

# Usefulness of BP230 and BP180-NC16a Enzyme-Linked Immunosorbent Assays in the Initial Diagnosis of Bullous Pemphigoid

## *A Retrospective Study of 138 Patients*

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**Objective:** To investigate the diagnostic value of commercially available BP230 and BP180-NC16a enzyme-linked immunosorbent assays (ELISAs) in routine practice in patients with bullous pemphigoid (BP).

**Design:** Single-center retrospective study.

**Setting:** French academic dermatology department.

**Patients:** The study population comprised 138 patients, who were admitted from January 1998 through December 2008.

**Interventions:** Sera samples were analyzed by ELISA; clinical and immunopathological data were recorded from the patients' medical charts.

**Main Outcome Measures:** BP230 and BP180-NC16a ELISA scores were evaluated with respect to clinical characteristics (number of blisters, mucosal involvement, localized or generalized disease, and outcome) and routine indirect immunofluorescence (IF).

**Results:** Of the 138 study patients, 81 (59%) had a positive BP230 ELISA result and 119 (86%) had a positive BP180 ELISA result. There was no relationship between a positive ELISA BP230 result and the disease extent at diagnosis or the presence of mucosal involvement. Serum anti-basement membrane zone autoantibodies (indirect IF) were more frequently detected when the BP230 ELISA result was positive ( $P < .001$ ). The median anti-basement membrane autoantibody titer as detected by indirect IF was higher in patients with a positive BP230 result ( $P < .001$ ). The BP180 ELISA result was associated with disease extent at diagnosis as estimated by both the percentage of patients with extensive BP ( $P = .01$ ) and the mean number of blisters ( $P = .03$ ) but was not associated with mucosal involvement.

**Conclusions:** The currently available BP230 ELISA is a reliable although less-sensitive test than BP180 ELISA in BP, and its diagnostic added value compared with BP180 ELISA alone is approximately 5%. Our results support the predominant contribution of the BP230-specific autoantibodies to anti-basement membrane zone antibody titer as detected by indirect IF.

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**B**ULLOUS PEMPHIGOID (BP) IS the most frequent subepidermal autoimmune blistering disorder of the skin that usually affects elderly persons.<sup>1,2</sup> Autoantibodies predominantly recognize BP230 (BPAG1) and BP180 (BPAG2 or type XVII collagen), 2 structural components of junctional adhesion

proteins and synthetic peptides suggested that BP sera react preferentially with C-terminal domains of BP230,<sup>5-10</sup> and a study using an eukaryotic expression system showed that multiple epitopes in various domains of BP230 are recognized by BP sera.<sup>11</sup> In contrast, BP180 is a transmembrane molecule with a collagenous extracellular domain involved in cell adhesion, and epitope mapping studies of human BP180 have shown that the NC16a domain of this protein harbors the major antigenic sites recognized by BP sera.<sup>12-14</sup>

### *See Practice Gaps at end of article*

complexes called hemidesmosomes.<sup>2,3</sup> BP230 is a cytoplasmic component belonging to the plakin protein family that consists of a central coiled-coil region flanked by 2 globular end domains.<sup>4</sup> Published studies using various recombinant

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Detection of serum autoantibodies can be assessed easily by standard indirect immunofluorescence (IF) techniques or indi-

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rect IF on salt-split skin. However, these methods are difficult to standardize and dependent on the operator's skills. Several enzyme-linked immunosorbent assays (ELISAs) using recombinant proteins encompassing various region of BP180 have been developed, with a sensitivity ranging from 60% to 95%.<sup>12,13,15-18</sup> The sensitivity of BP180 ELISA is higher when antibody detection is performed using several extracellular motifs, including the BP180-NC16a domain and other extracellular regions of BP180 recombinant protein.<sup>12</sup> A commercially available BP180 ELISA using bacterial recombinant protein of BP180-NC16a domain showed a sensitivity of 84% and a specificity of 99%.<sup>15</sup> The index values of the BP180 ELISA tended to fluctuate in parallel with the disease activity along the time course of patients with BP and reflected the disease activity much better than indirect IF.<sup>15,16,19</sup> Finally, a recent study from our group also showed that a high-titer anti-BP180 ELISA result is a good indicator of further relapse of BP after cessation of therapy.<sup>20</sup>

