Dermatological Health, 2024, Volume 2, Issue 1
http://ojs.bbwpublisher.com/index.php/DH

Diagnostic Value of Anti-Desmoglein 1 and 3 Antibodies and Anti-BP180 and 230 Antibodies in Autoimmune Bullous Dermatoses

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Abstract: *Objective:* To analyze and evaluate the value of the anti-epidermal intercellular desmosome antibodies, anti-desmoglein (Dsg) 1, and anti-Dsg3, as well as the anti-epidermal basement membrane hemidesmosomes antibodies, anti-BP180, and anti-BP230 in the diagnosis of pemphigus and bullous pemphigoid. *Methods:* Patients with pemphigus or bullous pemphigoid treated in the Department of Dermatology of Dalian Dermatosis Hospital from July 2019 to July 2021 were selected. They were clinically diagnosed with histopathological and indirect immunofluorescence methods. They were divided into the pemphigus group (n = 102) and the bullous pemphigoid group (n = 175). Additionally, 120 patients who were ruled out of pemphigus and bullous pemphigoid during the same period were selected as the control group. Enzyme-linked immunosorbent assay (ELISA) was used to detect anti-Dsg1, anti-Dsg3, anti-BP180, and anti-BP230 antibodies. Indirect immunofluorescence (IIF) was used to detect the IgG levels of the anti-epidermal intercellular desmosome antibodies and anti-epidermal basement membrane hemidesmosomes antibodies. The positive rate, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic rate of each antibody were evaluated and analyzed comprehensively. *Results:* In the pemphigus group, bullous pemphigoid group, and control group, the positive rates of anti-Dsg1 are respectively 83.3% (85/102), 0 (0/175), and 1.7% (2/120); the positive rates of anti-Dsg3 are 41.1% (42/102), 0 (0/175), and 0 (0/120), respectively; the positive rates of BP180 are 5.9% (6/102), 85.7% (150/175), and 5% (6/120), respectively; the positive rates of BP230 are 1.9% (2/102), 57.7% (101/175), and 1.7% (1/120), respectively. Meanwhile, the positive rates of anti-epidermal intercellular desmosome antibodies are 69.6% (71/102), 0 (0/175), and 0 (0/120), respectively; the positive rates of anti-epidermal basement membrane hemidesmosome antibodies are 0% (0/102), 51.4% (90/175), and 0 (0/120), respectively. Among the patients with pemphigus, the sensitivity, specificity, PPV, NPV, and diagnostic rates of the anti-epidermal intercellular desmosome antibody test were 65.1%, 100%, 100%, 85.2%, and 92.2%; for anti-Dsg1, respectively, 83.3%, 99.3%, 97.7%, 88.7%, and 95.2%; for anti-Dsg3, respectively, 41.1%, 100%, 100%, 83.1%, and 84.9%. Among the patients with bullous pemphigoid, the sensitivity, specificity, PPV, NPV, and diagnostic rates of the anti-epidermal basement membrane hemidesmosomes antibody test were 51.4%, 100%, 100%, 72.3%, and 78.6%; for anti-BP180, respectively, 85.7%, 94.6%, 92.6%, 89.4%, and 90.7%; for anti-BP230, respectively, 57.7%, 98.2%, 96.2%, 74.7%, and 80.1%. *Conclusion:* The detection of autoantibodies in serum and the confirmation of the specific target antigens could complement each other to reduce clinical missed diagnosis and increase the positive diagnostic rate if the two tests were conducted simultaneously. The positive result of the anti-epidermal intercellular desmosome antibody and the anti-epidermal basement membrane hemidesmosomes antibody has

better accuracy in diagnosing pemphigus and bullous pemphigoid. In contrast, the negative result is of great value in ruling out pemphigus and bullous pemphigoid.

Keywords: Pemphigus; Dsg1; Dsg3; Bullous pemphigoid; BP180; BP230

Online publication: March 29, 2024

1. Introduction

Pemphigus (P) and bullous pemphigoid (BP) are typical intraepidermal and subepidermal autoimmune bullous dermatoses (AIBD), respectively [1]. Autoantibodies mediate their onset, but the autoantibody profiles of the two diseases differ [2]. In patients with pemphigus, indirect immunofluorescence (IIF) can be used to detect the presence of antibodies against desmosomes between spiny cells in the patient's serum, and enzyme-linked immunosorbent assay (ELISA) can be used to detect autoantibodies such as anti-desmoglein (Dsg) 1 and anti-Dsg3. The IIF method in patients with bullous pemphigoid can detect the presence of hemidesmosomal antibodies against the epidermal basement membrane in the patient's serum. The ELISA method can detect specific anti-BP180 and anti-BP230 antibodies, and it is found that the serum of more than 90% of bullous pemphigoid patients contains anti-BP180 antibodies, which is related to the disease severity [3-5]. Previous literature shows that direct immunofluorescence (DIF) is the "gold standard" for diagnosing bullous dermatoses, but there are false negatives or false positives in clinical practice [3]. To explore the clinical application and significance of the detection of anti-Dsg1, anti-Dsg3, anti-BP180, and anti-BP230 antibodies in the serological diagnosis of bullous dermatoses, the IIF and ELISA test results of patients with bullous dermatoses in Dalian were collected and analyzed, providing the basis for the diagnosis and treatment of pemphigus and bullous pemphigoid.

