Risk Factors for Relapse in Patients With Bullous Pemphigoid in Clinical Remission

A Multicenter, Prospective, Cohort Study

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Objective: To identify prognostic factors for relapse in the first year after cessation of therapy in bullous pemphigoid (BP).

Design: Prospective, multicenter, cohort study (January 1, 2000, through December 31, 2006).

Setting: Fifteen French dermatology departments.

Patients: Patients with BP in remission under low doses of topical or systemic corticosteroids.

Interventions: Cessation of corticosteroid treatment (day 0) followed by a systematic clinical and immunologic follow-up.

Main Outcome Measures: The end point was clinical relapse within the first year after cessation of therapy. Associations of clinical, biological, and immunologic (including direct immunofluorescence, serum anti–basement membrane zone autoantibodies, and serum BP180 autoantibodies by enzyme-linked immunosorbent assay [ELISA] on day 0) variables with clinical relapse were assessed by means of univariate and multivariate analyses.

Results: On day 0, 30 of 114 patients (26.3%) still had a positive result of direct immunofluorescence, 63 of 112 (56.3%) had circulating anti–basement membrane zone autoantibodies, and 34 of 57 (60%) had anti-BP180 antibodies by ELISA. At month 12, 22 patients were dead (n=11) or lost to follow-up (n=11), 51 were in remission, and 45 had had relapses (mean interval to relapse, 3.2 months). Factors predictive of relapse within 12 months after cessation of therapy were a positive result of direct immunofluorescence microscopy (P=.02), a greater age (P=.01), and high-titer ELISA scores (P=.02) on day 0. In multivariate analysis, the only factor independently predictive of relapse was a high-titer ELISA score on day 0 (odds ratio, 11.00; 95% confidence interval, 1.29-93.76).

Conclusions: High-titer anti-BP180 ELISA score and, to a lesser degree, a positive direct immunofluorescence finding are good indicators of further relapse of BP. At least 1 of these tests should be performed before therapy is discontinued.

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BULLOUS PEMPHIGOID (BP) IS the most common subepidermal autoimmune bullous disease1,2 and is characterized by linear deposits of IgG and/or C3 along the epidermal basement membrane zone.3,4 Bullous pemphigoid autoantibodies react with 2 hemidesmosomal antigens, designated BP180 and BP230.4,5 It is usually a chronic disease, with spontaneous exacerbations and remissions, that typically affects elderly patients and is associated with substantial mortality.6-10 Old age and poor general condition have been shown to significantly affect prognosis.9,10

From these recent studies, it is likely that comorbidities and practice patterns (use of systemic corticosteroids and/or immunosuppressive drugs) also influence overall morbidity and mortality.9,10 So far, systemic corticosteroids have been used widely for management of the disease. Their efficacy has been established in uncontrolled and controlled studies.11-17 Although regularly used as first-line therapy, as corticosteroid-sparing adjuvants, or in rare instances of corticosteroid resistance, the effectiveness of the addition of immunosuppressive drugs, mainly azathioprine,14,18,19 to corticosteroids has not been established.17 Potent topical corticosteroids now represent more than an interesting alternative; they are a true first-line therapy for BP.16,17 Indeed, their efficacy in both limited and generalized

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blisters and erosions, or mucosal involvement), blood eosinophil count, titers of serum anti–basement membrane zone autoantibodies by standard indirect IF on normal human skin were systematically determined on day 0. An additional serum sample (stored at −70°C before use) was taken for further determination of titers of serum anti–BP180 autoantibodies by enzyme-linked immunosorbent assay (ELISA) using a specific commercial kit detecting antibodies against the NC-16A domain of BP180 (MBL Co Ltd, Nagoya, Japan) after forwarding to the study coordinator (P.B.).

Patients were then systematically followed up after cessation of corticosteroid therapy during at least 12 months to detect a further relapse. Visits were planned on days 90, 180, and 360. In addition, serum samples were taken for blood eosinophil count, ELISA, and indirect IF analysis on days 180 and 360. All samples were stored at −70°C before use. The date of any relapse that occurred was recorded. Missing information on systematic follow-up was minimized by letters and/or telephone calls to patients, families, or physicians. Relapse was defined as the occurrence of new bullae daily for several consecutive days along with the reappearance of pruritus and erythema. Patients who experienced relapse were usually treated with clobetasol cream or oral prednisone, but treatment modalities for the relapse were not recorded.