Similar to BP180, several groups developed the BP230 ELISA using various recombinant proteins. Kromminga et al<sup>21</sup> developed an ELISA system using 5 overlapping recombinant fragments covering the entire BP230 molecule, which showed a sensitivity of 63%. Thoma-Uszynski et al<sup>17</sup> also reported results on ELISAs for both BP180 and BP230 using baculovirus expression system, showing a sensitivity of 82% for the BP230 ELISA. To our knowledge, only 2 studies to date have evaluated the diagnostic accuracy of a commercially available ELISA for the detection of anti-BP230 antibodies in patients with BP,<sup>22,23</sup> reporting a sensitivity of 59% to 60% and a specificity of 98% to 99%. However, the relationship between BP230 ELISA values and clinical activity of BP was unclear in the study of Yoshida et al<sup>23</sup> and was not investigated in the small retrospective series reported by Tampoia et al.<sup>22</sup> The main objective of the present large retrospective study was to investigate the diagnostic value of this commercial BP230 ELISA used together with BP180-NC16a ELISA in routine dermatologic practice in patients with BP.

## METHODS

### STUDY PATIENTS

This retrospective, single-center, descriptive study was conducted in patients with a diagnosis of BP admitted (both as inpatients and outpatients) in our department of dermatology from January 1998 to December 2008, for whom a sera sample at the time of diagnosis was available.

Patients had been diagnosed as having BP using the following criteria: clinical features suggestive of BP according to the clinical criteria of Vaillant et al<sup>24</sup>; subepidermal blister on skin biopsy; and detection of IgG and/or C3 deposits in a linear pattern along the epidermal basement membrane zone by direct IF.

### CLINICAL AND ROUTINE LABORATORY DATA

For each patient, clinical and routine immunopathological data available at the time of the initial diagnosis were retrospectively recorded from the patients' medical charts. These data included demographic variables (sex and age at diagnosis), clinical characteristics of the disease (daily number of new blisters during the

3 days before starting therapy, localization of blisters and erosions, mucosal involvement, localized or generalized (ie, blisters involving  $\geq 2$  anatomic sites) disease, precise anatomic site if localized disease), titers of serum anti-basement membrane zone autoantibodies by standard indirect IF on monkey esophagus, and indirect IF using salt-split skin substrate.

Regarding clinical course, we recorded the following data: delay (in days) until clinical remission with therapy and clinical status (remission with or without therapy, active disease, or death) at the 12-month follow-up.

## BP230 AND BP180 ELISAs

To determine anti-BP230 and anti-BP180-NC16a antibody titers, specific commercial ELISA tests (MBL Co Ltd, Nagoya, Japan) were performed with patients' sera diluted at 1:100, following the manufacturer's instructions as previously described.<sup>15,18,23</sup> By standard indirect ELISA methods, 450-nm optical density values were obtained using a microplate reader, and results were evaluated as an index value calculated as  $(A_{450}[\text{sample}] - A_{450}[\text{negative control}])/(A_{450}[\text{positive control}] - A_{450}[\text{negative control}]) \times 100$ . Data are expressed as units per milliliter of serum. For the evaluation of sensitivity of ELISAs, the cutoff values proposed by the manufacturer (ie, 9 U/mL for both tests) were first considered.

## STATISTICAL ANALYSIS

Continuous variables were expressed as means (SDs).  $\chi^2$  Tests were used for comparison between qualitative variables (Fisher exact test for small samples) and nonparametric Mann-Whitney/Wilcoxon test for comparison between quantitative values. Kappa ( $\kappa$ ) coefficient test was used to evaluate concordance between qualitative variables. Two-sided *P* values less than .05 were considered statistically significant. Owing to absence of normal distribution, a nonparametric Spearman test was used for the calculation of correlation coefficient. Statistical computations were performed with SAS statistical software (version 8.2; SAS Institute Inc, Cary, North Carolina).

