

Anti-BP180 NC16A IgG Titres as an Indicator of Disease Activity and Outcome in Asian Patients with Bullous Pemphigoid

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Abstract

Introduction: Anti-BP180 IgG titres were observed to parallel disease activity in case series of bullous pemphigoid (BP). This study aimed to examine whether anti-BP180 titres are an indicator of disease severity, clinical course and outcome in Asian patients with BP. **Materials and Methods:** This was a prospective observational study conducted between March 2005 and March 2008 in the Immunodermatology Clinic at the National Skin Centre, Singapore. Disease activity and anti-BP180 IgG titres were measured 4-weekly for 12 weeks and during disease flares and clinical remission. Associations between anti-BP180 titres and disease activity, disease flare, clinical remission and cumulative prednisolone dose were examined. **Results:** Thirty-four patients with newly diagnosed BP were recruited. Median follow-up duration was 3 years. Notable correlations between disease activity and anti-BP180 titres were at baseline ($r = 0.51, P = 0.002$), and disease flare ($r = 0.85, P < 0.001$). Lower titres at Week 12 were associated with greater likelihood of clinical remission ($P = 0.036$). Post hoc, patients with anti-BP180 titres above 87.5 U/mL at time of diagnosis who reached remission within 2 years of diagnosis received significantly higher cumulative doses (mg/kg) of prednisolone (median, 72.8; range, 56.5 to 127.1) than those with titres <87.5 U/mL (median, 44.6; range, 32.5 to 80.8); $P = 0.025$). **Conclusion:** Anti-BP180 titres may be a useful indicator of disease activity at time of diagnosis and at disease flare. Lower titres at Week 12 may predict greater likelihood of clinical remission. Titres above 87.5 U/mL at time of diagnosis may suggest the need for higher cumulative doses of prednisolone to achieve remission within 2 years.

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Key words: Immunobullous disease, Subepidermal skin blistering, Tense skin blisters

Introduction

Bullous pemphigoid (BP) is a subepidermal blistering dermatosis characterised by circulating autoantibodies targeting BP180 and BP230 hemidesmosomal proteins. Anti-BP180 NC16A IgG antibodies have been demonstrated to be directly pathogenic in blister formation.^{1,2} Anti-BP180 IgG titres were noted to parallel disease activity in several case series,³⁻¹⁰ as well as reflect disease control and disease activity in larger studies.^{11,12} However, there are limited data regarding how anti-BP180 IgG titres vary over time with treatment, and whether antibody titres can be used to predict disease flares, remission, and cumulative prednisolone dose received by BP patients.

The aims of this prospective study were to determine

the temporal relationship between anti-BP180 IgG titres and disease activity, and whether antibody titres at specific follow-up time points were predictive of subsequent disease flare or remission. In addition, we investigated the association of anti-BP180 IgG titres with cumulative prednisolone dose received at remission.

Materials and Methods

Forty patients with newly diagnosed BP were recruited from the Immunodermatology Clinic at the National Skin Centre, Singapore between March 2005 and March 2008. BP was diagnosed based on clinical findings consistent with BP (tense bullae, urticarial plaques or erosions on the body, with or without oral mucosal involvement and

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without evidence of scarring) and at least 2 out of 3 of the following: subepidermal blister on histology, linear deposition of C3 and/or IgG along the basement membrane by direct immunofluorescence (DIF), and epidermal or epidermal and dermal binding of immunoglobulins on salt-split skin by indirect immunofluorescence (IIF). Exclusion criteria were treatment with systemic corticosteroids or immunosuppressants during the preceding 1 year, concurrent immune-related and neoplastic diseases, and negative anti-BP180 IgG titre (<9 U/mL) throughout the first 12 weeks of follow-up, pregnancy and lactation. Five patients were excluded due to persistently negative anti-BP180 IgG titres throughout the follow-up, and 1 patient was excluded due to underlying cancer. Thirty-four patients were included in the analysis. Informed consent was obtained from all study subjects and the study was approved by the National Healthcare Group Domain-Specific Review Board.

Disease activity of BP was evaluated using a standardised scoring method based on body surface area (BSA) involvement and number of non-epithelised erosions, blisters and urticarial plaques (Table 1). Serum anti-BP180 antibodies were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits detecting IgG antibodies against the NC16A domain of BP180 (MBL Co., Ltd, Japan). IgG antibodies ≥ 9 U/mL was interpreted as a positive ELISA result and <9 U/mL as a negative result. Disease activity scoring and blood sampling were performed at diagnosis, at monthly intervals during the first 3 months, at first disease flare after Week 12, and at clinical remission.

