DOI: 10.1111/jdv.18414 JEADV

ORIGINAL ARTICLE

Serum autoantibody reactivity in bullous pemphigoid is associated with neuropsychiatric disorders and the use of antidiabetics and antipsychotics: a large, prospective cohort study

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Abstract

Background Bullous pemphigoid (BP), the by far most frequent autoimmune blistering skin disease (AIBD), is immunopathologically characterized by autoantibodies against the two hemidesmosomal proteins BP180 (collagen type XVII) and BP230 (BPAG1 or dystonin). Several comorbidities and potentially disease-inducing medication have been described in BP, yet a systematic analysis of these clinically relevant findings and autoantibody reactivities has not been performed.

Objective To determine associations of autoantibody reactivities with comorbidities and concomitant medication.

Methods In this prospective multicenter study, 499 patients diagnosed with BP in 16 European referral centers were included. The relation between anti-BP180 NC16A and anti-BP230 IgG ELISA values at the time of diagnosis as well as comorbidities and concomitant medication collected by a standardized form were analysed.

Results An association between higher serum anti-BP180 reactivity and neuropsychiatric but not atopic and metabolic disorders was observed as well as with the use of insulin or antipsychotics but not with dipeptidyl peptidase-4 (DPP4) inhibitors, inhibitors of platelet aggregation and L-thyroxine. The use of DPP4 inhibitors was associated with less anti-BP180 and anti-BP230 reactivity compared with BP patients without these drugs. This finding was even more pronounced when compared with diabetic BP patients without DPP4 inhibitors. Associations between anti-BP180 and anti-BP230 reactivities were also found in patients using insulin and antipsychotics, respectively, compared with patients without this medication, but not for the use of inhibitors of platelet aggregation, and L-thyroxine.

Conclusion Taken together, these data imply a relation between autoantibody reactivities at the time of diagnosis and both neuropsychiatric comorbidities as well as distinct concomitant medication suggesting a link between the pathological immune mechanisms and clinical conditions that precede the clinically overt AIBD.

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Received: 3 March 2022; Accepted: 15 June 2022

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding sources

This work received infrastructural and financial support from the DFG-funded research consortium Clinical Research Unit 303 *Pemphigoid Diseases*, Collaborative Research Center CRC 1526 *Pathomechanisms of Antibody-mediated Autoimmunity (PANTAU)* and the Schleswig-Holstein Excellence Cluster *Precision Medicine in Chronic Inflammation* (DFG EXC 2167/1).

Introduction

Pemphigoid diseases are a group of autoimmune blistering skin disorders characterized by autoantibodies against structural proteins of the dermal-epidermal junction.[1–3] Bullous pemphigoid (BP) is by far the most common pemphigoid disorder and predominantly affects elderly individuals with an average age of 75–80 years at disease onset.[4–7]

BP180, also known as type XVII collagen, has been identified as the main target antigen in BP as well as in pemphigoid gestationis, mucous membrane pemphigoid, and linear IgA disease.[1] In BP, the extracellular part of the 16th non-collagenous domain (NC16A) is the immunodominant region. The pathogenic relevance of anti-BP180 NC16A antibodies has been shown in various *in vitro* and animal models including neonatal and adult mouse models.[8–10] These findings are corroborated by the clear correlation of anti-BP180 NC16A serum levels with disease activity.[11] In about 50%–60% of BP patients, reactivity against BP230 can be detected.[12–14] While serum levels of anti-BP230 IgG do not correlate with disease activity in BP,[15,16] the pathogenic relevance of BP230-specific antibodies has recently been demonstrated in mouse models.[17]

Multiple clinical associations of BP have been described including certain medication, most frequently aldosterone antagonists, dipeptidyl peptidase-4 (DPP4) inhibitors, anticholinergics, and dopaminergics[18,19] and comorbidities, most frequently, neuropsychiatric disorders that can be found in 30%–50% of patients.[20–25] Further comorbidities include cardiovascular disease, diabetes mellitus, psoriasis, ulcerative colitis and other autoimmune disorders, asthma, haematological malignancies, and atopic dermatitis.[20,26–33]

Despite ample data about associated disorders and medication in BP, only few studies investigated the relation of anti-BP180 and anti-BP230 autoantibodies with these parameters. The present cohort study included 499 patients from 16 referral centers for autoimmune blistering diseases and was aimed to relate anti-BP180 and anti-BP230 autoantibody levels at the time of diagnosis with associated diseases and concurrent medication. These data may be helpful to anticipate comorbidities at an early timepoint and shed light on the pathophysiological mechanisms behind potential trigger factors.

