

# Analysis of the autoimmune response against BP180 and BP230 in ethnic Poles with neurodegenerative disorders and bullous pemphigoid

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## Abstract

Recent studies postulated the association between bullous pemphigoid (BP) and neurodegenerative disorders (ND). The autoantibodies to BP180 and/or BP230 may be present not only in BP, but also in ND as neuronal isoforms of these proteins are identified in the central nervous system. However, there are only scant data about the precise pathogenetic mechanisms interlinking ND and BP as well as the immunologic profile in these patients. The aim is to analyze the serological immunopathological profiles (anti-BP180 IgG, anti-BP230 IgG) in BP patients with and without ND in order to identify the specific autoantibody(ies) and corresponding antigens responsible for ND development in BP patients.

Altogether, 82 ethnic Poles with BP and their medical records were examined (62 BP-ND; 20 BP+ND). Levels of serum anti-BP180/BP230 IgG in BP patients were evaluated with ELISAs. The statistical analyses involved Pearson chi-squared test, Mann-Whitney U-test and ranking of autoantibodies.

The prevalence of ND among BP patients was 24.4%. There were no statistically significant differences in autoantigens profiles (anti-BP180/anti-BP230 IgG) between BP+ND and BP-ND groups. There was no relationship between ND development and anti-BP180/anti-BP230 IgG level ( $p = 0.5933$ ,  $p = 0.4701$ , respectively).

The autoantibodies levels of BP+ND and BP-ND patients show insignificant differences suggesting that also in ethnic Poles a hypothetical pathogenetic association of BP and ND, but not only an aging-related epidemiological one, appears to be independent of a particular BP antigen. Nevertheless, it cannot be excluded that phenomena of epitopes spreading, immune cross-reaction and conformational changes in BP180/BP230 may underlie BP development in ND patients.

**Key words:** autoantibodies, neurodegenerative diseases, bullous pemphigoid.

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## Introduction

Bullous pemphigoid (BP) is the most common autoimmune subepidermal blistering disease of the skin and occurs mainly in an elderly population [1-3]. The clinical phenotype manifested by BP patients mostly demonstrated tense cutaneous blisters with no or transient involvement of mucosal surfaces and sparing the head and neck.

The molecular basis for BP is related to the development of IgG and IgE autoantibodies against hemidesmosomal proteins – BP180 (type XVII collagen, BPAG2) and/or BP230 (dystonin, BPAG1n, BPAG1a) [1-3].

The genes for both autoantigens encode different isoforms of BP180 and BP230 due to alternative splicing and multiple translation start sites. Expressed isoforms show a tissue-specific expression profile (epithelial, muscle, neuronal isoforms) [4]. The neuronal forms of BP180 and BP230 may occur physiologically in the neurons of the central nervous system (CNS), peripheral nervous system and in Schwann cells being fundamental for retaining of neuro-cytoskeleton organization [5, 6]. Thus, they may be associated with development of neurodegenerative disorders (ND) [5-8].

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Previous studies indicated the coincidence of BP and ND in various geographical regions as significant suggesting that these entities have a great deal in common [9-18]. Several epidemiological studies demonstrated that the risk of a BP event is doubled in the case of a stroke or epilepsy and tripled in patients with Parkinson's disease or dementia [9]. The level of incidences of ND in BP varies between 22% and 52.8% in these reports [9, 11, 13, 14, 16, 17, 18] postulating the existence of the neurocutaneous interval timing signaling/mechanism.

There are several possible hypotheses linking the ND and BP [9, 19]: (i) drug intake (myorelaxants, neuroleptics, aldosterone antagonists) as a BP trigger, (ii) immobility and decubitus ulcers as a cause of dermal-epidermal junction destruction with subsequent antigen exposition, (iii) trauma, (iv) age-related dysfunctions of the immune system. Also the aging immune system (*immunosenescence*) may be involved in the association between examined diseases. Innate immunity dysregulation, clonotypical immunity and presence of the chronic inflammatory process may accelerate tissue degeneration in both BP and ND [13]. Moreover, the skin and neural cells share a common origin and derive from the embryonic ectoderm layer.

The association of BP and ND was demonstrated in numerous recent studies showing that this association is statistically significant, however the data about the precise pathogenetic mechanisms interlinking ND and BP, as well as the immunologic profile in these patients still remain limited. Thus, this work attempts to characterize the immunopathological features of this relationship.

