Expression of Serum Anti-BP180/230 Antibodies in Bullous Pemphigoid Patients Combined with Nervous System Diseases and Relevant Factor Analysis

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ABSTRACT

Objective: To explore the anti-BP230/180 and anti-BP180 antibodies in patients with bullous pemphigoid (BP) combined with neurological diseases, and to analyse the relevant factors.

Study Design: Analytical study.

Place and Duration of the Study: Neurology Department, Cangzhou People's Hospital, Cangzhou, from April 2019 to June 2022.

Methodology: Eighty BP patients were chosen based on associated neurological diseases, they were split into single (n=42) and combined groups (n=38). Expression of anti-BP180/230 antibodies was compared between the two groups. Associations with neurological diseases were analysed and the factors affecting the expression of anti-BP180/230 antibodies were explored.

Results: Out of 80 patients, 61 were positive for anti-BP180 antibodies and 58 were positive for anti-BP230 antibodies. The proportion of patients with positive anti-BP230/180 antibodies in the single group was considerably lower than in the combined group ($p<0.05$). Presence of both nervous system diseases and BP was found to be associated with the presence of anti-BP230/180 antibodies ($p<0.001$). Univariate analysis showed statistically significant association with age (<70 years, total IgE (>100 IU/ml), and EOS count >0.5 x 10^9/L ($p<0.05$). Logistic analysis demonstrated that age, total IgE and EOS count were independent risk factors affecting the expression of anti-BP180 and anti-BP23 antibodies ($p<0.05$).

Conclusion: Serum anti-BP230/180 antibodies expression is abnormally high in BP patients having nervous system diseases. Combined nervous system diseases, age, total IgE and EOS count are independent risk factors affecting expression of anti-BP180/230 antibodies.

Key Words: *Anti-BP180 antibody, Anti-BP230 antibody, Bullous pemphigoid, Nervous system diseases.*

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INTRODUCTION

Bullous pemphigoid (BP) is a common autoimmune skin disease with clinical manifestations. Patients with BP mostly present erythematous papules, nodular plaques, blisters, etc., which can involve the hands and feet, and some are accompanied by obvious itching.[1] BP can be divided into the non-bullous stage characterised by a long course of the disease and nonspecific performance, and the bullous stage with typical symptoms, mainly skin erythema, urticaria and tension bullae. The clinical treatment of BP is mainly based on drugs. Patients will be significantly improved after potent glucocorticoid and antibiotic use. Its pathogenesis has not been thoroughly studied, but the mainstream view is that it is caused by abnormal antibody production. This statement highlights the two main autoantigens, BP230 and BP180, which are the key hemidesmosome components. These autoantigens facilitate the connection between keratinocytes in the basal layer of the skin and the basement membrane. Therefore, an inflammatory response can be triggered after the antibodies against hemidesmosomes binding to the antigens in the basement membrane.[2]

It has been shown that about 30% of BP patients may be accompanied by nervous system diseases, including Parkinson's disease and cognitive impairment.[3] While the majority believes that the presence of nervous system diseases raises the likelihood of developing BP, same scholars postulate that BP has an increased incidence of nervous system diseases.[4] At present, numerous studies have confirmed a correlation between BP and nervous system diseases. Consequently, some scholars consider that BP230 and BP180, as the pathogenic antigens of BP, also have a certain correlation with nervous system diseases.[5] Based on this view, the aim of this study was to analyse the expression of BP180 antibody and anti BP230 antibody and related factors in patients with BP combined with neurological diseases. This may help to improve the clinical management efficacy of BP combined with neurological diseases and find new therapeutic targets.

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METHODOLOGY

Using retrospective data, a total of 80 patients diagnosed with BP and treated at Cangzhou People's Hospital between April 2018 and June 2021 were included in the study. According to the presence of coexistent nervous system disease, they were segregated into two groups: the single group without neurologic symptoms (n = 42) and the combined group having neurologic symptoms (n = 38). This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. The participants provided written informed consent, and the Institutional Ethics Committee of Cangzhou People's Hospital approved the study.