Patients were treated based on standardised institutional guidelines and managed by a dedicated team of doctors in the Immunodermatology Clinic. Patients were started on oral prednisolone 0.3 to 0.8 mg/kg/day at diagnosis which was tapered gradually every 4 to 8 weeks depending on clinical

severity. Clinical remission was defined as a disease activity score of zero with a prednisolone dose of ≤ 3 mg/day for at least 1 month, after Week 12. Disease flare after Week 12 was defined as worsening of the prevailing disease activity requiring an escalation of treatment. Clinical remission and disease flare data were obtained from patient records from time of diagnosis until 31 November 2012.

Statistical Analysis

Spearman correlations were computed to assess the degree of association between disease activity, cumulative prednisolone dose, and anti-BP180 IgG titres. Wilcoxon signed-rank tests were performed to compare within-patient changes in anti-BP180 IgG titres and cumulative prednisolone dose at baseline, Week 4, 8 and 12. Wilcoxon rank-sum tests were used to compare anti-BP180 IgG titre levels and cumulative prednisolone doses between patient groups with and without disease flare, patients achieving and failing to achieve clinical remission, and patients receiving adjuvant therapy versus those receiving prednisolone alone. Cox regression was used to assess the influence of change (rise) in anti-BP180 IgG titres on time to clinical remission beyond the 12-week follow-up period. Linear regression was conducted to evaluate whether adjuvant therapy was a confounding factor in the relationship between cumulative prednisolone dose and anti-BP180 IgG titres. Simple linear regression was used to characterise the relationship of disease activity with anti-BP180 IgG titres at baseline, follow-up times, disease flare and remission.

Post hoc subgroup analysis was performed on patients achieving clinical remission within 2 years. The Wilcoxon rank-sum test was used to compare median anti-BP180 titres between patients who had disease flare after remission and those who did not, in the subgroup of patients with positive ELISA results (≥ 9 U/mL) at remission. Fisher's exact test was used to compare risk of disease flare after remission between patients with positive titres at clinical remission and those with negative titres. Statistical significance was set at $P < 0.05$ on a per test basis. P values are reported, thus leaving to reader prerogative any decision to adjust significance levels due to multiple tests and choice of adjustment method. All analyses were performed using SAS 9.2 (SAS® Carey, NC, USA).

Results

Demographics

Thirty-four patients were included in the analysis of which 21 were men and 13 were women (Table 2). Median age was 75 years (range, 27 to 96). The ethnic distribution of patients was 79.4% Chinese, 14.7% Indians and 5.9% Malays; 82.4% of patients had generalised BP, and median

Table 1. Components of the Disease Activity Score: Percentage Involvement of Body Surface Area (BSA), and Number of Non-Epithelised Erosions, Blisters or Urticarial Plaques

Disease Activity Score Component*		
Component Score	Percentage Involvement of Body Surface Area (% BSA)	Number of Non-Epithelised Erosions/ Blisters/Urticarial Plaques
5	>30	>30
4	16 – 30	21 – 30
3	11 – 15	11 – 20
2	5 – 10	5 – 10
1	1 – 4	1 – 4
0	0	0

*For each patient, component scores are summed to obtain the disease activity score.

Table 2. Patient Characteristics at Baseline and Outcomes during Follow-up*

Variable	All Patients n = 34	Flare n = 27	No Flare n = 7	P Value	Remission n = 25	No Remission n = 9	P Value
Age	75 (27,96)	77 (27, 96)	68 (51, 81)	0.063	77 (27,96)	75 (59,85)	0.368
Gender							
Male	21 (61.8)	16 (59.3)	5 (71.4)	0.682	15 (60.0)	6 (66.7)	1.000
Female	13 (38.2)	11 (40.7)	2 (28.6)		10 (40.0)	3 (33.3)	
Ethnicity							
Chinese	27 (79.4)	21 (77.8)	6 (85.7)	0.260	20 (80.0)	7 (77.8)	0.788
Malay	2 (5.9)	1 (3.7)	1 (14.3)		1 (4.0)	1 (11.1)	
Indian	5 (14.7)	5 (18.5)	0 (0)		4 (16.0)	1 (11.1)	
Type of BP at diagnosis							
Localised	2 (5.9)	2 (7.4)	0 (0)	0.720	2 (8.0)	0 (0)	0.447
Generalised	28 (82.4)	21 (77.8)	7 (100)		19 (76.0)	9 (100)	
Acral	4 (11.8)	4 (14.8)	0 (0)		4 (16.0)	0 (0)	
Beyond 12 weeks of follow-up	32 (94.1)	27 (100)	5 (71.4)	0.037	25 (100)	7 (77.8)	0.064
Duration of follow-up (year)	3.0 (0.1, 7.3)	3.7 (0.4, 7.3)	0.5 (0.1, 1.8)	<0.001	3.7 (1.3, 7.3)	0.5 (0.1, 5.0)	0.001
Concomitant immunosuppressant	12 (35.3)	10 (37.0)	2 (28.6)	1.000	8 (32.0)	4 (44.4)	0.687
Azathioprine	2 (5.9)	2 (7.4)	0 (0)	1.000	2 (8.0)	0 (0.0)	1.000
Methotrexate	1 (2.9)	1 (3.7)	0 (0)	1.000	1 (4.0)	0 (0.0)	1.000
Dapsone	7 (20.6)	6 (2.2)	1 (14.3)	1.000	5 (20.0)	2 (22.2)	1.000
Nicotinamide/tetracycline	4 (11.8)	3 (11.1)	1 (14.3)	1.000	1 (4.0)	3 (33.3)	0.048

