



# Autoantibodies against Desmoglein 1 and 3 in South Tunisian pemphigus

## Anticorps anti-desmogléine 1 et 3 dans le pemphigus Sud Tunisien

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### RÉSUMÉ

**Introduction :** Les desmogléines (Dsg) 1 et 3 sont les 2 principales cibles antigéniques dans le pemphigus superficiel (PS) et le pemphigus vulgaire (PV). Nous avons cherché à déterminer l'intérêt des anticorps (Ac) anti-Dsg1 et 3 dans le diagnostic du pemphigus et à étudier la corrélation de ces Ac avec le phénotype et l'activité de la maladie chez des patients du Sud Tunisien.

**Méthodes :** Nous avons analysé rétrospectivement 131 prélèvements de 82 patients (52 avec PS et 30 avec PV) au cours du suivi. Les Ac-anti-Dsg1 et 3 ont été mesurés par ELISA. Les prélèvements consécutifs ont été corrélés avec l'activité de la maladie. Les courbes ROC ont été réalisées pour déterminer les seuils des Ac-anti-Dsg1 et 3 avec une sensibilité et une spécificité optimales pour l'activité de la maladie.

**Résultats :** Les taux des Ac-anti-Dsg1 et 3 étaient associés au PS et PV respectivement ( $p < 0,001$ ).

Les Ac-anti-Dsg1 et 3 étaient associées aux lésions cutanées (95%) et muqueuses (60%) respectivement.

Une diminution significative des taux des Ac-anti-Dsg1 a été observée chez les patients atteints de PF en rémission  $36 \pm 62$  U/mL; ( $p=0,04$ ). Aucune corrélation n'a été trouvée entre les Ac-anti-Dsg3 et l'évolution des lésions muqueuses dans le PV ( $p=0,3$ ). Au cours du suivi, les Ac anti-Dsg1 étaient corrélés aux rechutes ( $177 \pm 60$  U/mL;  $p=0,04$ ). Le seuil de 161,5 U/mL pour les Ac-anti-Dsg1 fournissait une spécificité de 100% et une sensibilité de 86,4%. La valeur seuil de 30,7U/mL pour les Ac-anti-Dsg3 fournissait une sensibilité de 89,5% et une spécificité 100% dans le PV.

**Conclusion :** Des valeurs élevées des Ac-anti-Dsg3 ne sont pas toujours associées à l'activité du PV. Les Ac-anti-Dsg1 ont montré une relation étroite avec les lésions cutanées du PS et devraient donc être pris en compte dans la gestion thérapeutique des patients pemphigiques.

**Mots clés :** anticorps, desmogléine 1 et 3, pemphigus, sud tunisien, ELISA

### Abstract

**Background :** Desmoglein (Dsg) 1 and 3 are the 2 major autoantigens in pemphigus foliaceus (PF) and pemphigus vulgaris (PV).

**Aim :** We aimed to determine anti-Dsg1 and 3 Abs usefulness in the diagnosis of pemphigus and to assess the correlation of these antibodies (Abs) with clinical phenotype and disease activity in south Tunisian patients.

**Methods :** We retrospectively analyzed 131 samples from 82 patients (52 with PF and 30 with PV) during follow-up. Anti-Dsg1 and 3 Abs were measured by ELISA. Consecutive anti-Dsg1 and 3 Abs were correlated with disease activity. Receiver operating characteristics (ROC) curve were calculated to determine anti-Dsg1 and 3 Abs cut-offs with optimal sensitivity and specificity for disease activity.

**Results :** Anti-Dsg1 and 3 levels were associated to in PF and PV patients respectively ( $p < 0,001$ ). Anti-Dsg1 and 3 Ab were associated to PV and PV patients with skin (95%) and mucosal (60%) lesions, respectively. A significant decrease of anti-Dsg1 Abs was observed in patients with PF in clinical remission ( $36 \pm 62$  U/mL; ( $p=0,04$ )). No correlation was found between anti-Dsg3 Abs and the course of mucosal lesions in PV ( $p=0,3$ ). During follow-up, anti-Dsg1 Abs correlated with relapses ( $177 \pm 60$  U/mL;  $p=0,04$ ). The 161,5 U/mL cut-off for anti-Dsg1 Abs provided 100% specificity and 86,4% sensitivity in PF disease activity. The 30,7U/mL cut-off for anti-Dsg3 provided 89,5% sensitivity and 100% specificity in PV.

