Demographics and Autoantibody Profiles of Pemphigoid Patients with Underlying Neurologic Diseases

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Bullous pemphigoid (BP) is an autoantibody-mediated blistering disease that is often associated with neurologic disease. BP antibodies target two epidermal adhesion molecules, known as BP180 and BP230. Homologues to these proteins are found in the brain, and it is hypothesized that neurologic disease leads to the production of autoantibodies that can cross-react with their cutaneous forms. To better understand the link between BP and neurologic disease, we evaluated primary demographic features (age, sex, race, ethnicity, and elapsed time between onset of skin symptoms and BP diagnosis), severity of BP, and IgG and IgE autoantibody levels in BP control individuals and patients with BP with preceding Parkinson disease, dementia, and stroke. The main findings of this study are that patients with BP with preceding neurologic disease have a shorter elapsed time between onset of skin disease and BP diagnosis and that patients with preceding Parkinson disease or dementia, but not stroke, are significantly older than patients with BP without neurologic disease. However, no significant differences in clinical presentation, BP severity scores, or autoantibody (IgG and IgE) responses were observed among the groups. These findings suggest that, despite the age difference, the clinical phenotype of BP is not affected by preceding neurologic disease.

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INTRODUCTION

Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by autoantibodies targeting epidermal adhesion molecules BP180 (collagen XVII), and BP230 (Diaz et al., 1990; Stanley et al., 1988). BP primarily affects individuals of age 70 years and older, and disease risk increases with age (Hubner et al., 2016; Langan et al., 2008). Worldwide, there is an increasing incidence of BP that varies by geographic location; reported rates range from 2.4 to 50 cases per million individuals per year (Brick et al., 2014; Hubner et al., 2016; Joly et al., 2012; Langan et al., 2008; Marazza et al., 2009). An association of BP with neurologic disease (ND) was first reported in a 1985 case series of three patients with multiple sclerosis who developed severe, generalized BP (Simjee et al., 1985). Subsequently, several large case-control and population-based studies estimated that individuals with ND, such as multiple sclerosis, dementia (DEM) and stroke (STR), are 1.8–10.7 times more likely to develop BP than the general population (Bastuji-Garin et al., 2011; Chen YJ et al., 2011; Cordel et al., 2007; Försti et al., 2016; Kibsgaard et al., 2017; Langan et al., 2011; Ren et al., 2017; Taghipour et al., 2010; Yu Phuan et al., 2017). Based on these studies, ND is now recognized as one of the most common BP comorbidities, affecting 30%–60% of patients.

The link between ND and BP suggests a common mechanism of disease susceptibility or pathogenesis. One possibility is that progressive neurodegeneration and inflammation leads to exposure of neuronal isoforms of the BP180 and BP230 proteins, which facilitates cross-reactivity to the skin isoforms (Brown et al., 1995; Kunzli et al., 2016; Li et al., 2009; Seppänen, 2013; Seppänen et al., 2006). Evidence in support of this hypothesis is provided by studies showing serum IgG reactivity to the skin isoforms of BP180 and BP230 in patients who have ND but not BP (Chen J et al., 2011; Foureur et al., 2006; Kokkonen et al., 2017; Messingham et al., 2016). Similarly, IgG reactivity to a 230-kDa protein of human brain extract was detected in the serum of patients with BP who did not have ND (Chen J et al., 2011).

The significance of the BP180- and BP230-specific antibodies in the pathogenesis of ND, and their role in the eventual development of BP, remains unclear. Serum IgG antibody reactivity to skin antigens is reported only in a subset (≤20%) of patients with ND (Foureur et al., 2006; Messingham et al., 2016; Recke et al., 2016). Furthermore, IgE antibodies have not been assessed in patients with BP and ND, despite the critical role of this autoantibody subclass in the pathogenesis of BP (Fairley et al., 2009). To better understand the association between ND and BP, we examined patient demographics and circulating IgG and IgE

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Abbreviations: BP, bullous pemphigoid; BPDAI, bullous disease area index; CON, control; DEM, dementia; ND, neurologic disease; STR, stroke; PD, Parkinson disease

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autoantibody levels in patients with BP with and without ND and further determined if particular features were associated with preceding ND.

RESULTS

Of the 112 patients with BP initially identified in our database, 35 (31%) were diagnosed with a preexisting ND; two of these were excluded because of an unspecified ND that could not be classified. The remaining 110 patients with BP were categorized as follows: 77 patients with BP without ND (BP control [CON]) and 33 with preceding ND in addition to BP (ND + BP), which could be subdivided as 11 with Parkinson disease (PD) + BP, 11 with DEM + BP, and 11 STR + BP. Analysis of patient demographics (see Supplementary Table S1 online) showed a similar male-to-female incidence when ND + BP (43% male) and BP CON (45% male) were compared. When broken down by type of ND, the STR + BP group was also 45% male, the PD + BP was 90% male, and the DEM + BP was only 27% male, but these differences were not statistically significant based on a chi-square test. Additionally, no differences in race or ethnicity were noted among the study groups, which is typical of BP (see Supplementary Table S1).