BP: Bullous pemphigoid

*Continuous variables are summarised using median (min, max); categorical variables using count (%). Wilcoxon rank-sum test used for continuous variables, Fisher's exact test for categorical variables.

disease activity score at diagnosis was 5 (range, 2 to 10). All patients received prednisolone as first-line therapy. Twelve (35.3%) patients received adjuvant therapies: dapsone (5), azathioprine (2), nicotinamide/tetracycline only (2); methotrexate only (1), with 2 patients receiving both dapsone and nicotinamide/tetracycline.

Duration of Follow-up and Time to Disease Flare or Remission

Median duration of follow-up was 3 years (range, 0.1 to 7.3) (Table 2). Thirty-two (94.1%) patients were followed up beyond Week 12, of which 25 (73.5%) achieved clinical remission. Twenty-seven (79.4%) patients experienced a flare after Week 12. Median elapsed time from diagnosis to first disease flare was 0.9 years (range, 0.3 to 6.6) and from diagnosis to first clinical remission was 1.2 years (range, 0.6 to 3.2).

Antibody Titres and Disease Activity during 12-week Follow-up

A total of 177 serum samples were analysed for an average of 5.2 samples per patient. Anti-BP180 IgG titres and disease activity decreased in unison over the first 12 weeks but increased during disease flare (Fig. 1). Within-patient reductions in anti-BP180 IgG titres and decline in disease activity scores from baseline to Week 4 ($P < 0.0001$), Week 4 to 8 ($P < 0.0001$), and Week 8 to 12 ($P < 0.0001$) were all statistically significant. Within-patient decline in disease activity scores from baseline to Week 4 ($P < 0.0001$), Week 4 to 8 ($P = 0.013$), and Week 8 to 12 ($P = 0.042$) were also statistically significant.

Correlations between anti-BP180 IgG titres and disease activity scores at study follow-up times were: baseline, $r = 0.51$ ($P = 0.002$); Week 4, $r = 0.36$ ($P = 0.053$); Week 8, $r = 0.40$ ($P = 0.024$); and Week 12, $r = 0.22$ ($P = 0.243$). Trend

lines showing relationships between disease activity and anti-BP180 IgG titres at study follow-up times are plotted in Figure 2. The falling lines and shrinking line lengths from baseline through remission reflect progressive reductions over time in both disease activity and anti-BP180 IgG titres. This is indicative of treatment efficacy with passing time. Trend line parallelism indicates that regardless of disease activity level, the expected change in disease activity as a function of change in BP180 IgG titre remains constant.

Disease Flare after 12-week Follow-up

A statistically significant correlation was observed between anti-BP180 IgG titres and disease activity score at disease flare ($r = 0.85$, $P < 0.001$). Anti-BP180 IgG titres within the 12-week follow-up period were not significantly different between patients with disease flare after Week 12 and those without (Table 3).

Only at the time of disease flare was a significant difference in anti-BP180 titres observed between patients who needed adjuvant therapy and those who did not ($P = 0.030$). Median (min, max) anti-BP180 titres at disease flare were 65.5 (10.8, 224.5) in patients who needed adjuvant therapy and 27.6 (7, 48.7) in those receiving prednisolone alone.

Clinical Remission after 12-week Follow-up

Patients who achieved clinical remission after 12 weeks had significantly lower antibody titres at Week 12 (median, 17.8; range, 3.9 to 88.7) compared to those who did not achieve remission (median, 62.3; range, 24.5 to 142.1) ($P = 0.036$) (Table 3). Furthermore, using the 4 to 12 week remission rate as reference, the post 12-week remission rate decreased on average 24% for every 1% weekly increase in anti-BP180 IgG titres (hazard ratio, 0.76; 95% CI, 0.58 to 0.93).

Cumulative Prednisolone Dose Received

A statistically significant positive correlation was observed between cumulative prednisolone dose received (mg/kg/patient) and anti-BP180 IgG titres at Week 4 ($r = 0.37$, $P = 0.048$); i.e. higher antibody titres at Week 4 were associated with higher cumulative doses of prednisolone. Correlations at other study time points were: Week 8, $r = 0.32$ ($P = 0.074$); Week 12, $r = 0.29$ ($P = 0.117$), disease flare, $r = 0.42$ ($P = 0.093$) and clinical remission, $r = 0.42$ ($P = 0.060$).