**Conclusions :** High anti-Dsg3 Abs values are not always associated with PV disease activity. Anti-Dsg1 Abs showed a closer relationship with skin activity in PS and should be therefore taken into account in management of pemphigus patients.

**Keywords:** antibodies, desmoglein 1 and 3, pemphigus, South Tunisian, ELISA

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## INTRODUCTION

Pemphigus is a group of autoimmune bullous diseases characterized by the loss of cell adhesion and formation of blisters within the epidermis in skin and/or the mucosal surfaces (1). The two main subtypes of pemphigus are pemphigus vulgaris (PV) and pemphigus foliaceus (PF). In addition to sporadic cases, endemic types of PF have been described in Brazil, Columbia as well as in south Tunisia (2-7). In all subtypes, the common histological feature is acantholysis, which is the consequence of intercellular glycolalce destruction, mediated by auto-antibodies (auto-Abs) (8). Major auto-antigens for PV and PF have been identified as desmoglein3 (Dsg3) and desmoglein1 (Dsg1) respectively (9). Both Dsg1 and Dsg3 are extracellular domains of desmosomal adhesion molecules that belong to the cadherin family. They are expressed in stratified squamous epithelia and play pathogenic roles in blister formation in PF and PV respectively (9). Patients with mucosal dominant PV typically demonstrate anti-Dsg3 Abs only and patients with mucocutaneous PV have both anti-Dsg3 and anti-Dsg1 Abs. Patients with PF only have circulating anti-Dsg1Abs although few cases of PF with anti-Dsg3 Abs have been reported (10). Many studies have examined the relationship between the severity of the disease and serum Ab levels using indirect immunofluorescence (IIF) but data have been conflicting (11). IIF is known to be a subjective technique and Abs values vary according to both epithelial substrate used and the quantities of anti-Dsg1 and 3 in tested sera (12). Besides, IIF is not able to differentiate between anti-Dsg1 and 3 Abs. In contrast, detecting anti-Dsg1 and 3 Abs by enzyme linked immunosorbent assay (ELISA) using recombinant Dsg1 and 3 molecules is reported to be a sensitive and specific tool for diagnosing PV and PF (13-15). Usefulness of Dsg ELISA tests in the immunological follow-up of the disease remains controversial (8,16,17). To the best of our knowledge, correlation of anti-Dsg1 and 3 Abs with disease activity have not been assessed in a Tunisian population.

The aim of this study was to determine anti-Dsg1 and 3 Abs usefulness in the diagnosis of subtypes of pemphigus and to assess the correlation of these Abs with clinical phenotype and disease activity during follow-up in south Tunisian patients with PV and PF.

## METHODS

From the database of our Immunology laboratory, we retrospectively included pemphigus patients with one or two consecutive sera samples who underwent anti-Dsg1 and 3 ELISA dosage during follow-up between 1992 and 2015. All patients were followed at the Department of Dermatology, Hedi Chaker University Hospital of Sfax, Tunisia.

All patients fulfilled the following inclusion criteria: (1) diagnosis of pemphigus based on the presence of mucosal erosions and/or superficial cutaneous blisters, an histologic picture of intraepidermal acantholysis; and deposition of IgG associated or not to complement component 3 on the keratinocyte membrane detected by direct immunofluorescence (DIF); (2) available anti-Dsg1 and 3 Abs ELISA results; and (3) in consecutive anti-Dsg samples, a minimal period of 5 months between 2 samples with clinical evaluations of disease severity over the follow-up period .

Paraneoplastic pemphigus was excluded. Clinical data were retrieved from medical charts: age, gender, type of pemphigus (PV or PF) and follow-up.

