CLINICAL REPORT

Clinical Significance of Anti-desmoglein-1 and -3 Circulating Autoantibodies in Pemphigus Patients Measured by Area Index and Intensity Score

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Detection of anti-desmoglein-1 (anti-DSG-1) and anti-DSG-3 autoantibodies is widely used in the diagnosis of pemphigus. Two validated scoring systems, Pemphigus Disease Area Index (PDAI) and Autoimmune Bullous Skin Disorder Intensity Score (ABSIS), are used for the evaluation of clinical severity. The aim of this cross-sectional study was to interpret the titres of pemphigus autoantibodies in correlation with either total or location-dependent PDAI scores and ABSIS. A total of 35 pemphigus patients were selected and evaluated at 3 time points. Total PDAI and ABSIS seemed useful in pemphigus with cutaneous lesions or in the mucocutaneous form, while location-dependent PDAI and ABSIS scores were useful in the mucosal form. Anti-DSG-1 autoantibodies titres better showed the disease extent in pemphigus with cutaneous only or with mucocutaneous lesions. Anti-DSG-3 autoantibodies titres did not correlate to disease activity. *Key words: anti-DSG-1; anti-DSG-3; pemphigus; PDAI; ABSIS.*

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Detection of circulating autoantibodies by specific enzyme-linked immunoassay (ELISA) kits has become a preferred method for the diagnosis and follow-up of patients with autoimmune bullous diseases. In particular, in pemphigus patients, detection of anti-desmoglein-1 (anti-DSG-1) and/or anti-DSG-3 serum autoantibodies has been reported to coincide with the clinical type and severity of the disease.

During the last decade, efforts to evaluate the clinical extent and severity of pemphigus, in order to better estimate the therapeutic efficacy of different modalities, have led to the establishment of scoring systems. There are currently 2 validated scoring systems for pemphigus; the Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) and the Pemphigus Disease Area Index (PDAI) (1).

ABSIS is a clinical score introduced in 2007 by Pfutze et al. (2), in order to achieve improved evaluation and monitoring of the status of both oral and cutaneous lesions in patients with pemphigus. Total ABSIS score consists of ABSIS score for skin involvement, mucosal extent score and mucosal severity score.

PDAI was developed by the International Pemphigus Definitions Committee in 2008, to evaluate both mucosal and cutaneous lesions (3). Total PDAI score consists of PDAI-skin score, PDAI-scalp score and PDAI-mucous membranes score.

The aim of the present study was to examine the titres of anti-DSG-1 and anti-DSG-3 autoantibodies in correlation with the scores of either total or location-dependent ABSIS and PDAI indexes.

MATERIALS AND METHODS

A total of 35 pemphigus patients were consecutively selected to participate in this longitudinal study. For all patients, diagnosis was confirmed by histology, direct and indirect immunofluorescence, as well as by detection of circulating anti-DSG-1 and anti-DSG-3 autoantibodies. ELISA was performed using MBL kits (Nagoya, Japan). Patients with paraneoplastic pemphigus or pemphigus herpetiformis were excluded. Titres of circulating autoantibodies were measured at 3 different time-points: baseline (the time of initial diagnosis), month 6, and month 12. At the same time-points, the clinical picture was evaluated using both ABSIS and PDAI, total and location-dependent scores (Table I). Location-dependent scores were the ABSIS score for skin involvement, mucosal extent score, mucosal severity score, PDAI score for cutaneous lesions and PDAI-mucous membranes score. PDAI score for cutaneous lesions was defined as the sum of PDAI-skin score and PDAI-scalp score. Both

Table I. *Definition of both Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) and Pemphigus Disease Area Index (PDAI) total and location-dependent scores*

Total ABSIS score	ABSIS score for skin involvement + Mucosal extent score + Mucosal severity score
Total PDAI score	PDAI-cutaneous lesions score + PDAI-mucous membranes score
PDAI-cutaneous lesions score	PDAI-skin score + PDAI-scalp score
PDAI-skin score	Activity score + Damage score
PDAI-scalp score	Activity score + Damage score

PDAI-skin score and PDAI-scalp score are defined as the sum of activity and damage score. All scores were measured by one specific dermatologist of the research group in order to avoid measurement bias. Ethics board approval and patients' informed consent were provided.

Patients were studied and analysed after they had been divided into 3 subgroups regarding the location of lesions at baseline: cutaneous lesions, mucosal lesions, both cutaneous and mucosal lesions.