The aim of this study was the comparative molecular analysis of serological immunopathological profiles (anti-BP180 IgG, anti-BP230 IgG) in ethnic Poles with BP with and without ND in order to identify the specific autoantibody(ies) and corresponding antigens responsible for ND development in BP patients.



## Material and methods

### Specimens and patients

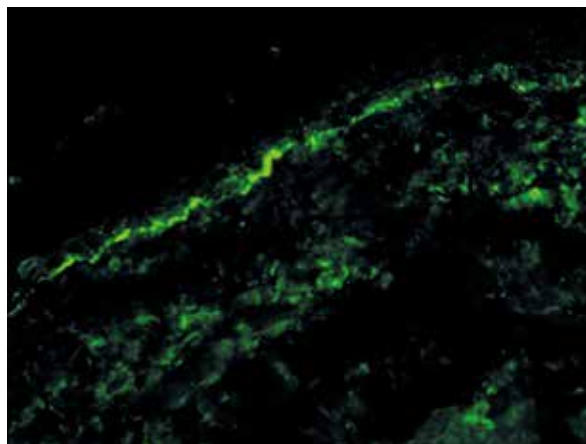
Medical history of 82 BP patients, ethnic Poles, diagnosed at a molecular level with direct immunofluorescence (DIF of perilesional skin demonstrating deposits of IgG/IgG1/IgG4 and/or 3<sup>rd</sup> component of complement – linear N-serrated/undefined patterns of staining at the dermal-epidermal junction were determined), indirect immunofluorescence mosaic (IIFm) (Euroimmun, Germany) and ELISA (Euroimmun, Germany) hospitalized in the university dermatology department between December 2006 and November 2014 was reviewed for the presence of ND records. ND included: Parkinson's disease – 5 cases, dementia – 3 cases, stroke – 8 cases, and other – 5 cases (neurosyphilis, epilepsy).

Altogether, 82 sera samples from those untreated BP patients, including 20 BP patients with coincidence of ND (BP+ND) and 62 BP patients without coincidence of ND (BP-ND), were studied. There were 11 men and 9 women in the BP+ND group (male/female ratio was 1.2), and the mean age at diagnosis was 78.5 years (range 64-91). The representative patient of BP+ND was shown in Fig. 1.

There were 17 men and 45 women in the BP-ND group (male/female ratio was 0.38), and the mean age at diagnosis was 74 years (range 50-96).

### ELISA

The specific circulating serum autoantibodies were detected with commercially available ELISAs. ELISAs were performed using the Euroimmun (Lübeck, Germany) ELISA kits, utilizing recombinant protein BP180, BP230, with the manufacturer's cut-off 20 RU/ml. Anti-BP180-



**Fig. 1.** Itchy blisters and vesicles with their evolutionary lesions on wheal-like background on medial and frontal surfaces of thighs (A) as well as IgG4 linear deposits along the dermal-epidermal junction and dotted deposits at the periphery of certain basal keratinocytes detected with laser scanning confocal microscopy ZEISS LSM 510 (B) in an elderly debilitated man with BP who underwent brain stroke more than a decade before (level of serum anti-BP180 IgG > 200 RU/ml, anti-BP230 IgG 44.255 RU/ml with ELISA)

NC16A-4X ELISA includes four copies of domain NC16A fused to a polyhistidine tag to enhance protein expression. Anti-BP230-CF ELISA contains an amplified fragment of the C-terminal globular domain. All measurements were made in the ELISA plate reader (Asys Expert 96) equipped with Microwin 2000 software by a single operator following the manufacturer's instructions.

### Statistical analysis

The Pearson  $\chi^2$  test was used to check the relationship between ND development and the anti-BP180/anti-BP230 IgG level and to compare the frequencies of antigen recognition and antibody levels. The Mann-Whitney U-test was calculated to find the differences in autoantigens profiles (anti-BP180/anti-BP230 IgG) between groups BP+ND and BP-ND.

Data were analyzed by ranking specimens according to antibody levels.

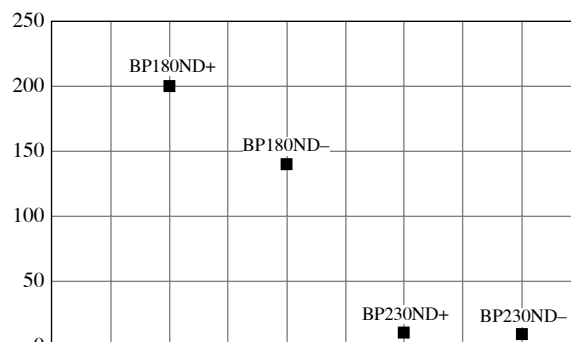
A  $p < 0.05$  was arbitrarily considered statistically significant. Statistical analyses were performed using statistical analysis software Statistica PL 10.0 (StatSoft Inc.).