Complete remission (CR) was defined according to the consensus statement as the absence of new and/or established lesions while the patient was receiving minimal therapy (19). Relapse was defined as the reappearance of lesions that do not heal spontaneously or by the extension of established lesions in a patient who has achieved disease control (1).

For each sample, we measured anti-Dsg1 and 3 Abs by ELISA according to the manufacturer's instructions using a commercial kit (Euroimmun®, Germany).Anti-Dsg1 values above 14 U/ml and anti-Dsg3 values above 7 U/ml were considered positive.

### Statistical analysis

We first compared levels of anti-Dsg1 and 3 Abs at baseline (time of first available Dsg ELISA results) and after initial treatment (between 5-9 months, at 15 or 24 months according to available sera) between patients who achieved complete remission with therapy and those who did not. The Wilcoxon signed rank test was used for the comparison of paired data. Two-sided P values less than 0,05 were considered statistically significant. Quantitative variables were presented as mean  $\pm$  SD, and qualitative variables as frequency and percentage.

A receiver operating characteristics (ROC) curve was calculated to determine a cut-off value for anti-Dsg1 and anti-Dsg3 Abs with the best combination of sensitivity and specificity of disease activity in skin or mucosa. The positive and negative predictive values (VPP and VPV) for these cut-off values were calculated using contingency tables.

All data were analyzed using SPSS 20.0.

## RESULTS

### Baseline characteristics and epidemiological data of patients:

We included 82 patients: 52 with PF and 30 with PV (mucocutaneous n=19, pure mucosal n=6 and pure cutaneous n=5). The sex-ratio (female/male) was 9/1 in PF and 5/1 in PV. The mean age was 42,8±14 years. Demographic data as well as description of mucosal and/ or cutaneous involvement of lesions in patients with PV or PF are shown in table 1.

**Table 1.** Patients' demographic and clinical data

	Pemphigus foliaceus (n=52)	Pemphigus vulgaris (n=30)
Mean age in years ± SD	41,2 ± 13	45,6 ± 15
Sex	47 Female 5 Male	26 Female 4 Male
Exclusive skin involvement	52	5
Exclusive mucous involvement	NA*	6
Mucocutaneous involvement	NA*	19

\*NA: not applicable

We analyzed 131 serum samples collected during the course of the disease for anti-Dsg1 and anti-Dsg3, 51 corresponding to patients with PV and 80 to patients with PF. The number of samples for each patient ranged from 1 to 4. The mean period of follow-up was 21 months.

### Pretreatment

A total of 31 samples were taken from newly-diagnosed patients (21 with PF and 10 with PV) before starting of the treatment.

Anti-Dsg1 and 3 values in PF patients were 178,44±44 U/ml and 2±1,27 U/ml respectively. In PV, anti-Dsg1 and 3 values were 58,65 [13,77–106,97 U/ml] and 162±78 U/ml respectively. Anti-Dsg1 and 3 Abs were associated to PS

and PV respectively (p<0,001).

Abs patterns at the time of the diagnosis are summarized in table 2.

**Table 2.** Anti Dsg1 and 3 antibodies profile in pretreatment patients (n=31)

Type of pemphigus	Anti-Dsg1 - Anti-Dsg3 -	Anti-Dsg1 + Anti-Dsg3 -	Anti-Dsg1 - Anti-Dsg3 +	Anti-Dsg1 + Anti-Dsg3 +
PF n=21 (%)	1 (5)	20 (95)	-	-
PV n=10 (%)				
Mucocutaneous n= 5	0	1 (20)	0	4 (80)
Mucous n=4	0	0	2 (50)	2 (50)
Cutaneous n=1	0	0	0	1 (100)

Anti-Dsg1 and 3 Abs were correlated to skin (95%) and mucosal (60%) lesions respectively: in PF, 20 patients among 21 (95%) with exclusive skin lesions had only positive anti-Dsg1 Abs. In PV, 6 patients among 10 (60%) with mucous lesions associated or not to cutaneous lesions had positive anti-Dsg3-abs.

