Positive Direct Immunofluorescence Is of Better Value than ELISA-BP180 and ELISA-BP230 Values for the Prediction of Relapse after Treatment Cessation in Bullous Pemphigoid: A Retrospective Study of 97 Patients

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**Key Words**
Autoantibodies · BP180 · BP230 · Bullous pemphigoid · Direct immunofluorescence · ELISA

**Abstract**

**Background:** ELISA-BP180 values and direct immunofluorescence (DIF) are prognostic factors for relapse after treatment cessation in bullous pemphigoid (BP). **Objective:** To determine the relevance of ELISA-BP230 antibodies for predicting relapse 6 months after treatment cessation. **Methods:** We retrospectively selected patients with BP and available data from ELISA-BP180 and -BP230 and DIF performed at treatment cessation. The rate of relapse was calculated at 6 months. We compared ELISA-BP180 and -BP230 values and DIF in patients with relapse and remission. **Results:** We included 97 patients. At 6 months, 25.6% of patients showed relapse. The proportion of patients with an ELISA-BP230 value ≥27 UA/ml was higher, but not significantly, for those with relapse than for those with remission (p = 0.11). The frequency of positive DIF findings was significantly higher for patients with relapse (p = 0.005). **Conclusion:** DIF is of better value than ELISA-BP180 and -230 tests to predict relapse after treatment cessation in BP.

**Introduction**

Bullous pemphigoid (BP) is the most frequent subepidermal autoimmune bullous disease. It occurs in older adults and is characterized by the absence of head and neck and mucosal involvement and the absence of atrophic scars [1]. The first-line treatment in France is based on prolonged high-dose superpotent topical corticosteroids (TCSs) [2, 3]. However, immunosuppressive agents (methotrexate, mycophenolate mofetil) may be necessary with TCS dependence or relapse, and their usefulness as first-line therapy was recently suggested [4, 5]. Clinical
relapse occurs in 50% of patients with BP treated with TCSs in the 12 months after treatment cessation [6]. The mortality rate in France is high – about 25% in patients receiving TCSs or systemic steroids, depending on age and Karnofsky status – and patients are frequently lost to follow-up in routine clinical practice [4, 7].

Autoantibodies mostly target two major components of the dermal-epidermal junction: BP230 (BPAG1), an intracellular protein of the hemidesmosomal plaque, and BP180 (BPAG2), a transmembrane protein whose dominant epitope is contained within the NC16A domain located extracellularly close to the transmembrane domain [8]. Anti-BP180 autoantibodies have a pathologic role in BP [9, 10]. In contrast, anti-BP230 autoantibodies seem to result from an epidermal epitope-spreading mechanism [11], and their pathologic role remains to be demonstrated. Circulating antibodies against dermal-epidermal junction components are routinely detected by indirect immunofluorescence (IIF), whereas autoantibodies against BP180 and BP230 may be identified by ELISA [12].

Detection of serum anti-BP180 autoantibodies by commercial ELISA (ELISA-BP180) is useful for detecting BP at different stages of the disease. The test is sensitive and specific (>90%) at the time of diagnosis [13, 14] and results parallel the clinical activity of BP during treatment [15–18]. Values ≥27 arbitrary units (AU)/ml (3 times the 9 AU/ml threshold of the MESACUP BP180 and BP230 kit [MBL, Nagoya, Japan]) represent a major prognostic factor for predicting relapse after treatment cessation similar to persistent positive direct immunofluorescence (DIF) [6]. Several studies involving the detection of serum anti-BP230 autoantibodies with ELISA (ELISA-BP230) at diagnosis showed a positive correlation with IIF titers and lower sensitivity than ELISA-BP180 (about 60%), but high specificity (>90%) [14, 18, 19]. In contrast with ELISA-BP180, ELISA-BP230 values do not correctly correlate with disease activity at diagnosis [15], and their usefulness for immunological follow-up of the disease is poorer than that of ELISA-BP180 [15, 17, 18].

Considering the disease’s high relapse rate, management after treatment cessation is a challenge but remains non-consensual. In contrast with the previously published value of ELISA-BP180 and DIF as prognostic factors for relapse, the value of ELISA-BP230 at the end of treatment remains unknown.