## RESULTS

### CHARACTERISTICS OF PATIENTS AND CLINICAL COURSE

A total of 138 patients with a clinical and immunological diagnosis of BP were included in the study. The mean age at diagnosis was 78.9 years, and the male to female ratio was 0.5:1. The number of blisters at diagnosis was available from patients' files in 126 of the 138 patients. Among these 126 patients, 68 (54%) had an extensive disease (ie,  $>10$  daily blisters), and the mean (SD) number of blisters at diagnosis was 20 (24). Only 14 of the 138 patients (10%) presented with a localized clinical variant (ie, with blisters involving only 1 anatomic site: pretibial in 7 cases, palmo-plantar in 4 cases, and perineal in 3 cases). Mucosal involvement, mainly affecting oral mucosa, was observed in 18 of 138 patients (13%).

At the time of diagnosis, among the 138 patients, serum autoantibody detection had been performed using standard indirect IF in 132 and indirect IF on salt-split skin in 113. Anti-basement zone antibodies as detected by standard indirect IF were found positive in 90 of 132 patients (68%), with a median titer of 1/100 (range, 0-1/

**Table 1. Results of BP230 and BP180 ELISAs in 138 Patients With Bullous Pemphigoid**

BP180 ELISA Result	BP230 ELISA		Total
	Positive, No.	Negative, No.	
Positive	74	45	119
Negative	7	12	19
<b>Total</b>	<b>81</b>	<b>57</b>	<b>138</b>

Abbreviation: ELISA, enzyme-linked immunosorbent assay.

3200). Anti-basement zone antibodies as detected by indirect IF using salt-split skin substrate were found positive in 70 of 113 patients (62%), with a labeling on the epidermal side only in 68 of 70 cases and on both dermal and epidermal sides in 2 of 70 cases.

The mean (SD) delay until clinical remission with therapy was 24 (17) days. One year after starting therapy, 92 patients (67%) were still alive, 41 (30%) died, and 5 (4%) were lost to follow-up. Among 85 patients who were still alive and were followed up over 12 months, 33 (39%) were in remission without any treatment, 49 (58%) were in remission while receiving therapy, and 3 patients (4%) still had active disease while receiving therapy.

#### DETECTION OF ANTI-BP230 AND ANTI-BP180 AUTOANTIBODIES BY ELISA

Among the 138 patients, 81 (59%) had a positive BP230 ELISA result (ie, titer >9 U/mL), with a mean (SD) titer of 36 (41) U/mL, and 119 (86%) had a positive BP180 ELISA result, with a mean (SD) titer of 75 (57) U/mL. A total of 126 patients (91%) had a positive ELISA result with at least 1 of these tests (**Table 1**). The concordance rate between anti-BP180 and anti-BP230 autoantibody detection using specific commercial ELISAs was 62% ( $\kappa=0.13$ ;  $P=.05$ ). Only 7 patients had anti-BP230 autoantibodies without anti-BP180 autoantibodies, whereas 45 patients had anti-BP180 autoantibodies without anti-BP230 autoantibodies. Thus, the diagnostic added value of BP230 ELISA compared with BP180 ELISA alone was 5%.

#### COMPARISON OF ELISA SCORES WITH CLINICAL FEATURES, IF TITER, AND 1-YEAR SURVIVAL

##### BP230

Results are given in **Table 2**. At the time of diagnosis, there was no significant relationship between a positive BP230 ELISA score and (1) the mean number of daily blisters ( $P=.85$ ), (2) the proportion of patients with initial extensive disease ( $P=.14$ ), or (3) the proportion of patients with a localized BP ( $P=.20$ ). There was no correlation between BP230 ELISA values and the number of new daily blisters at diagnosis ( $r=-0.06$ ;  $P=.51$ ) (data not shown). In addition, a positive ELISA BP230 score was not associated with the presence (or absence) of mucosal involvement ( $P=.82$ ) or with 1-year survival ( $P=.69$ ).