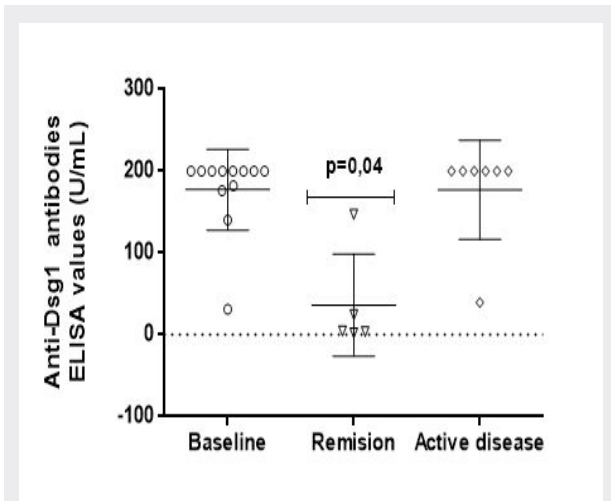
Only one patient with PF showed a double negative anti-Dsg1-/anti-Dsg3- phenotype. There was a double positive phenotype anti-Dsg1+/anti-Dsg3+ in 3 patients with only mucous (n=2) or cutaneous (n=1) involvement of lesions. Another patient with mucocutaneous PV had only positive anti Dsg1 Abs.

### Evolution after initial treatment

All patients received systemic corticosteroids (prednisone) throughout the course of the disease. Different drugs were associated: azathioprine in 23 patients, cyclophosphamide in 10, dapsone in 9 and mycophenolate mophetil in 2.

For 21 patients (12 with PF and 9 with PV), 38 samples were collected in active disease and 7 during remission. Complete remission was achieved within a mean period of 16 months.

Mean anti-Dsg1 and anti-Dsg3 Abs ELISA values at baseline and after initial treatment are given in figures 1, 2&3. Mean anti-Dsg1 Abs ELISA values of the 5 patients with PF who achieved complete remission of their skin lesions decreased from 177,42 (± 49)U/mL to 36 (± 62) U/mL after initial treatment (p=0,04). In contrast, anti-Dsg1 Abs ELISA values of the 7 patients with active PF remained stable (177 ± 49 U/mL at baseline vs 177 ± 60 U/mL) (Figure 1).



**Figure 1.** Correlation between PF disease course and anti- Desmoglein 1 Abs sera levels

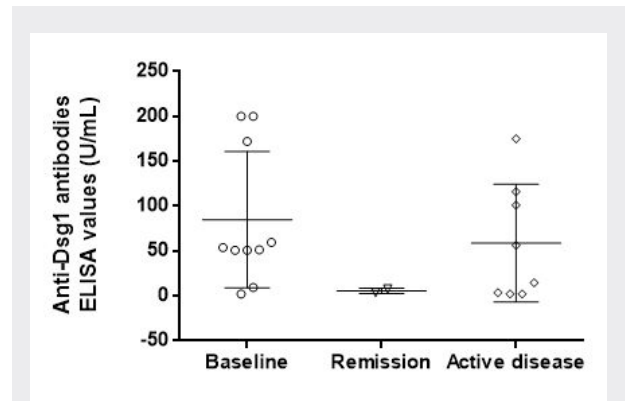
Anti-Dsg1 and 3 Abs ELISA values of the only patient with mucocutaneous PV who achieved complete remission decreased from 200 U/mL and 39,4U/mL respectively at baseline to 7,8 U/mL and 7,3 U/mL after initial treatment. Anti-Dsg1 and 3 Abs values of patients with active mucocutaneous PV remained high ( $83 \pm 72$  U/mL and  $149 \pm 73$  U/mL respectively) (Figure 2 & 3).

Anti-Dsg1 Abs ELISA values of the 2 patients with persistent active cutaneous PV remained positive ( $101 \pm 140$  U/mL at baseline vs  $59 \pm 80$  U/mL after initial treatment). The disease remained active in the 2 patients with mucous PV. Anti Dsg3 Abs ELISA values from these patients remained high ( $151 \pm 57$  U/mL at baseline vs  $172 \pm 38$  U/mL after initial treatment) (Figure 2 & 3).