The development of the diagnostic criteria for BP was guided by the Manual for Diagnosis and Treatment of Dermatological Diseases: The clinical manifestation was normal appearance or erythema, accompanied by tension blisters, negative Nikolsky's sign and itching; Histopathological examination showed that the blister fluid was dominant by eosinophils (EOS), but contained few lymphocytes and neutrophils. The dermis was dominant by EOS and neutrophils; Direct immunofluorescence (DIF) of the skin tissue 1 cm away from the blisters displayed mostly positive results; Indirect immunofluorescence (IIF) presented anti-basement membrane antibodies in the serum, mainly IgG; Specific antibody detection could detect anti-BP230 and anti-BP180 antibodies in the serum.

The inclusion criteria were patients meeting the above diagnostic criteria, age within 40-95 years, receiving specific antibody detection and diagnosis of nervous system diseases; clear detection results, and complete clinical data. The participants were informed of the research content, benefits, and risks, and provided their written consent. The exclusion criteria were a history of glucocorticoid, immunosuppressant and antibiotic therapy before admission, complicated with other skin diseases and autoimmune diseases, complicated with malignant tumours, hepatic and renal dysfunction, and abnormal thyroid hormone metabolism, low communication and understanding abilities, difficult to communicate with medical staff; poor compliance, either withdrew from the research or moved to another hospital before completing the treatment.

Patients were instructed to fast prior to venous blood collection of 5 ml in the morning. After collection, the blood underwent centrifugation at 3,500 r/min for 5 minutes, and the resulting supernatant was stored at -20°C until required. The presence of anti-BP230 and anti-BP180 antibodies was determined in serum using an enzyme-linked immunosorbent assay (ELISA). The samples and control serum were diluted at 1: 101, with replicates set up. The absorbance was read at the wavelength of 450 nm, the results were obtained by averaging two wells, and the antibody indexes were calculated according to the formula. Antibody index = (absorbance of sample - absorbance of control serum 1) / (absorbance of control serum 2 - absorbance of control serum 1) * 100. Antibody index ≥20 U/mL was considered as positive, and <20 U/mL as negative. Clinical data, including gender, age, hypertension, diabetes, cardiovascular diseases, etc., and laboratory indicators, including the expression of serum anti-BP230 and anti-BP180 antibodies, antibody index (reference range, 0~20 RU/mL), EOS count in the peripheral blood measured by ELISA (reference range, 0.02-0.52 × 10^9/L), and total serum anti-IgE antibody (reference range, 0-100 IU/L) was noted. The data obtained were statistically analysed using SPSS 22.0. The qualitative data was presented in the form of numbers and percentages and their comparison was performed using the χ^2 test. The quantitative data such as serum anti-BP230 and anti-BP180 antibody indexes and EOS count were expressed as ($\bar{x}\pm s$) and compared with the t-test. Spearman's correlation analysis was used for ranked data, and Pearson's correlation analysis for non-ranked data. Binary logistic regression analysis was utilised to identify the independent risk factors that had an impact on the serum levels of anti-BP230 and anti-BP180 antibodies. A significance level of $p < 0.05$ was considered for statistical analyses.

RESULTS

Within the single group, the age range of participants was 47-93 (average 71.07 ± 10.34) years, consisting of 21 women and 21 men with rashes distributed on the limbs in 27 patients and the whole body in 15 patients. The group with neurological symptoms consisted of 18 women and 20 men, whose ages ranged from 49 to 94 (average 71.61 ± 10.47) years, including 24 cases of rashes on the limbs and 14 on the whole body. The gender, age, disease severity and combined underlying diseases exhibited no significant difference between the two groups ($p > 0.05$). Significant differences were found in the expression of the two specific antibodies between the two groups. The anti-BP230 and anti-BP180 antibody-positive patients in the single group were prominently less than those in the combined group, with a statistically meaningful contrast ($p<0.05$). Anti-BP180 and anti-BP230 antibody indices were positively correlated with the occurrence of BP combined with nervous system diseases ($r=0.444$, 0.557, $p<0.001$). The risk of BP combined with nervous system diseases increases with higher antibody indices. Notably, there was a strong positive correlation ($p<0.001$, $r=0.895$) between anti-BP180 and anti-BP230 antibody indices, as depicted in Figure 1.

