Circulating bullous pemphigoid autoantibodies in the setting of negative direct immunofluorescence findings for bullous pemphigoid: A single-center retrospective review

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Background: Bullous pemphigoid (BP) autoantibody levels are generally elevated in patients with BP but can be present nonspecifically in patients without BP.

Objective: To analyze the clinical findings of patients with elevated BP180 or BP230 autoantibody levels and negative direct immunofluorescence (DIF) study findings.

Methods: We retrospectively reviewed records of patients seen at our institution during January 1, 2005-December 31, 2015, who were positive for BP180 or BP230 autoantibodies and had a negative DIF study finding. These patients' demographic characteristics and BP180 and BP230 levels were compared with those of a BP control group who were positive for BP180 or BP230 autoantibodies and had positive DIF study findings.

Results: We identified 208 patients with BP autoantibodies but without positive DIF study findings. These patients' mean age and enzyme-linked immunosorbent assay values were significantly lower than those of the control group. Dermatitis was the most common final clinical diagnosis. Of the 208 patients, 41 (19.7%) had at least 2 years' follow-up. Four patients had positive DIF results upon repeating the test and ultimately received pemphigoid diagnoses.

Limitations: Retrospective design with limited follow-up.

Conclusion: Patients might harbor serum BP autoantibodies in the context of a wide range of dermatoses. Low positive BP180 and BP230 autoantibody levels should not be overinterpreted as evidence for BP in the setting of a negative DIF. (J Am Acad Dermatol 2019;81:472-9.)

Key words: autoantibody; basement membrane zone; bullous diseases; immunobullous; immunofluorescence; medical dermatology; pemphigoid.

Bullous pemphigoid (BP) is an acquired autoimmune blistering dermatosis associated with circulating autoantibodies targeting the basement membrane zone (BMZ) proteins BP180 (BP antigen 2 or type XVII collagen) and BP230 (BP antigen 1).[1] This disease is associated with increased mortality,[2,3] neurologic disorders,[4] and decreased quality of life.[5] Accurate diagnosis of BP is essential for guiding its management because certain systemic treatments for BP are also associated with substantial risk of infection and mortality.[6-8]

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BP commonly presents with characteristic tense bullae. However, the disease might also manifest as nonbullous urticarial and pruritic eruptions.[9] Hence, the enzyme-linked immunosorbent assay (ELISA) that detects BP180 and BP230 autoantibodies and the indirect immunofluorescence (IIF) and direct immunofluorescence (DIF) assays that detect autoantibodies, as well as histopathologic examination, provide valuable diagnostic information. Among these tests, DIF has the highest sensitivity (90.8% and 95.7%)[10,11] and negative predictive value (NPV; 95.4% and 97.3%)[10,11] and has been proposed to be an obligate criteria for diagnosing BP.[9] The BP180 and BP230 ELISAs are among the tests with the highest specificities and positive predictive values (PPVs).[11,12] The specificity of the BP180 ELISA has been reported to be 94% and its PPV 95% and 96%.[11,12] The specificity of the BP230 ELISA has been reported to be 94% and 99%[11,12] and its PPV 94% and 99%.[11,12]

CAPSULE SUMMARY

- Autoantibodies BP180, BP230, or both are elevated in bullous pemphigoid (BP) but can occur in patients without BP, causing diagnostic uncertainty.
- Low-positive BP180 or BP230 autoantibody levels might be present in various clinical presentations and should not be overinterpreted as BP in the setting of a negative direct immunofluorescence.

Because of the high NPV of DIF, patients with a negative DIF result are unlikely to have BP. Furthermore, because of the high PPV of the BP180 and BP230 ELISAs, patients with a positive ELISA result are likely to have BP. However, there are situations in which patients might have a positive BP180 or BP230 ELISA result and a negative DIF test result. This discrepancy in laboratory results might cause uncertainty in the diagnosis of BP and management of patient care. Unfortunately, little guidance from published experience exists regarding this clinical dilemma. Therefore, the purpose of this study was to retrospectively review 10 years of our institution's experience with patients who had positive BP180 ELISA results, positive BP230 ELISA results, or both, but negative DIF results. Our cohort includes a series of patients who were initially positive for BP180 or BP230 autoantibodies with negative DIF results who were later found to have positive DIF test results.