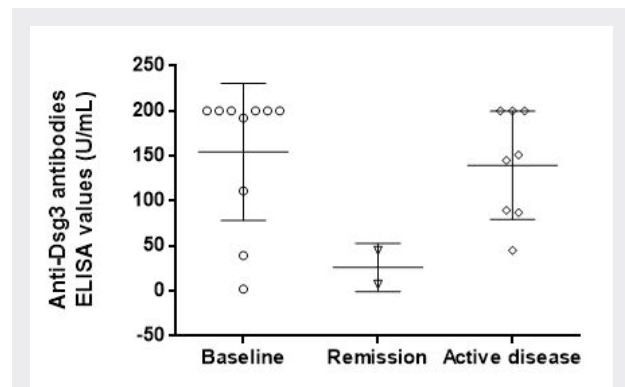
**Sensitivity, specificity, positive and negative predictive values of anti Dsg1 and 3 Abs for disease activity**

We calculated sensitivity, specificity, positive and negative predictive values of anti-Dsg1 and 3 Abs ELISA values for the course of skin and/or mucosal lesions in patients with PV or PF (ongoing remission vs active disease). When using the cut-off values proposed by the manufacturer (14 U/mL for anti Dsg1 Abs and 7 U/mL for anti Dsg3 Abs), anti-Dsg1 Abs ELISA values higher than 14 U/mL had a 100% sensitivity and a 60% specificity for the occurrence of activity in cutaneous forms whereas anti-Dsg3 Abs ELISA values higher than 7 U/mL had a 89,5% sensitivity but a specificity of 50% for the occurrence of activity in

mucosal forms.



**Figure 2.** Correlation between PV disease course and anti-Desmoglein 1 Abs sera levels

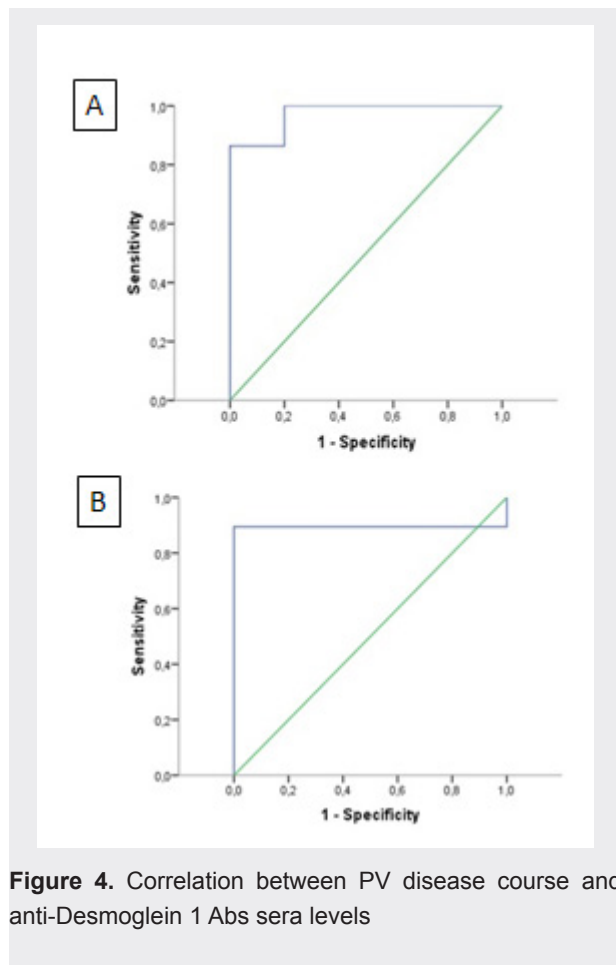


**Figure 3.** Correlation between PV disease course and anti-Desmoglein 3Abs sera levels

A receiver operating characteristics (ROC) curve was calculated to determine a cut-off value for anti-Dsg1 and 3 Abs with the best combination of sensitivity and specificity for of pemphigus activity in skin or mucosa (figure 4). The area under curve (AUC) of the ROC curve was 0,973 ( $p=0,01$ ) for anti-Dsg1 Abs and 0,947 ( $p=0,042$ ) for anti-Dsg3 Abs.

