Prevalence and Clinical Significance of Anti–Laminin 332 Autoantibodies Detected by a Novel Enzyme-Linked Immunosorbent Assay in Mucous Membrane Pemphigoid

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Importance: A rare variant of mucous membrane pemphigoid (MMP) is characterized by circulating anti-laminin 332 (Lam332) autoantibodies and seems to be associated with concurrent malignant neoplasms.

Objective: To determine the prevalence and clinical significance of anti-Lam332 autoantibody detection from a large series of patients with MMP.

Design: Multicenter retrospective study.

Setting: Four French national centers for autoimmune bullous diseases.

Participants: One hundred fifty-four patients with MMP and 89 individuals serving as controls were included.

Interventions: Serum samples were analyzed by a new Lam332 enzyme-linked immunosorbent assay (ELISA); clinical and immunopathologic data were obtained from the patients' medical records.

Main Outcome Measures: The Lam332 ELISA scores were evaluated with respect to clinical characteristics, standard and salt-split indirect immunofluorescence, and bullous pemphigoid (BP) 230 and BP180-NC16A ELISAs.

Results: The Lam332 ELISA score was positive (≥9 U/mL) in 20.1% of serum samples from patients with MMP, 1 of 50 patients with bullous pemphigoid (BP), none of 7 with pemphigus, and 3 of 32 other controls. No relationship was evidenced between a positive ELISA Lam332 score and age; sex ratio; oral, ocular, genital, skin, or esophageal/laryngeal involvement; internal malignant neoplasm; or BP180 ELISA score. Salt-split skin indirect immunofluorescence and ELISA BP230 results were more frequently positive when Lam332 ELISA results were positive ($P= .04$ and .02, respectively). Patients with a positive Lam332 ELISA score frequently had more severe MMP (67.8% vs 47.2%; $P= .04$).

Conclusions and Relevance: Results of this novel ELISA showed that serum anti-Lam332 autoantibodies are detected in 20.1% of patients with MMP. Anti-Lam332 autoantibodies are mainly detected in patients with severe MMP but not preferentially in those with a malignant neoplasm. The association between anti-Lam332 and anti-BP230 autoantibodies might arise from an epitope-spreading phenomenon.


MUCOUS MEMBRANE PEMPHIGOID (MMP) is a heterogeneous group of autoimmune diseases encompassing chronic, inflammatory, subepithelial blistering diseases that predominantly affect mucous membranes and lead to scarring. On the basis of the 2002 international consensus, MMP includes those blistering diseases, as well as cicatricial pemphigoid, mucous membrane–dominant epidermolysis bullosa acquisita, and linear IgA bullous dermatosis with preferential mucous membrane involvement. Immunologically, MMP is characterized by linear deposits of immunoglobulins (IgG and/or IgA) and/or complement fragments at the dermal-epidermal and/or chorioepithelial basement membrane zone (BMZ). Mucous membrane pemphigoid is associated with significant morbidity, potentially leading to definitive functional sequelae, especially in ocular or upper aerodigestive tract manifestations. Patients with MMP exhibit, although inconsistently, circulating autoantibodies directed against various components of the dermal-epidermal/chorioepithelial BMZ including bullous pemphigoid (BP) 180 antigens and BP230 antigens, laminin (Lam) 332 an-
tigens,12-14 the 97/120-kDa linear IgA bullous disease antigen, type VII collagen, and the integrin β₃ antigen.15 The 2 major autoantigens of MMP are BP180, mainly its extracellular domain,16,17 and Lam332; both are components of anchoring filaments and reach into the lamina densa of the BMZ. Previously termed nicin/BM600,18 p155,19,20 epiligrin,21 and laminin-5,22 Lam332 is an heterotrimeric glycoprotein consisting of α3, β3, and γ1 subunits that are covalently linked by disulfide bonds and bind to α6β4 and α6β1 integrins.19 The subgroup of patients with anti-Lam332 autoantibodies,21,23 previously termed anti–laminin 5 or anti–epiligrin MMP, cannot be distinguished clinically from patients with other variants of MMP. Patients with anti-Lam332 autoantibodies display circulating IgG autoantibodies that bind to the dermal side of split skin (SSS) by indirect immunofluorescence (IF) and react with the α3 chain (G domain) and, less frequently, the β3 and/or γ1 chain of Lam332 (α6β3γ1). Occasionally, binding to the α3 chain of laminin 311, a heterotrimer of α3β1γ1 chains, is also observed, because of the presence of cross-reacting antibodies. In vitro and in vivo studies24 have provided convincing evidence that anti-Lam332 autoantibodies are pathogenic. This form of MMP seems to be associated with an increased relative risk for cancer, which enhances the need to identify patients with anti-Lam332 MMP.23 Traditional immunoprecipitation and immunoblot studies were initially used to detect IgG anti-Lam332 autoantibodies in patients with MMP.12,14,21,20,27 More recently, enzyme-linked immunosorbent assays (ELISAs) have been developed for such purposes, and early studies22,23 showed a variable but rather low specificity, since up to 40% of patients with BP may have IgG that is reactive with Lam332. In fact, the prevalence of anti-Lam332 among patients with MMP varied from 31% to 70% in 2 studies.22,28 Using a novel ELISA assay, we analyzed serum samples from 154 patients with MMP and 89 individuals serving as controls. We correlated the results of Lam332 ELISA with immunologic and clinical features to establish the prevalence and clinical significance of Lam332 autoantibody detection in MMP.