METHODS

This retrospective study of patients seen at our institution during January 1, 2005-December 31, 2015, was approved by the Mayo Clinic Institutional Review Board, and informed consent was waived for those patients who provided research authorization (approval no. 16-008138). The electronic health records database was searched for patients >18 years of age who were positive for BP180, BP230, or both by ELISA. For these patients, DIF reports were reviewed, and a clinical diagnosis was determined on the basis of codes from the International Classification of Diseases, Tenth Revision (ICD-10), as well as by review of clinic notes and communication letters.

BP180 and BP230 ELISAs were performed on serum samples with a commercially available test (MBL, Nagoya, Japan). As recommended by the manufacturer, values were expressed as units per milliliter of serum, and 9 U/mL served as a cutoff for both the BP180 and BP230 ELISAs. The IIF test substrate was monkey esophagus.

Patient groups

The primary study cohort comprised patients positive for BP180, BP230, or both by ELISA who also had negative DIF test results (hereafter referred to as the ELISA^{+}, DIF^{-} cohort). As our institution is a tertiary referral center, and BP180 and BP230 ELISA results are often obtained upon initial consultation in recalcitrant skin disease. Age at the time of testing, sex, BP180 and BP230 ELISA values, IIF test results, antinuclear antibody (ANA) values, serum eosinophil counts, duration of follow-up, morphologic characteristics on physical examination, extent of cutaneous involvement, and final or working diagnosis were determined. Because of the diagnostic ambiguities within this cohort, patients of all diagnoses were included if the conditions of positive ELISA result and negative DIF test result were satisfied.

Patients with mucous membrane pemphigoid (MMP) and BP can have overlapping immunologic profiles, including their BP180 and BP230 ELISA values.[13-15] IIF test is less reliable for the diagnosis of MMP than it is for BP because lower titers of detectable circulating autoantibodies are present for MMP.[15,16] Thus, within the study cohort, the IIF test was analyzed with and without the inclusion of MMP patients. The BP180 and BP230 ELISA values and IIF results were analyzed for patients with MMP.

Demographic characteristics of the study cohort were compared with those of a control group of 49 patients with BP. The control group consisted of all patients with BP established by use of the ICD-10

Abbreviations used:

ANA:	antinuclear antibody
BMZ:	basement membrane zone
BP:	bullous pemphigoid
DIF:	direct immunofluorescence
ELISA:	enzyme-linked immunosorbent assay
ICD-10:	International Classification of Diseases, Tenth Revision
IIF:	indirect immunofluorescence
IQR:	interquartile range
MMP:	mucous membrane pemphigoid
NPV:	negative predictive value
PPV:	positive predictive value

code documented during January 1, 2005-December 31, 2015. All patients in the control group were also positive for BP180, BP230, or both by ELISA but also had positive DIF test results (or positive for antibody deposition along the BMZ) and are hereafter referred to as the ELISA$^+$, DIF$^+$ cohort. In this group, the information collected included age at the time of ELISA, sex, and BP180 and BP230 ELISA values.

Statistical analysis

Data for variables with continuous values were summarized by stating the means and standard deviations when values were distributed approximately normally and by the medians and interquartile ranges (IQRs) when values were distributed otherwise; categorical variables were summarized with frequency counts and percentages. Comparisons of demographic features between patients with negative and positive DIF test results were evaluated by using 2-sample *t*, Wilcoxon rank sum, and χ^2 tests. Associations of BP180 and BP230 with additional features of interest among patients with negative DIF test results were evaluated by using Spearman rank correlation coefficients and Kruskal-Wallis and Wilcoxon rank sum tests. Statistical analyses were performed by using SAS version 9.4 (SAS Institute Inc, Cary, NC) and R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). All tests were 2-sided, and *P* values $<.05$ were considered statistically significant.