Regarding therapy algorithm, all patients were initially treated with systemic prednisolone at a dose of 1–1.5 mg/kg/BW for 4 weeks and, after tapering, remained to a maintenance dose of 5 mg of prednisolone. In some patients, an immunosuppresive drug (azathioprine or cyclophosphamide) was added to enhance the remission (Table SI[1]).

Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS 15.0). All tests were 2-sided, and the significance level was set at $\alpha = 0.05$. Descriptive statistics, including the mean, the standard deviation (SD), the median, the minimum and the maximum values were used in order to present continuous variables, while frequency distributions and percentages were used for categorical data. The normality of the continuous variables was tested with the Shapiro–Wilk test. Spearman's rank test was used to explore relationships between continuous variables. Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity and homoscedasticity.

RESULTS

Subjects were divided into 3 subgroups regarding the location of lesions at baseline: cutaneous lesions ($n=7$), mucosal lesions ($n=11$), both cutaneous and mucosal lesions ($n=17$). No patients were lost during the 12-month follow-up. Patients' clinical characteris-

tics at baseline, month 6 and month 12 regarding each subgroup are summarized in Table SII[1].

In the subgroup of patients presenting only cutaneous lesions (boxplots shown in Fig. 1), weakly positive correlations were detected at baseline between anti-DSG-1 autoantibodies and total ABSIS, ABSIS for skin involvement, total PDAI, and PDAI for cutaneous lesions (Table SIII[1]). On the other hand, no significant correlations were found at baseline between anti-DSG-3 autoantibodies and any of the clinical scores. At month 6 and 12 the disease was in total remission and no correlations were detected between clinical scores) and titres of autoantibodies.

In the subgroup of patients presenting only mucosal lesions (Fig. 2), no correlations were found between anti-DSG-1 and -3 autoantibodies and any of the clinical scores at baseline, nor at 6 and 12 months (see Table SIII[1]).

In the subgroup of patients presenting both cutaneous and mucosal lesions (Fig. 3), statistically significant correlations were detected at baseline between anti-DSG-1 autoantibodies and (*i*) total ABSIS ($p=0.010$), (*ii*) ABSIS for skin involvement ($p=0.005$), (*iii*) total PDAI ($p=0.013$), and (*iv*) PDAI for cutaneous lesions ($p=0.001$) (see Table SIII[1]). No significant correlations were detected between anti-DSG-3 autoantibodies in the clinical scores at baseline. At 6 and 12 months, anti-DSG-1 autoantibodies were still significantly correlated with total ABSIS, ABSIS for skin involvement, total PDAI, and PDAI for cutaneous lesions (see Table SIII[1]).

DISCUSSION

During the past decade, sensitive and specific ELISA systems for diagnosis and monitoring of patients with pemphigus have been developed (4).

There is increasing evidence that the clinical phenotype is strongly related to the antibody profile in most pemphigus patients (5, 6). Each subtype has its own anti-DSG autoantibody profile, indicating that the clinical phenotype of pemphigus is defined by the targeted desmoglein. The severity of skin lesions is generally correlated with anti-DSG-1 autoantibody levels, and the severity of oral lesions with anti-DSG-3 autoantibody levels (7, 8).

In accordance with the above, in the group of our patients with only cutaneous
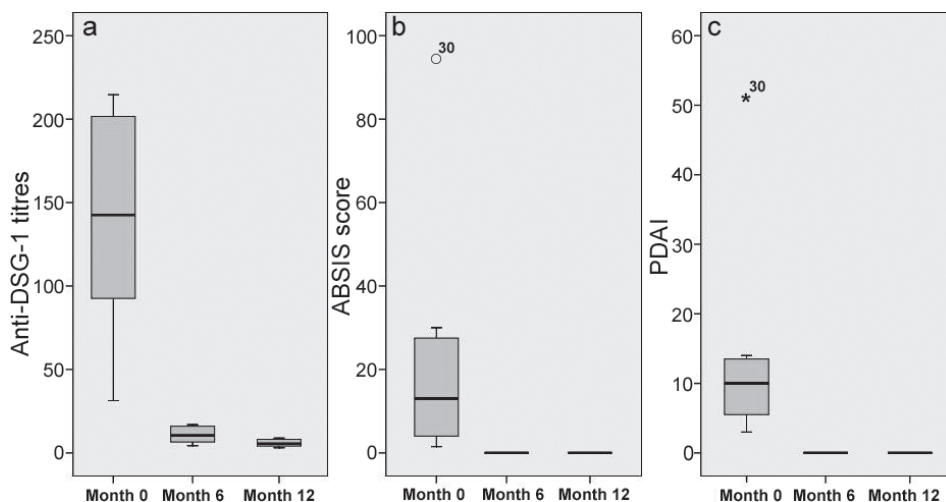