Although preceding ND increases individual risk of developing BP (Bastuji-Garin et al., 2011; Chen YJ et al., 2011; Ren et al., 2017), it is not established how it affects the progression to clinical BP. Studies investigating the association of ND and BP are problematic. The main issue is that it is difficult to estimate the time between the onset of ND and the development of BP because cognitive decline is gradual and, often, self-reported. In this study, this is further complicated because most of the patients were referred by outside physicians specifically for treatment of BP, but their neurologist was offsite. Based on the information available, the time between the onset of skin disease and BP diagnosis (days elapsed) and the age at BP onset were evaluated (Figures 1a and b). The days elapsed were calculated based on the date of first skin symptoms, as perceived by the patient. This information was available for 41 BP CON, 10 PD + BP, 8 DEM + BP, and 6 STR + patients with BP. Overall, patients with all types ND had a significantly ($P < 0.0001$) shorter time between symptom onset and definitive diagnosis of BP (Table 1). In addition, patients with PD had a significantly shorter ($P < 0.0001$) interval to diagnosis of BP than patients with preceding DEM or STR. When the age of patients at the time of BP diagnosis was compared (Table 1), patients with preceding PD (82.7 ± 5.6 years) or DEM (82.6 ± 4.9 years) were significantly older than those in either the BP CON (71.6 ± 10.8 years) or STR+ BP (69.6 ± 8.2 years) groups (*P*-values range from 0.0030 to 0.0052). Upon clinical examination, no differences in morphologic presentation of BP, such as localization of lesions, or Bullous Disease Area Index (BPDAI) (Murrell et al., 2012) scores were observed with any type preceding ND (Figure 1c and Table 1).

If preceding ND facilitates the development of autoantibodies reactive to cutaneous antigens, this could affect the autoantibody response observed in BP. Thus, circulating antibody levels were measured in patient sera by ELISA. Comparison of BP180 and BP230 IgG levels did not uncover any significant differences in the incidence or specificity of the IgG response in patients with BP with or without ND;