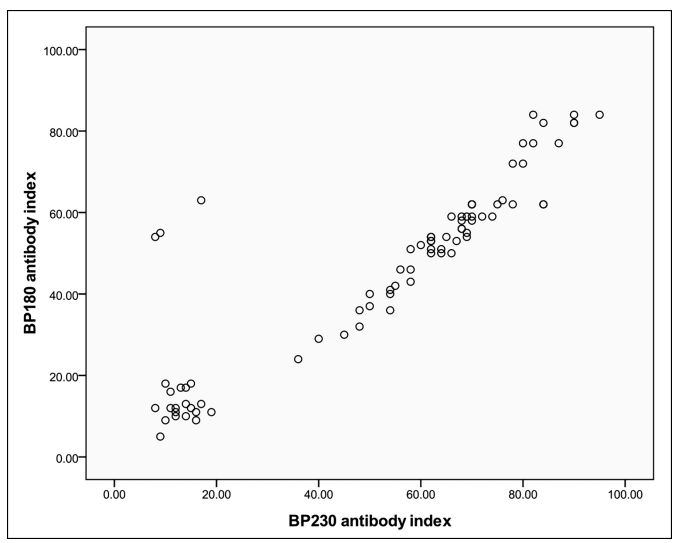

Figure 1: Correlation between anti-BP230 antibody index and anti-BP180 antibody index.

Table I: Univariate analysis of factors affecting the expression of anti-BP230 and anti-BP180 antibodies [($\bar{x}\pm s$)/n (%)].

Baseline data	N	Anti-BP180 antibody-positive (n = 61)	χ^2/ t	p	Anti-BP230 antibody-positive (n = 58)	χ^2	p
Gender			21.092	<0.001		0.019	0.890*
Male	41	40 (65.57)			30 (51.72)		
Female	39	21 (34.43)			28 (48.28)		
Age (year)			2.533	0.112		18.379	<0.001*
<70	38	32 (52.46)			19 (32.76)		
≥70	42	29 (47.54)			39 (67.24)		
Rash distribution			0.235	0.628		0.258	0.612*
Limbs	51	38 (62.30)			36 (62.07)		
Whole body	29	23 (37.70)			22 (37.93)		
Hypertension			1.127	0.288		2.090	0.148*
Yes	20	17 (27.87)			17 (29.31)		
No	60	44 (72.13)			41 (70.69)		
Diabetes			0.366	0.545		0.016	0.898*
Yes	21	15 (24.59)			15 (25.86)		
No	59	46 (75.41)			43 (74.14)		
Total serum IgE			31.498	<0.001		24.159	<0.001
<100 IU/mL	20	6 (9.84)			6 (10.34)		
≥100 IU/mL	60	55 (90.16)			52 (89.66)		
EOS count			34.871	<0.001		32.291	<0.001
$<0.52 \times 10^9$/L	24	8 (13.11)			7 (12.07)		
$\geq 0.52 \times 10^9$/L	56	53 (86.89)			51 (87.93)		

*Note: *χ^2 test. p <0.05*

Table II: Logistic regression analysis of factors affecting the expression of anti-BP180/230 antibody.

		Age	IgE	EOS count	Constant
Anti-BP180	β	-0.348	-8.422	-3.549	40.105
	Standard error S.E	0.140	3.540	1.303	15.196
	Wald	6.19	5.660	7.412	6.965
	P	0.013	0.017	0.006	0.008
	OR	0.706	0.000	0.029	2.165
	95% CI	0.537~0.929	0.000~0.227	0.002~0.370	/
Anti-BP230	β	-0.157	-3.569	-2.001	18.371
	Standard error S.E	0.048	1.318	0.903	4.986
	Wald	10.965	7.333	4.913	13.578
	P	0.001	0.007	0.027	0.000
	OR	0.854	0.028	0.135	9.518
	95% CI	0.778~0.938	0.002~0.373	0.023~0.793	/

Among the 80 patients, there were 58 anti-BP230 antibody positive patients and 61 anti-BP180 antibody positive patients. In addition to nervous system diseases, univariate analysis showed statistical significance in age, total IgE and EOS count ($p<0.05$). With combined nervous system diseases, age, total IgE and EOS count as independent variables, logistic regression analysis demonstrated that age, total IgE and EOS count were independent risk factors affecting the expression of anti-BP180 and anti-BP23 antibodies ($p<0.05$), as displayed in Table I and II.