RESULTS

Over a 10-year period, 208 patients were identified who had an evaluation that included positive ELISA and negative DIF test results. Among the 208 patients, 10 patients (4.8%) had MMP, 41 (19.7%) had at least 2 years of follow-up at our institution, and 23 (11.1%) had repeated DIF tests that remained negative. In total, 4 patients who were initially ELISA positive and DIF negative were retested, and this subsequent DIF result was positive.

Several significant differences were shown between age and sex of the groups in the study (Table I). The control ELISA$^+$, DIF$^+$ BP patients (mean 73.5 years) were significantly older than the study cohort (mean 67.3 years, $P = .009$), but their age ranges overlapped. The median values of the BP180 and BP230 ELISAs were significantly higher in the ELISA$^+$, DIF$^+$ patients (38.0 U/mL BP180 and 69.9 U/mL BP230) than the ELISA$^+$, DIF$^-$ patients (14.5 U/mL BP180 and 17.5 U/mL BP230) across all age and sex groups ($P < .001$). The data remained statistically significant when the assessment was repeated excluding the 10 patients with MMP from the ELISA$^+$, DIF$^-$ group.

The clinical presentation and diagnoses of the ELISA$^+$, DIF$^-$ patients varied, and a patient could have >1 clinical presentation and diagnosis (Table II). The top 5 clinical presentations were eczematous patches and plaques; prurigo nodules, excoriated papules, or both; ulcers, erosions, or both; erythematous patches; and urticarial lesions. Only 11.1% of the patients in this group had vesiculobullous lesions. The most frequent working diagnoses were dermatitis (34.6%), essential pruritus (8.7%), and BP (9.6%); For 13.9%, no diagnosis was given. The rash distribution of ELISA$^+$, DIF$^-$ patients was generalized in 83 patients (39.9%), multisite in 86 patients (41.3%), and localized in 11 patients (5.3%). Four patients (1.9%) did not have any findings or pruritus on dermatologic examination; 31 patients (14.9%) had mucosal disease, 7 of whom had concurrent cutaneous rashes.

The median BP180 value for the 8 patients with MMP and positive BP180 ELISA results was 30.6 (IQR 15.8-60.9) U/mL. The median BP230 value for the 6 patients with MMP and positive BP230 ELISA results was 25.9 (IQR 14.4-39.8) U/mL. Of the 10 ELISA$^+$, DIF$^-$ patients with MMP, the outcomes of the IIF test were BMZ deposition (1 patient), intracellular deposition (1 patient), and negative results (6 patients), and 2 patients were not tested by IIF.

Clinical signs (Table III) and laboratory results (Table IV) for patients from the primary study cohort who were positive for BP180, BP230, or both are shown. Patients positive for BP230 autoantibodies with BMZ deposition displayed on their IIF test had significantly higher BP230 levels ($P = .003$). When excluding patients with MMP, the median BP230 concentration for patients with negative IIF results (n = 66) was 13.0 (IQR 10.3-23.7) U/mL, compared with 25.2 (IQR 13.4-41.0) U/mL for patients with BMZ deposition seen on their IIF test (n = 29,

