg3 autoantibody titer at diagnosis, respectively.

§§ Among relapsing patients, 25 had a positive anti-Dsg1 autoantibody titer, and 43 had a positive anti-Dsg3 autoantibody titer at clinical remission, respectively. Among non-relapsing patients, 8 had a positive anti-Dsg1 autoantibody titer, and 19 had a positive anti-Dsg3 autoantibody titer at clinical remission, respectively.

§§§ Among relapsing patients 45 had a positive anti-Dsg1 autoantibody titer, and 53 had a positive anti-Dsg3 autoantibody titer at relapse, respectively.

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) for relapse according to clinical and immunopathological parameters in pemphigus patients with different serological subsets at diagnosis.

		Anti-Dsg1 positive and anti-Dsg3 negative patients (n=37)		Anti-Dsg1 negative and anti-Dsg3 positive patients (n=47)		Anti-Dsg1 positive and anti-Dsg3 positive patients (n=59)		All patients (n=143)	
		OR (95% CI)	**P value**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Age (years)	<=45	1*	0.6944	1*	0.7375	1*	0.1006	1*	0.1674
	46-60	0.47 (0.07-3.34)		0.92 (0.20-4.31)		0.22 (0.05-0.88)		0.43 (0.18-1.04)	
	>60	0.80 (0.12-5.41)		0.59 (0.15-2.36)		0.42 (0.11-1.57)		0.58 (0.25-1.35)	
Sex	M	1*	0.5653	1*	0.9793	1*	0.3278	1*	0.3332
	F	1.52 (0.37-6.30)		1.02 (0.31-3.36)		1.69 (0.59-4.85)		1.40 (0.71-2.79)	
Pemphigus subtype	mcPV	1*	0.8563	1*	0.9769	1*	0.1780	1*	0.5180
	cPV	††		††		0.63 (0.15-2.68)		1.51 (0.46-4.88)	
	mPV	1.00 (0.03-29.81)		1.15 (0.32-4.08)		6.26 (0.72-54.75)		1.80 (0.69-4.64)	
	Foliaceus	2.22 (0.27-18.37)		††		††		1.68 (0.67-4.19)	
Mucosal, cutaneous or mucocutaneous involvement	Mucosal	1*	0.6156	1*	0.9769	1*	0.1780	1*	0.3247
	Cutaneous	2.44 (0.14-43.47)		††		0.10 (0.01-1.17)		0.90 (0.32-2.56)	
	Mucocutaneous	1.01 (0.03-29.81)		0.87 (0.25-3.11)		0.16 (0.02-1.40)		0.56 (0.22-1.44)	
BSA	0-2	1*	0.0794	†		1*	0.1537	1*	**0.0240**
	3	4.62 (0.84-25.49)				2.80 (0.68-11.52)		3.30 (1.17-9.38)	
OSA	0-2	†		1*	0.8506	1*	0.4134	1*	0.4824
	3			0.83 (0.13-5.56)		1.73 (0.47-6.44)		1.44 (0.52-4.01)	
Nasal/laryngeal involment	Yes	†		1*	0.8770	1*	0.8539	1*	0.9829
	No			0.87 (0.14-5.31)		0.89 (0.25-3.14)		0.99 (0.36-2.69)	
Anogenital involvement	Yes	†		1*	0.4663	1*	0.4924	1*	0.9829
	No			1.67 (0.42-6.59)		0.55 (0.10-3.08)		0.99 (0.36-2.69)	
Ocular involvement	Yes	†		1*	0.5522	1*	0.3448	1*	0.8111

	No			1.87 (0.24-14.61)		0.34 (0.04-3.22)		0.84 (0.20-3.51)	
Therapy at diagnosis	Systemic CS monotherapy	1*	0.4164	1*	0.6795	1*	0.3939	1*	0.2361
	Systemic CS plus immunosuppressive adjuvant therapy	1.78 (0.44-7.18)		1.29 (0.39-4.24)		1.58 (0.55-4.48)		1.51 (0.76-2.99)	
Anti-Dsg1 positivity at diagnosis	Yes	NA		NA		NA		1*	0.8774
	No							1.06 (0.51-2.19)	
Anti-Dsg3 positivity at diagnosis	Yes	NA		NA		NA		1*	0.4988
	No							1.31 (0.60-2.90)	
Anti-Dsg1 negativity at remission	Yes	1*	0.9093	NA		1*	**0.0331**	1*	0.0864
	No	1.08 (0.27-4.29)				5.74 (1.15-28.61)		2.16 (0.90-5.23)	
Anti-Dsg3 negativity at remission	Yes	NA		1*	**0.0026**	1*	0.8211	1*	0.1083
	No			7.89 (2.06-30.21)		1.13 (0.40-3.21)		1.78 (0.88-3.59)	
Anti-Dsg1 and anti-Dsg3 negativity at remission§	Yes	†		†		†		1*	**0.0130**
	No							2.42 (1.21-4.85)	

cPV= cutaneous pemphigus vulgaris; mcPV= mucocutaneous pemphigus vulgaris; mPV= mucosal pemphigus vulgaris; BSA=body surface area; Dsg= desmoglein; NA= Not Appropriate; OSA=oral surface area; CS= corticosteroid. Multivariate analysis estimates for "All patients": Anti-Dsg1 and anti-Dsg3 negativity at remission (No vs Yes), OR=2.39 (1.17-4.85), **p=0.0164**; BSA (3 vs 0-2), OR=3.24 (1.13-9.27), **p=0.0285**.

*Reference category. † Model fit was not possible because of too sparse data. †† Odds ratio estimates were not obtained because of too sparse data. § Parameter considered only in the analysis performed on all the 143 patients