By indirect IF, serum anti-basement membrane zone autoantibodies were more frequently detected in pa-

tients with a positive ELISA BP230 result compared with those with a negative result (83% vs 49%;  $P<.001$ ; **Table 2**). In addition, the median anti-basement membrane autoantibodies as detected by indirect IF was significantly higher in patients with a positive BP230 result ( $P<.001$ ; **Table 2** and **Figure**).

##### BP180

Results are given in **Table 2**. At the time of diagnosis, the BP180 ELISA result was significantly associated with disease extent as estimated both by the percentage of patients with more than 10 daily blisters ( $P=.01$ ) and by the mean number of blisters at diagnosis ( $P=.003$ ). In addition, a localized BP was less frequent in patients with a positive BP180 ELISA result ( $P=.03$ ), and BP180 ELISA results were correlated with the number of new daily blisters at diagnosis ( $r=0.37$ ;  $P<.001$ ) (data not shown). None of the patients with a negative BP180 ELISA result had mucosal involvement. A positive BP180 ELISA result was not associated with 1-year survival ( $P=.76$ ).

Serum anti-basement membrane zone autoantibodies as detected by indirect IF were positive in 78 of 113 patients (69%) with a positive BP180 ELISA result and in 12 of 19 patients (63%) with a negative result ( $P=.61$ ), and the median titer was not different in these 2 groups of patients ( $P=.24$ ; **Table 2**).

#### COMMENT

To our knowledge, this study is the first to attempt to determine the significance, in terms of clinical presentation, extent of disease, and outcome of BP230 autoantibody detection compared with BP180 autoantibody detection using commercial ELISAs on a large series of patients with BP seen in routine dermatologic practice. We found a sensitivity of 59% for the detection of anti-BP230 autoantibodies in patients with active BP at the time of diagnosis. This value is identical to the values found in 2 previous retrospective studies performed with the same commercial ELISA, which showed that 58% and 60% of BP sera samples were positive in active stage,<sup>22,23</sup> thus demonstrating the excellent reproducibility of this test in routine practice.

In other previous studies describing noncommercial BP230 ELISAs in BP, results were more heterogeneous. Kromminga et al<sup>21</sup> reported a sensitivity of 63% in 56 sera samples from patients with BP, using 5 overlapping complementary DNA constructs covering the entire length of BP230 expressed in baculovirus-infected Sf21 insect cells. In this study, 94% of the BP sera samples that were positive by ELISA reacted with the C-terminal portion of BP230.<sup>21</sup> Thoma-Uszynski et al<sup>17</sup> reported a sensitivity of 81.5% using baculovirus-encoded recombinant proteins in 127 patients with BP. Other reported ELISA-based detection systems for autoantibodies to BP230 appeared less sensitive and specific. These assays used either synthetic peptides<sup>8</sup> or bacterial fusion proteins<sup>9</sup> covering only small portions of the molecule or a fragment of murine BP230,<sup>7</sup> respectively. Despite the reliability of the currently available BP230 ELISA, we did not find evidence of a positive or negative relationship between BP230 ELISA

**Table 2. Disease Extent, Indirect IF Titer, and 1-Year Survival in Patients With BP According to BP230 and BP180 ELISA Results**

