Anti–Bullous Pemphigoid 180 and 230 Antibodies in a Sample of Unaffected Subjects

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Objective: To evaluate the prevalence of autoantibodies against 2 hemidesmosomal proteins typically found in patients with bullous pemphigoid (BP), BP antigen II (BP180) and BP antigen I (BP230), in persons without BP.

Design: Cross-sectional study.

Setting: Academic medical center.

Patients: An age- and sex-stratified, random, population-based sample of local county patients seen during 2007: 20 men and 20 women per decade of age (from age 20 to 89 years) and 57 patients (33 women and 24 men) aged 90 to 99 years.

Intervention: Stored serum samples were retrieved for analysis by enzyme-linked immunosorbent assay and indirect immunofluorescence.

Main Outcome Measure: Presence of circulating autoantibodies to BP180 and BP230.

Results: Of the 337 study patients, 25 (7.4%) were positive for 1 or both autoantibodies; these 25 samples all tested negative with indirect immunofluorescence. Autoantibody levels did not vary by age or sex.

Conclusions: Bullous pemphigoid has a higher incidence in the elderly population, but the prevalence of antibodies to BP180 and BP230 did not increase significantly with age or vary by sex in this population-based sample. Other exogenous factors may affect the development of these autoantibodies in a population without clinically evident immunobullous disease, including limitations inherent to the test (false-positive rate).

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Bullous pemphigoid (BP) is an acquired autoimmune bullous disorder characterized clinically by tense subepidermal bullae arising on normal or erythematous skin. The pathogenesis of BP has been characterized by circulating autoantibodies directed against basement membrane zone hemidesmosomal proteins. Diagnosis is established by a combination of studies including histopathologic assessment by routine microscopy, direct immunofluorescence studies to

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Intracellular component of the hemidesmosomal plaque, and BP antigen II (BP180), a 180-kDa transmembrane protein. Antibodies against BP180 most commonly react with epitopes in the 16th noncollagenous domain (NC16a). Serum levels of autoantibodies to BP180-NC16a have been shown to parallel disease activity. Furthermore, subepidermal blisters have been reproduced in animal models and in cryosections of human skin when they were exposed to serum samples of patients with BP, which confirms the pathogenicity of BP180 autoantibodies.

Epidemiologic studies show that BP has a higher incidence in the elderly population. A study of the incidence of BP in 3 French populations found the mean age at the onset of BP to be 82 years, and a Polish study found the mean age to be 69 years for women and 67 years for men. These findings parallel those of a study of the German population, which showed that the risk of BP rapidly increases in patients older than 60 years, with patients older than 90 years having the highest incidence of the disease.

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Abundant evidence supports the presence of autoantibodies to BP180 and BP230 in patients with BP, along with the established pathogenicity of the autoantibodies and their relationship to disease activity. Little is known, however, about the prevalence of BP autoantibodies in the general population. Therefore, our main objective was to determine the prevalence of these autoantibodies in a population-based sample of patients without underlying immunobullous disease. Studies have shown an enzyme-linked immunosorbent assay (ELISA) for BP180 and BP230 autoantibodies to be both sensitive and specific for the disease. We used this ELISA technique to examine serum samples of patients without BP for the presence of autoantibodies against BP180 and BP230.

This study was approved by the Mayo Clinic Institutional Review Board. The source population for the study was patients residing in Olmsted County, Minnesota, who were seen at Mayo Clinic in Rochester, Minnesota, during 2007 and had a serum sample taken.

Study participants were identified from a “waste-blood” list that is compiled daily, which includes all excess serum samples originally collected for clinical purposes but subsequently made available for research use. We used a series of previously developed automated programs to search the waste-blood lists for subjects meeting the study criteria.

The study included Olmsted County residents who had provided research authorization and were aged 20 to 99 years at the time of sample collection. Persons younger than 20 years and those with a history of clinically diagnosed celiac disease or who previously had a blood sample taken as part of clinical evaluation for celiac disease were excluded from the study. The HICDA (Hospital International Classification of Diseases Adapted) codes for all samples were subsequently reviewed to exclude all cases of diagnosed pemphigoid or pemphigus syndromes.

