Observational Study

Medicine®

OPEN

Evaluation of anti-desmoglein-1 and anti-desmoglein-3 autoantibody titers in pemphigus patients at the time of the initial diagnosis and after clinical remission

Irene Russo, MD, Francesco Paolo De Siena, MD, Andrea Saponeri, BSc, Mauro Alaibac, MD, PhD*

Abstract

It has been suggested that anti-desmoglein autoantibody titers could be helpful in follow-up and therapeutic management of pemphigus patients. However, there is no consensus regarding the relationship between anti-desmoglein autoantibody titers and clinical activity of pemphigus.

The aim of our study was to evaluate if clinical remission of pemphigus relates to the presence of anti-desmoglein autoantibodies.

Thirty patients with pemphigus vulgaris and 7 patients with pemphigus foliaceous were included in the study. Assessment of autoantibody titers was carried out at the time of the initial diagnosis and after the clinical remission using an enzyme-linked immunosorbent assay-based assay.

Our results indicate that pemphigus clinical remission did not necessarily imply a serological remission, and consequently it is necessary to establish if withdrawal of the immunosuppressive regimen in pemphigus should be based exclusively on the achievement of clinical remission or also on the serological findings.

Abbreviations: DSG = desmoglein, ELISA = enzyme-linked immunosorbent assay, PF = pemphigus foliaceous, PV = pemphigus vulgaris.

Keywords: desmoglein-1, desmoglein-3, ELISA, pemphigus

1. Introduction

Pemphigus is a rare autoimmune intraepithelial blistering skin disease characterized by the presence of circulating autoantibodies directed against surfaces of keratinocytes, resulting in a process called acantholysis, which is responsible for loss of the normal epithelial cell-to-cell adhesion.[1] Two main subtypes of pemphigus can be distinguished on the basis of clinical, histological, and immunopathological features: pemphigus vulgaris (PV) and pemphigus foliaceous (PF).[2] PV is associated with autoantibodies against desmoglein[3] (DSG)3 and, sometimes, DSG1,[1] whereas PF is associated with autoantibodies against DSG1[1]. In PV, blisters develop just above the basal cell layer, resulting in chronic painful oral erosions and multiple flaccid blisters arising from healthy skin, whereas in PF, blisters are just below the stratum corneum, causing scaly, crusted cutaneous erosions, often on an erythematous base, without clinically apparent mucosal involvement.[3]

Diagnosis of pemphigus is generally based on clinical features, histology, and immunological tests as direct and indirect immunofluorescence and enzyme-linked immunosorbent assays (ELISAs).[4] The aim of treatment of pemphigus is to induce a complete remission with minimum side-effects. Standard treatment involves systemic corticosteroids, azathioprine and other immunosuppressive agents, dapsone and other immunomodulating drugs, plasmapheresis, immunoadsorption, and rituximab.[5–7] The response to therapy vary greatly from patient to patient, and clinical relapses are frequent.[3] Therefore, patients should be closely followed up by a clinical point of view and also by monitoring serum anti-DSG antibody levels. Indeed, it has been suggested that autoantibody titers could be helpful in follow-up and therapeutic management of pemphigus patients.[8–10] In our clinical experience, many pemphigus patients still have positive ELISA index values during clinical remission. This observation raises questions for clinicians and their patients about the effectiveness of the ELISA assay as a follow-up tool for the management of pemphigus therapy. Hence, the aim of this study was to evaluate the real usefulness of anti-DSG autoantibody titers as a parameter to determine pemphigus remission to evaluate if clinical remission coincides with a negative serology for these autoantibodies.

2. Materials and methods

Thirty patients with PV and 7 patients with PF were included in the study (Table 1). The mean age of patients was 61. The diagnosis of PV was established on the basis of clinical features, histology, and immunopathological findings, notably positive

Editor: Sergio Gonzalez Bombardiere.

The authors report no conflicts of interest.

Unit of Dermatology, University of Padua, Padova, Italy.

* Correspondence: Mauro Alaibac, Unit of Dermatology, University of Padua Via V. Gallucci 4, 35128 Padova, Italy (e-mail: mauro.alaibac@unipd.it).