The values with best sensitivity and specificity were defined as the theoretical cut-off values to be used. According to the ROC curve, the cut-off value of anti-Dsg1 Abs was 161,5 U/mL. It provided 100% specificity, 86,4% sensitivity, and 100% positive and 62,5% negative predictive value for skin activity. The cut-off of anti Dsg3 Abs calculated from the ROC curve was 30,7 U/mL, providing 100% specificity,

89,5% sensitivity, and 100% positive and 50% negative predictive value for the mucosal activity.



**Figure 4.** Correlation between PV disease course and anti-Desmoglein 1 Abs sera levels

**Evolution during long term follow-up:**

In PF and PV, 36 (69%) and 19 (63%) patients had occurrence of relapse respectively. Relapses ranged from 1 to 12 with a mean of 2 relapses per patient.

In PF, pretreatment mean (SD) anti-Dsg1 Abs values in patients with occurrence of relapse ( $177 \pm 58$  U/ml) were similar to those with no relapse ( $179 \pm 28$  U/ml) ( $p=0,5$ ). Similarly, difference between anti-Dsg3 Abs values of pretreatment PV patients was found to be not significant between those who relapsed ( $136 \pm 96$  U/ml) and those who did not (200 U/ml) ( $p=0,3$ ). Interestingly, in PF patients, there was a significant positive correlation between number of relapses and anti-Dsg1 levels after initial treatment ( $r=0,466$ ;  $p=0,04$ ). In PV patients, no correlation was found between anti-Dsg1 and 3 levels after initial treatment and number of relapses.

**DISCUSSION**

Pemphigus is a group of autoimmune bullous diseases where Dsg1 and 3 are the two major autoantigens.

In our study we established once again the previously reported relationship (14,18,19) between the type of pemphigus (foliaceus or vulgaris) and the presence of circulating anti-Dsg1 or 3 Abs.

According to the compensation theory (9), anti-Dsg1 and 3 Ab profiles in patients sera and the epidermal distributions of Dsg1 and 3 determine the sites of blister formation. In the superficial epidermis of PF patients, where only Dsg1 is expressed, anti-Dsg1 Abs cause superficial skin lesions. Dsg3 Abs cause suprabasal split in the oral mucosa of PV patients that lacks Dsg1; and skin lesions in PV patients are developed when both Dsg1 and Dsg 3 Abs are absent.

In our study, the Ab profile was in correlation with the clinical phenotype in the majority of patients. However, we found a case of PF with no anti-Dsg Abs, a mucocutaneous PV case with only positive anti-Dsg1 Abs, 2 cases of pure mucosal PV and a case of pure cutaneous PV where both anti-Dsg1 and 3 Abs were raised. Several authors have questioned the compensation theory since they found that Abs profile do not always correlate to the clinical phenotype (20-21). Variable pathogenic potential of anti-Dsg1 and 3 Abs as well as other antigen targets may explain these observations (8,23).

The relationship between anti-Dsg1 and 3 Abs and the activity of pemphigus is still controversial. According to Amagai *et al.* (19) and Harman *et al.* (11), there is a correlation between anti-Dsg1 Abs and skin activity, and between anti-Dsg3 Abs and mucosal severity. In our study, we found a better correlation between anti-Dsg 1 and skin lesions. Abasq *et al.* (18) and Patsatsi *et al.* (24) also have found that anti-Dsg 1 Abs ELISA values correlate closely to PF skin activity, whereas anti-Dsg3 Abs ELISA values did not necessarily parallel the course of mucosal lesions in patients with PV. Moreover, during the long-term follow-up, we found that persistent high levels of anti-Dsg1 Abs after initial treatment was correlated to number of relapses. This was not the case with anti-Dsg3 Abs in PV.