Table I. Characteristics of the study and control groups

Characteristic	ELISA$^+$*, DIF$^-$ group, n = 208	ELISA$^+$*, DIF$^+$ group, n = 49	*P* value
Age, y, mean (SD)†	67.3 (15.8)	73.5 (10.8)	.009
Women, U/mL, median (IQR)			
BP180, study n = 61, control n = 24	14.3 (11.0-26.7)	48.2 (27.5-97.6)	<.001
BP230, study n = 71, control n = 15	13.7 (11.0-27.7)	44.2 (38.0-82.7)	<.001
Men, U/mL, median (IQR)			
BP180, study n = 68, control n = 20	14.5 (11.2-24.2)	38.0 (21.1-134.2)	<.001
BP230, study n = 52, control n = 9	17.5 (10.7-27.1)	69.9 (56.7-105.9)	<.001
Age, y, n (%)†			.03
≤50	30 (14.4)	2 (4.1)	
51-60	34 (16.3)	5 (10.1)	
61-70	42 (20.2)	11 (22.4)	
71-80	53 (25.5)	16 (32.7)	
≥81	49 (23.6)	15 (30.6)	
Sex, n (%)			.78
Female	110 (52.9)	27 (55.1)	
Male	98 (47.1)	22 (44.9)	

BP, Bullous pemphigoid; *DIF*, direct immunofluorescence; *ELISA*, enzyme-linked immunosorbent assay; *IQR*, interquartile range; *SD*, standard deviation.
*Positive for BP180, BP230, or both.
†Age at the time of testing.

P = .002). Among patients for whom eosinophil counts were performed, the median was 0.23 (IQR 0.1-0.4) × 10^9 cells/L. Of the 26 ELISA$^+$, DIF$^-$ patients with BMZ deposition on their IIF tests, 11 had a clinically determined diagnosis of BP, 9 had dermatitis, 5 had a history of BP, 1 had MMP, 1 had prurigo nodularis, and 4 had no final diagnosis.

The features of the 4 patients with positive BP180 or BP230 ELISA results who initially had negative DIF tests that became positive on repeat testing are shown in Table V. The range for time to repeat positive DIF test result was 3 days-9 months. Review of the DIF test reports suggested that a suboptimal initial specimen (such as lack of epithelium) was a contributing factor to the initial false-negative DIF test result in 1 of 4 patients. Clinical presentations of disease with these patients included prurigo nodules or papules, dermatitis, and oral erosions. Of the 4 patients, 2 had BMZ deposition on IIF testing, 2 had negative IIF test results, 1 had low-positive ANA levels (1.1 U/mL, reference range <1.0 U/mL), 2 had ANA levels within the reference range, and 1 was not tested for ANA.

DISCUSSION

This study showed significant age and ELISA value differences between ELISA$^+$, DIF$^+$ patients with BP and ELISA$^+$, DIF$^-$ patients. We studied the ELISA$^+$, DIF$^-$ patient cohort precisely because it mirrors the real-life clinical conundrum of how to interpret positive ELISA results in the setting of a negative DIF test result. The mean age of the ELISA$^+$, DIF$^+$ patients was 73.5 years, similar to the mean ages in previously reported BP cohorts (ie, 73-83 years).[3,17-19] In ELISA$^+$, DIF$^+$ patients with BP in our study, the lower limits of the IQRs for BP180 and BP230 were 26.6 U/mL and 41.7 U/mL, respectively, which were over 2-fold higher than the upper reference limit (9 U/mL) for both laboratory tests at our institution. This suggests that the clinical suspicion for BP is reinforced by age >70 years and BP180 and BP230 ELISA values 3 times over the upper limit of normal. BP should be diagnosed with caution among patients with low-positive BP180 or BP230 autoantibody levels (in the 10-20 U/mL range) if there is a negative DIF test result or the DIF test is not performed.