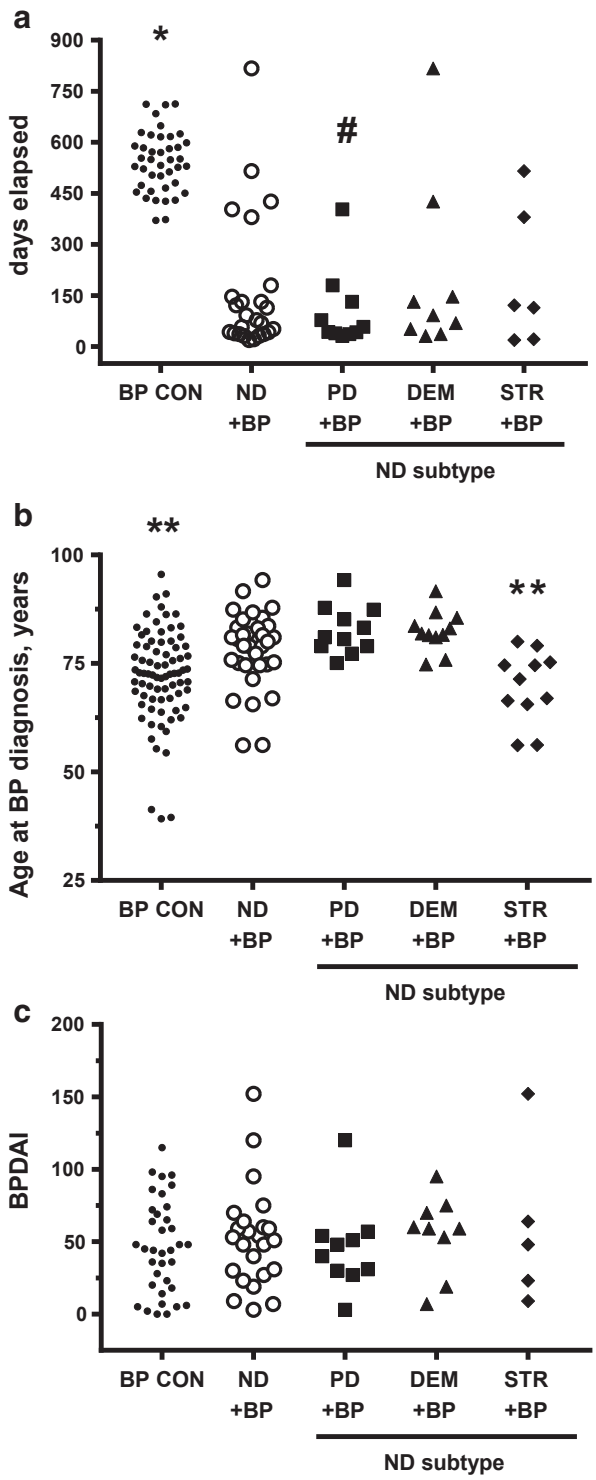

Figure 1. Initial presentation of BP with or without preceding neurologic disease. Participants were BP control individuals (BP CON) and patients with BP with preceding neurologic disease (ND) Parkinson disease (PD + BP), dementia (DEM + BP), and stroke (STR + BP). **(a)** Days elapsed were calculated based on the time between the onset of skin symptoms and BP diagnosis. **(b)** Age at BP onset, years. **(c)** Disease severity calculated with the Bullous Disease Area Index (BPDAI). Each point represents an individual patient. By using generalized linear models with a Bonferroni correction, $P \leq 0.0071$ was required for significance; *vs. all other groups, #vs. DEM or STR, **= vs. PD or DEM. BP, bullous pemphigoid; CI, confidence interval; CON, control; DEM, dementia; ND, neurologic disease; PD, Parkinson disease; STR, stroke.

Table 1. Analysis of days elapsed, age at diagnosis, and BPDAI scores in study participants

	Days Elapsed[2]		Age at Diagnosis, years[3]		BPDAI[3]	
Comparison[1]	Mean Ratio[4] (95% CI)	*P*	Mean Ratio (95% CI)	*P*	Mean Ratio (95% CI)	*P*
ND + BP vs. BP CON	0.515 (0.496–0.534)	**<0.0001**	1.085 (1.021–1.124)	0.0085	1.059 (0.707–1.587)	0.7762
PD + BP vs. BP CON	0.333 (0.312–0.355)	**<0.0001**	1.146 (1.048–1.252)	**0.0030**	0.935 (0.543–1.610)	0.8042
DEM + BP vs. BP CON	0.661 (0.627–0.696)	**<0.0001**	1.143 (1.046–1.249)	**0.0036**	1.120 (0.635–1.974)	0.6911
STR + BP vs. BP CON	0.624 (0.587–0.663)	**<0.0001**	0.965 (0.883–1.055)	0.4297	1.200 (0.582–2.476)	0.6154
PD + BP vs. STR + BP	0.534 (0.491–0.581)	**<0.0001**	1.187 (1.056–1.335)	**0.0046**	0.779 (0.340–1.783)	0.5474
DEM + BP vs. STR + BP	1.060 (0.982–1.144)	0.1337	1.184 (1.053–1.331)	**0.0052**	0.933 (0.401–2.168)	0.8693
PD + BP vs. DEM + BP	0.504 (0.466–0.545)	**<0.0001**	1.003 (0.892–1.128)	0.9643	0.835 (0.417–1.672)	0.6045

Abbreviations: BP, bullous pemphigoid; BPDAI, Bullous Disease Area Index; CI, confidence interval; CON, control; DEM, dementia; ND, neurologic disease; PD, Parkinson disease; STR, stroke.

[1]Participants included BP control individuals (BP CON) and patients with BP with preceding neurologic disease (ND), Parkinson disease (PD + BP), dementia (DEM + BP), and stroke (STR + BP).

[2]Calculated based on the interval between the onset of skin symptoms and BP diagnosis. n = 41 BP CON, 10 PD + BP, 8 DEM + BP, and 6 STR + BP.

[3]n = 77 BP CON, 11 PD + BP, 11 DEM + BP, and 11 STR + BP.

[4]Mean ratios, 95% CIs, and *P*-values are indicated for each comparison. A *P*-value less than 0.0071 was considered significant (bold font).

however, it is of interest that the highest levels of both antibodies were found in the STR + BP sera (Figure 2 and Table 2, and see Supplementary Table S2 online). Total IgE and BP-antigen-specific IgE, which have not been previously described in patients with BP with ND, were also evaluated (Figure 3 and Table 3, and see Supplementary Table S2). Consistent with previous studies of BP examining IgE in BP (Iwata et al., 2008; Messingham et al., 2009), patients with preexisting ND exhibited robust production of both total and antigen-specific IgE. Alhtough no significant differences in the incidence or specificity of the IgE response were observed, the IgE antibody profiles did vary among the groups. Specifically, the median BP180 IgE concentration was highest in the BP CON group, whereas BP230 and total IgE were the highest in the STR + BP group (Figure 3, and see Supplementary Table S2).

A Spearman correlation matrix was performed to evaluate the relationships between circulating antibodies, symptom duration, age at BP onset, and BPDAI score. Patients with ND were evaluated as a group and also by their individual diagnoses. The duration of skin symptoms (days elapsed) was not associated with any other measures in the BP CON group, but it was correlated ($P = 0.036$, $r = 0.459$) with total IgE levels in the ND + BP group. Although the patients with preceding ND were significantly older when diagnosed with BP, no correlations with age were observed in this group. However, in the BP CON group, age at BP diagnosis was inversely related ($P = 0.0003$, $r = -0.5086$) to BP180 IgG levels.