Copyright © 2017 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the Creative Commons Attribution-NoDerivatives License 4.0, which allows for redistribution, commercial and non-commercial, as long as it is passed along unchanged and in whole, with credit to the author.

Medicine (2017) 96:46(e8801)

Received: 15 May 2017 / Received in final form: 13 October 2017 / Accepted: 30 October 2017

http://dx.doi.org/10.1097/MD.0000000000008801

Table 1

Summary of clinical data.

Characteristic	Result
Patients	37
Sex, M/F	15/22 (40.5%–59.5%)
Subtype, PV/PF	30/7 (81.1%–18.9%)
Age, M + F, y*	61 (23–91)
Duration of clinical remission, mos*	11 (6–27)

F = female, M = male, PF = pemphigus foliaceus, PV = pemphigus vulgaris.
* Data are mean.

direct immunofluorescence and serum detection of anti-DSG3 and anti-DSG1 autoantibodies by ELISA. In particular, the specific circulating autoantibodies were detected with an ELISA assay utilizing recombinant proteins DSG1 and DSG3 (MBL, Nagoya, Japan), consisting of the entire extracellular domain of DSG1 and DSG3, respectively, and produced by Amagai et al using a baculovirus expression system.[11,12] The cut-off values were 20 U/mL both for DSG1 and DSG3. In this study, we measured antibody titers at the time of the initial diagnosis and after achieving complete clinical remission. Complete clinical remission was defined as a period greater than 6 months, during which the patient was lesion-free and on no systemic therapy. The mean duration of clinical remission was 28 months (from minimum of 6 months to maximum of 27 months). Furthermore, in 13 cases of PV in clinical remission, we evaluated the titers of anti-DSG3 antibodies against pathogenetic Ca^{2+}-dependent epitopes from nonpathogenic non-Ca^{2+}-dependent epitopes. The pathogenic Ca^{2+}-dependent epitopes were modified by treatment with 0.5 mM EDTA for 30 minutes at room temperature. After washing 4 times with the ELISA assay wash buffer, circulating autoantibodies were detected using the conventional ELISA approach.[11,12] Ethical approval was waived as we used in the study serum samples obtained for diagnostic purposes.

3. Results

Pemphigus vulgaris and PF autoantibody titers measured at the time of the initial diagnosis and after complete clinical remission are reported in Table 2. Among 30 patients with PV, ELISA identified positive values of anti-DSG3 autoantibodies in all of

Table 2

Autoantibody titers in patients with PV and PF at the time of diagnosis and in clinical remission (unit/mL).

Patient	Subtype	Sex	Age	DSG1 titer (diagnosis)	DSG3 titer (diagnosis)	DSG1 titer (clinical remission)	DSG3 titer (clinical remission)	Systemic therapy
1	PV	F	51	18.66	98.32	18.59	22.16	PRD, AZA, TCN/NAM
2	PV	F	46	21.33	137.82	0	40.5	PRD, AZA
3	PV	F	23	131.62	76.18	32.42	23.79	PRD, TCN/NAM
4	PV	F	53	68.88	311.8	2.85	102.23	PRD, AZA
5	PV	F	70	41.41	210.57	0.36	166.36	PRD, AZA
6	PV	M	59	16.8	21.76	53.62	108.13	PRD
7	PV	F	62	32	110	164.1	0.31	PRD, AZA, TCN/NAM
8	PV	F	68	1	30.66	4.08	223.64	PRD, AZA
9	PV	M	84	86.92	168.43	98.42	198.97	PRD
10	PV	F	64	1	140.9	0	36.73	PRD
11	PV	F	75	48.3	143.86	19.31	8.99	PRD, AZA
12	PV	M	61	40.95	97.67	16.53	0	PRD
13	PV	M	49	100.72	80.65	6.35	3.77	PRD
14	PV	M	66	95.31	178.93	100.72	80.65	PRD, TCN/NAM
15	PV	F	77	264.31	100	6.37	6.74	PRD, AZA
16	PV	F	91	107.13	167.59	0.65	81.75	PRD, AZA
17	PV	F	52	5.5	142.98	4.74	28.06	PRD, TCN/NAM
18	PV	M	23	0.16	53.1	0	8.53	PRD, AZA
19	PV	F	76	45.34	230.95	5.01	204.73	PRD, AZA
20	PV	M	46	53.09	97.93	1.02	20.21	PRD, AZA, TCN/NAM
21	PV	M	71	74.52	161.04	0	129.64	PRD, AZA
22	PV	F	70	3.96	169.69	0	32.71	PRD, AZA
23	PV	M	67	50.88	114.4	6.49	117.16	PRD, AZA, RTX, TCN/NAM
24	PV	F	62	3.28	198.98	0	0.72	PRD, TCN/NAM
25	PV	F	42	0	20.62	1.76	106.28	PRD, TCN/NAM
26	PV	M	62	3.84	323.35	1,62	220.29	PRD, TCN/NAM
27	PV	F	78	3.35	38.34	1	26.58	PRD, AZA
28	PV	M	53	1.18	102.51	1	184.2	PRD, AZA
29	PV	M	70	0.86	44.13	0	35.6	PRD
30	PV	F	53	126.74	41.08	80.48	11.87	PRD, AZA, TCN/NAM
31	PF	M	57	149.63	0.32	6.59	0	PRD, TCN/NAM
32	PF	F	86	47.3	0	7.51	0	PRD
33	PF	F	65	39.58	0	41.61	0	PRD, TCN/NAM
34	PF	M	62	176.1	9.8	20	16.26	PRD, TCN/NAM
35	PF	F	56	123.45	4.85	14	10.2	PRD, TCN/NAM
36	PF	M	65	26.17	0	29	0.82	PRD
37	PF	F	58	54.07	0	108.42	1.22	PRD