Material and methods

Patients

This multicentric cohort study included data from patients diagnosed with BP between February 3rd, 2015 and July 28th, 2020 at referral centers for autoimmune blistering diseases including the dermatology departments of the Universities of Berlin, Dresden, Düsseldorf, Erlangen, Freiburg, Hamburg, Kiel, Lübeck, Munich, Würzburg (all Germany), Budapest (Hungary), Oulu (Finland), Reims, Rouen (both France), Sofia (Bulgaria), and Thessaloniki (Greece). The recruitment of the patients was performed in a consecutive manner after taking their informed consent. Data about the clinical picture, Bullous Pemphigoid Disease Area Index (BPDAI), immunopathological parameters, comorbidities, and concurrent medication were prospectively collected according to a standardized data sheet (Figure S1). Medication and comorbidities were grouped prior to correlation analyses into DPP4 inhibitors (sitagliptin, vildagliptin, linagliptin), antipsychotics (risperidone, pipamperone), insulin, L-thyroxine, inhibitors of platelet aggregation (acetylsalicylic acid, clopidogrel) as well as neuropsychiatric disorders (including dementia, multiple sclerosis, Parkinson disease, psychosis, and stroke), atopic disorders (including atopic dermatitis, allergic asthma and/or rhinoconjunctivitis), and metabolic disorders (including arterial hypertension, diabetes mellitus type 2, psoriasis). The study was performed according to the Declaration of Helsinki and approved by the ethics committee of the university of Lübeck (15-051, 18-046) and the local ethics committees of the other centers.

Diagnosis of BP was made following current guidelines in patients with (i) compatible clinical picture without predominant mucosal involvement, (ii) linear deposits of IgG and/or C3 at the dermal-epidermal junction by direct immunofluorescence (IF) microscopy of a perilesional biopsy, and (iii) labelling of serum IgG at the epidermal side of 1 M NaCl-split human skin by indirect IF microscopy, or circulating IgG against BP180 NC16A or

BP230 by ELISA (Euroimmun, Lübeck, Germany; MBL, Nagoya, Japan). If direct IF microscopy was negative, epidermal binding of IgG by indirect IF microscopy on salt-split skin and BP180 NC16A IgG ELISA reactivity was present.[34,35] Only patients with anti-BP180 and anti-BP230 ELISA results were included in the correlation analyses. Disease severity was quantified by BPDAI score.[36] In 140 out of 499 (28.1%) patients, BPDAI scores were not available. In line with a large multicenter international prospective study, mild disease activity was defined as BPDAI <20, moderate as BPDAI ≥20 and ≤56, and severe as BPDAI >56.[37]

Clinical and immunopathological data

Clinical and immunopathological parameters included age, sex, BPDAI, comorbidities, and concomitant medication as well as results of indirect IF microscopy on salt-split skin, anti-BP180 and anti-BP230 ELISA values.

Statistical analyses

Quantitative data is shown using mean (standard deviation, SD) and median (range). Qualitative data is expressed as numbers (*n*) and percentage (%). To describe possible relationships between qualitative variables, descriptive *P*-values from $\chi 2$-tests are reported. The statistical analysis was performed with the SAS System (SAS Institute, Cary, NC, USA).

Results

Demographics of the study population

The study cohort included 499 patients with BP, 241 (48.3%) males and 258 (51.7%) females. The mean age (±SD) at diagnosis was 78.3 (±9.5) years, the median was 80 years with a range between 37 and 98 years (Table 1). 153 additional BP patients diagnosed within the study period were excluded since not both BP180 and BP230 ELISA results were available.

Serum anti-basement membrane zone antibody levels

Indirect IF microscopy on monkey oesophagus showed anti-basement membrane zone IgG in 247 of 462 (53.5%) patients. IgG against the epidermal side of human salt-split skin was present in 316 of 462 (68.4%) patients by indirect IF microscopy. Both anti-BP180 NC16A and anti-BP230 ELISA values were available from 499 (76.5%) patients. Anti-BP180 NC16A IgG ranged from negative to 4593 U/mL with a mean (±SD) of 257 (±478) U/mL and a median of 115 U/mL. Accordingly, patients with anti-BP180 NC16A ELISA values ≤115 U/mL were classified as "low anti-BP180 IgG", patients with values >115 U/mL as "high anti-BP180 IgG". 369 (74%) patients had a positive BP180 NC16A ELISA value (cut-off, >20 U/mL; Table 1). Analysed anti-BP230 IgG ranged from negative to 1549 U/mL with a mean (±SD) of 150 (±201) U/ml and a median of 91 U/mL. 205 (41.1%) patients had a positive BP230 ELISA value (cut-off, >20 U/mL; Table 1).