Studies evaluating autoantibody profiles in BP show that disease severity often correlates with IgG and/or IgE autoantibody levels (Hashimoto et al., 2017; Iwata et al., 2008). In agreement with this, BPDAI scores of all study participants strongly correlated with BP180 IgG levels ($P = 0.002$, $r = 0.5850$ for BP CON; $P = 0.0212$, $r = 0.468$ for ND + BP). In participants without ND, BPDAI scores also correlated moderately with BP180 IgE ($P = 0.0173$, $r = 0.3944$).

The relationship between the different classes and specificities of the antibodies were also evaluated. Although several correlations were identified in the BP CON group, none were observed when patients with ND were considered as a whole, nor were they consistent when broken down by type of ND. Briefly, BP180 IgG levels correlated moderately with BP180 IgE ($P = 0.0187$, $r = 0.3491$) in the BP CON group, but they correlated strongly ($P = 0.0279$, $r = 0.7857$) with BP230 IgG in the STR + BP group. In contrast, BP230 IgG correlated ($P = 0.292$, $r = 0.3183$) only with total IgE in the BP CON group. Finally, BP180 IgE levels were inversely associated with both BP230 IgG ($P = 0.0202$, $r = -0.7333$) and BP230 IgE ($P = 0.034$, $r = -0.6848$) only in participants with PD + BP.

DISCUSSION

Preceding ND is one of the most common comorbidities of BP (Bastuji-Garin et al., 2011; Chen YJ et al., 2011; Ren et al., 2017). However, it has not been established whether the clinical phenotype of BP differs in the presence or absence of ND. In this report, patient demographics and circulating autoantibody levels were examined in patients with BP with PD, DEM, or STR and in patients with BP without ND. The main findings of this study are that patients with BP with preceding ND have a shorter elapsed time between onset of skin disease and BP diagnosis and that patients with preceding PD or DEM, but not STR, are significantly older than patients with BP without ND. However, no significant differences in clinical presentation, BP severity scores, or autoantibody (IgG and IgE) responses were observed among the groups.

It is hypothesized that the association between ND and subsequent BP results from antibodies initially generated in response to the inflammatory processes associated with ND (Chen J et al., 2011; Li et al., 2009; Messingham et al., 2016; Seppänen, 2013). In this scenario, generation of cutaneous autoantibodies is dependent on transit of neuronal proteins and/or sensitized immune cells through the blood-brain barrier. Thus, the progression to BP is likely dictated by the nature and timing of these events. We propose that the progression to BP is influenced by a combination of neurodegeneration and inflammation that is unique to each type of ND. This idea is consistent with a relatively short gap (5.5 years) between cerebral infarction and development of BP compared with a much longer gap (3–18 years) between the initial diagnosis of PD or DEM and the development of BP (Chen J et al., 2011; Försti et al., 2016; Simjee et al., 1985;

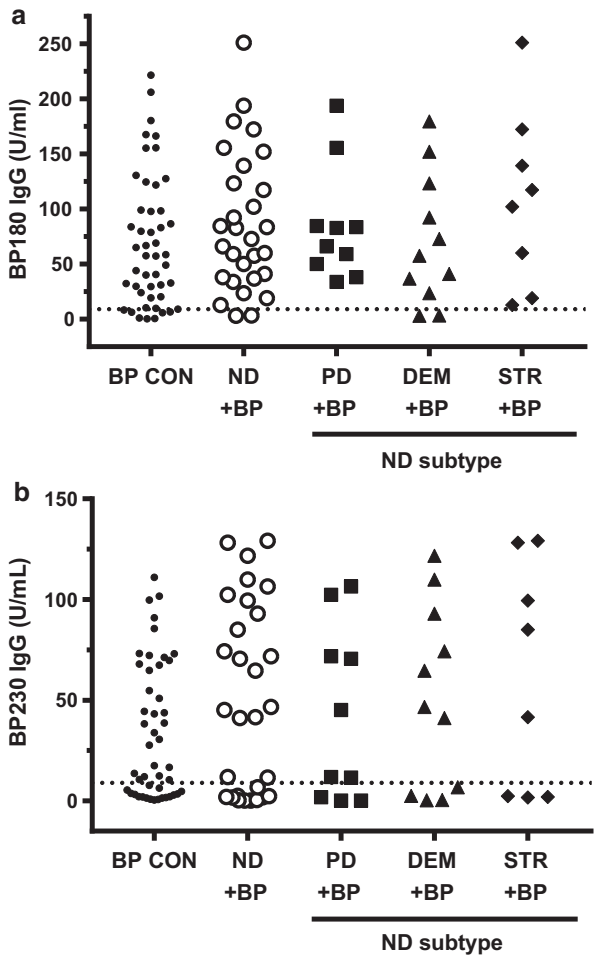

Figure 2. Serum IgG autoantibody levels in patients with BP with and without neurologic disease. Participants were BP control individuals (BP CON) and patients with BP with preceding neurologic disease (ND), Parkinson disease (PD + BP), dementia (DEM + BP), and stroke (STR + BP). **(a)** BP180- and **(b)** BP230-specific IgG were measured by ELISA. The dashed line indicates the minimum value for a positive test, ≥ 9 units/ml. Each point represents an individual patient. No statistically significant differences were observed. BP, bullous pemphigoid; CI, confidence interval; CON, control; DEM, dementia; ND, neurologic disease; PD, Parkinson disease; STR, stroke.