For all identified patients meeting the study criteria, stratified sampling was applied to ensure age and sex balance. The final study sample was assembled by randomly selecting 40 patients (20 women and 20 men) per decade of age from 20 to 89 years and 57 patients (33 women and 24 men) aged 90 to 99 years. For these 337 patients, serum samples were retrieved for analysis. These serum samples had been stored, and were no longer needed after being held in the central laboratory for the standard 6 days, and were still available for testing (waste serum samples).

Commercially available BP180-NC16a and BP230 ELISA kits (Medical and Biological Laboratories Co Ltd, Nagoya, Japan) were used to test the serum samples for the presence of autoantibodies to the BP antigens. Reactivity to BP180 and BP230 was analyzed by ELISA using purified recombinant forms of antibodies to the BP antigens. Reactivity to BP180 and BP230 were used to test the serum samples for the presence of autoantibodies. Reactivity to BP180 and BP230 (amino and carboxy terminals) was analyzed by ELISA using purified recombinant forms of antibody to the BP antigens.

Autoantibodies were further tested with IIF. Indirect immunofluorescence was performed with monkey esophagus as a tissue substrate, which was overlaid with the serum samples, washed, and subsequently overlaid with fluorescein isothiocyanate–tagged antihuman IgG. Antibody titer is determined using monkey esophagus with serial dilutions of the serum samples. This method will detect the presence of circulating antibodies against basement membrane zone components including BP180 and BP230 (Michael J. Camilleri, MD, unpublished data, 2009).

The relationship of autoantibody levels to age and sex was assessed graphically with scatter plots. A smooth curve was fit to each scatter plot to summarize the pattern separately for men and women. The smoothed curves were generated by fitting a nonparametric smoother to the data using a cubic spline routine. Given that the distributions of the autoantibody levels were positively skewed, logarithmic transformations were applied and the average results were summarized using the geometric mean. General linear regression models were fitted to assess the relationship of autoantibody levels (after logarithmic transformation) to age and sex. All analyses were performed using the SAS software package, version 9.0 (SAS Institute Inc, Cary, North Carolina). P < .05 was considered statistically significant.

Of the 337 patients without known bullous disease who were included in the sample, 25 (7.4%; 95% confidence interval, 4.6%-10.2%) had positive results by ELISA (≥9 U/mL) for autoantibodies against BP180 only (n=11), BP230 only (n=11), or both (n=3) (Table). Neither sex nor age was significantly associated with a positive test result (χ² test, P=.24; 2-sample t test, P=.88). Among these 25 patients with positive results, 15 (60.0%) were men and the mean (SD) age was 61.2 (24.9) years. Among the 312 patients with negative results, 149 (47.8%) were men and the mean (SD) age was 62.0 (23.1) years. The incidence of a positive result was 7.4% among all patients, 7.3% among patients younger than 60 years, and 7.6% among patients 60 years or older.

BP180 and BP230 autoantibody levels did not appear to vary significantly with age or sex (Figure). Levels of both antibodies remained stable across the age groups. After adjusting for age, the geometric mean of the levels did not vary significantly by sex (women vs men, BP180, 1.44 vs 1.56 U/mL [P=.47]; BP230, 1.47 vs 1.52 U/mL [P=.77]).

To further rule out a history of another autoimmune bullous disorder or undetected BP, all 25 ELISA-positive serum samples were tested with IIF, and the medical records of those patients were reviewed for any potentially relevant data that could suggest an underlying clinically occult autoimmune bullous disorder. All serum samples showed negative IIF results (Table). Antibodies to the NC16a domain of BP180 are seen in herpes gestationsis as well as BP. Therefore, the clinical histories of female patients with positive BP180 or BP230 ELISA results were reviewed for a history of pregnancy or use of hormone therapy. In this subgroup, nothing in the history suggested the presence of clinical or laboratory features typical of herpes gestationsis. One woman had a newly diagnosed connective tissue disorder. Several patients had a reported history of dermatitis, but none of these cases were noted to be urticarial or suggestive of BP. In addition a review of the patient-completed family history forms was available for 24 of 25 patients did not reveal any family history of immunobullous disease, including BP or herpes gestationsis. Information in the medical history that was...
considered irrelevant was reviewed but excluded from the Table.