2. Materials and methods

2.1. Clinical data

Patients diagnosed and treated from July 2019 to July 2021 at Dalian Dermatosis Hospital were selected. Based on clinical manifestations and pathological results, 102 cases of diagnosed pemphigus were recorded as group P; 175 patients diagnosed with bullous pemphigoid were recorded as group BP. During the same period, 120 patients who were clinically ruled out of having autoimmune bullous dermatoses were selected as the control group. The sera of all the patients were collected for testing. In the control group, there were 70 cases of eczema, 7 cases of dermatitis, 10 cases of prurigo nodularis, 4 cases of urticaria, 8 cases of psoriasis, 2 cases of epidermolysis bullosa, 3 cases of oral lichen planus, 1 case of erythroderma, 12 cases of erythema multiforme, 2 cases of fixed-drug eruption, and 1 case of folliculitis. There is no clinical subdivision of pemphigus, so the patients were classified into the pemphigus group (group P) in this study. Since epidermolysis bullosa has pathological manifestations on the dermal side and the general target antigen is collagen VII, the cases were classified in the control group instead of the BP group.

2.2. Methods

Serum anti-Dsg1, anti-Dsg3, anti-BP180, and anti-BP230 antibodies were measured using ELISA kits (Oumeng Medical Experimental Diagnostics AG, Germany) according to the kit's instructions. 100 μl of serum specimen, Dsg1, Dsg3, BP180, and BP230 antibody standards was each added to the reagent wells and incubated at

room temperature for 30 minutes. After washing three times, 100 μl enzyme-labeled antibodies was added to each well and incubated at room temperature for 30 minutes; washing was repeated for 3 times, 100 μl substrate solution was added to each well and incubated at room temperature for 15 minutes, and then 100 μl stop solution was added. A fully automated microplate reader (TECAN fully automated enzyme immunoassay instrument) was used for the measurement, with 450 nm as the detection wavelength and 620 nm as the reference wavelength. The absorbance value was recorded and data processing was performed according to the calculation method provided in the instruction manual. According to the kit's instructions, antibody quantitative value of ≥ 20 RU/ml is positive and < 20 RU/ml is negative.

The indirect immunofluorescence method was used to determine the serum antibody IgG level using the IIF kit (Oumeng Medical Experimental Diagnostics AG, Germany), and it was performed according to the kit's instructions. The anti-epidermal basement membrane hemidesmosome antibodies and anti-epidermal intercellular desmosome antibodies IgG subtypes in the patient's serum were detected using the monkey esophagus and tongue tissue sections as the detection matrix. Fluorescein-labeled goat anti-human IgG was used as the secondary antibody, and the results were observed under a fluorescence microscope. Positive and negative serum controls were set for each batch.

2.3. Statistical processing

The data were collected using the SPSS22.0 statistical software package. The positivity rate, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic rate were calculated for anti-Dsg1, anti-Dsg3, anti-BP180, anti-BP230, anti-epidermal intercellular desmosome antibodies, and anti-epidermal basement membrane hemidesmosome antibodies, and the four-grid table χ^2 test was used to determine the test level $\alpha = 0.05$.

3. Results

3.1. Overall detection status of anti-Dsg1, anti-Dsg3, anti-BP180, anti-BP230, anti-epidermal intercellular desmosome antibodies, and anti-epidermal basement membrane hemidesmosome antibodies

In the pemphigus group, bullous pemphigoid group, and control group, the positive rates of anti-Dsg1 antibodies were 83.3% (85/102), 0 (0/175), and 1.7% (2/120) respectively; the positive rates of anti-Dsg3 antibodies were 41.1% (42/102), 0 (0/175), and 0 (0/120) respectively; the positive rates of anti-BP180 antibodies were 5.9% (6/102), 85.7% (150/175), and 5% (6/120) respectively; the positive rates of anti-BP230 antibodies were 1.9% (2/102), 57.7% (101/175), and 1.7% (1/120) respectively; the positive rates of anti-epidermal intercellular desmosome antibodies were respectively 69.6% (71/102), 0 (0/175), and 0 (0/120); and the positive rates of anti-epidermal basement membrane hemidesmosome antibodies were respectively 0% (0/102), 51.4% (90/175), and 0 (0/120). In the pemphigus group, the positive rate of anti-Dsg1 and anti-Dsg3 antibodies, and anti-intercellular desmosome antibodies was statistically significant compared with the bullous pemphigoid and control groups ($P < 0.01$). In the pemphigoid group, the positive rate of anti-BP180 and anti-BP230 antibodies, and anti-epidermal basement membrane hemidesmosome antibodies was statistically significant compared to the pemphigoid and control groups ($P < 0.01$), as shown in **Table 1**.