Taghipour et al., 2010). We were unable to reliably ascertain the onset of ND in this study; however, based on the established age of onset of PD at 60 years (Pagano et al., 2016) and the onset of BP at a mean age of 82.7 ± 5.6 years, a gap of 17–27 years is observed here.

Although serum IgG reactivity to brain and/or skin isoforms of BP180 and BP230 has been reported in patients with ND, it is not known if or how the specificity of these antibodies changes over time or what changes are associated with the development of BP (Chen J et al., 2011; Foureur et al., 2006; Kokkonen et al., 2017; Messingham et al., 2016). Furthermore, differences in the immunologic mechanisms leading to BP (with or without ND) could influence the manifestation of disease, autoantibody profiles, or both.

This study found a significantly shorter duration of skin symptoms before BP diagnosis in patients with preceding ND. Although this observation may be biologically significant, it must be interpreted with caution. In patients with ND, the duration of skin symptoms, such as itch, erosions, or eczema, could be easily underestimated or go unnoticed by their representatives or caretakers. Indeed, the fact that preceding ND was not associated with any differences in clinical presentation or severity of BP suggests that this may be the case.

In this report, preceding ND was not associated with any significant differences in the incidence or specificity of the IgG or IgE antibody response, which is in agreement with a previous study that examined only IgG (Gornowicz-Porowska et al., 2017). Additionally, BP180 IgG levels correlated with both age at BP onset and disease severity regardless of ND. Thus, although the events leading to the development of cutaneous antibodies might differ, preceding ND may not alter the pathogenic mechanisms driving BP. With regard to the IgE autoantibody profiles, a larger number of patients are needed to tease out some potential differences associated with type of ND. Going forward, it would also be interesting to compare serum IgE reactivity to brain isoforms of BP180 and BP230 based on the relative abundance of BP230 in the central nervous system (Brown et al., 1995) and the robust BP230-IgE response found here.

The possibility remains that preceding ND results in differences in autoantibody specificity that are not evident when the

Table 2. Analysis of serum IgG autoantibodies

	BP180 IgG[2]		BP230 IgG[2]	
Comparison[1]	Mean Ratio[3] (95% CI)	*P*	Mean Ratio (95% CI)	*P*
ND + BP vs. BP CON	1.234 (0.788–1.933)	0.3536	1.493 (0.823–2.708)	0.1840
PD + BP vs. BP CON	1.029 (0.626–2.337)	0.5675	1.241 (0.516–2.981)	0.6257
DEM + BP vs. BP CON	1.020 (0.541–1.922)	0.9510	1.500 (0.646–3.487)	0.3408
STR + BP vs. BP CON	1.560 (0.756–3.217)	0.2249	1.798 (0.687–4.711)	0.2284
PD + BP vs. STR + BP	0.775 (0.316–1.092)	0.5735	0.690 (0.209–2.277)	0.5373
DEM + BP vs. STR + BP	0.654 (0.271–1.575)	0.3387	0.834 (0.259–2.688)	0.7585
PD + BP vs. DEM + BP	1.186 (0.519–2.711)	0.6827	0.827 (0.275–2.484)	0.7314

Abbreviations: BP, bullous pemphigoid; CI, confidence interval; CON, control; DEM, dementia; ND, neurologic disease; PD, Parkinson disease; STR, stroke.
[1]Patients included BP control individuals (BP CON) and patients with BP with preceding neurologic disease (ND), Parkinson's disease (PD + BP), dementia (DEM + BP), and stroke (STR + BP).
[2]IgG antibodies specific for BP180 and BP230 were measured by ELISA.
[3]Mean ratios, 95% CIs, and *P*-values are indicated for each comparison. A *P*-value less than 0.0071 was considered significant.