Fig. 1. Boxplots of data from patients presenting only cutaneous lesions from baseline to month 12. (a) Anti-desmoglein-1 (anti-DSG-1) autoantibodies titres, (b) Autoimmune Bullous Skin Disorder Intensity Score (ABSIS), and (c) Pemphigus Disease Area Index (PDAI). Circles and * stand for outliers.
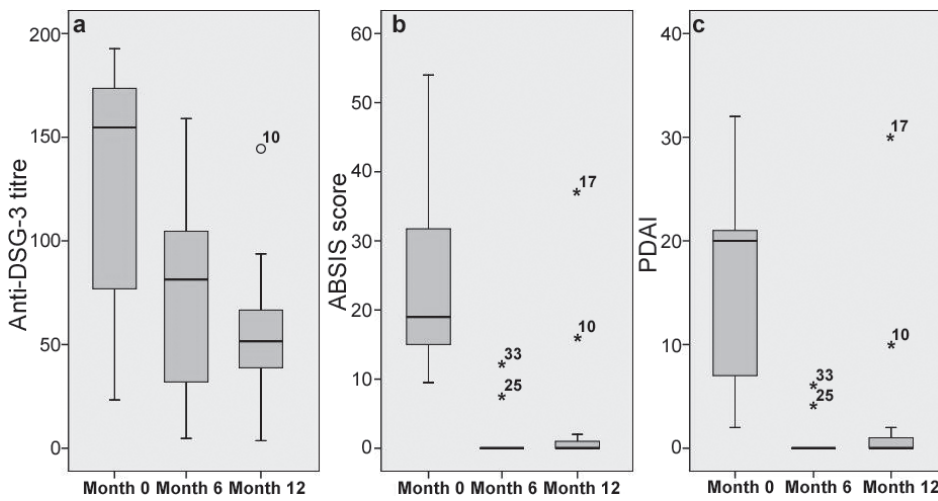
Fig. 2. Boxplots of data from patients presenting only mucosal lesions from baseline to month 12. (a) Anti-desmoglein-3 (anti-DSG-3) autoantibodies titres, (b) Autoimmune Bullous Skin Disorder Intensity Score (ABSIS), and (c) Pemphigus Disease Area Index (PDAI). Circles and * stand for outliers.

anti-DSG-3 autoantibodies, as the lesions were located only on the oral mucosa. The mucosal extent ABSIS and PDAI scores were more indicative and showed a medium positive correlation with the circulating anti-DSG 3 autoantibodies at baseline and after 6 months. There was a slow reduction in the above titres, while the clinical picture was almost in remission, as shown in Fig. 2.

A similar observation was reported in the study by Pfütze et al. (2), wherein ABSIS scoring truly reflected clinical disease activity, while autoantibody titres did not, as autoantibodies decreased, but were still detectable, after 6 months, in discordance with the observed clinical remission. Whether this persistence of anti-DSG 3 titres, despite the clinical improvement of patients with mucosal lesions, indicates that there is an upcoming recurrence, needs to be proved in a large cohort of patients.