There were no significant differences in cumulative prednisolone dose received (mg/kg/patient) at any study time point between patients who experienced a flare and those who did not: Week 4 ($P = 0.766$), Week 8 ($P = 0.469$), and Week 12 ($P = 0.781$). Similarly, there were no significant differences in cumulative prednisolone dose received at

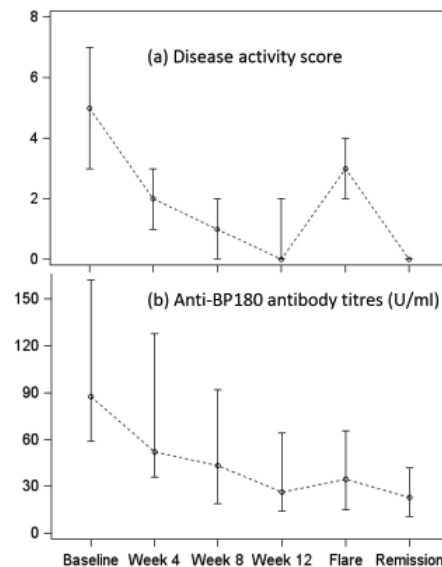


Fig 1. (a) Mean disease activity scores and (b) mean anti-BP180 antibody levels (95% CI) at follow-up times, disease flare and clinical remission.

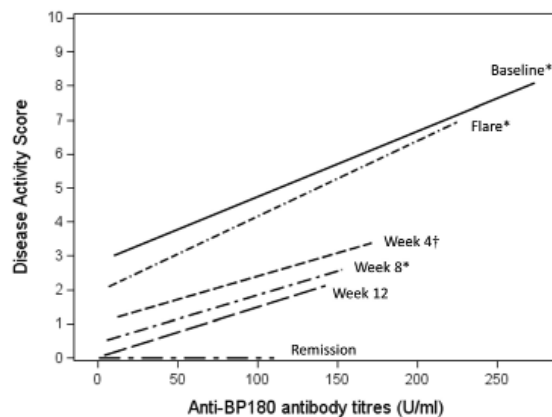


Fig 2. Trend lines showing relationships between disease activity and anti-BP180 IgG titres at baseline, 4-, 8- and 12-week follow-up times, and at remission and disease flare. Note: *Correlations between anti-BP180 titres and BP disease activity were statistically significant at baseline ($P = 0.002$), Week 8 ($P = 0.024$) and disease flare ($P < 0.001$); †nearly significant at Week 4 ($P = 0.053$); and at Week 12, $P = 0.243$. Falling trend lines and shrinking lengths over time is indicative of treatment efficacy. Parallelism indicates that regardless of disease activity, the expected change in disease per unit change in BP180 IgG titre remains constant.

any time between patients who achieved clinical remission and those who did not (Table 4).

We found no evidence that use of adjuvant therapy modified the relationship between anti-BP180 titres and cumulative prednisolone dose received. After adjusting for length of follow-up, there was no difference in cumulative

Table 3. Anti-BP180 Titres during the 12 Week Follow-up Period in Patients with and without Flare and in Patients with and without Remission*

Follow-up	Flare (n = 27)	No Flare (n = 7)	P Value	Remission (n = 25)	No Remission (n = 9)	P Value
Baseline	81.3 (9.9,273.5)	137.8 (59.1, 208.7)	0.125	81.3 (9.9, 273.5)	103.5 (50.1, 208.7)	0.640
Week 4	48.1 (12.5,171.2)	139.7 (50.2, 145.8)	0.075	48.1 (12.5, 171.2)	65.4 (45.6, 145.0)	0.303
Week 8	28.4 (5.4, 136.3)	63.6 (28.2, 152.7)	0.154	28.2 (5.4, 136.3)	72.2 (43.1, 152.7)	0.062
Week 12	22.4 (3.9, 88.7)	63.9 (16.2, 142.1)	0.095	17.8 (3.9, 88.7)	62.3 (24.5, 142.1)	0.036

*Results are reported in median (min, max); P values from Wilcoxon rank-sum test.

Table 4. Cumulative Prednisolone Dose Received (mg/kg/patient) over 12 Weeks Starting from Baseline*

Time Period	Entire Cohort (n = 34)	Flare (n = 27)	No Flare (n = 7)	P Value	Remission (n = 25)	No Remission (n = 9)	P Value
Till Week 4	13.6 (0, 34.4)	13.2 (0, 26.4)	14.0 (0, 34.4)	0.766	13.2 (0, 34.4)	14.0 (0, 21.1)	0.740
Till Week 8	23.6 (0, 50.9)	23.3 (0, 50.9)	28.7 (0, 48.1)	0.469	23.3 (0, 50.9)	24.7 (0, 39.6)	0.828
Till Week 12	31.2 (7.7, 64.6)	30.9 (7.7, 63.1)	34.3 (17.5, 64.6)	0.781	31.3 (7.7, 64.6)	31.1 (17.5, 54.8)	0.897

*Results are reported in median (min, max), P values from Wilcoxon rank-sum test.

prednisolone dose at any time point between those who received adjuvant therapy and those who did not.