We had only one patient with mucocutaneous PV who achieved complete remission. Although anti-Dsg3 Abs levels of this patient decreased, we should mention that they remained positive (upper limit of manufacturer's cut-off). Persistent positive Abs, particularly anti-Dsg3

Abs, have been reported in the sera of patients in clinical remission (20,25). It has been suggested that anti-Dsg3 Abs disappearance maybe delayed during clinical improvement of PV compared with anti-Dsg1 Abs (8,15,17,23,24). On the other hand, Abasq *et al.* (18) and Harman *et al.* (12) suggested that positive results of anti-Dsg3 Abs seen in patients with PV in clinical remission may be attributed to nonpathogenic Abs. Recently, Kamiya *et al.* (28) introduced EDTA-treated Dsg ELISA to overcome this limitation by detecting nonpathogenic Dsg3 Abs against the non-calcium-dependent epitopes. The differences between EDTA-untreated and EDTA-treated ELISA index values were defined as conformational ELISA index values (28) with a much closer correlation to the disease activity (29). Despite occasional discordance, ELISA test is considered to be a good substitute for DIF to evaluate immunological remission in PV patients (30).

We plotted ROC curves of anti-Dsg1 and anti-Dsg3 values for the analysis of cut-off for disease activity. To the best of our knowledge, 3 authors have focused on determining cut-off values for anti-Dsg1 and 3 Abs with optimal sensitivity and specificity for disease activity in their respective countries (8,18,25) For anti-Dsg1 Abs, our cut-off (27,7 U/mL) was similar to the cut-off found in Abasq *et al.* report (18) (20 U/mL) but far from Barnadas *et al.* (25) cut-off (112 U/mL). In patients with PV, our anti-Dsg3 cut-off value (30,7 U/ml) was close to Anand *et al.* (5) cut-off (50U/mL) but quite far from the cut-off calculated by Abasq *et al.* (18) and Barnadas *et al.* (25) (130 U/ml and 134,4 U/ml respectively). Therefore, we join Barnadas *et al.* (25) in the fact that detection of anti-Dsg1 and anti-Dsg3 Abs is not always associated with activity of the disease. Besides, we should mention that in spite of the large group of patients included in Abasq *et al.* (18) and Barnadas *et al.* (25) studies there were only 7 and 9 PF patients respectively. Cut-off values for prediction of disease activity seem to differ from a population to another. PF is a particular endemic form of the disease where genetic and environmental factors are involved (31-36). Previously, Abida *et al.* (37) demonstrated that anti-Dsg1 Abs (IgG2) are prevalent in healthy subjects and in PF patient' relatives in particular. Besides anti-Dsg1 Abs values in patients (mainly IgG4) were found to be lower in regions where PF is endemic (southern Tunisia). These findings are compatible with our low cut-offs compared to other reports (18,25).

Our study strengthens the previously published data on anti-Dsg1 Abs in south Tunisian PF patients (37). Besides, we provide new data concerning anti-Dsg3 Abs in PV. We focused on anti-Dsg1 and 3 Abs at pretreatment, remission and relapse of the disease with a long-term follow-up. We have also determined the cut-off value for disease activity in skin and mucosa.

One limitation of our study is the lack of serial ELISA sampling and evaluation in all patients. Moreover, we did not distinguish between pathogenic subclasses of IgG anti-Dsg1 and 3 Abs and those who are not since Abida *et al.* (37) showed previously that south Tunisian patients had mainly IgG4 Abs.

Overall, this study demonstrated that anti-Dsg1 and 3 Abs could be a useful tool for the diagnosis of PF and PV respectively. Clinical phenotype was related to the Ab profile in the majority of patients. Abnormal values of anti-Dsg Abs are not always associated with disease activity. Anti-Dsg1 Abs are closely correlated with the skin course of lesions in PF. Persistent high values or a rise in anti-Dsg1 Abs ELISA values should be taken into account for the management of PF patients. In contrast, anti-Dsg3 Abs should be interpreted along with DIF and clinical findings to make a therapeutic decision in patients with PV.

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