Table 1 Demographic, clinical and immunological characteristics of patients

Age at diagnosis; years	
Mean (SD)	78.3 (9.5)
Median (range)	80 (37–98)
Sex, *n* (%)	
Male	241 (48.3%)
Female	258 (51.7%)
Disease severity*, *n* (%)	
Mild (BPDAI <20)	92 (25.6%)
Moderate (BPDAI ≥20 ≤ 56)	177 (49.3%)
Severe (BPDAI >56)	90 (25.1%)
BP180 NC16A ELISA (IgG)	
Seropositivity, *n* (%)	369/499 (74%)
ELISA value, median; U/mL	115
BP230 ELISA (IgG)	
Seropositivity, *n* (%)	205/499 (41.1%)
ELISA value, median; U/mL	91

n, number; SD, standard deviation; BPDAI, bullous pemphigoid disease area index.
*Indicated by Masmoudi *et al.*[37]

Serum anti-BP180 reactivity correlates with disease severity

To test the validity of our approach, we initially assessed the well-known association of serum anti-BP180 NC16A IgG with disease activity. Disease activity was classified according to Masmoudi *et al.* as detailed above.[37] The anti-BP180 reactivity was categorized according to the BP180 NC16A ELISA titers as "without" for sera with ELISA titers below the diagnostic cut-off value of 20 U/mL and as "low" and "high" for sera below and above the median ELISA titre, respectively. Based on these classifications, an association between circulating anti-BP180 IgG and the extent of disease was seen ($P = 0.0011$; Table 2).

Higher serum-anti-BP180 reactivity is associated with neuropsychiatric disease

Subsequently, the cohort was analysed for the association between serum anti-BP180 IgG and various comorbidities including neuropsychiatric, atopic, and metabolic disorders. Using the same classification for anti-BP180 serum IgG as outlined above, an association between higher BP180-specific IgG and the presence of neuropsychiatric disorders was observed ($P = 0.0319$; Table 2). No associations were observed between anti-BP180 NC16A reactivity and metabolic disorders or atopic disorders (Table S1).

Higher serum-anti-BP180 reactivity is associated with the use of insulin and antipsychotics

Additionally, we analysed the cohort for the association between serum anti-BP180 IgG levels and the use of different medications, i.e. insulin, antipsychotics, DPP4 inhibitors, inhibitors of platelet aggregation, and L-thyroxine. An association between

Table 2 Association of serum anti-BP180 NC16A reactivity with disease activity, neuropsychiatric disorders as well as use of insulin and antipsychotics

	n (%) patients without anti-BP180 IgG	*n* (%) patients with low levels of anti-BP180 IgG	*n* (%) patients with high levels of anti-BP180 IgG
Mild disease activity* (BPDAI < 20), *n* = 92	27 (29.4%)	43 (46.7%)	22 (23.9%)
Moderate disease activity* (BPDAI ≥ 20 und ≤ 56), *n* = 177	41 (23.2%)	61 (34.5%)	75 (42.4%)
Severe disease activity* (BPDAI > 56), *n* = 89	13 (14.6%)	28 (31.5%)	48 (53.9%)
With neuropsychiatric disorders, *n* = 148	32 (21.6%)	49 (33.1%)	67 (45.3%)
Without neuropsychiatric disorders, *n* = 342	98 (28.7%)	131 (38.3%)	113 (33%)
With insulin, *n* = 59	7 (11.9%)	26 (44.1%)	26 (44.1%)
Without insulin, *n* = 431	123 (28.5%)	154 (35.7%)	154 (35.7%)
With antipsychotics, *n* = 19	3 (15.8%)	0 (0%)	16 (84.2%)
Without antipsychotics, *n* = 471	127 (27.0%)	180 (38.2%)	164 (34.8%)

n, number; low ELISA levels, ≤115 U/mL; high ELISA levels, >115 U/mL; *defined by Masmoudi *et al.*[37]; association with BPDAI, $P = 0.0011$; association with neuropsychiatric disorders, $P = 0.0319$; with and without insulin, $P = 0.0247$; with and without antipsychotics, $P < 0.0001$.