Subgroup Analyses

The majority of patients (19/25, 76%) achieved clinical remission within 2 years. This provided a reasonable basis for a post hoc analysis exploring association between level of anti-BP180 IgG titres at the time of diagnosis and cumulative prednisolone dose at the time of clinical remission, within 2 years of diagnosis. The linear regression slope relating cumulative prednisolone dose at remission to anti-BP180 IgG titres at time of diagnosis was positive and statistically significant ($P=0.0037$). Using the median value of anti-BP180 IgG titres at time of diagnosis (87.5 U/mL) as a cutoff value, it was noted that 9 patients with antibody titres above 87.5 U/mL at time of diagnosis, and in remission within 2 years of diagnosis, had received significantly higher cumulative doses of prednisolone at the time of clinical remission (median, 72.8; range, 56.5 to 127.1), compared to the 10 patients with titres below 87.5 U/mL (median, 44.6; range, 32.5 to 80.8; $P=0.025$) (Fig. 3).

In a second subgroup analysis, 17 of 25 patients (68%)

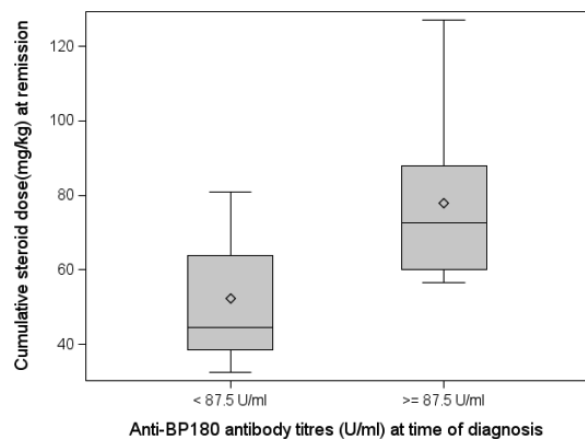


Fig 3. Cumulative prednisolone dose received (mg/kg/patient) at clinical remission within 2 years using an anti-BP180 antibody level cutoff of 87.5 U/mL at time of diagnosis. Note: The bottom and top edges of the box indicate the interquartile range (25th to 75th percentile). The line within the box and the diamond symbol represent the median and mean value, respectively. The whiskers extend from the box in both directions to represent the minimum and maximum observations.

were found to have positive anti-BP180 IgG titres despite achieving clinical remission. The median antibody titre in this subgroup was 33.9 (range, 9.2 to 109.9). Thirteen (76.5%, 95% CI, 50.1% to 93.2%) of the 17 patients with elevated titres at clinical remission had subsequent disease flare after remission. However, we did not find a significant difference in median antibody titres at clinical remission in patients who had subsequent disease flares, compared to those who did not. Among the 8 patients with negative titres at clinical remission (median 4.9, range, 0.6 to 8.2), 4 (50%, 95% CI, 15.7% to 84.3%) had disease flare after remission. However, comparing patients with positive titres at remission versus those with negative titres, we found no significant difference in the subsequent risk of flare ($P = 0.36$, Fisher's exact test).

Discussion

This study showed a statistically significant positive correlation between anti-BP180 IgG titres and BP disease activity at time of diagnosis (baseline) and at disease flare. Anti-BP180 IgG titres exhibited a “nearly” statistically significant correlation with disease activity at follow-up Week 4, a statistically significant correlation at Week 8 and a non-significant correlation at Week 12. The non-significant correlation at Weeks 4 and 12 are likely a consequence of the simultaneous reduction of both disease activity and anti-BP180 IgG titres. Correlation is a function of the range of data values, and the shrinking range in outcome values for both variables would have the effect of reducing statistical power for detecting correlation. The simultaneous diminution of antibody titres and disease activity scores with the passage of time is evidence of treatment efficacy.