**COMMENT**

To our knowledge, this is the first study to examine the prevalence of anti-BP180 and anti-BP230 antibodies in a nondisease (non-BP) population-based sample of patients stratified by age and sex. Although studies have shown a higher incidence of BP in the elderly population,

Table. Summary of the 25 Patients With Positive ELISA Results for Autoantibodies to BP180 and/or BP230

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>ELISA, U/mL</th>
<th>IIF</th>
<th>Relevant Medical History (Since 1994)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/22</td>
<td>11.3</td>
<td>4.3</td>
<td>None</td>
</tr>
<tr>
<td>2/F/25</td>
<td>16.3</td>
<td>12.4</td>
<td>Recent diagnosis of connective tissue disease (elevated ANA and RNP levels)</td>
</tr>
<tr>
<td>3/F/29</td>
<td>13.0</td>
<td>8.8</td>
<td>History of OC use</td>
</tr>
<tr>
<td>4/M/30</td>
<td>20.2</td>
<td>0.8</td>
<td>None</td>
</tr>
<tr>
<td>5/M/35</td>
<td>4.2</td>
<td>15.3</td>
<td>None</td>
</tr>
<tr>
<td>6/F/35</td>
<td>5.5</td>
<td>11.1</td>
<td>G2, P2, history of OC use</td>
</tr>
<tr>
<td>7/F/38</td>
<td>3.8</td>
<td>9.2</td>
<td>G0, infertility with IVF attempts, history of OC use</td>
</tr>
<tr>
<td>8/M/42</td>
<td>3.4</td>
<td>10.4</td>
<td>Allergic contact dermatitis (1999)</td>
</tr>
<tr>
<td>9/F/44</td>
<td>9.2</td>
<td>1.1</td>
<td>G5, P3 (potential miscarriage history unknown)</td>
</tr>
<tr>
<td>10/M/46</td>
<td>18.5</td>
<td>4.3</td>
<td>None</td>
</tr>
<tr>
<td>11/M/48</td>
<td>1.7</td>
<td>11.0</td>
<td>None</td>
</tr>
<tr>
<td>12/F/55</td>
<td>12.3</td>
<td>1.8</td>
<td>G0, HT for 10 y</td>
</tr>
<tr>
<td>13/M/68</td>
<td>1.8</td>
<td>10.3</td>
<td>None</td>
</tr>
<tr>
<td>14/F/68</td>
<td>16.3</td>
<td>5.5</td>
<td>G3, P unknown, HT for &gt;5 y</td>
</tr>
<tr>
<td>15/F/76</td>
<td>1.4</td>
<td>9.7</td>
<td>None</td>
</tr>
<tr>
<td>16/M/77</td>
<td>5.3</td>
<td>15.2</td>
<td>History of palm psoriasis/dyshidrotic eczema</td>
</tr>
<tr>
<td>17/M/77</td>
<td>13.1</td>
<td>12.5</td>
<td>Psoriasis (scalp)</td>
</tr>
<tr>
<td>18/M/78</td>
<td>12.4</td>
<td>3.9</td>
<td>None</td>
</tr>
<tr>
<td>19/M/79</td>
<td>8.5</td>
<td>9.1</td>
<td>None</td>
</tr>
<tr>
<td>20/F/86</td>
<td>14.8</td>
<td>3.6</td>
<td>None</td>
</tr>
<tr>
<td>21/F/91</td>
<td>4.6</td>
<td>9.2</td>
<td>Localized dermatitis (1996)</td>
</tr>
<tr>
<td>22/M/91</td>
<td>9.8</td>
<td>4.5</td>
<td>None</td>
</tr>
<tr>
<td>23/M/92</td>
<td>2.4</td>
<td>12.1</td>
<td>Asteatotic dermatitis (2005), cutaneous eruption NOS (2003)</td>
</tr>
<tr>
<td>24/M/93</td>
<td>25.8</td>
<td>9.3</td>
<td>None</td>
</tr>
<tr>
<td>25/M/97</td>
<td>33.1</td>
<td>1.8</td>
<td>Truncal cutaneous eruption (guttate psoriasis, 2005), nummular dermatitis (1997)</td>
</tr>
</tbody>
</table>

Abbreviations: ANA, antinuclear antibody; BP180, BP antigen II; BP230, BP antigen I; ELISA, enzyme-linked immunosorbent assay; G, gravida, pregnancies; HT, hormone therapy; IIF, indirect immunofluorescence; IVF, in vitro fertilization; NOS, not otherwise specified; OC, oral contraceptive; P, para, live births; RNP, ribonucleoprotein complex; −, negative result. Bold numbers represent positive ELISA values (≥9 U/mL).