higher BP180-specific IgG and the use of insulin ($P = 0.0247$) and antipsychotic medication was observed ($P < 0.0001$; Table 2). In contrast, no association was detected between serum anti-BP180 IgG titre and application of DPP4 inhibitors, inhibitors of platelet aggregation, and L-thyroxine (Table S2). When patients with neuropsychiatric disorders without antipsychotics were compared with patients with these disorders taking antipsychotics, latter group of patients showed higher anti-BP180 IgG compared with patients with neuropsychiatric disorders without antipsychotics ($P = 0.006$; Fig. 1). No differences were observed between patients without neuropsychiatric disorders and without antipsychotics compared with patients with neuropsychiatric disorders without antipsychotics (Fig. 1).

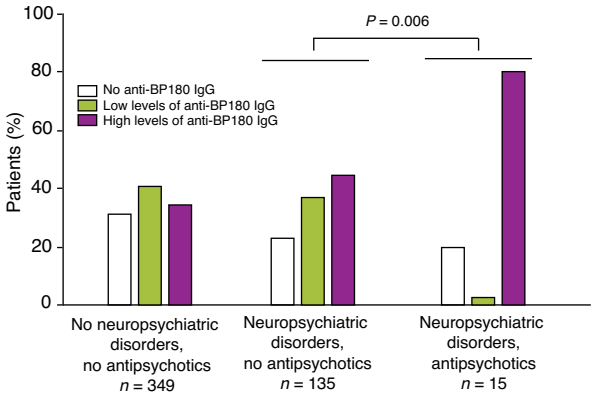

Figure 1 Percentage of bullous pemphigoid patients with no serum anti-BP180 NC16A IgG as well as low and high levels of anti-BP180 IgG in the three groups of patients (i) without neuropsychiatric disorders and without antipsychotics, (ii) with neuropsychiatric disorders but without antipsychotic medication and (iii) with neuropsychiatric disorders and antipsychotics. While the three groups of patients revealed different anti-BP180 autoantibody levels (Chi^2-test for all three categories, $P = 0.001$), higher anti-BP180 IgG levels were observed in patients with neuropsychiatric disorders and antipsychotics (right panel) compared with those with neuropsychiatric disorders but no antipsychotic medication (middle panel; $P = 0.006$).

Use of DPP4 inhibitors is associated with lack of autoantibody reactivity against both BP180 NC16A and BP230 and lack of combined anti-BP180 and anti-BP230 antibodies

For further analyses, autoantibody reactivity was categorized according to BP180 NC16A and BP230 ELISA reactivities in patients (i) without ELISA reactivity against both BP180 and BP230 (BP180 neg., BP230 neg.), (ii) with anti-BP230 IgG alone (BP180 neg., BP230 pos.), (iii) with anti-BP180 IgG alone (BP180 pos., BP230 neg.) and (iv) with both antibodies against BP230 and BP180 (BP180 pos., BP230 pos.; Table 3). The use of DPP4 inhibitors was associated with less frequent anti-BP180 and anti-BP230 reactivities compared with patients without DPP4 inhibitors ($P = 0.002$). Patients on DPP4 inhibitors had twice as frequently no anti-BP180 and anti-BP230 IgG and considerably less combined anti-BP180 and anti-BP230 reactivity compared with patients without DPP4 inhibitors (Table 3). Anti-BP230 IgG reactivity without anti-BP180 IgG reactivity was not found in patients taking DPP4 inhibitors, whereas 7.7% of the patients not taking these drugs had this serological constellation (Table 3). When autoantibody reactivities in patients using these drugs were compared with patients with diabetes mellitus not treated with DPP4 inhibitors, similar associations were seen ($P = 0.0447$; Table 3).