AZA = azathioprine, DSG = desmoglein, EDTA = ethylene diamine tetraacetic acid, ELISA = enzyme-linked immunosorbent assay, PF = pemphigus foliaceous, PRD = prednisone, PV = pemphigus vulgaris, RTX = rituximab, TCN/NAM = tetracycline, doxycycline, or minocycline plus niacinamide.

Table 3

Presence of anti-DSG3 and anti-DSG1 antibodies in patients with PV at diagnosis and in remission (cut-off >20 unit/mL).

	Anti-DSG3		Anti-DSG1	
	Diagnosis	Remission	Diagnosis	Remission
>20	30	22	17	6
≤20	0	8	13	24
Total (patients)	30	30	30	30

DSG = desmoglein.

them at the time of the initial diagnosis and in 22/30 (73.3%) after complete remission (Table 3). Positive values of anti-DSG1 autoantibodies were detected in 17/30 (56,6%) patients with pemphigus vulgaris at the time of diagnosis and in 6 out of 30 patients (20%) in complete remission (Table 3). In PF, anti-DSG1 autoantibody positivity was observed in 7/7 patients at diagnosis time-point and in 3/7 (42,8%) at remission time-point (Table 4). In 10 out of 13 patients with PV in complete remission, a large proportion of nonpathogenic anti-DSG3 antibodies were detected, whereas in the remaining 3 cases, pathogenic anti-DSG3 antibodies were the most frequently observed autoantibodies (Table 5).

4. Discussion

Pemphigus is a life-threatening disease that requires an early diagnosis and a timely initiation of treatment. ELISA assay is considered as the gold standard for the serological diagnosis of pemphigus and allows to discriminate between PV and PF.[13] Because ELISA is a good qualitative and quantitative test for the detection of serological autoantibody titers, the correlation between anti-DSG titers, and both disease activity and severity have been studied by several authors.[10,14–23] The clinical course of pemphigus is extremely variable and it is difficult to be predicted only by the clinical evaluation. Thus, serial detection of anti-DSG titers could be helpful in monitoring disease activity and in managing therapy. However, contrasting results have been observed. Some authors[15,16] reported that anti-DSG3 titer seems to be related to the severity of both cutaneous and mucous lesions, whereas anti-DSG1 titer relates only to the severity of mucous lesions. On the contrary, Harman et al[17] found a correlation between anti-DGS3 titer and the severity of mucous involvement, and between anti-DSG1 titer and the severity of cutaneous involvement. Moreover, Abasq et al[10] observed that anti-DSG3 titer is not necessarily linked to the clinical course of mucous lesions in PV patients. Kwon et al[18] even observed these antibodies in the sera of several patients in clinical remission. These different findings could be due to the small number of patients included in each study, ethnical and racial differences, and to different criteria used to evaluate the disease severity.[19]

Table 4

Presence of anti-DSG1 antibodies titer in patients with pemphigus foliaceus at diagnosis and in remission (cut-off >20 unit/mL).