Clinical findings and diagnoses were diverse in the ELISA$^+$, DIF$^-$ cohort. The most common clinical presentation was eczema, but various lesion morphologies were present. In patients with nonbullous BP or preclinical BP diagnoses or for whom nonbullous BP or preclinical BP is suspected, possible lesion morphologies included erythema, papules or nodules, eczematous patches or plaques, and essential pruritus with excoriations.[20,21] Approximately 10% (20/208, Table II) of the ELISA$^+$, DIF$^-$ patients were given a working diagnosis of BP because of a combination of clinical presentation, positive IIF test results, and highly elevated BP180 and BP230 levels. Given multiple factors, including limited follow-up and treatment, progression to more classical BP cannot be confirmed or negated in this group. Hence, it is difficult to diagnose nonbullous BP and

Table II. Clinical presentations and diagnoses for ELISA^{+}*, DIF^{-} patients

Feature	n (%), N = 208
Clinical presentations at initial evaluation†	
Eczematous, scaly erythematous patches or plaques or both	73 (35.1)
Prurigo nodules, excoriated papules, or both	66 (31.7)
Ulcers, erosions, or both	55 (26.4)
Erythematous patches	43 (20.7)
Urticarial, not otherwise specified	24 (11.5)
Other presentations	23 (11.1)
Vesiculobullous lesions	23 (11.1)
Essential pruritus	16 (7.7)
Urticarial dermatitis	10 (4.8)
Purpura, petechiae, or both	7 (3.4)
Blisters, not otherwise specified	6 (2.9)
Clinical diagnoses†	
Dermatitis	72 (34.6)
No diagnosis given	29 (13.9)
Bullous pemphigoid	20 (9.6)
Essential pruritus	18 (8.7)
Lichen planus	12 (5.8)
Mucous membrane pemphigoid	10 (4.8)
Prurigo nodularis	10 (4.8)
Urticarial eruption	8 (3.8)
History of bullous pemphigoid	6 (2.9)
Erythema multiforme	3 (1.4)
Other‡	27 (13.0)

BP, Bullous pemphigoid; *DIF*, direct immunofluorescence; *ELISA*, enzyme-linked immunosorbent assay.
*Positive for BP180, BP230, or both.
†Some patients had multiple clinical presentations and diagnoses.
‡Two cases each: Stevens–Johnson syndrome/toxic epidermal necrolysis, scabies, lichenoid mucositis, aphthous stomatitis, and folliculitis. One case each: pemphigoid gestationis, polymorphic eruption of pregnancy, bullous lupus, connective tissue disease, paraneoplastic pemphigus, exanthematous eruption, exfoliative erythroderma, erythrodermic psoriasis, neutrophilic pustulosis, subcorneal pustular dermatosis, Sweet syndrome, urticarial vasculitis, cutaneous T-cell lymphoma, dermatomyositis, porphyria cutanea tarda, postoperative nonhealing ulcer, and vancomycin-induced blistering disease.

Table III. Clinical signs and laboratory results for the study patients with positive BP180 ELISA* and negative DIF test results

Feature	n	BP180, U/mL, median (IQR)	*P* value†
IIF test result			.50
Basement membrane deposition	12	15.2 (11.5-32.0)	
Intracellular deposition	5	19.5 (17.7-22.0)	
Negative	89	13.6 (11.0-25.7)	
Not done	23	14.7 (12.7-23.4)	
Antinuclear antibodies			.27
Positive	21	18.7 (13.2-27.0)	
Negative	45	12.9 (11.0-21.9)	
Not done	63	15.8 (11.2-26.8)	
Extent of rash			.78
Localized	7	12.9 (9.8-29.5)	
Multifocal	55	14.4 (11.2-31.8)	
Generalized	47	16.0 (11.2-23.4)	
Eczematous rash			.53
Yes	39	15.8 (11.2-27.1)	
No	90	13.8 (11.2-25.0)	
Prurigo nodule			.97
Yes	39	14.1 (11.2-22.6)	
No	90	15.2 (11.2-27.0)	
Ulcer, erosion			.79
Yes	33	13.6 (12.5-24.0)	
No	96	14.6 (11.0-26.2)	
Erythematous patch			.50
Yes	29	13.2 (11.5-20.6)	
No	100	15.4 (11.0-26.7)	
Urticaria			.72
Yes	9	15.8 (11.2-27.0)	
No	120	14.2 (11.2-25.4)	

BP, Bullous pemphigoid; *DIF*, direct immunofluorescence; *ELISA*, enzyme-linked immunosorbent assay; *IIF*, indirect immunofluorescent; *IQR*, interquartile range.
*Includes patients with elevated BP230 levels and BP230 levels within the reference range of the ELISA.
†Significance of BP180 levels for the features listed.

preclinical BP on the basis of clinical morphology alone in ELISA^{+}, DIF^{-} cases, and the authors still consider the DIF test to be the gold standard for diagnosis of nonbullous or atypical BP.

