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

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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, crusty 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
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 20U/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 Ca²⁺-dependent epitopes from nonpathogenic non-Ca²⁺-dependent epitopes. The pathogenic Ca²⁺-dependent epitopes were modified by treatment with 0.5mM 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 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)     |

* = Data are mean.

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                           | 8.31                           | 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      | M   | 76  | 45.34                  | 230.95                 | 5.01                            | 204.73                         | PRD, TCN/NAM     |
| 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  | 20                     | 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, TCN/NAM     |
| 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.

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.

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.[23] 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, serological remission was 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. Additionally, these 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]

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.

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