Use of insulin is associated with more frequent combined anti-BP180 NC16A and anti-BP230 IgG

Anti-BP180 NC16A and anti-BP230 IgG reactivities were significantly different between patients treated or not treated with insulin compared with those without ($P = 0.0456$). In detail, lack of both BP180- and BP230-specific IgG was observed twice

Table 3 Anti-BP180 NC16A and anti-BP230 IgG reactivities were different between bullous pemphigoid patients using DPP4 inhibitors, insulin and antipsychotics, respectively, compared with those without these medications

	n (%) patients, reactivity against					
	BP180 neg. & BP230 neg.	**BP180 neg. & BP230 pos.**	**BP180 pos. & BP230 neg.**	**BP180 pos. & BP230 pos.**	**BP180 pos.**	**BP230 pos.**
With DPP4i, *n* = 56	20 (35.7%)	0 (0%)	22 (39.3%)	14 (25%)	36 (64.3%)	14 (25.7%)
Without DPP4i, *n* = 443	76 (17.2%)	34 (7.7%)	176 (39.7%)	157 (35.4%)	333 (75.2%)	191 (43.1%)
Diabetics without DPP4i, *n* = 60	9 (15%)	2 (3.3%)	29 (48.3%)	20 (33.3%)	49 (81.7%)	22 (36.7%)
With insulin, *n* = 47	5 (10.6%)	0 (0%)	20 (42.6%)	22 (46.8%)	42 (89.4%)	22 (46.8%)
Without insulin, *n* = 452	91 (20.1%)	34 (7.5%)	178 (39.4%)	149 (33%)	327 (72.3%)	183 (40.5%)
Diabetics without insulin, *n* = 69	24 (34.8%)	2 (2.9%)	31 (44.9%)	12 (17.4%)	43 (62.3%)	14 (20.3%)
With antipsychotics, *n* = 15	2 (13.3%)	1 (6.7%)	1 (6.7%)	11 (73.3%)	12 (80%)	12 (80%)
Without antipsychotics, *n* = 484	94 (19.4%)	33 (6.8%)	197 (40.7%)	160 (33.1%)	357 (73.8%)	193 (39.9%)

n, number; DPP4i, dipeptidylpeptidase-4 inhibitor; with and without DPP4i: all patients, $P = 0.002$, diabetics, $P = 0.0447$; with and without insulin: all patients, $P = 0.0456$, diabetics, $P = 0.001$; with and without antipsychotics: all patients, $P = 0.0097$.

as frequently in patients not using insulin compared with BP patients treated with insulin (Table 3). In line, patients using insulin developed more frequently a combined anti-BP180 and anti-BP230 response than patients not treated with insulin irrespective of being diabetic. When autoantibody reactivities in insulin-treated patients with insulin were compared with non-insulin treated ones, the described differences were considerably more pronounced ($P = 0.001$; Table 3).

Use of antipsychotic medication is associated with reactivity against BP230 as well as combined anti-BP180 NC16A and anti-BP230 IgG

Different autoantibody reactivities were observed between patients taking or not taking antipsychotics ($P = 0.0097$). Combined anti-BP180 and anti-BP230 IgG reactivity as well as BP230 seropositivity was twice as frequent in BP patients using antipsychotics compared with patients without these drugs (Table 3).

Discussion

Autoantibodies against the two hemidesmosomal antigens, BP180 (collagen type XVII) and BP230 are immunopathological hallmarks of BP. Their direct pathogenic relevance has been clearly demonstrated though BP180 is regarded as main target autoantigen since (i) in nearly all BP patients, antibodies against BP180 can be detected,[15,38] (ii) antibodies against the immunodominant NC16A domain of BP180 but not against BP230 were found to correlate with disease severity,[11,15,16] and (iii) patients with antibodies restricted to BP230 are rare and present with low disease activity.[39,40] A clinical characteristic of BP is its high association with concomitant diseases as well as, in small but well-described subgroups, the association with certain medications. So far, only a limited number of studies have analysed associations between autoantibody reactivities and clinical outcomes in BP. In these studies, high serum anti-BP180 IgG levels at the time of diagnosis were indicative for an early death and a higher number of infections.[41–44] In contrast, lower serum anti-BP180 IgG levels were described in DPP4 inhibitor-associated BP,[45,46] whereas BP230 reactivity was linked with neuropsychiatric comorbidities.[47]

The aim of the present study was to explore the association of autoantibody reactivities with comorbidities and the medication of BP patients in a large multicentric cohort. Therefore, data on comorbidities and concomitant medication were collected prospectively in a large cohort of patients diagnosed on well-established criteria in 16 dermatological referral centers for autoimmune blistering diseases from six European countries.[34,35] To characterize this cohort, basic epidemiological and immunopathological parameters were collected. The mean age of 78 years and the relation of male to female patients corresponded well to published data from Europe.[6,48,49] Disease activity was classified into mild, moderate and severe BP based on the BPDAI according to Masmoudi *et al.*[37] This classification was based on the BPDAI of a large international cohort of BP patients in which the quarters of patients with the lowest and highest BPDAI, that is, <20 and >56, were classified as mild and severe disease, respectively, and the middle two quarters as patients as moderate disease. In the present study, this classification nearly exactly matched the one of the original study with 26%, 49% and 25% of patients with mild, moderate and severe BP, respectively.[37] These findings indicate that the basic epidemiological data in the present study well reflect previously described patient cohorts.