## Statistical Analysis

Our estimation of the necessary number of patients was based on an expected rate of 25% of patients with a positive direct IF result on day 0 because this was the main suspected prognostic factor for relapse from previous studies in patients with pemphigus vulgaris.21 We determined the accrual of 120 patients to identify a 20% increase in the relapse rate in the group with a positive direct IF result (95% 1-sided confidence interval; β risk = 0.05). The main outcome variable was clinical relapse during the first year after the cessation of therapy as observed during systematic follow-up or reported by the patient or physician (dermatologist or general practitioner). From the testing sample, associations of clinical (age, initial number of blisters and mucosal involvement at diagnosis, and treatment duration), biological (blood eosinophilia at diagnosis and day 0), and immunologic (direct IF skin testing on day 0, circulating anti–basement membrane zone autoantibodies by indirect IF both at diagnosis and on day 0, and serum BP180 autoantibodies by ELISA on day 0) factors with clinical relapse were assessed by univariate analysis. A forward stepwise multivar-
late logistic regression was then performed to introduce into the model any variables showing significance on univariate analysis. The entering threshold was set at $P = .10$, and variables were maintained in the model if $P < .05$.

Qualitative variables were expressed as percentages. The chi-squared test or Fisher exact test was used to compare percentages between the 2 groups of patients (ie, with or without relapse at 12 months). Continuous variables were expressed as mean (SD). The $t$ test was used to compare quantitative variables in patients with or without relapse at 12 months. For all tests, a 2-sided $P < .05$ was considered statistically significant. Data analyses were conducted with SAS statistical software 8.2 (SAS Institute Inc, Cary, North Carolina).

RESULTS

PATIENTS

A total of 118 patients with BP in clinical remission were included in the study between January 1, 2000, and December 31, 2006. Of these patients, 57 (48.3%) were male. The age distribution ranged from 46 to 97 years. The mean age at entry in the cohort was 81.0 (9.1) years (median, 82 years). Of these 118 patients, 97 were treated with low doses of topical corticosteroids and 21 with low doses of oral prednisone before cessation of therapy and enrollment in the study. The mean duration of treatment before enrollment in the study was 11.5 (11.2) months (median, 8 months). Of these 118 patients, 27 (22.9%) had been initially treated with systemic corticosteroids alone or in association with topical corticosteroids, 85 (72.0%) with topical corticosteroids alone, and 2 (1.7%) with methotrexate and topical corticosteroids; the initial treatment regimen was not known in the remaining 4 patients. The mean blood eosinophil count on entry into the study was 276 (29) cells/µL (to convert to number of cells x10$^9$ per liter, multiply by 0.001).

At diagnosis, all patients had cutaneous blisters and/or erosions, and the mean number of blisters was 28.2 (48.7). In addition, 11 patients (9.3%) had involvement of the oral mucosa. The mean blood eosinophil count was 1374 (1207) cells/µL. Circulating autoantibodies against the epidermal basement membrane zone were detected in 94 of 117 patients (80.3%) by indirect IF on monkey or guinea pig esophagus and/or human sodium chloride-split skin. The mean anti–basement membrane zone titer was 1343 (2778).

At cessation of therapy, 30 of 114 patients (26.3%) in clinical remission exhibited a positive result of direct IF testing, 63 of 112 patients (56.3%) had circulating anti–basement membrane zone autoantibodies by indirect IF, and 34 of 57 (60%) had positive anti-BP180 autoantibodies by ELISA (ie, titer $>9$ IU/mL).

CLINICAL RELAPSE

Among the 118 patients, 11 (9.3%) died within the first year and 11 (9.3%) were lost to follow-up. Of the 96 who were still followed up 1 year after the cessation of corticosteroid therapy, 45 (47%) remained in clinical remission and 51 (53%) experienced at least 1 relapse of BP. The distribution of BP relapses within 1 year after cessation of therapy is illustrated in the Figure. Most relapses occurred within the first 3 months of the study. The mean interval to relapse was 3.2 (3.3) months.