Table 1. Results analysis of the pemphigus, bullous pemphigoid, and control groups

Antibody	Positive rate (%)		
	Group P (n = 102)	Group BP (n = 175)	Control group (n = 120)
Anti-Dsg1*	83.3 (85/102)	0 (0/175)	1.7 (2/120)
Anti-Dsg3*	41.1 (42/102)	0 (0/175)	0 (0/120)
Anti-epidermal intercellular desmosome antibodies*	69.6 (71/102)	0 (0/175)	0 (0/120)
Anti-BP180**	5.9 (6/102)	85.7 (150/175)	5 (6/120)
Anti-BP230**	1.9 (2/102)	57.7 (101/175)	1.7 (2/120)
Anti-epidermal basement membrane hemidesmosome antibodies**	0 (0/102)	51.4 (90/175)	0 (0/120)

*$P = 0.007$, $P < 0.01$; **$P = 0.004$, $P < 0.01$

3.2. Test evaluation of anti-Dsg1, anti-Dsg3, anti-BP180, anti-BP230, anti-epidermal intercellular desmosome antibodies, and anti-epidermal basement membrane hemidesmosome antibodies

The sensitivity, specificity, PPV, NPV, and diagnostic rate of anti-epidermal intercellular desmosome antibody detection test for pemphigus patients were 65.1%, 100%, 100%, 85.2%, and 92.2%, respectively; for anti-Dsg1, respectively, 83.3%, 99.3%, 97.7%, 88.7%, and 95.2%; and for anti-Dsg3, respectively, 41.1%, 100%, 100%, 83.1%, and 84.9%. The sensitivity, specificity, PPV, NPV, and diagnostic rate of the anti-epidermal basement membrane hemidesmosome antibody test for bullous pemphigoid patients were 51.4%, 100%, 100%, 72.3%, and 78.6%, respectively; for anti-BP180 antibody, 85.7%, 94.6%, 92.6%, 89.4%, and 90.7%, respectively; and for anti-BP230 antibody, 57.7%, 98.2%, 96.2%, 74.7%, and 80.1%, respectively (**Tables 2** and **3**).

Table 2. The analysis and evaluation of anti-Dsg1 and anti-Dsg3 antibodies and anti-epidermal intercellular desmosome antibody in pemphigus group

Pemphigus group	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic rate (%)
Anti-Dsg1	83.3	99.3	97.7	88.7	95.2
Anti-Dsg3	41.1	100	100	83.1	84.9
Anti-epidermal intercellular desmosome antibodies	65.1	100	100	85.2	92.2

Table 3. The analysis and evaluation of anti-BP180 and anti-BP230 antibodies and anti-epidermal basement membrane hemidesmosomes antibodies in bullous pemphigoid group

Bullous pemphigoid group	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic rate (%)
Anti-BP180	85.7	94.6	92.6	89.4	90.7
Anti-BP230	57.7	98.2	96.2	74.7	80.1
Anti-epidermal basement membrane hemidesmosome antibodies	51.4	100	100	72.3	78.6

3.3. Evaluation of detection results of indirect immunofluorescence method and enzyme-linked immunosorbent assay

The tissues used for indirect immunofluorescence detection were monkey esophagus and tongue tissue sections

while the enzyme-linked immunosorbent assay used purified or genetically recombinant specific antigens as the detection matrix, detecting the antibody levels in the patient's serum from different aspects. This part analyzed the results of dual-method detection of pemphigus and bullous pemphigoid-related antibodies, as shown in **Tables 4** and **5**.

Table 4. The results analysis of pemphigus by the two methods

Test methods	Results		Total
	+	–	
ELISA	102	0	102
IIF	45	57	102

Among 102 patients with pemphigus, 102 were positive for anti-Dsg1 or anti-Dsg3 antibodies, with a positive rate of 100%. Among the 102 patients, 45 had positive IIF test results at the initial diagnosis, with a positive rate of 44.1%. The positive rate of the IIF method is lower than that of the ELISA method.