Anti-DSG1 titer	Diagnosis	Remission
>20	7	3
≤20	0	4
Total (patients)	7	7

DSG = desmoglein.

Table 5

Titers of anti-DSG3 autoantiboides in patients with PV in clinical remission using EDTA-ELISA and conventional ELISA.

Patient	EDTA-ELISA	Conventional ELISA
4	30.04	85.75
6	91.81	108.79
5	169.13	163.44
14	37.09	109.96
9	141.61	151.34
16	98.86	113.87
21	56.24	118.98
8	221.88	232.96
26	108.89	154.02
23	88.66	113.79
25	81.02	93.81
28	169.88	198.24
2	74.42	85.61

DSG = desmoglein, EDTA = ethylene diamine tetraacetic acid, ELISA = enzyme-linked immunosorbent assay, PV = pemphigus vulgaris.

The aim of our study was to evaluate if clinical remission of pemphigus relates to the presence of anti-DSG autoantibodies. There is only another study which has specifically addressed the relationship between clinical and serological remission in patients with pemphigus.[24] In this study, Daneshpazhooh et al included patients who were still under treatment, whereas in our investigation, we included only patients whose immunosuppressants had been discontinued. Daneshpazhooh et al showed that 17 of 46 patients (37%) with PV in clinical remission were positive for anti-DSG3 antibodies, and only 2 out of 46 patients were positive for anti-DSG1 antibodies. Patients involved in our study were tested for titers of anti-DSG3 and anti-DSG1 antibodies, firstly at diagnosis time-point and then after complete clinical remission for at least 6 months. Our data show that anti-DSG3 antibody levels were found to be above the cut-off index values (20 m/UL) in the majority of PV patients (73.3%) that achieved complete clinical remission. Similarly, anti-DSG1 antibodies which were positive in 56.6% of patients at the time of the initial diagnosis were still positive in 20% after complete remission. This may be due to the fact that our patients were all without immunosuppressive therapy, and this may have influenced the titers of autoantibodies. On the contrary, our results confirm that antibody titers determined by ELISA do not fluctuate in parallel with disease activity and is consistent with the view that there is a lack of correlation between antibody titers and clinical course of pemphigus.[23] Moreover, in most of our patients with PV in clinical remission nonpathogenic autoantibodies against DSG3 were the most frequently observed autoantibodies, whereas it is well-established that pathogenic anti-DSG3 autoantibodies are the most abundant antibodies during the active phase of PV.[25]

5. Conclusions

In conclusion, it is not clear how long immunosuppressive maintenance therapy should be continued in patients without serological remission, and, moreover, if discontinuation of the immunosuppressive therapy in patients without a serological remission may represent a risk factor for relapse. Future studies may help to understand if withdrawal of the immunosuppressive regimen in pemphigus should be based exclusively on the

achievement of clinical remission or also on the serological findings.