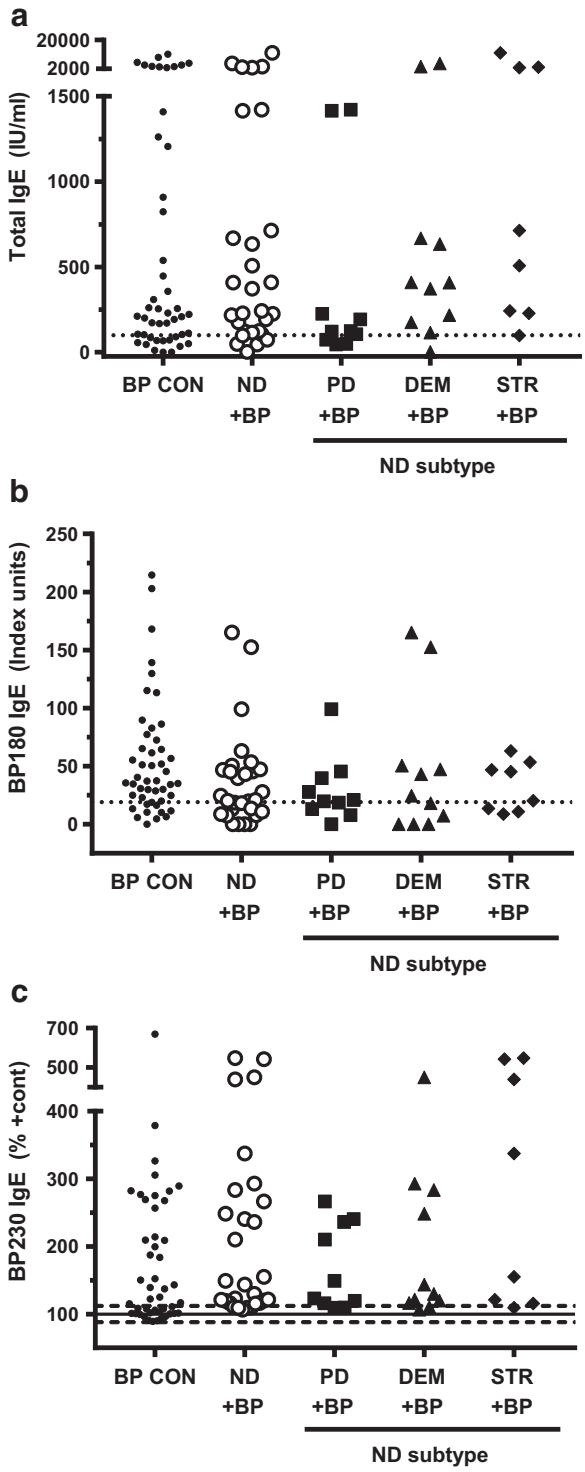

Figure 3. Serum IgE antibody levels in patients with BP with and without neurologic disease. Participants were BP control individuals (BP CON) and patients with BP with preceding neurologic disease (ND), Parkinson disease (PD + BP), dementia (DEM + BP), and stroke (STR + BP). **(a)** Circulating total IgE was measured with electrochemiluminescence; normal level ≤ 100 U/ml (dashed line). **(b)** BP180-specific IgE was measured by ELISA, positive test ≥ 19 units/ml. **(c)** BP230-specific IgE was measured by ELISA; mean ± standard deviation is indicated for n = 23 healthy control individuals. Each point represents an individual patient. No statistically significant differences were observed. BP, bullous pemphigoid; CI, confidence interval; CON, control; DEM, dementia; ND, neurologic disease; PD, Parkinson disease; STR, stroke.

commercially available ELISAs are used. These kits detect only antibodies targeting a relatively small domain of the protein, known as NC16A, because their pathogenicity in BP is well established (Giudice et al., 1993; Zillikens et al., 1997). However, it is widely accepted that patients with BP often have serum reactivity to regions of BP180 that are outside of NC16A (Fairley et al., 2013). Furthermore, the epitope specificity may depend on the protein isoform driving the antibody response. In agreement, differences in serum reactivity to brain- or skin-derived proteins suggest that patients with ND (and not BP) recognize different autoepitopes than patients with BP (Foureur et al., 2006; Kokkonen et al., 2017; Messingham et al., 2016). Indeed, a recent study (Tuusa et al., 2019) showed, via epitope mapping of recombinant proteins, that sera from participants with multiple sclerosis or Alzheimer disease recognize different regions of the BP180 protein than sera from patients with BP. These findings explain why skin symptoms are not observed when BP180 antibodies are observed in patients with ND but not BP. To determine if BP180- and BP230-reactive antibodies play a role in the progression to BP, and if epitope spreading is required for the development of BP, longitudinal studies using detailed epitope mapping of reactive sera from patients with well-characterized ND are needed.

Because only a fraction of individuals with ND go on to develop BP, the goal of this study was to determine if any specific characteristics of these patients could be discerned. However, other than the timing of the disease, we did not see any clinical or serologic phenotype that differentiates patients with ND compared with those who do not. Furthermore, the medications for ND are commonly used and have not themselves been associated with BP at this point. Thus, additional studies are needed to further characterize these patients, and as personalized medicine advances, we may be able to identify predictive factors for the development of BP in the context of ND.