The seropositivity for anti-BP180 IgG and anti-BP230 IgG in the present study were with 74% and 41% at the lower ends of the reported ranges of 65%–97% and 50%–60%, respectively.[12–14,40,50] This may be explained by the retrospective design of latter studies that applied well characterized but most likely preselected patient cohorts. In line with previous reports,[11,15,16] anti-BP180 but not anti-BP230 reactivity was found to be associated with disease activity in the present study.

Based on these results, we analysed the association of anti-BP180 NC16A reactivity with various comorbidities including

neuropsychiatric, atopic and metabolic disorders. Higher anti-BP180 reactivities were found in patients with neuropsychiatric diseases. A similar association has been reported in a single center study with 47 BP patients.[51] Our results are also in agreement with the finding that serum levels of BP180 NC16A-specific IgG correlated with cognitive decline in patients with Alzheimer disease.[52] In addition, in the past, IgG against BP180 was shown in patients with Parkinson disease and stroke, while another study failed to detect BP180 NC16A-specific IgG in patients with Parkinson disease and multiple sclerosis.[53–55] Compatible with our data, it has been shown that BP180 is expressed in the central nervous system and, while the exact mechanisms behind the observed associations remain elusive, it is an attractive hypothesis that degenerative processes in the central nervous system associated with the mostly preceding neuropsychiatric diseases may trigger the autoimmune skin disease.[22] Differentiating between the impact of the underlying neuropsychiatric disorders and the concomitant antipsychotic medication has been debated previously without conclusive results.[19,56–60] Our finding of higher levels of anti-BP180 IgG in patients with neuropsychiatric disorders and concomitant antipsychotic medication compared with patients with these disorders but without antipsychotics points to an additional impact of the antipsychotic medication for the induction of BP. Although the association of BP with metabolic and atopic disorders, such as diabetes mellitus, allergic asthma and atopic dermatitis, has been well recognized,[26,29,30,33] no correlation between these comorbidities and serum levels of anti-BP180 NC16A IgG has been observed in the present study.

Although sound data about disease-inducing medication in BP are available, only few studies investigated the relation of anti-BP180 and anti-BP230 autoantibodies with these medications. When we analysed the association of serum anti-BP180 NC16A IgG titers with various medications, an association with higher titers was seen in patients using insulin and antipsychotics, respectively. This finding is in line with the association of BP with neuropsychiatric medication.[60] In contrast, lower serum anti-BP180 IgG levels were described in DPP4 inhibitor-associated BP,[45,46,61] a finding that could not be confirmed in our study. Furthermore, no association between serum anti-BP180 IgG levels and inhibitors of platelet aggregation or L-thyroxine was revealed in our study.

Next, we were interested in the relation of anti-BP180 and anti-BP230 reactivities with the medication most commonly used by BP patients; that is, DPP4 inhibitors, insulin, antipsychotics, inhibitors of platelet aggregation and L-thyroxine. Following anecdotal evidence for the association of BP with autoantibody response restricted to BP230 and dementia,[62] Ständer *et al.* reported anti-BP230 seropositivity to be linked to neuropsychiatric comorbidities. In the latter monocentric study with 273 patients that also included patients from the present investigation, only 83 (30%) patients with anti-BP230 IgG levels were evaluated. Although the low rate of available anti-BP230 IgG results may have been prone to bias, the present multicenter prospective study confirmed the reported link with a twofold higher rate of anti-BP230 IgG in patients using antipsychotics compared with patients without these drugs.