In a previous study of 17 patients with BP, we found that ELISA-BP230 values did not seem to be a prognostic factor for relapse within 3 months after treatment cessation [18], but no larger series have been published to confirm this preliminary result. Therefore, we conducted a retrospective multicenter study to investigate the interest of ELISA-BP230 for predicting relapse in BP.

Patients and Methods

Inclusion Criteria

In two tertiary French referral centers for autoimmune bullous diseases (Henri Mondor Hospital, Créteil, and Reims University Hospital, Reims), we retrospectively selected patients followed for BP who had available data from ELISA-BP180 and -BP230 tests performed at treatment cessation, which occurred between October 2010 and December 2013. According to the organization of each referral center, data were collected from the immunological laboratory database after excluding patients with a diagnosis other than BP and those with tests performed at a time other than treatment cessation (Henri Mondor Hospital) or from BP patient files selected with a specific register (Reims University Hospital).

Patients

The disease was diagnosed by usual clinical and histological criteria [1]. The following clinical data were recorded from the medical charts: age and gender at the time of BP diagnosis, extent of the disease at diagnosis (<10 or ≥10 new blisters per day), type and duration of treatment, need for immunosuppressive agents (methotrexate, mycophenolate mofetil), and clinical status 6 months after treatment cessation: relapse (≥3 new blisters a month [20]), alive without relapse (remission), died, or lost to follow-up. Age and gender of included patients were compared to those of 64 non-included patients seen during the same time.

Immunological Data

For each patient, we recorded the results of ELISA-BP180 and -BP230 tests at diagnosis (when available) and at cessation of therapy (see inclusion criteria). Both centers used the same commercial assays (BP180-NC16A and BP230 ELISA kits; MESACUP BP180 and BP230, MBL) with a positivity threshold of 9 AU/ml. Sera with antibody values >100 AU/ml were not diluted, and the values were considered as >100 AU/ml. When available, the result for DIF performed at the time of treatment cessation was also recorded.

Statistical Analysis

Categorical variables are reported as number (percentage) and continuous variables as median (range). Medians from immunological tests were calculated for all negative and positive titers or values, ranging from 0 to >100 AU/ml for ELISA tests. A p value ≤0.05 indicated statistical significance. The sensitivity of ELISA tests, defined as the proportion of positive test results for all sera from BP patients, was assessed at diagnosis. The median differences between results at treatment cessation for patients with relapse or remission and diagnosis were compared by Wilcoxon’s rank-sum test. The median results for both ELISA tests at treatment cessation were compared for patients with relapse and remission at 6 months after treatment cessation by Wilcoxon’s rank-sum test. Data analysis involved use of Microsoft Excel and the online Biostat TGV software (Institut Pierre Louis d’Epidémiologie et de Santé Publique [iPLESP], Paris).
Results

Characteristics of Patients at Diagnosis and Treatment

We included 97 patients. Clinical and immunological characteristics are shown in table 1. At the time of diagnosis, patients included and those not included (n = 64) did not differ in mean age (79.4 vs. 79.9 years, range 43–101) or sex (55 [56.7%] vs. 35 [54.7%] female); 54/90 patients (55.6%, missing data n = 7) had extensive disease (i.e. >3 new daily blisters). Within the 6 months following treatment cessation, 8 patients (8.2%) were lost to follow-up and 3 (3.1%) died. Therefore, 86 patients (88.7%) were assessable. In all, 22/86 patients (25.6%) showed disease relapse within 6 months after treatment cessation (fig. 1). Most of the relapses occurred within the first 3 months (16/22 cases [72.7%]) (fig. 2).

At the time of diagnosis, 87.2% (median 54 UA/ml) and 65.4% (median 36 UA/ml) of patients were positive for ELISA-BP180 and -BP230, respectively (table 1). After treatment cessation, patients with relapse and remission did not differ in antibody positivity at diagnosis.

First-line treatment was high-dose TCS (clobetasol propionate) in all cases but one (first treatment with oral prednisone), according to the French recommendations for the management of BP [21]. During treatment, 47 patients (48.5%) also received immunosuppressive agents, mostly methotrexate (39 cases). The median duration of treatment was 317 days. In all, 40 patients (41.2%) received an immunosuppressive agent (with or without TCS) at treatment cessation. Patients with relapse and remission at 6 months did not differ in clinical or immunological characteristics at diagnosis or in treatment schedules.