References

[1] Sitaru C, Zillikens D. Mechanisms of blister induction by autoantibodies. Exp Dermatol 2005;14:861–75.
[2] Joly P, Litrowski N. Pemphigus group (vulgaris, vegetans, foliaceus, herpetiformis, brasiliensis). Clin Dermatol 2011;29:432–6.
[3] Bystryn JC, Rudolf JL. Pemphigus. Lancet 2005;366:214–23.
[4] Schmidt E, Zillikens D. Modern diagnosis of autoimmune blistering skin diseases. Autoimmun Rev 2010;10:84–9.
[5] Mutasim DF. Management of autoimmune bullous diseases: pharmacology and therapeutics. J Am Acad Dermatol 2004;51:859–77.
[6] Harman KE, Albert S, Black MM. Guidelines for the management of pemphigus vulgaris. Br J Dermatol 2003;149:926–37.
[7] Hammers CM, Lunardon L, Schmidt E, et al. Contemporary management of pemphigus. Expert Opin Orphan Drugs 2013;1:295–314.
[8] Sharma VK, Prasad HR, Khandpur S, et al. Evaluation of desmoglein enzyme-linked immunosorbent assay (ELISA) in Indian patients with pemphigus vulgaris. Int J Dermatol 2006;45:518–22.
[9] Anand V, Khandpur S, Sharma VK, et al. Utility of desmoglein ELISA in the clinical correlation and disease monitoring of pemphigus vulgaris. J Eur Acad Dermatol Venereol 2012;26:1377–83.
[10] Abasq C, Mouquet H, Gilbert D, et al. ELISA testing of anti-desmoglein 1 and 3 antibodies in the management of pemphigus. Arch Dermatol 2009;45:529–35.
[11] Amagai M, Hashimoto T, Green KJ, et al. Antigen-specific immunoadsorption of pathogenic autoantibodies in pemphigus foliaceus. J Invest Dermatol 1995;104:895–901.
[12] Ishii K, Amagai M, Hall RP, et al. Characterization of autoantibodies in pemphigus using antigen-specific ELISAs with baculovirus-expressed recombinant desmogleins. J Immunol 1997;159:2010–7.
[13] Amagai M, Tsunoda K, Zillikens D, et al. The clinical phenotype of pemphigus is defined by the anti-desmoglein autoantibody profile. J Am Acad Dermatol 1999;40:167–70.
[14] Cheng SW, Kobayashi M, Tanikawa A, et al. Monitoring disease activity in pemphigus with enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3. Br J Dermatol 2002;147:261–5.
[15] Kumar B, Arora S, Kumaran MS, et al. Study of desmoglein 1 and 3 antibody levels in relation to disease severity in Indian patients with pemphigus. Indian J Dermatol Venereol Leprol 2006;72:203–6.
[16] Akman A, Uzun S, Alpsoy E. Immunopathologic features of pemphigus in the east Mediterranean region of Turkey: a prospective study. Skinmed 2010;8:12–6.
[17] Harman KE, Seed PT, Gratian MJ, et al. The severity of cutaneous and oral pemphigus is related to desmoglein 1 and 3 antibody levels. Br J Dermatol 2001;144:775–80.
[18] Kwon EJ, Yamagami J, Nishikawa T, et al. Anti-desmoglein IgG autoantibodies in patients with pemphigus in remission. J Eur Acad Dermatol Venereol 2008;22:1070–5.
[19] Harman KE, Gratian MJ, Bhogal BS, et al. A study of desmoglein 1 autoantibodies in pemphigus vulgaris: racial differences in frequency and the association with a more severe phenotype. Br J Dermatol 2000;143:343–8.
[20] Avgerinou G, Papafragkaki DK, Nasiopoulou A, et al. Correlation of antibodies against desmogleins 1 and 3 with indirect immunofluorescence and disease status in a Greek population with pemphigus vulgaris. J Eur Acad Dermatol Venereol 2013;27:430–5.
[21] Schmidt E, Dahnrich C, Rosemann A, et al. Novel ELISA systems for antibodies to desmoglein 1 and 3: correlation of disease activity with serum autoantibody levels in individual pemphigus patients. Exp Dermatol 2010;19:458–63.
[22] Daneshpazhooh M, Chams-Davatchi C, Khamesipour A, et al. Desmoglein 1 and 3 enzyme- linked immunosorbent assay in Iranian patients with pemphigus vulgaris: correlation with phenotype, severity, and disease activity. J Eur Acad Dermatol Venereol 2007;21:1319–24.
[23] Belloni-Fortina A, Faggion D, Pigozzi B, et al. Detection of autoantibodies against recombinant desmoglein 1 and 3 molecules in patients with pemphigus vulgaris: correlation with disease extent at the time of diagnosis and during follow-up. Clin Dev Immunol 2009;2009:187864.
[24] Daneshpazhooh M, Kamyab K, Kalantari MS, et al. Comparison of desmoglein 1 and 3 enzyme-linked immunosorbent assay and direct immunofluorescence for evaluation of immunological remission in pemphigus vulgaris. Clin Exp Dermatol 2013;39:41–7.
[25] Kamiya K1 , Aoyama Y, Shirafuji Y, et al. Detection of antibodies against the non-calcium-dependent epitopes of desmoglein 3 in pemphigus vulgaris and their pathogenic significance. Br J Dermatol 2012;167: 252–61.