MATERIALS AND METHODS

Study participants

Patients with clinical and histologic characteristics of BP were recruited from the University of Iowa hospitals and clinics, and written informed consent was obtained in compliance with the guidelines of the institutional review board (#201106752) and the Declaration of Helsinki. A BP diagnosis was confirmed by detection of cutaneous autoantibodies via direct immunofluorescence, BP180/BP230 ELISA, or immunoblot against the recombinant extracellular domain of BP180 (Fairley et al., 2013).

A self-reported history and medical records were used to identify preceding ND. Of the 112 patients with BP initially identified, two patients diagnosed with an unspecified autoimmune central nervous system disease were excluded. The remaining 110 patients with BP were included: 77 BP CON, 11 PD + BP, 11 DEM + BP, and 11 STR + BP. The type of DEM, location and number of STRs, and elapsed time from the STR to the onset of BP were heterogeneous. None of the patients in our database had multiple sclerosis.

Disease severity

Disease severity was scored with the BPDAI (Murrell et al., 2012). Because some patients were enrolled before validation of the BPDAI, scores were available on only a subset of patients: 37 BP CON, 11 PD + BP, 9 DEM + BP, and 7 STR + BP.

Table 3. Analysis of serum IgE antibodies

	BP180 IgE[2]		BP230 IgE[2]		Total IgE[3]	
Comparison[1]	Mean Ratio[4] (95% CI)	*P*	Mean Ratio (95% CI)	*P*	Mean Ratio (95% CI)	*P*
ND + BP vs. BP CON	0.760 (0.504–1.139)	0.1801	1.195 (0.934–1.530)	0.1540	0.889 (0.443–1.787)	0.7383
PD + BP vs. BP CON	0.581 (0.325–1.038)	0.0661	0.944 (0.665–1.340)	0.7429	0.290 (0.107–0.787)	0.0159
DEM + BP vs. BP CON	1.135 (0.617–2.089)	0.6789	1.084 (0.774–1.519)	0.6331	0.778 (0.297–2.035)	0.6040
STR + BP vs. BP CON	0.586 (0.318–1.077)	0.0804	1.663 (1.132–2.443)	0.0100	1.792 (0.598–5.372)	0.2933
PD + BP vs. STR + BP	0.992 (0.458–2.150)	0.9844	0.568 (0.353–0.914)	0.0205	0.162 (0.041–0.631)	0.0094
DEM + BP vs. STR + BP	1.939 (0.875–4.296)	0.1013	0.652 (0.409–1.040)	0.0720	0.434 (0.114–1.648)	0.2164
PD + BP vs. DEM + BP	0.512 (0.236–1.109)	0.0884	0.870 (0.561–1.350)	0.5299	0.372 (0.106–1.306)	0.1209

Abbreviations: BP, bullous pemphigoid; CI, confidence interval; CON, control; DEM, dementia; ND, neurologic disease; PD, Parkinson disease; STR, stroke.
[1]Participants included BP control individuals (BP CON) and patients with BP with preceding neurologic disease (ND); Parkinson disease (PD + BP), dementia (DEM + BP), and stroke (STR + BP).
[2]IgE autoantibodies specific for BP180 and BP230 were measured by ELISA.
[3]Total IgE was measured electrochemiluminescence.
[4]Mean ratios, 95% CIs, and *P*-values are indicated for each comparison. A *P*-value less than 0.0071 was considered significant.

Serum antibody detection

Serum antibodies were measured only patients who had not received any prior corticosteroids or immunomodulatory treatment: 47 BP CON, 10 PD + BP, 11 DEM + BP, and 8 STR + BP. IgG antibodies specific for BP180 and BP230 were evaluated with commercial ELISAs (MBL International, Woburn, MA). This study included nine participants (7 BP CON, 2 DEM + BP) whose results were negative from the BP180 ELISA that targets the immunodominant NC16A region of BP180. BP180 ELISA-negative patients were included in the study if serum IgG reactivity to recombinant full-length BP180 was verified with immunoblot (Fairley et al., 2013). Additionally, IgE autoantibodies specific for BP180 and/or BP230 were typically observed in these patients.

Total serum IgE levels were quantified with electrochemiluminescence performed by the institutional pathology laboratory. BP180 (NC16A)-specific IgE was evaluated by ELISA as described (Messingham et al., 2009). BP230-specific IgE was evaluated with the IgG kit with the substitution of an anti-IgE detection antibody that was previously verified as IgE-specific via immunoblot and ELISA.

Statistical analysis

Statistical analysis was performed with assistance from Patrick Ten Eyck at the Institute for Clinical and Translational Science Biostatistics Core at the University of Iowa. A chi-square test was used to assess differences in sex ratios. Generalized linear models were used to compare the values of several outcome measures between different groups. Because all outcomes followed a right-skewed distribution, a gamma distribution and log link were specified to provide pairwise group estimates for the ratio of group means, along with *P*-values. SAS, version 9.4 (SAS Institute, Cary, NC) was used for all generalized linear models analyses. We decided on a type I error rate, α, of 0.05, which necessitates a *P*-value of 0.0071 or less for significance, based on the Bonferroni correction for seven separate comparisons.