Variable	Positive, No. (%)	Negative, No. (%)	OR (95% CI)	P Value
<b>BP230 ELISA</b>				
No. of blisters at diagnosis, mean (SD)	24 (3)	25 (5)	NA	.85
Patients with initial extensive disease <sup>a</sup>	44 (59)	24 (46)	0.58 (0.28-1.19)	.14
Patients with localized BP <sup>b</sup>	6 (7)	8 (14)	0.49 (0.16-1.50)	.20
Mucosal involvement	11 (14)	7 (12)	0.89 (0.32-2.46)	.82
Indirect IF titer, median	1/400	0	NA	<.001
Patients with a positive indirect IF result	63 (83)	27 (49)	2.21 (2.20-12.5)	<.001
Patients deceased within 1 y	23 (29)	18 (33)	0.86 (0.41-1.81)	.69
<b>BP180 ELISA</b>				
No. of blisters at diagnosis, mean (SD)	27 (3)	7 (2)	NA	.003
Patients with initial extensive disease <sup>a</sup>	64 (58)	4 (25)	0.24 (0.07-0.79)	.01
Patients with localized BP <sup>b</sup>	9 (8)	5 (26)	0.23 (0.07-0.78)	.03
Mucosal involvement	18 (15)	0	ND (0.00-1.05)	.06
Indirect IF titer, median	1/100	1/50	NA	.24
Patients with a positive indirect IF result	78 (69)	12 (63)	1.30 (0.42-3.95)	.61
Patients deceased within 1 y	36 (31)	5 (28)	1.18 (0.39-3.57)	.76

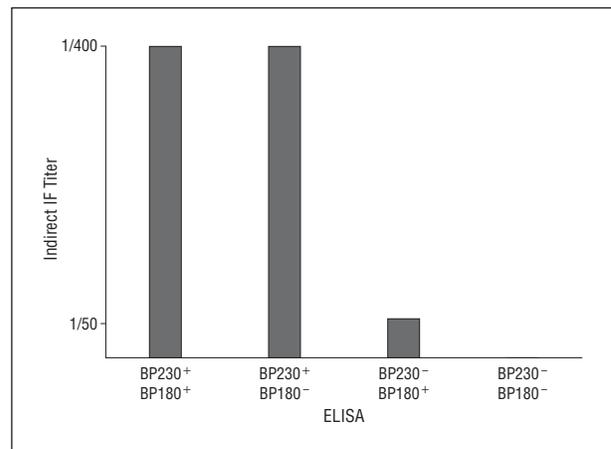
Abbreviations: BP, bullous pemphigoid; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IF, immunofluorescence; NA, not applicable; ND, not defined; OR, odds ratio.

<sup>a</sup>Patients with more than 10 daily blisters at the time of diagnosis.

<sup>b</sup>Patients with blisters localized on only 1 anatomical site.

values and the clinical extent of BP as estimated by the number of new daily blisters at the time of diagnosis. The positivity of BP230 ELISA was slightly higher in patients with initial extensive disease, ie, with more than 10 daily blisters, without reaching statistical significance (Table 2). This contrasts with the previous results of Thoma-Uszynski et al,<sup>17</sup> who found that BP230 IgG reactivity detected by ELISA using baculovirus-encoded recombinant proteins was significantly associated with the outcome “focal” BP, which was defined as only few bullae at limited areas of the body involving maximum 20% of total body surface. In the study by Yoshida et al,<sup>23</sup> sequential BP230 ELISA values were compared with clinical outcome in 10 patients with BP whose serial sera and clinical data were available, and BP230 values fluctuated in parallel with the disease activity in only 5 of 10 patients. Therefore, the present study completes previous reports<sup>17,23</sup> regarding the clinical characterization of patients with BP according to BP230 antibodies by establishing that BP230 ELISA result were not associated with the presence or absence of mucosal involvement.