Notably, our study showed that lower anti-BP180 IgG titres at Week 12 were associated with a higher likelihood of achieving subsequent clinical remission. This is consistent with other studies which found that antibody titres significantly decreased after remission,^{5,8,12,13} or during treatment toward disease control.⁹⁻¹¹ However, an interesting finding was that 68% of patients had persistent elevated antibody titres despite achieving clinical remission and that the antibody titres in these patients were not predictive of subsequent disease flare. A previous study also reported that there was no significant decrease in anti-BP180 titres in a group of BP patients whose lesions had been resolved by oral corticosteroid treatment.¹⁴ This seems to indicate that a clinical remission may not always accompany with serological remission.¹⁵ In our practice, the management of such patients needs to be individualised and the risks and benefits of long-term immunosuppression should be discussed with the patients and family. The decision to withdraw, taper or maintain long-term, low dose immunosuppressive agents depends on several factors such

as patient comorbidities, severity of initial disease, number of recent flares, duration and sustainability of remission, and the presence of any adverse effects of the medications used. In general, most patients can be continued on very low dose prednisolone if there are no contraindications. It also appears that a disease flare may not always be reflected in a rise in antibody titres. It has been reported that anti-BP180 titres could fail to show a rise during disease flare.⁷ Nonetheless, 2 previous studies found that a higher anti-BP180 titre at remission was a risk factor for relapse within the subsequent 3 months¹¹ or 1 year.¹² However, these 2 studies included patients with negative titres, in contrast to our analysis which included only patients who had positive titres (≥ 9 U/mL).

The limited usefulness of anti-BP180 IgG titres for predicting flares may be related to several factors. First, downstream factors such as complement activation, mast cell degranulation, macrophage activation and neutrophilic chemotaxis, which are not measured by antibody levels, play an important role in causing subepidermal splitting, after the initial binding of the antibodies to BP180 antigens.^{1,16,17} Second, specific IgG subclasses such as IgG1 and IgG4, as well as anti-BP180 IgE antibodies,¹⁸⁻²⁰ have been shown to have differential disease-causing potential and may be differentially associated with disease activity and remission respectively, and these were not measured by the current ELISA assay.²¹⁻²³ Third, epitope spreading over time may generate pathogenic antibodies against other epitopes of BP180 (including the non-NC16A domain) and BP230.²⁴⁻²⁸ Finally, long-lived memory plasma cells may produce non-pathogenic anti-BP antibodies that would not result in disease flare.^{29,30}

In this study, we also found a positive and statistically significant correlation between the cumulative prednisolone dose received and anti-BP180 titres at week 4 ($r = 0.37$, $P = 0.048$). This is consistent with a previous study which showed that the dose of prednisolone necessary to suppress new blister formation was significantly correlated with the antibody titre³ as well as another recent study which found that high anti-BP180 titres correlated with increased effective prednisolone dose ($r = 0.37$, $P < 0.05$).³¹ Of note, we recognise that the relationship between disease activity, antibody titres and cumulative prednisolone dose are not independent parameters. Hence, patients with higher antibody titres are more likely to have higher disease activity, and hence, receive greater cumulative doses of prednisolone over time.

While there was no difference in cumulative prednisolone dose between patients who received adjuvant therapy and those who did not, across all follow-up times, after controlling for cumulative prednisolone dose at disease flare, we noted that at disease flare patients with adjuvant

therapy had higher anti-BP180 titres compared to patients without adjuvant therapy. This may be explained by the fact that adjuvant therapy was added during flares or in cases with poor disease control, when the anti-BP180 titres were expectedly high.

We observed that patients who achieved clinical remission within 2 years and had antibody titres above a threshold of 87.5 U/mL at time of diagnosis, exhibited cumulative prednisolone doses at clinical remission that were significantly higher than in patients with titres below that threshold. Thus, antibody titres at time of diagnosis may be useful in prognosticating the cumulative prednisolone dose needed to achieve clinical remission and hence guide dosing in patients. Indeed, as antibody titres correlate with disease activity, early detection and therefore timely treatment are important in the management of BP. However, because this finding was based on post hoc analysis it must be viewed as hypothesis generating. Thus future studies would be essential to confirm this finding.

Strengths of our study include a relatively large cohort of patients compared to previous case series, the prospective tracking of antibody titres and disease activity over fixed time points, and a long follow-up duration (median 3 years, maximum 7.3 years) compared to previous studies where the follow-up ranged from several months to a year.³⁻¹² We were also able to assess the cumulative prednisolone dose received in relation to antibody titres and disease course. A limitation is that we used a simplified but standardised lesion scoring method, as no published validated scoring system was available at the time this study was initiated. Our scoring system is similar to the recently developed Autoimmune Bullous Skin Disorder Intensity Score (ABSIS)³² and assesses BSA involvement and extent of active lesions but not the involvement of oral mucosa. However, we believe our scoring system was sufficiently comprehensive to adequately reflect disease burden, as most of our study patients did not have oral involvement and the gradation in the quantification of lesions was sensitive enough to reflect changes in disease activity. Our scoring system was similar to those used in previous published studies^{33,34} before the availability of the ABSIS scoring method. The other limitation was that it was not possible to standardise the duration of follow-up after the first 3 months, and there were differences noted in the follow-up duration for patients with and without disease flare, as well as those with and without remission. This may have affected antibody titres and total cumulative prednisolone doses received. Finally, high antibody titres with values above 150 U/mL, which only accounted for 8% of all results, will need to be interpreted with caution as the linearity of the ELISA assay cannot be ascertained beyond this level, since no further sera dilutions were performed. However, we have verified that our key results

and conclusions remained unchanged with no significant differences detected after repeat analysis, using 150 U/mL as the upper limit of detection (data not shown).