Immunological Tests at Treatment Cessation

At treatment cessation, 47 patients (48.4%) had detectable anti-BP180 antibodies (median ELISA-BP180 value...
in all patients 8 UA/ml) and 57 patients (58.8%) detectable anti-BP230 antibodies (median ELISA-BP230 value in all patients 18 UA/ml). The median decrease between ELISA tests at treatment cessation and diagnosis was –33 and –3 UA/ml, respectively (table 2). At treatment cessation, the proportion of patients with ELISA values ≥27 UA/ml (i.e. 3 times the threshold) was 20.6% (n = 20) and 38.1% (n = 37) for anti-BP180 and -BP230 antibodies, respectively. The proportion of patients with ELISA-BP230 values ≥27 UA/ml was higher, but not significantly, for those with relapse than for those with remission (50% [n = 11] vs. 31.2% [n = 20], p = 0.11) (table 2).

At treatment cessation, DIF results were positive for 23/67 patients (34.3%) and was more frequently positive for patients with relapse than remission (58.9% [n = 10] vs. 22.2% [n = 10], p = 0.005) (table 2). For predicting relapse, sensitivity, specificity, positive and negative predictive values for DIF were 59.0, 78.0, 50.0 and 83.3%, respectively.

### Discussion

Our series is one of the largest describing the follow-up of BP patients after treatment cessation in routine practice. Therefore, we could define the place of auto antibodies as predictive markers of clinical relapse after treatment cessation. We found that ELISA-BP230 do not seem to have value for predicting BP relapse after treatment cessation as compared with the previously demonstrated interest of ELISA-BP180 test. Furthermore, we confirm the interest of DIF results as a predictor of relapse.

In this study, the rate of patients lost to follow-up and death at 6 months after treatment cessation (11.3%) was lower than previously published rates from a retrospective series of 96 patients followed in one center of the current series (lost to follow-up 14% and deaths 17.7% at 6 months) [4]. A better management of the follow-up after treatment cessation, implemented for a few years in our referral centers, with a systematic visit at 3 and 6 months, could explain the lower rate of loss to follow-up. Selection bias seems excluded as shown by a similar profile between included and non-included patients. Patients in the current series being younger than those in our previous study (mean age 79 vs. 84 years) could explain the lower rate of death, as it was previously shown that old age (>83 years) is a major risk factor for death in BP [7].

In our study, only 22 patients (25.6%) showed relapse after treatment cessation, especially in the first 3 months. This relapse rate is much lower than the 50% in our previous series at 12 months [6]. In the present study, the lower relapse rate could be explained by a modification of routine practices following the results of a previous study published in 2009, with a prolongation of treatment in

### Table 2. Immunological results at treatment cessation and within 6 months for patients with relapse and remission

<table>
<thead>
<tr>
<th></th>
<th>Relapse (n = 22)</th>
<th>Remission (n = 64)</th>
<th>All patients (n = 86)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ELISA-BP180</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive result (≥9 AU/ml)</td>
<td>12 (54.5%)</td>
<td>28 (42.6%)</td>
<td>47 (48.4%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Value ≥27 AU/ml</td>
<td>6 (27.3%)</td>
<td>10 (15.6%)</td>
<td>20 (20.6%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Median value, AU/ml</td>
<td>10.6 (&lt;1 to 99)</td>
<td>7 (&lt;1 to 53)</td>
<td>8 (&lt;1 to 99)</td>
<td>0.28</td>
</tr>
<tr>
<td>Median difference between treatment cessation and diagnosis, AU/ml</td>
<td>–15 (–87 to 1)</td>
<td>–35 (–140 to 41)</td>
<td>–33 (–140 to 41)</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>(n = 19)</td>
<td>(n = 59)</td>
<td>(n = 86)</td>
<td></td>
</tr>
<tr>
<td><strong>ELISA-BP230</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive result (≥9 AU/ml)</td>
<td>14 (63.6%)</td>
<td>34 (53.1%)</td>
<td>57 (58.8%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Value ≥27 AU/ml</td>
<td>11 (50.0%)</td>
<td>20 (31.2%)</td>
<td>37 (38.1%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Median value, AU/ml</td>
<td>25.5 (&lt;1 to &gt;100)</td>
<td>14 (&lt;1 to &gt;100)</td>
<td>18 (&lt;1 to &gt;100)</td>
<td>0.31</td>
</tr>
<tr>
<td>Median difference between treatment cessation and diagnosis, AU/ml</td>
<td>–7 (–50 to 15)</td>
<td>–2.5 (–93 to 30)</td>
<td>–3 (–93 to 30)</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>(n = 19)</td>
<td>(n = 54)</td>
<td>(n = 81)</td>
<td></td>
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<tr>
<td><strong>DIF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive skin result</td>
<td>10 (58.9%)</td>
<td>10 (22.2%)</td>
<td>23 (34.3%)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>(n = 17)</td>
<td>(n = 45)</td>
<td>(n = 67)</td>
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</tr>
</tbody>
</table>