FACTORS PREDICTIVE OF RELAPSE

Factors predictive of relapse within 1 year after cessation of therapy could be studied only in the 96 patients who were still being followed up on day 360. In univariate analysis (Table), factors associated with relapse were a positive result of direct IF microscopy (35% vs 16% in the group without relapse; $P = .02$), greater age (82 vs 77 years; $P = .01$), and high-titer ELISA scores, ie, the first titer greater than the 75th percentile (32% vs 4%; $P = .02$). This high-titer ELISA score was 27 IU, ie, 3 times higher than the normal cutoff value. No other clinical or biological factors were predictive of disease relapse within the first year after cessation of corticosteroids. In multivariate analysis, the only factor remaining indepen-
had relapses and these relapses were treated by moderate French population because only half of the patients. Mortality after cessation of therapy was nearly that of the general French population at the time of the study (according to 2003 French census data).24 Indeed, French patients with BP died mainly during the first 6 months of the treatment of their disease, owing to side effects of therapy rather than the skin disease itself.26,27 Therefore, it is not surprising that mortality after cessation of therapy was nearly that of the general French population because only half of the patients had relapses and these relapses were treated by moderate

to low doses of topical corticosteroids, which are well tolerated in elderly patients. Strikingly, the clinical relapse of BP occurs very early in most cases; the median interval to relapse was 2.1 months in our study (Figure). Although late relapses may be observed, even after disease-free intervals of several years,6 our results indicate that, in routine practice, the period of follow-up (when performed) after weaning of corticosteroids may be limited to 6 months, which seems sufficient to detect most relapses of BP.

Clinical characteristics at the time of diagnosis of the patients included in the present study (age, sex ratio, mucosal involvement, and number of daily blisters) and duration of therapy are similar to those in recent large series of patients with BP,8,10,25 in France or elsewhere, suggesting that they were representative of the disease. Indeed, the results of this study could probably be extrapolated to patients with BP in general. Notably, the mean number of new daily blisters and blood eosinophil count at the time of diagnosis were identical to those reported in our previous randomized studies in which patients with various extents of cutaneous lesions were included.10,20 Indeed, most (72%) of the patients with BP included in the present cohort had been previously included either in a randomized therapeutic trial comparing topical and systemic corticosteroids20 or in a further trial comparing 2 regimens of topical corticosteroids.22 Interestingly, the latter randomized study by our French Bullous Disease Group indicates that the doses of topical clobetasol required to control the disease may be adapted according to the disease’s severity and patient’s weight. Dosages of 10 to 30 g/d frequently are sufficient to control the disease, with rapid tapering and cessation of therapy within 4 months.21 In fact, in patients with BP, corticosteroids are generally the most common maintenance therapy11 and the preferred treatment of relapses,26 although recent studies favor methotrexate23 or immunosuppressive agents19 as maintenance therapy. In a recent retrospective study, only 10% of patients re-

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<th>Table. Univariate Analysis of Potential Factors Predictive of Relapse of BP During the Year After Cessation of Therapy</th>
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<td>Patients with positive anti-BMZ antibodies, day 0, No. (%)b</td>
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Abbreviations: BMZ, basement membrane zone; BP, bullous pemphigoid; IF, immunofluorescence.
SI conversion factor: To convert eosinophil count to number of cells ×10⁹ per liter, multiply by 0.001.
a More than 10 new daily blisters at the time of diagnosis.
b Titers greater than 1:10 by indirect IF on normal human skin, or monkey or guinea pig esophagus.
c Titers greater than 9 IU/mL by enzyme-linked immunosorbent assay. Determined in 31 patients with relapse and 23 in remission.
d Titers of 27 IU/mL or greater by enzyme-linked immunosorbent assay. Determined in 31 patients with relapse and 23 in remission.

To our knowledge, this is the first prospective study to assess risk factors of relapse after cessation of maintenance therapy in patients with BP. High-titer ELISA scores (3 times higher than the normal cutoff value) at the time of cessation of therapy represent the main factor for predicting the risk of relapse of BP within the following year. Taking into consideration the expected difficulties of follow-up of elderly patients without active disease, the proportion of patients lost to follow-up in our study (9%) may be considered more than acceptable. Eleven additional patients (9%) died during the year after cessation of therapy, and their precise dermatologic status regarding a possible recurrence of BP could not be established with certainty. This mortality rate of 9% is largely, but not exclusively, age-related because the probability of survival at 1 year in a French population of the same median age was 91.9% for men and 53.3% for women at the time of the study (according to 2003 French census data).24 Indeed, French patients with BP died mainly during the first 6 months of the treatment of their disease, owing to side effects of therapy rather than the skin disease itself.26,27 Therefore, it is not surprising that mortality after cessation of therapy was nearly that of the general French population because only half of the patients had relapses and these relapses were treated by moderate
quired prolonged systemic corticosteroid treatment with a mean follow-up of 26 months. In any case, the present results apply to patients with BP who had been treated and further maintained in remission with low doses of corticosteroids alone during the preceding 6 months.