### Results

The prevalence of ND among BP patients was 24.4%.

There were no statistically significant differences in autoantigens profiles between BP+ND and BP-ND groups (anti-BP180  $p = 0.0871$ ; anti-BP230  $p = 0.4625$ ). The comparison of anti-BP180 IgG and anti-BP230 IgG median value in BP+ND and BP+ND is shown in Fig. 2.

The statistical analysis of obtained results is presented in Tables 1 and 2.



**Fig. 2.** The comparison of anti-BP180 IgG and anti-BP230 IgG median value in BP+ND and BP-ND

The subjects were ranked according to the level of circulating autoantibodies, what is presented in Tables 3 and 4 demonstrating lack of relationship between ND development and the anti-BP180/anti-BP230 IgG level ( $p = 0.5933$ ,  $p = 0.4701$ , respectively).

### Discussion

There are strong data about a relationship between ND and BP [9-18], however the interval between the diagnosis of ND and BP ranges from months to years. Nevertheless, the findings regarding a common molecular signaling network in these disorders are very scant. It is known that sera of BP patients and accompanying ND recognize BP antigens in the human brain. It is postulated [4] that the neurodegenerative process, synaptic damage, persistent

**Table 1.** Statistical data regarding anti-BP180/anti-BP230 IgG obtained in BP+ND and BP-ND

	GROUP	N	Median (RU/ml)	Min (RU/ml)	Max (RU/ml)	p value (Mann – Whitney U-test)
Anti-BP180 IgG	BP+ND	20	200.00	35.56	> 200.00	0.0871
	BP-ND	62	139.99	8.04	> 200.00	
Anti-BP230 IgG	BP+ND	20	11.03	0.00	> 200.00	0.4625
	BP-ND	62	9.77	0.00	> 200.00	

*n* – number of cases; *min.* – minimum; *max.* – maximum

**Table 2.** Target antigens (BP180, BP230) detected with ELISAs in BP+ND and BP-ND

	All BPs ( <i>n</i> = 82) <i>n</i> (%)	BP-ND ( <i>n</i> = 62) <i>n</i> (%)	BP+ND ( <i>n</i> = 20) <i>n</i> (%)	p value (Pearson $\chi^2$ test)
BP180	78 (95%)	58 (94%)	20 (100%)	0.2441
BP230	20 (24%)	15 (24%)	5 (25%)	0.9706
BP180+BP230	18 (22%)	13 (21%)	5 (25%)	
Negative	2 (2%)	2 (3%)	0 (0%)	

BP – bullous pemphigoid; ND – neurodegenerative disorders; *n* – number of cases

**Table 3.** Ranking of serum samples according to anti-BP180 IgG level in BP+ND and BP-ND

	Anti-BP180 IgG ranking					Line together
	< 50 RU/ml	50-100 RU/ml	100-150 RU/ml	150-200 RU/ml	≥ 200 RU/ml	
BP-ND	10	12	9	13	18	62
% of column	83.33	85.71	75.00	92.86	60.00	
% of line	16.13	19.35	14.52	20.97	29.03	
%o of total	12.20	14.63	10.98	15.85	21.95	75.61
BP+ND	2	2	3	1	12	20
% of line	16.67	14.29	25.00	7.14	40.00	
% of column	10.00	10.00	15.00	5.00	60.00	
% of total	2.44	2.44	3.66	1.22	14.63	24.39
Total	12	14	12	14	30	82
% of total	14.63	17.07	14.63	17.07	36.59	100.00

**p = 0.5933**

**Table 4.** Ranking of serum samples according to anti-BP230 IgG level in BP+ND and BP-ND

	Anti-BP230 IgG ranking					Line together
	< 50 RU/ml	50-100 RU/ml	100-150 RU/ml	150-200 RU/ml	≥ 200 RU/ml	
BP-ND	50	5	1	1	5	62
% of column	76.92	71.43	33.33	100.00	83.33	
% of line	80.65	8.06	1.61	1.61	8.06	
% of total	60.98	6.10	1.22	1.22	6.10	75.61
BP+ND	15	2	2	0	1	20
% of column	23.08	28.57	66.67	0.00	16.67	
% of line	75.00	10.00	10.00	0.00	5.00	
% of total	18.29	2.44	2.44	0.00	1.22	24.39
Total	65	7	3	1	6	82
% of total	79.27	8.54	3.66	1.22	7.32	100.00

**p = 0.4701**

neuroinflammation resulting in the blood-brain barrier disruption may lead to impairment of the ‘immune privilege’ of the brain. Therefore, it was speculated that neurological episodes in BP patients causing brain antigens exposure with consequently autoantibodies production, what may contribute to the ND development.