One might expect that if BP was caused by the presence of autoantibodies alone, a higher incidence of these antibodies would be found in the older age groups. Overall, the incidence of positive ELISA results was 7.3% among patients younger than 60 years and 7.6% among patients 60 years and older. From this, we can speculate that secondary triggering or initiating factors may be involved in the induction of BP in susceptible persons. In a multicenter case-control study by Bastuji-Garin et al,10 the long-term use of aldosterone-antagonist diuretics...
may be necessary for the development of BP. Much focus has been placed on the BP180 autoantibody reactivity to the NC16a domain. However, Mariotti et al\textsuperscript{22} identified a subgroup of BP180-NC16a-negative patients in whom antibodies reacted with different BP180 epitopes. Perhaps a greater number of elderly patients have antibodies to other epitopes or additional antibody targets completely different from BP180 and BP230. In addition to a lack of difference in antibody profiles among decades, sex differences were not seen in our study. Because antibodies to the NC16a domain of BP180 are seen in herpes gestations (pemphigoid of pregnancy) as well as BP,\textsuperscript{15,16} an increased prevalence of antibodies may be expected among women. Because we did not find this result, a role for potentially undiagnosed cases of herpes gestationis or a hormonal association in our patients with positive ELISA test results is not likely. Pemphigoid of pregnancy is a rare disease, and its confirmation requires careful clinicopathologic correlation.

The incidence rate of 7.4\% might represent the false-positive rate of the test in our hands, but it also could constitute an aggregate of false-positive and true-positive test results, which did not change significantly across the age groups. In contrast, the prevalence of BP is so low that the prevalence of autoantibodies may increase significantly, but the number of cases studied was too small or the study was not sufficiently powered to measure this change. The latter explanation is more likely and represents a significant limitation of this study, given the high sensitivity of the ELISA test, low incidence of BP, and the total number of cases. Despite the high levels of sensitivity and specificity reported for the BP180 and BP230 ELISAs, the ultimate and most accurate diagnosis of BP depends on careful correlation of the clinical findings with histopathologic, immunopathologic, and serologic features. Patients who have negative results in any of these parameters will present challenges to diagnosis and may require close clinical follow-up with serial testing to establish the diagnosis. As we learn more about BP susceptibility and induction patterns, additional factors, other than the presence of anti-basement membrane antibodies, that have a role in disease pathogenesis will emerge. These factors could then potentially be used to aid in diagnosis, particularly in early stages of disease.

Ultimately, our findings raise questions for future scientific study. A multifactorial pathogenetic model for BP could be considered, one that requires not just the presence of specific pathogenic autoantibodies but also additional triggers (eg, environmental factors, comorbid conditions, drug exposures, concurrent or preceding infections) that may have a role in disease initiation within a susceptible person. In addition, there may be as yet unidentified antigenic epitopes that are necessary for disease development and that the current ELISA tests are unable to identify.

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Author Contributions: Dr Comfere had full access to all the data in the study and takes responsibility for the

Figure. Enzyme-linked immunosorbent assay value for BP180 (BP antigen II) (A) and BP230 (BP antigen I) (B) autoantibodies vs patient age. Smooth curves were fit separately for men (solid line) and women (dotted line). Dashed line shows the cutoff value of 9 U/mL for a positive result (\( \geq 9 \) U/mL).
integrity of the data and the accuracy of the data analysis. Study concept and design: Comfere, Gibson, and Krause. Acquisition of data: Wieland, Comfere, and Murray. Analysis and interpretation of data: Wieland, Comfere, Gibson, and Weaver. Critical revision of the manuscript for important intellectual content: Comfere, Gibson, Weaver, Krause, and Murray. Statistical analysis: Weaver. Administrative, technical, and material support: Gibson and Murray. Study supervision: Comfere and Gibson.

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REFERENCES