The most common clinical diagnosis in our study cohort was eczema; however, the patients also had mucosal diseases, interface dermatoses and connective tissue diseases, non-BP autoimmune bullous diseases, pregnancy-related dermatoses, neutrophilic dermatoses, and other diagnoses. Previous reports have shown that BP180 or BP230 positivity is also associated with prurigo, eczema, lichen planus, scabies, essential pruritus, MMP, bullous systemic lupus erythematosus, pemphigus gestationis, and linear immunoglobulin A bullous disease, which our findings corroborate.[22-28] However, BP180 and BP230 positivity has not previously been reported in neutrophilic dermatoses. A previous study by Wieland et al[29] showed that 7.4% of dermatologically normal-appearing patients with negative IIF test results also had positive BP180 or BP230 ELISA results. Therefore, a wide differential diagnosis should be considered in an ELISA^{+}, DIF^{-} patient. Our findings support a thorough clinical examination, including mucosal surfaces, and a complete history, including a review of systems, pregnancy,

Table IV. Clinical signs and laboratory results for study patients with positive BP230 ELISA and negative DIF test results*

Feature	n	BP230, U/mL, median (IQR)	*P* value†
IIF test result			.003
Basement membrane deposition	23	36.0 (16.7-51.1)	
Intracellular deposition	7	18.7 (9.9-35.5)	
Negative	70	13.7 (10.4-24.0)	
Not done	23	13.7 (10.7-22.5)	
Antinuclear antibodies			.78
Positive	194	13.0 (10.9-32.0)	
Negative	4	15.7 (10.8-23.9)	
Not done	60	17.3 (10.9-29.3)	
Extent of rash			.50
Localized	7	22.5 (9.6-39.1)	
Multifocal	43	13.7 (10.7-27.7)	
Generalized	53	17.3 (12.4-29.4)	
Eczematous rash			.34
Yes	44	17.0 (10.9-34.7)	
No	79	14.4 (10.9-25.2)	
Prurigo nodule			.36
Yes	37	16.8 (11.0-35.5)	
No	86	14.1 (10.9-24.9)	
Ulcer, erosion, or both			.46
Yes	34	16.9 (11.3-30.9)	
No	89	14.8 (10.8-26.5)	
Erythematous patch			.08
Yes	21	11.5 (10.4-14.4)	
No	102	17.4 (11.2-27.7)	
Urticaria			.10
Yes	18	11.5 (9.6-26.5)	
No	105	16.7 (11.2-27.1)	

BP, Bullous pemphigoid; *DIF*, direct immunofluorescence; *ELISA*, enzyme-linked immunosorbent assay; *IIF*, indirect immunofluorescent; *IQR*, interquartile range.

*Includes patients with elevated BP180 levels and BP180 levels within the reference range of the ELISA.

†Significance of BP230 levels for the features listed.

and medications. In addition, clinical criteria established by Vaillant et al[19] and Joly et al,[30] which include age >70 years, no atrophic scars, no head and neck involvement, or no mucosal involvement, can be helpful for making decisions for retesting with the BP180 or BP230 ELISA or the DIF test.

Sárdy et al[11] reported that BP230 results significantly correlated with the detection of BMZ deposition on IIF test, and this observation was confirmed by our study. The presence of eczema, prurigo, ulcers, erythema, and urticaria, as well as the extent of the rashes, were not correlated with BP180 or BP230 ELISA values. Furthermore, ANA positivity was also not associated with BP180 or BP230 ELISA values.