Until now, 2 retrospective studies were unable to identify any clinical features that could prospectively identify particular subgroups of patients with BP with predictable outcomes, including relapses after discontinuation of treatment. Similarly, our prospective study, no clinical or biological characteristics at the time of diagnosis were predictive of outcome after cessation of therapy. On the contrary, we identified 2 immunologic characteristics at the time of cessation of therapy associated with an increasing risk of further relapse of BP on univariate analysis, ie, a positive finding on direct IF microscopy and a high titer of BP180 antibodies by ELISA. In the multivariate model, a positive finding on direct IF microscopy did not appear to be an independent significant prognostic factor, probably because it is linked to high ELISA scores of BP180 antibodies, which represent the prominent prognostic factor for relapse. However, detection of BP180 antibodies by ELISA is not available in all centers in routine practice and was actually performed in only 57 of the 96 patients who were available for evaluation 1 year after the cessation of corticosteroid therapy in the present study (because of loss of some serum samples for technical reasons or absence of sampling in some centers, without any link to either severity of BP or occurrence of relapse). This resulted in a lack of statistical power in the multivariate analysis for the 2 other variables showing significance at univariate analysis (greater age and positive direct IF microscopy at day 0), which both were available in 92 of those 96 patients. Therefore, we also performed a forward stepwise multivariate logistic regression introducing only 2 variables into the model: greater age (with the median age of 82 years as the threshold) and positive direct IF microscopy at day 0. In this multivariate analysis without ELISA scores, a positive result of direct IF microscopy was the only factor independently predictive of relapse within the 12 months after cessation of treatment (odds ratio, 3.37; 95% confidence interval, 1.24-9.14). In univariate analysis, a positive direct IF at cessation of therapy had a positive predictive value of 72% and a negative predictive value of 53% for a clinical relapse of BP during the next year. This finding suggests that, in patients with BP in whom BP180 antibody detection by ELISA is not available, a positive result of direct IF microscopy may represent a valid predictive factor for relapse after cessation of therapy. In fact, our study is, to our knowledge, the first prospective assessment of the usefulness of direct IF microscopy in patients with BP in clinical remission receiving maintenance therapy, whereas it was previously suggested that a negative direct IF finding represents a good indicator of remission in pemphigus.

In the present study, we demonstrate for the first time that high levels of circulating autoantibodies to BP180 as detected by ELISA using a recombinant form of NC16A before cessation of treatment in patients with BP were the main factor predictive of further relapse, in both univariate and multivariate analysis. To date, it is well established that serum levels of auto antibodies to BP180 as detected by ELISA using either a recombinant form of NC16A or the entire extracellular domain of BP180 have been shown to parallel disease activity at the acute initial phase of the disease. In addition, the commercially available ELISA kit using the NC16A domain recombinant protein was shown to more closely follow disease activity than the indirect IF titers. However, the practical value of these ELISA indexes in guiding therapy when compared with other obvious symptoms and findings (eg, presence of itch, skin lesions, and/or blood eosinophilia) still remains controversial. Our present results suggest that measuring titers of circulating BP180 autoantibodies by means of a commercially available ELISA is helpful to predict relapses after cessation of therapy in patients with BP who underwent clinical remission with low doses of either topical or systemic corticosteroids. Performing skin direct IF microscopy before cessation of therapy is probably redundant because a high-titer ELISA score (ie, ≥27 IU/mL, corresponding to the 75th percentile) is the only factor in multivariate analysis independently predictive of relapse. On the contrary, when ELISA BP180 tests are not available in a dermatologic center in routine practice, direct IF microscopy should be performed instead.

In conclusion, high titers of anti-BP180 circulating autoantibodies as detected by ELISA and, to a lesser degree, a positive direct IF microscopy finding are good indicators of relapse of BP. At least 1 of these tests should therefore be performed before therapy is discontinued. Performing those immunologic tests in patients with BP in clinical remission for several months under maintenance therapy should contribute to better management of the disease in preventing further relapses and their consequent attack treatment regimen with corticosteroids and/or immunosuppressants. However, that continuing low-dose corticosteroid therapy actually prevents relapse in patients with BP with persistent positive direct IF results or high-titer anti-BP180 antibodies requires further confirmation.

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