Conclusion

This is the first study to investigate correlations of anti-BP180 antibody titres with disease activity and cumulative prednisolone doses received at fixed time points of disease evolution in a collective cohort of patients. The study showed positive and statistically significant correlations of antibody titres with disease activity at baseline and at disease flare. Thus, anti-BP180 antibody titres may be a useful indicator of disease activity at the time of diagnosis and at disease flare. Importantly, we demonstrated that antibody titres at 12 weeks after diagnosis may be useful in predicting clinical remission. We also found a significant correlation between the cumulative prednisolone dose received and antibody titres at Week 4. Notably, titres above 87.5 U/mL at time of diagnosis may be an indicator that higher cumulative prednisolone doses are needed to reach clinical remission within 2 years.

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REFERENCES

1. Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DJ, Fairley JA, et al. A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. *J Clin Invest* 1993;92:2480-8.
2. Liu Z, Diaz LA, Swartz SJ, Troy JL, Fairley JA, Giudice GJ. Molecular mapping of a pathogenically relevant BP180 epitope associated with experimentally induced murine bullous pemphigoid. *J Immunol* 1995;155:5449-54.
3. Schmidt E, Obe K, Bröcker EB, Zillikens D. Serum levels of autoantibodies to BP180 correlate with disease activity in patients with bullous pemphigoid. *Arch Dermatol* 2000;136:174-8.