Among ELISA$^+$, DIF$^-$ patients, only a minor fraction might become DIF test positive on subsequent retesting. There were 4 patients in this study positive for BP180, BP230, or both and a patient with an initial negative DIF test result who subsequently had positive DIF test results showing BMZ deposition. Ultimately, these patients were given BP or MMP diagnoses; 3 of the 4 patients had either elevated autoantibody levels or histopathologic findings suspicious for pemphigoid (including eosinophilic spongiosis or subepithelial clefting with eosinophils). Therefore, if there is high clinical suspicion for BP, the results of this study support repeating the DIF test within a few months, especially if there was any concern about a suboptimal initial specimen (such as minimally intact BMZ or nonperilesional skin). We also reported data for 23 ELISA$^+$, DIF$^-$ patients whose repeat DIF test result remained negative.

This study has limitations. Because only existing health records were reviewed, laboratory results for

Table V. Patients positive for BP180, BP230, or both by ELISA with an initial negative DIF test result with a subsequent positive DIF test result

Pt	Age, y	Sex	Clinical presentation	BP180 level, U/mL	BP230 level, U/mL	Time to repeat positive DIF test result	Histologic findings on initial biopsy with negative DIF test result	Histologic findings on repeat biopsy with positive DIF test result	Final diagnosis
1	72	F	Dermatitis, prurigo nodules	92.63	27.97	1 mo	Acanthosis, diffuse dermal mixed inflammation with eosinophils	Eosinophilic spongiosis, subepidermal cleft, diffuse dermal eosinophils, and perivascular lymphocytes	BP
2	94	M	Erythematous papules and nodules	<5.00	15.48	1 mo	Eosinophilic spongiosis, subepidermal cleft, diffuse dermal mixed inflammation	Eosinophilic spongiosis, diffuse dermal mixed inflammation with eosinophils	BP
3	73	M	Erythematous macules and papules	10.80	<5.00	3 d	Perivascular lymphocytic inflammation	Eosinophilic spongiosis, subepidermal cleft, diffuse dermal mixed inflammation with eosinophils	BP
4	47	F	Oral erosions, conjunctival adhesion	11.36	5.35	9 mo*	Submucosal cleft, diffuse dermal mixed inflammation with eosinophils	Subepidermal cleft, diffuse dermal mixed inflammation with eosinophils	MMP

BP, Bullous pemphigoid; *DIF*, direct immunofluorescence; *ELISA*, enzyme-linked immunosorbent assay; *MMP*, mucous membrane pemphigoid; *Pt*, patient.

*The initial DIF test was performed on a suboptimal specimen without epidermis. A subsequent DIF test performed 9 months later was negative; a third DIF test was positive another 2 weeks later.

tests, such as the ANA and IIF tests, were not available for every patient. For the ELISA$^+$, DIF$^-$ cohort, the clinical diagnoses were interpreted by clinicians at the time patients were examined. Because our institution is a tertiary care center and patients often follow up with local dermatologic providers, long-term follow-up was not available for every patient.

In summary, ELISA$^+$, DIF$^-$ patients had lower mean ages and lower BP180 or BP230 ELISA values than ELISA$^+$, DIF$^+$ patients. ELISA$^+$, DIF$^-$ patients had a wide range of clinical morphologies and potential diagnoses. Most ELISA$^+$, DIF$^-$ patients did not have positive results on BMZ staining on repeat DIF testing. Low-positive BP180 and BP230 autoantibody levels should not be overinterpreted as evidence for BP in the setting of a negative DIF test results. However, higher BP180 or BP230 autoantibody levels, advanced age, and suspicious histopathologic features, such as eosinophilic spongiosis, support a diagnosis of BP, and repeating a biopsy for DIF testing should be considered.

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