Interestingly, usually BP follows neurological pathology (BP is diagnosed after neurologic disease) suggesting that neurological pathology promotes accelerated BP in ND patients. This feature led to the proposal that the autoimmune response against neuronal isoforms of BP180/BP230 may be extended to epithelial isoforms of these proteins.

The concept of epitope spreading and immune cross-reaction has been recently proposed [20].

Therefore, the excessive risk of BP observed in ND patients appears to be driven by cross-reactivity of skin autoantibodies against BP180 and BP230, which isoforms are expressed in CNS. The alteration of CNS in the course of the neurodegenerative process could trigger a specific immunological reaction resulting in BP development.

Furthermore, BP is a type of autoimmune disease with conformation-dependent epitopes. Thus, the changes in structure/conformation of BP180 and/or BP230 may induce the skin pathology specific for BP. It is possible that either both antigens or their isoforms, may conduct switching through conformational changes.

Here, anti-BP180/anti-BP230 IgG autoantibodies levels of BP+ND patients display a similar ranking compared to BP-ND patients. Thus, the probable pathogenetic as-

sociation of BP and ND, not only epidemiology-related to the elderly, seems to be independent of a particular BP antigen. The current study demonstrated that ND in BP patients is not directly associated with the levels of sera anti-BP180 and anti-BP230 IgG antibodies. The lack of differences in levels of anti-BP180 IgG and anti-BP230 IgG observed in this work in a Slavic population is similar to previously reported findings [20, 21]. Taghipour *et al.* [20] investigated immunopathological correlations with the presence and absence of ND in BP patients. This group of researchers found that the humoral response in BP+ND and BP-ND did not differ in terms of antibody titers and target antigens with the use of indirect immunofluorescence, immunoblotting and ELISA. They also showed no specific correlation between the presence of anti-BP180 or anti-BP230 antibodies and ND. However, Taghipour *et al.* [20] used ELISA from MBL (Nagoya, Japan) utilizing N- and C-terminal regions of BP230. Still, although the molecular constructs of both BP180 and BP230 in those two ELISA kits (MBL and Euroimmun) are not identical, the obtained results are consistent.

Interestingly, Laffitte *et al.* [22] found that cerebrospinal fluid (CSF) samples obtained from patients with multiple sclerosis showed reactivity to BPAG1. Ideally, in order to cast more light on the speculative issue of the immunopathogenetic link between ND and BP, *in vivo* (using brain tissue, CSF and serum in comparative way), *ex vivo* and animal model studies should be performed [23]. Still, it has to be emphasized that CSF can be unobtainable as lumbar puncture can be a challenging procedure to perform even by a practicing neurologist in elderly people having spine degenerative lesions. Thus, further experimental investigations, especially with CSF of BP+ND patients, are still required in the future in order to establish a definite relationship between these disorders.

The anti-BP180 and anti-BP230 IgG autoantibodies levels in ethnic Poles with BP+ND and BP-ND show insignificant differences suggesting that probably a pathogenetic association of BP and ND, but not only aging-related epidemiological one, appears to be independent from a particular BP antigen regardless of the ethnic background.

Nevertheless, it cannot be excluded that phenomena of epitopes spreading, immune cross-reaction and conformational changes in BP180/BP230 may underlie BP development in ND patients.

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*The Figure 1B laser scanning confocal fluorescence microscopy image was taken at the Laboratory of Electron and Confocal Microscopy, Adam Mickiewicz University in Poznan.*