DISCUSSION

BP has an annual incidence of about 13 million in China; it is also common in Western Europe, Northern Europe, and Singapore. Compared with young people, BP is more common in the elderly. The incidence of BP in people over 90 years old is 300 times that in young people.[6] At present, the main argument about the pathogenesis of BP is that the body produces antibodies against its own components, such as BP antigen 1 (BP230) and BP antigen 2 (BP180).[7] After the above two specific antibodies bind to skin basement membrane antigens, complements are activated and inflammatory cells are more likely to invade, resulting in inflammatory damage to the skin.[8] The correlation theory of BP and nervous system diseases was first put forward in 1989. In the following ten years, it was reported that the probability of nervous system diseases in patients with BP is dramatically more frequencies than that in the control group of the same age, and the probability of BP occurrence in patients with nervous system diseases is much higher than that in people without nervous system diseases. Therefore, it can be speculated that BP and nervous system diseases may have common antigens.[9-11]

Zhu *et al*. investigated the expression of anti-BP180 antibody in the serum of patients with BP and nervous system diseases.[12] They observed that the anti-BP180 antibody was not detected in 20 healthy individuals, while the anti-BP180 antibody titer was (128.38 ± 54.68) in 20 BP patients and (143.48 ± 72.57) in patients with BP combined with nervous system diseases. Anti-BP180 antibody titer in patients with BP combined with nervous system diseases was significantly

higher, suggesting that for patients with nervous system diseases, increased anti-BP180 antibody may indicate the possibility of BP. This research yielded a comparable outcome, demonstrating that patients in the combined group had significantly more prominent levels of anti-BP230 and anti-BP180 antibodies than those in the single group. This suggests that individuals with both bullous pemphigoid (BP) and nervous system disorders exhibit abnormal expression of these antibodies compared to patients with BP alone. In addition, correlation analysis revealed that anti-BP239 and anti-BP180 antibodies were positively correlated with the occurrence of BP combined with nervous system diseases.

Li *et al*. investigated the relationship between peripheral blood eosinophils and total serum IgE levels with serum anti-BP230 and anti-BP180 antibody levels in bullous pemphigoid (BP).[13] They discovered that both the EOS count and total IgE level were positively linked to anti-BP230 and anti-BP180 IgG antibodies, which aligns with our findings. This research revealed that age, total IgE, and EOS count were also identified as independent risk factors for positive expression of anti-BP230 and anti-BP180 antibodies, on the basis of the consequences of our logistic regression analysis. The main cause is speculated to be degenerative processes in older patients, thus exposing some neural isomers, which is more likely to lead to abnormally positive expression of anti-BP230 and anti-BP180 antibodies through the neuroimmune process, ultimately resulting in BP. Additionally, EOS count and total IgE level can reflect the activity of BP. During its pathogenesis, the IgE antibody is produced prior to the IgG antibody. Hence, IgE can bind to BP230 earlier, thereby playing a pathogenic role.[14-16]

However, there are still shortcomings in this study; no long-term follow-up was performed and a limited sample size was included. In this regard, corresponding measures should be taken in the future to improve this study in order to further explore the relationship between serum anti-BP180 antibodies, anti-BP230 antibodies and BP.

CONCLUSION

Abnormal high expression of serum BP180 antibodies and anti BP230 antibodies in patients with BP associated neurological diseases, and a positive correlation between BP180 antibodies and anti BP230 antibodies and the incidence of BP associated neurological diseases. Associated neurological diseases, age, total IgE value, and EOS count are independent risk factors affecting the positive expression of BP180 antibodies and BP230 antibodies.

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ETHICAL APPROVAL:
This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. The participants provided written informed consent, and the Institutional Ethics Committee of Cangzhou People's Hospital granted approval for the study.

PATIENTS' CONSENT:
The author declared that they have obtained informed consent from the patients to publish the data concerning this case.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
GL, BL: Designed this study, prepared this manuscript, and accountable for the accuracy or integrity of the work.
ZZ, LQ: Collected and analysed the data.
YZ, SF: Participated in the acquisition, analysis and interpretation of data, and drafted the manuscript.
All authors read and agreed to the final version of the manuscript to be published.

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