Data availability statement

Primary research data, including deidentified patient demographics and classification, disease severity scores, and raw ELISA values, are available free of charge to all researchers wherever possible and with minimal reuse restrictions.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: KNM, NSN, JAF; Data Curation: JAF; Formal Analysis: KNM; Funding Acquisition: KNM, NSN, JAF; Investigation: KNM, NSN, SJC, JAF; Methodology: JAF; Project Administration: KNM, JAF; Resources: NSN, JAF; Supervision: KNM, JAF; Validation: SJC, JAF; Visualization: KNM; Writing - Original Draft Preparation: KNM, JAF; Writing - Review and Editing: KNM, NSN, JAF

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.01.034.

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Supplementary Table S1. Sex and ethnicity of patients with BP grouped by type of preceding neurologic disease[1]

			Type of Neurologic Disease (n = 33)		
	BP CON (n = 77)	ND + BP (n = 33)	PD + BP (n = 11)	DEM + BP (n = 11)	STR + BP (n = 11)
Sex					
Male/female[2]	35/42	14/19	7/4	3/8	5/6
Ethnicity[3]					
Caucasian	71 (92%)	30 (91%)	11 (100%)	9 (90%)	10 (91%)
Jewish	4 (5%)	0	0	0	0
Black	1 (1%)	1 (3%)	0	0	1 (9%)
Hispanic	2 (3%)	0	0	0	0
Asian	2 (3%)	0	0	0	0
Native American	0	0	0	0	0
Mixed	0	0	0	0	0
Unspecified	1 (1%)	1 (3%)	0	1 (10%)	0

Abbreviations: BP, bullous pemphigoid; CON, control; DEM, dementia; ND, neurologic disease; PD, Parkinson disease; STR, stroke.

[1]Groups were BP control individuals (BP CON) or patients with BP and neurologic disease (ND + BP), Parkinson disease (PD + BP), dementia (DEM + BP), and stroke (STR + BP).

[2]No significant differences in sex ratios or ethnicity were observed.

[3]Expressed as the number (percentage) of individuals in each group.

Supplementary Table S2. Incidence and concentration of circulating antibodies in patients with BP with and without neurologic disease[1]

			Type of Neurologic Disease (n = 29)		
	BP CON (n = 47)	BP + ND (n = 29)	PD + BP (n = 10)	DEM + BP (n = 11)	STR + BP (n = 8)
BP180 IgG [2]					
Positive, n (%)	40 (85)[6]	27 (93)	10 (100)	9 (82)	8 (100)
Median, IU (range)	57.7 (0.5–221.7)	72.9 (3.2–251.1)	74.4 (33.8–193.8)	57.6 (3.2–179.6)	109.8 (12.9–251.1)
BP230 IgG[2]					
Positive, n (%)	30 (64)	20 (69)	7 (70)	7 (64)	6 (75)
Median, IU (range)	17.6 (0.4–111)	45.2 (0.1–129.2)	28.6 (0.1–106.5)	46.6 (0.4–121.7)	63.3 (1.7–129.2)
Total IgE[3]					
ANR, n (%)	28 (60)	24 (83)	7 (70)	10 (91)	7 (78)
Median, IU/ml (range)	222 (1–10,924)	243.1 (2.7–11,835)	122.5 (46–1,422)	408 (2.7–5,123)	609.8 (98–11,835)
BP180 IgE[4]					
Positive, n (%)	36 (77)	19 (66)	7 (70)	6 (55)	6 (75)
Median, index units (range)	34.8 (0–214.6)	21.1 (0–165.0)	20.4 (0–99.2)	24.6 (0–165.0)	32.8 (8.8–63.2)
BP230 IgE[5]					
ANR, n (%)	28 (60)	24 (83)	7 (70)	10 (91)	7 (86)
Median, index units (range)	131.2 (89–670)	143.8 (106.7–547.5)	6.2 (109–266)	129.9 (106.7–451)	246.2 (109–547.5)

Abbreviations: ANR, above normal range; BP, bullous pemphigoid; CON, control; DEM, dementia; IU, international units; ND, neurologic disease; PD, Parkinson disease; STR, stroke.

[1]Groups were BP control individuals (BP CON) or patients with BP and neurologic disease (ND + BP), Parkinson disease (PD + BP), dementia (DEM + BP), and stroke (STR + BP).

[2]Minimum cutoff for positive ELISA test result = 9 U/ml.

[3]Measured using electrochemiluminescence, normal level = 100 IU/ml.

[4]Minimum cutoff for positive ELISA test = 19 units/ml.

[5]ELISA expressed as percent control supplied with kit (average optical density = 100%). Normal range, 96.4 ± 11.3 index units (mean ± standard deviation), was established using 23 healthy control individuals.

[6]Chi-square analysis did not show any differences in the percentage positive for any of the antibodies examined.