In the present study, our major aim was to investigate the diagnostic value of the commercial BP230 together with the BP180-NC16a ELISA in routine practice in patients with BP, without evaluating the specificity of those tests on control sera samples, since this had already been investigated.<sup>15-23</sup> Indeed, the specificity of the commercially available BP230 ELISA was demonstrated to rise as high as 99.5% or 98.8% in previous studies in which negative control sera samples were taken from healthy subjects, patients with pemphigus vulgaris or foliaceus, or patients with unrelated dermatological conditions (eg, discoid lupus erythematosus, lichen planus).<sup>22,23</sup> The specificity of other ELISAs developed with baculovirus-expressed BP230 was 93% for Kromminga et al,<sup>21</sup> who used healthy blood donors and patients with pemphigus vulgaris or epidermolysis bullosa acquisita as negative controls, and 64.8% for Thoma-Uszynski et al,<sup>17</sup> who used healthy volunteers and



**Figure.** Median indirect immunofluorescence (IF) titers according to the detection of autoantibodies to BP230 and BP180 by specific enzyme-linked immunosorbent assays (ELISAs).

patients with unrelated conditions as controls.<sup>17</sup> Interestingly, a recent study showed that the prevalence of anti-BP230 and/or anti-BP180 serum antibodies in individuals without BP was 7.4%,<sup>25</sup> giving an overall specificity of approximately 92% when those tests are combined. However, the assessment of the specificity of ELISAs in a group of healthy subjects or in patients with pemphigus is not necessary, since both standard direct IF and indirect IF are sufficient for that in routine practice. To fully complete specificity analysis, the specificity of BP230 or BP180 ELISAs should be calculated in the future with patients with mucous membrane pemphigoid serving as controls, whose exact seroprevalence of anti-BP230 or anti-BP180 antibodies has not been determined to date. In a French study currently in progress by members in our group, BP230 and BP180-NC16A autoantibodies as detected using commercial ELISAs were found in 7% and 37%, respectively, of patients with mucous membrane pemphigoid (unpublished

data, 2010). These preliminary results confirm the good specificity of BP230 ELISA for the diagnosis of BP, whereas BP180-NC16A ELISA is less specific.

The clinical usefulness of BP180 ELISA for the diagnosis of BP has been demonstrated for years,<sup>14-16</sup> and serum levels of antibodies to BP180-NC16a have been shown to parallel disease activity at the acute initial phase of the disease.<sup>3,18,19</sup> In that respect, our present results confirm (1) the high sensitivity of this immunological test (86% in patients with active disease at the time of diagnosis), which is in accordance with previous studies that showed sensitivity between 79% and 94%,<sup>14-16,18,23,26</sup> and (2) the good relationship between the BP180 ELISA value and disease activity at the time of diagnosis.<sup>18,19</sup> However, in routine practice, the commercially available BP180 ELISA is probably not superior, as a diagnostic tool, to indirect IF microscopy studies using salt-split normal skin<sup>2</sup> and even to the validated clinical criteria that were used in the present study.<sup>24</sup> Indeed, our French study group has showed that in patients who have a subepidermal blistering disorder associated with linear deposits of IgG and/or C3 along the epidermal basement membrane, the presence of 3 of the 4 following clinical criteria indicates a diagnosis of BP with 90% sensitivity and 83% specificity: absence of skin atrophy, absence of mucosal involvement, absence of head and neck involvement, and age older than 70 years.<sup>24</sup> In our present study, BP180 ELISA values were not associated with 1-year survival, contrary to our previous results using immunoblot analysis, which showed that detection of anti-BP180 autoantibodies, but not autoantibodies against BP230, was more frequent in patients with BP who died within the first year of treatment.<sup>27</sup> A possible explanation for this apparent discrepancy is that, in the latter study, a majority of patients with BP (most of them had an extensive disease) had been treated with systemic corticosteroids.<sup>2</sup> In contrast, most patients in the present series had been treated by potent topical corticosteroids as a first-line therapy, which have been shown to increase survival of patients with extensive BP compared with oral prednisone, 1 mg/kg/d.<sup>28-30</sup> Finally, the difference between the relation of clinical features (number of blisters, disease extension, and mucosal lesions) and the results of ELISAs (BP180 and BP230) may be explained by the pathogenic role of these autoantibodies (with BP180 autoantibodies being pathogenic, and BP230 autoantibodies, probably not).<sup>2,3</sup>