Associations with anti-BP180/BP230 IgG were also significant when we compared DPP4 inhibitor- or insulin-treated patients with untreated ones. As such, DPP4 inhibitors and insulin appear to influence the autoantibody specificities in BP, without regard to the underlying diabetic condition. Insulin has not been described as a pathogenic factor for BP in a previous study.[63] While anti-DPP4 inhibitor-associated BP is a well-recognized BP subgroup,[18,61,64,65] clinical and immunopathological features that differ from those of DPP4 inhibitor-unrelated BP were contradictory in different patient populations.[46] In Japanese and Hungarian BP patients, DPP4 inhibitor use tended to reveal a non-inflammatory phenotype, a low number of lesional infiltrated eosinophils, less anti-BP180 NC16A IgG reactivity and an HLA-DQB1*03:01 haplotype.[61,66–68] In contrast, in another cohort of Japanese BP patients, patients without BP180 NC16A reactivity showed no increased use of DPP4 inhibitors.[69] Alike, the present study showed less frequent anti-BP180 NC16A and anti-BP230 IgG reactivity in patients on these drugs compared with both all BP patients without DPP4 inhibitors and diabetic BP patients without these drugs. In line, the rate of patients with double anti-BP180/BP230 reactivity and patients with anti-BP230 IgG were lower in the group using DPP4 inhibitors compared with BP patients without DPP4 inhibitors. A lower seropositivity rate for BP230-specific IgG in patients applying a DPP4 inhibitor was also observed by Mai *et al.* that found predominant IgG reactivity against the cell-derived 97 kDa extracellular part of BP180 in this patient population.[70] Studies from France and Greece did not find serological differences between DPP4 inhibitor-associated BP and patients without use of these drugs.[64,71] In the study by Ständer *et al.* that also included about 100 patients of the present cohort, DPP4 inhibitor-associated BP revealed lower serum levels of anti-BP180 NC16A and anti-BP230 IgG compared with other BP patients,[45] findings that were not seen in the present study. These differences may be explained by the considerably lower number of patients with available anti-BP180 NC16A IgG levels using DPP4 inhibitors in the latter study (n = 20) compared with the present cohort (n = 56) or the retrospective and single-center approach by Ständer *et al.*[45]

The mechanisms how insulin and DPP4 inhibitors modulate the pathophysiology of BP are unclear. A common aspect of both DPP4 inhibition and insulin substitution is an increase of insulin. It can be speculated that the inhibited expression of DPP4 (CD26) on T-cells or higher levels of insulin may trigger a tolerance break of BP180. One possible mechanism for this includes an aberrant expression of BP180 due to an abnormal homeostasis.[46] Furthermore, DPP4 inhibition has been shown to increase the CLC11-mediated infiltration of eosinophils in the skin of rats.[72] The expression of DPP4 (CD26) in various cells,

that is, melanocytes, keratinocytes and fibroblasts indicates the critical impact of its inhibition.[73] Insulin causes *in vitro* an increase of protein synthesis and a decrease of protein degradation in muscle cells.[74,75] It is possible that this phenomenon also applies to the skin causing more antigen exposure through less degradation of basement membrane zone proteins.

This prospective multicenter study included a high number of well characterized patients. Age, sex, disease severity and anti-BP180 and BP230 antibody levels reflected figures reported from other European cohorts. However, the study was not without limitations. It was performed in tertiary referral centers which may have resulted in lower numbers of patients with mild BP. Furthermore, the multicenter design of the study may have led to variations how BPDAI was scored. Statistical analysis was performed with descriptive *P* values for associations not following predetermined hypotheses without correction for multiple testing or confounding factors.

Taken together, the present study sheds new light on the link between autoantibody reactivities and both comorbidities and concomitant medication in BP patients. These results may be a further step towards individualized autoantibody-based risk assessment in BP and may encourage research to define the pathophysiological mechanisms linking comorbidities and preceding medication with the autoantibody profile in this disease.

Acknowledgements

We thank Kerstin Saalmüller, Frank Sandig and Johanna Otten, Lübeck for the assistance during the documentation and data analysis. Furthermore, we thank Dr. Annette Baratay, Hamburg and Carina Palak, Kiel for their contribution to the sample collection and documentation.

Author contributions

D.Z., N.B., C.D.S. and E.S. designed the project. H.O.D. and I.R.K. analysed the data. H.O.D., K.Y. and E.S. wrote the manuscript. The other authors assembled patient data and sera and reviewed the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. No association of anti-BP180 NC16A IgG serum levels and atopic or metabolic disorders observed.
Table S2. No association of anti-BP180 NC16A IgG serum levels with preceding medication observed.
Appendix S1. Standardized data sheet.