Figures are number with percentage in parentheses or median with range in parentheses.
patients with high ELISA-BP180 values. Indeed, in our series, the median values of ELISA-BP180 at treatment cessation (8 AU/ml, below the positivity threshold) and the median duration of treatment longer than previously described (317 vs. 240 days) favor this modification of practice. A higher use of immunosuppressive agents (48.5 vs. 1.7% [6]) may also explain the low relapse rate. Methotrexate was found to be effective as first-line treatment with short-term TCS in a retrospective study [5], and a prospective trial is being conducted in France. Methotrexate is also effective as a steroid-sparing agent in BP and is now recommended in France as second-line therapy after excluding contraindications, in contrast with other countries, where azathioprine is the drug of choice [4, 5, 21–24].

Several studies have attempted to evaluate the clinical relevance of anti-BP180 autoantibodies in BP. Anti-BP180 antibodies detected by immunoblotting were found to be associated with death in the first year [16]. The role of ELISA-BP180 test for predicting relapse during the first year of treatment [17] or after treatment cessation was previously described, and a threshold of 27 UA/ml was found to be associated with relapse at 12 months after treatment cessation. Yet, our current data did not confirm the association of ELISA-BP180 and relapse. As we hypothesized above, the results of this previous study, published a few years earlier, modified our routine practice of management of patients with BP and could explain why, in a number of cases, the treatment was not stopped with high ELISA-BP180 values (and consequently patients not included in our study). The mean duration of treatment in our series (317 days, 10.5 months), longer than the 4- to 9-month treatment according to national guidelines from referral centers, could be explained by these modifications in practices [21]. In contrast, we confirm as previously shown [6] that positive DIF at treatment cessation is significantly associated with relapse (p = 0.005). Consequently, we suggest that the treatment should be prolonged with low-dose topical steroids or immunosuppressive drugs with persistent positive DIF results, but the duration of the prolongation remains unknown.

As a serological marker of BP, the role of anti-BP230 antibodies in the management of BP at diagnosis and during follow-up remains to be determined. At diagnosis, the sensitivity of ELISA-BP230 is lower than that of ELISA-BP180 [14, 18, 25]. We previously showed that during treatment, ELISA-BP230 values followed the clinical evolution, especially in patients with disease control, and in 17 patients, values at the end of treatment did not differ between patients with relapse and remission at 3 months [18]. However, the low number of patients was a limitation for a definite conclusion. Here, we found no significant difference in patients with relapse and remission (p = 0.11). No other significant threshold could be identified (data not shown). In our study, although ELISA-BP230 values were higher, but not significantly, for patients with relapse than for those with remission, we confirm that ELISA-BP230 does not seem to be a useful or reliable test to predict relapse as compared with ELISA-BP180.

To conclude, ELISA-BP230 does not seem to be a useful test to predict relapse after treatment cessation in BP. According to previous studies, but not confirmed here due to modifications of routine practices, ELISA-BP180 may be of interest. Furthermore, DIF keeps a high interest in predicting relapse and should be performed before treatment cessation. In patients with high risk of relapse, the benefit-to-risk ratio of prolonged TCS or systemic treatment must be considered. Treatment modalities according to immunological results, especially DIF, and the clinical profile of the patient and a better knowledge of the severity of relapses remain to be determined in further dedicated, long-term prospective studies.

**Disclosure Statement**

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References


DIF and ELISA to Predict Relapse in Bullous Pemphigoid

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