Table 5. The results analysis of bullous pemphigoid by the two methods

Test methods	Results		Total
	+	–	
ELISA	175	0	175
IIF	112	63	175

Among 175 patients with bullous pemphigoid, 175 were positive for anti-BP180 or anti-BP230 antibodies, with a positive rate of 100%. Among the 175 patients, 112 had positive IIF test results at the initial diagnosis, with a positive rate of 64.0%. The positive rate of the IIF method is lower than that of the ELISA method.

4. Discussion

Autoimmune bullous dermatoses (AIBD) are a group of organ-specific autoimmune diseases. The patient's serum has antibodies (desmosomes, hemidesmosomes) against structures of skin junctions. Autoantibodies damage the connections between epidermal cells and between the epidermis and the dermis, causing intraepidermal blisters or subepidermal blistering diseases. AIBD can be clinically divided into pemphigus and bullous pemphigoid. In addition to typical clinical manifestations, specific antibodies often appear in patients' serums. For example, anti-epidermal intercellular desmosome antibodies, anti-Dsg1 antibodies, and anti-Dsg3 antibodies are detected in the serum of patients with pemphigus; anti-epidermal basement membrane hemidesmosome antibodies, anti-BP180 antibodies, and anti-BP230 antibodies are detected in the serum of patients with bullous pemphigoid [6].

Clinical diagnosis of patients with bullous dermatoses mainly relies on skin pathology, and serological testing is not widely used. However, the detection of skin pathology is invasive, with slow wound healing when patients undergo immunosuppressive and hormonal treatments, and the location from which the skin specimen is collected, etc., can also easily lead to false negative results. Domestic expert recommendations [7,8] clearly stated that the detection of serum antibodies can be used as a diagnostic standard for these types of diseases and that the antibody titer is positively correlated with the severity assessment and treatment monitoring. The methods for the detection of antibodies in serum include IIF and ELISA, but most laboratories

do not fully utilize these two methodologies for detection, which may lead to certain deficiencies in diagnosis [9]. The indirect immunofluorescence method detects total antibodies and cannot classify antibody target antigens. Evaluating antibody titer changes is not as intuitive and sensitive as the ELISA method, and treatment monitoring is poor [10].

102 patients with clinically diagnosed pemphigus and 175 patients with bullous pemphigoid were selected for serum antibody tests, and 120 patients without bullous dermatoses were collected as a control group. The anti-epidermal intercellular desmosome antibodies, anti-epidermal basement membrane hemidesmosome antibodies, anti-Dsg1, anti-Dsg3, anti-BP180, and anti-BP230 antibodies in the patient's serum were statistically analyzed respectively, and the value of each antibody in disease diagnosis was evaluated. The research results showed that anti-epidermal intercellular desmosome antibodies, anti-Dsg1, and anti-Dsg3 antibodies have better specificity, sensitivity, PPV, NPV, and diagnostic rate in patients with pemphigus, among which the diagnostic rates are respectively 92.2%, 95.2%, and 84.9%, which are similar to the research results of Chen *et al.* [11]. The research results of Li *et al.* [12] demonstrated that the ELISA method detecting anti-Dsg antibodies can be used as a screening method for bullous diseases. No further testing is needed if the results are consistent with the clinical diagnosis. Further pathological and immunofluorescence testing can be performed if the results show discrepancy with the clinical diagnosis. Anti-epidermal basement membrane hemidesmosome antibodies, anti-BP180, and anti-BP230 antibodies also show good characteristics in patients with bullous pemphigoid, with diagnostic rates of 78.6%, 90.7%, and 80.1%, respectively. It can be seen from the data that there are differences in the specificity and sensitivity between the indirect immunofluorescence method and the enzyme-linked immunosorbent assay. The specificity of the indirect immunofluorescence method is almost 100%, but the sensitivity is lower than that of the enzyme-linked immunosorbent method. They play a complementary role in the diagnosis of diseases and avoid missed diagnoses due to a single methodology. We can see from the test results of the dual-method detection that the positive rate of the ELISA method is significantly higher than the IIF method. This test result is slightly different from the research results of Huang *et al.* [13,14]. From the matrix analysis of the test, the monkey esophageal or tongue tissues are used to detect anti-epidermal intercellular desmosome antibodies and anti-epidermal basement membrane hemidesmosome antibodies, the target antigens are anti-Dsg1, anti-Dsg3, anti-BP180, and anti-BP230 antibodies, and the consistency of the two methods should be better. From the perspective of human subjective interpretation of IIF, the difference between the results of this study and that of Huang *et al.* may be caused by fluorescence interpretation. Our laboratory will further standardize the interpretation and observe the consistency of the dual-method detection results to better inform clinical practice.

Disclosure statement

The authors declare no conflict of interest.

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