*The authors declare no conflict of interest.*

## References

- Gornowicz-Porowska J, Bowszyc-Dmochowska M, Dmochowski M (2012): Autoimmunity-driven enzymatic remodeling of the dermal-epidermal junction in bullous pemphigoid and dermatitis herpetiformis. *Autoimmunity* 45: 71-80.
- Lo Schiavo A, Ruocco E, Brancaccio G, et al. (2013): Bullous pemphigoid: etiology, pathogenesis, and inducing factors: facts and controversies. *Clin Dermatol* 31: 391-399.
- Perivier S, Trelle LT (2013): Bullous pemphigoid: a cutaneous disorder to be known in aging persons]. *Rev Med Suisse* 9: 2049-2053.
- Chen J, Li L, Chen J, et al. (2011): Sera of elderly bullous pemphigoid patients with associated neurological diseases recognize bullous pemphigoid antigens in the human brain. *Gerontology* 57: 211-21.
- Seppänen A, Suuronen T, Hofmann SC, et al. (2007): Distribution of collagen XVII in the human brain. *Brain Res* 1158: 50-56.
- Li L, Chen J, Wang B, et al. (2009): Sera from patients with bullous pemphigoid (BP) associated with neurological diseases recognized BP antigen 1 in the skin and brain. *Br J Dermatol* 160: 1343-1345.
- Seppänen A (2013): Collagen XVII: a shared antigen in neurodermatological interactions? *Clin Dev Immunol* 2013: 240570.
- Bastuji-Garin S, Joly P, Lemordant P, et al. (2011): French Study Group for Bullous Diseases. Risk factors for bullous pemphigoid in the elderly: a prospective case-control study. *J Invest Dermatol* 131: 637-643.
- Langan SM, Groves RW, West J (2011): The relationship between neurological disease and bullous pemphigoid: a population-based case-control study. *J Invest Dermatol* 131: 631-636.
- Taghipour K, Chi CC, Bhogal B, et al. (2014): Immunopathological characteristics of patients with bullous pemphigoid and neurological disease. *J Eur Acad Dermatol Venereol* 28: 569-573.
- Tarazona MJM, Mota ANC de M, Gripp AC, et al. (2015): Bullous pemphigoid and neurological disease: statistics from a dermatology service. *An Bras Dermatol* 90: 280-282.
- Brick KE, Weaver CH, Savica R, et al. (2014): A population-based study of the association between bullous pemphigoid and neurologic disorders. *J Am Acad Dermatol* 71: 1191-1197.
- Pietkiewicz P, Gornowicz-Porowska J, Bowszyc-Dmochowska M, et al. (2016): Bullous pemphigoid and neurodegenerative diseases: A study in a setting of a Central European university dermatology department. *Aging Clin Exp Res* 28: 659-663.
- Gambichler T, Segert H, Höxtermann S, et al. (2015): Neurological disorders in patients with bullous pemphigoid: clinical and experimental investigations. *J Eur Acad Dermatol Venereol* 2015; 29: 1758-1762.
- Casas-de-la-Asunción E, Ruano-Ruiz J, Rodríguez-Martín AM, et al. (2014): Association between bullous pemphigoid and neurologic diseases: a case-control study. *Actas Dermosifiliogr* 105: 860-865.
- Taghipour K, Chi C-C, Vincent A, et al. (2010): The association of bullous pemphigoid with cerebrovascular disease and dementia: a case-control study. *Arch Dermatol* 2010; 146: 1251-1254.

17. Jedlickova H, Hlubinka M, Pavlik T, et al. (2010): Bullous pemphigoid and internal diseases – a case-control study. *Eur J Dermatol* 20: 96-101.
18. Cordel N, Chosidow O, Hellot M-F, et al. (2007): French Study Group of Bullous Diseases. Neurological disorders in patients with bullous pemphigoid. *Dermatol Basel Switz* 215: 187-191.
19. Stinco G, Codutti R, Scarbolo M, et al. (2005): A retrospective epidemiological study on the association of bullous pemphigoid and neurological diseases. *Acta Derm Venereol* 85: 136-139.
20. Taghipour K, Chi CC, Bhogal B, et al. (2014): Immunopathological characteristics of patients with bullous pemphigoid and neurological disease. *J Eur Acad Dermatol Venereol* 28: 569-573.
21. Gornowicz-Porowska J, Pietkiewicz P, Bowszyc-Dmochowska M, et al. (2015): Schorzenia neurodegeneracyjne a pemphigoid pęcherzowy – retrospektywne badanie immunopatologiczne (abstract). *Przegląd Dermatologiczny* 102: 89-90.
22. Laffitte E, Burkhard PR, Fontao L, et al. (2005): Bullous pemphigoid antigen 1 isoforms: potential new target autoantigens in multiple sclerosis? *Br J Dermatol* 152: 537-540.
23. Messingham K, Narayanan N, Aust N, et al. (2015): Collagen XVII autoantibodies are present in Parkinson's Disease patients and co-localize with tyrosine hydroxylase in the substantia nigra. 2015 Annual Meeting of the SID, Atlanta, USA, 6-9 May 2015. *J Invest Dermatol* 135 Suppl: S14.