In the mucocutaneous form, total PDAI and ABSIS scores were strongly correlated with the titres of anti-DSG-1 autoantibodies at all time-points and less with the anti-DSG-3 autoantibodies at baseline and at 6 months. The only time point when all scores were strongly correlated with anti-DSG-3 autoantibodies was month 12, probably because the oral lesions show a slow rate of recovery. Although the rate of reduction of anti-DSG-3 and anti-DSG-1 autoantibodies is similar, the values of anti-DSG-3 autoantibodies remained at higher levels, as shown in Fig. 3.
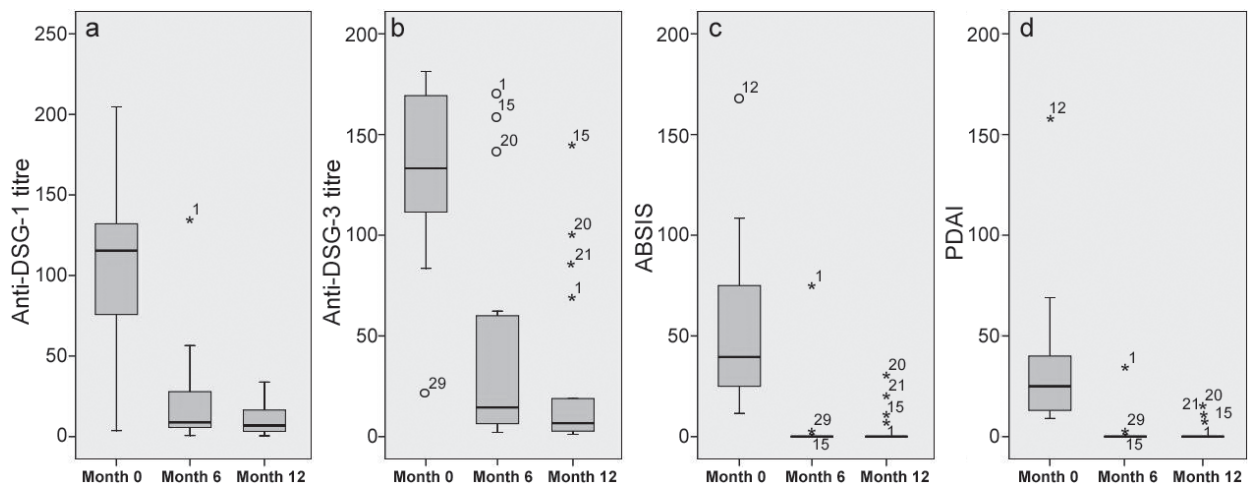
lesions the total and the cutaneous ABSIS and PDAI scores were positively correlated only with the titres of anti-DSG-1 autoantibodies and not with the anti-DSG-3 autoantibodies by the time of initial diagnosis. A total and lasting remission (maintenance dose of 5 mg prednisolone) was achieved in this group and, since the clinical scores were zero (0) and the titres of autoantibodies negative, no correlations were detected. The course of circulating anti-DSG-1 autoantibodies was similar to the course of scoring indexes, as shown in Fig. 1.

Pemphigus vulgaris is most commonly preceded by the development of oral mucosal lesions and in a great number of patients it remains limited to such lesions. It was expected that no correlation would be found between the clinical scores and the titres of anti-DSG-1 autoantibodies in this subgroup at baseline. Total scores (both PDAI and ABSIS) did not correlate with titres of

Fig. 3. Boxplots of data from patients presenting both cutaneous and mucosal lesions from baseline to month 12. (a) Anti-desmoglein-1 (anti-DSG-1) autoantibodies titres, (b) anti-DSG-3 autoantibodies titres, (c) Autoimmune Bullous Skin Disorder Intensity Score (ABSIS), and (d) Pemphigus Disease Area Index (PDAI). Circles and * stand for outliers.

The type of circulating autoantibodies did not change throughout the study period in any of our pemphigus patients. According to a study by Ohyama et al. (9), the antigenic epitopes in both anti-DSG-3 mucosal dominant-type pemphigus vulgaris and anti-DSG-3/DSG-1 mucocutaneous-type pemphigus vulgaris remain unchanged over the course of the disease and the rare epitope spreading among extracellular domains on DSG-3 and DSG-1 has no correlation with the disease course. Differences have been observed in paraneoplastic pemphigus and in pemphigus herpetiformis, in which the epitope distributions are unique (9).

Location-dependent clinical scoring is useful, since, due to the clinical variability of pemphigus lesions, there is a different impact of each location, which is under-represented in a single total score (2).

In conclusion, total PDAI and ABSIS are more useful in pemphigus with only cutaneous lesions or in the mucocutaneous forms. Location-dependent PDAI and ABSIS scores are more useful in pemphigus with mucosal lesions. Anti-DSG-1 autoantibodies titres seem to better show the disease extent and activity in pemphigus with cutaneous only or with mucocutaneous lesions. Anti-DSG-3 autoantibodies titres are significant for setting the diagnosis, but appear not to indicate disease activity.

Measurement of the titres of specific circulating autoantibodies and the use of clinical scoring systems are therefore new tools for the diagnosis and follow-up of patients with pemphigus. Multi-centre studies including large numbers of patients with long-term follow-up may clarify the means of interpretation of findings in association with the disease course.

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