4. Döpp R, Schmidt E, Chimanovitch I, Leverkus M, Brocker EB, Zillikens D. IgG4 and IgE are the major immunoglobulins targeting the NC16A domain of BP180 in bullous pemphigoid: serum levels of these immunoglobulins reflect disease activity. *J Am Acad Dermatol* 2000;42:577-83.
5. Amo Y, Ohkawa T, Tatsuta M, Hamada Y, Fujimura T, Katsuoka K, et al. Clinical significance of enzyme-linked immunosorbent assay for the detection of circulating anti-BP180 autoantibodies in patients with bullous pemphigoid. *J Dermatol Sci* 2001;26:14-8.
6. Izumi T, Ichiki Y, Esaki C, Kitajima Y. Monitoring of ELISA for anti-BP180 antibodies: clinical and therapeutic analysis of steroid-treated patients with bullous pemphigoid. *J Dermatol* 2004;31:383-91.
7. Kobayashi M, Amagai M, Kuroda-Kinoshita K, Hashimoto T, Shirakata Y, Hashimoto K, et al. BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and monitoring tool for bullous pemphigoid. *J Dermatol Sci* 2002;30:224-32.
8. Tsuji-Abe Y, Akiyama M, Yamanaka Y, Kikuchi T, Sato-Matsumura KC, Shimizu H. Correlation of clinical severity and ELISA indices for the NC16A domain of BP180 measured using BP180 ELISA kit in bullous pemphigoid. *J Dermatol Sci* 2005;37:145-9.
9. Feng S, Wu Q, Jin P, Lin L, Zhou W, Sang H, et al. Serum levels of autoantibodies to BP180 correlate with disease activity in patients with bullous pemphigoid. *Int J Dermatol* 2008;47:225-8.
10. Damoiseaux J. Bullous skin diseases: classical types of autoimmune diseases. *Scientifica (Cairo)* 2013;2013:457982.
11. Le Saché-de Peufeilhoux L, Ingen-Housz-Oro S, Hue S, Sbidian E, Valeyrie-Allanore L, Ortonne N, et al. The value of BP230 enzyme-linked immunosorbent assay in the diagnosis and immunological follow-up of bullous pemphigoid. *Dermatol* 2012;224:154-9.
12. Bernard P, Reguiai Z, Tancrede-Bohin E, Cordel N, Plantin P, Pauwels C, et al. Risk factors for relapse in patients with bullous pemphigoid in clinical remission: a multicenter, prospective, cohort study. *Arch Dermatol* 2009;145:537-42.
13. Kusajima E, Akiyama M, Sato M, Natsuga K, Shimizu H. Type XVII collagen ELISA indices significantly decreased after bullous pemphigoid remission. *Int J Dermatol* 2011;50:233-40.
14. Iwata H, Kamio N, Aoyama Y, Yamamoto Y, Hirako Y, Owaribe K, et al. IgG from patients with bullous pemphigoid depletes cultured keratinocytes of the 180-kDa bullous pemphigoid antigen (type XVII collagen) and weakens cell attachment. *J Invest Dermatol* 2009;129:919-26.
15. Leuci S, Gurcan HM, Ahmed AR. Serological studies in bullous pemphigoid: a literature review of antibody titers at presentation and in clinical remission. *Acta Derm Venereol* 2010;90:115-21.
16. Li Q, Ujiie H, Shibaki A, Wang G, Moriuchi R, Qiao HJ, et al. Human IgG1 monoclonal antibody against human collagen 17 noncollagenous 16A domain induces blisters via complement activation in experimental bullous pemphigoid model. *J Immunol* 2010;185:7746-55.
17. Liu Z, Giudice GJ, Swartz SJ, Fairley JA, Till GO, Troy JL, et al. The role of complement in experimental bullous pemphigoid. *J Clin Invest* 1995;95:1539-44.
18. Iwata Y, Komura K, Koderia M, Usuda T, Yokoyama Y, Hara T, et al. Correlation of IgE autoantibody to BP180 with a severe form of bullous pemphigoid. *Arch Dermatol* 2008;144:41-8.
19. Ishiura N, Fujimoto M, Watanabe R, Nakashima H, Kuwano Y, Yazawa N, et al. Serum levels of IgE anti-BP180 and anti-BP230 autoantibodies in patients with bullous pemphigoid. *J Dermatol Sci* 2008;49:153-61.
20. Dresow SK, Sitaru C, Recke A, Oostingh GJ, Zillikens D, Gibbs BF. IgE autoantibodies against the intracellular domain of BP180. *Br J Dermatol* 2009;160:429-32.
21. Hofmann SC, Thoma-Uszynski S, Hunziker T, Bernard P, Koebnick C, Stauber A, et al. Severity and phenotype of bullous pemphigoid relate to autoantibody profile against the NH2- and COOH-terminal regions of the BP180 ectodomain. *J Invest Dermatol* 2002;119:1065-73.
22. Liu Z. Are anti-BP180 IgG1 or IgG4 autoantibodies pathogenic? *J Invest Dermatol* 2002;119:989-90.
23. Sitaru C, Mihai S, Zillikens D. The relevance of the IgG subclass of autoantibodies for blister induction in autoimmune bullous skin diseases. *Arch Dermatol Res* 2007;299:1-8.
24. Miner A, Kirsner RS. Epitope spreading in bullous pemphigoid: what does it mean? *J Invest Dermatol* 2011;131:2165.
25. Hashimoto T, Tsuruta D, Dainichi T, Hamada T, Furumura M, Ishii N. Demonstration of epitope spreading in bullous pemphigoid: results of a prospective multicenter study. *J Invest Dermatol* 2011;131:2175-77.
26. Di Zenzo G, Thoma-Uszynski S, Fontao L, Calabresi V, Hofmann SC, Hellmark T, et al. Multicenter prospective study of the humoral autoimmune response in bullous pemphigoid. *Clin Immunol* 2008;128:415-26.
27. Fairley JA, Bream M, Fullenkamp C, Syrbus S, Chen M, Messingham KN. Missing the target: characterization of bullous pemphigoid patients who are negative using the BP180 enzyme-linked immunosorbent assay. *J Am Acad Dermatol* 2013;68:395-403.
28. Di Zenzo G, Thoma-Uszynski S, Calabresi V, Fontao L, Hofmann SC, Lacour JP, et al. Demonstration of epitope-spreading phenomena in bullous pemphigoid: results of a prospective multicenter study. *J Invest Dermatol* 2011;131:2271-80.
29. Slifka MK, Ahmed R. Long-lived plasma cells: a mechanism for maintaining persistent antibody production. *Curr Opin Immunol* 1998;10:252-5.
30. Leyendeckers H, Tasanen K, Tuderman-Bruckner L, Zillikens D, Sitaru C, Schmitz J, et al. Memory B cells specific for the NC16A domain of the 180kDa bullous pemphigoid autoantigen can be detected in peripheral blood of bullous pemphigoid patients and induced in vitro to synthesize autoantibodies. *J Invest Dermatol* 2003;120:372-8.
31. Miida H, Fujiwara H, Ito M. Association between effective dose of prednisolone, alone or in conjunction with other immunosuppressants, and titre of anti-bullous pemphigoid 180 antibody: a retrospective study of 42 cases. *Clin Exp Dermatol* 2011;36:485-88.
32. Daniel BS, Hertl M, Werth VP, Eming R, Murrell DF. Severity score indexes for blistering diseases. *Clin Dermatol* 2012;30:108-13.
33. Agarwal M, Walia R, Kochhar AM, Chander R. Pemphigus Area and Activity Score (PAAS)—a novel clinical scoring method for monitoring of pemphigus vulgaris patients. *Int J Dermatol* 1998;37:158-60.
34. Harman KE, Albert S, Black MM, British Association of Dermatologists. Guidelines for the management of pemphigus vulgaris. *Br J Dermatol* 2003;149:926-37.