Interestingly, we showed for the first time to our knowledge that the median titer of anti-basement membrane autoantibodies as detected by indirect IF was higher in BP sera with positive BP230 ELISA results, whereas it did not vary according to the positivity of BP180 ELISA results. These results are in accordance with a previous study by Pas et al,<sup>31</sup> who compared indirect IF titers of anti-basement membrane antibodies on monkey esophagus substrate in 13 patients with anti-BP230 antibodies alone and in 9 with anti-BP180 alone when tested using immunoblotting. Indirect IF titers of the circulating antibodies determined displayed, for the BP230-specific group, a mean of 1:1102 (maximal titer, 1:5120), whereas in the BP180-specific group, it was only 1:29 (maximal titer, 1:160). Those and the present results together support that the contribution of the BP230-specific autoantibod-

ies to the total epidermal anti-basement membrane zone antibody titer by indirect IF is much higher than that of the BP180-specific antibodies. For years, it has been established that the titers of indirect IF do not correlate with the disease activity in BP.<sup>32-34</sup> Our study provides a possible explanation for this, since indirect IF not only detects anti-BP180 antibodies, which parallel disease activity despite being at a low titer, but also anti-BP230 antibodies, which are associated with high indirect IF titers without correlating properly with disease activity.

One of the drawbacks of our study is that it is retrospective and based on a single center, which makes the results of this study probably less informative and powerful compared with a prospective multicenter study. Nevertheless, although monocentric, our study included up to 138 patients with BP, which represents a series large enough to investigate the sensitivity of ELISAs and their relationship with clinical and indirect IF findings. Our present data on currently available ELISAs in BP clearly show that BP230 ELISA is a reliable but less-sensitive test than BP180 ELISA and essentially have confirmatory diagnostic value. Indeed, the sensitivity of the 2 combined ELISAs in patients with BP at the time of diagnosis was 91% compared with 86% for ELISA BP180 alone.

Based on the present results and clinical experience, the diagnosis of BP in routine practice relies first on a positive direct IF finding and histologic and clinical criteria.<sup>24</sup> In routine practice, indirect IF on salt-split skin and BP180-NC16A are recommended when clinical findings are not typical for BP (mucous membrane involvement, lower age, and anatomic distribution of skin eruption) but are systematically performed in our tertiary care practice. Besides, performing standard indirect IF studies with more diluted patient sera to obtain a precise titer is nowadays probably not clinically relevant and cost and labor consuming. Also, because of the weak diagnostic added value of BP230 ELISA of 5% and a lack of correlation with disease activity, we believe that BP230 ELISA should not be systematically performed in routine practice but should preferentially be performed in cases of BP with atypical clinical features or in typical BP with a negative BP180 ELISA result. With the usefulness of BP180 ELISA in predicting relapses after stopping treatment now established,<sup>19</sup> the practical value of BP230 ELISA results in guiding therapy, especially during tapering or after cessation of corticosteroids, remains to be determined. To address this question, a French multicenter, prospective study by our group is currently in progress.

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**Author Contributions:** Dr Bernard had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Bernard. *Acquisition of data:* Charneau, Lorin, Reguiat, and Barbe. *Analysis and interpretation of data:* Vitry, Antonicelli, Barbe, Tabary, Grange, and Bernard. *Drafting of the manuscript:* Charneau, Lorin, Reguiat, and Bernard. *Critical revision of the manuscript for important intellectual*

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## PRACTICE GAPS

# ELISA Instead of Indirect IF in Patients With BP

Charneux et al raise a practice gap regarding the appropriateness and effectiveness of ELISAs for confirming the initial diagnosis of BP, the most common autoimmune blistering skin disease. The diagnosis of BP is traditionally based on a combination of evi-

dence: tense bullae on normal-appearing skin or erythematous and urticarial plaques on clinical morphologic examination, subepidermal blister with eosinophil infiltration on histopathologic examination, linear IgG or C3 deposits along the epidermal basement membrane of per-