**STUDY PATIENTS**

Serum samples from patients with a new diagnosis of MMP seen between January 1, 1990, and December 31, 2011, in 4 departments of dermatology belonging to the French Referral Centers for Autoimmune Bullous Diseases (Île-de-France/Bobigny, Limoges, Reims, and Rouen, France) were used. All patients with MMP were included if they fulfilled the following criteria: (1) predominant mucous membrane involvement, including active (erosions and/or blisters) and/or cicatricial involvement of mucous membranes (eg, oral, ocular, genital, laryngeal, and esophageal), and (2) positive results of direct IF with linear IgG and/or C3 deposits along the BMZ of perilesional skin or mucous membrane.

Control serum samples were obtained from 50 patients with BP, 7 patients with pemphigus vulgaris, and 32 nondonor-logic hospital patients without any autoimmune bullous disease. All serum samples were stored at −80°C until analysis. The study was performed in accordance with the Declaration of Helsinki and the guidelines of the local ethics committee of the Reims University Hospital.

**CLINICAL AND ROUTINE IMMUNOLOGIC DATA**

For each patient with MMP, clinical and routine immunopathologic data available at the time of the initial diagnosis were obtained from the patients’ medical records using a standardized form. Data included demographic variables (sex, age at diagnosis), clinical characteristics (number and location of involved mucous membrane and skin areas), history of an internal malignant neoplasm, serum anti-BMZ autoantibody by standard indirect IF on monkey esophagus and by indirect IF on SSS,29 and ELISA scores of anti-BP180 and anti-BP230 autoantibodies. Anti-BP230 and anti–BP180-NC16A autoantibodies were detected using commercially available ELISA tests (MBL Co Ltd).30,31 Additional data on diagnoses of internal cancer made after the initial diagnosis of MMP were systematically recorded, including the type of solid tumors or lymphomas and the time between the diagnoses of MMP and the cancer.

The severity of MMP was scored in each patient according to the number of the involved anatomic sites and extension of active and cicatricial lesions using the conjunctival Tauber32 grade for ocular involvement. The MMP severity was scored as “low” for patients having low activity in 1 or 2 sites other than the eye. Severity was scored as “intermediate” for patients having 2 active sites, including a conjunctival Tauber grade of I/II when ocular lesions were present. The severity was scored as “high” in patients with (1) active or cicatricial lesions in the larynx/esophagus, (2) a conjunctival Tauber grade of III/IV (whatever the number of other involved sites), or (3) at least 3 active sites.

**LAM332 ELISA**

The Lam332 ELISA was developed using purified Lam332 from SCC25 cells.30 Briefly, the conditioned medium from SCC25 cells was harvested and centrifuged. The supernatant was then filtered and stored at 4°C. After clarification, Lam332 was purified by affinity chromatography using a mouse monoclonal anti-Lam332 antibody (anti-B3 6F12 monoclonal antibody; Santa Cruz Biotechnology) coupled-Sepharose column (Amersham Bioscience). With a rabbit polyclonal anti-Lam332 antibody (L132) raised against the Lam332 purified native form, the eluted protein fraction revealed bands (200-165, 155, and 140-105 kDa) corresponding to the unprocessed and processed forms of the α3, β3, and/or γ1 chains of Lam332, respectively.33

Optimal ELISA conditions were addressed by analyzing the amount of coated Lam332 (from 0.1 to 0.8 μg), material of microtiter plaques (MaxiSorp [Nunc] and standard untreated plaque), blocking buffers (barbital buffer, pH 8.6; sodium bicarbonate buffer, pH 9.2; and phosphate buffer, pH 7), and concentration of L132 used as the detection antibody (from 1:2000 to 1:128 000). Finally, microtiter plate wells were coated overnight at 4°C with 0.5 μg of Lam332 diluted in 100 μL of phosphate-buffered saline (PBS), then blocked with PBS-Tween containing 5% bovine serum albumin for 45 minutes at 37°C. Wells were incubated for 1 hour at 37°C with the L132 antibody (1: 8000). Determination of anti-Lam332 autoantibodies in patient serum was performed by replacing the L132 antibody with human serum (1:100) in blocking buffer. Bound antibodies were detected using horseradish peroxidase–conjugated donkey anti-rabbit or goat anti-human IgG antibodies (30 minutes at 37°C) (Amersham Bioscience and Sigma, respectively) in 0.05% PBS-Tween (1:1000). At that time, 100 μL of a 3’,3’’,5’,5’’-tetramethylbenzidine (Sigma) was added and the reaction was...
stopped by the addition of 50 μL sulfuric acid (2N). Color change was measured spectrophotometrically at a wavelength of 450 nm (Biotech Instruments). Between all steps, wells were washed (5 times) with PBS-Tween. The anti-Lam332 ELISA was validated using the following controls: omission of Lam332 coating (negative control) and use of 5 positive serum samples (positive control) from patients with MMP containing anti-Lam332 autoantibodies detected by immunoprecipitation. All serum samples were tested in duplicate. The score of anti-Lam332 autoantibodies in patients' serum was then calculated as an index value = (A450sample − A450negative control)/(A450positive control − A450negative control) × 100. Data are expressed as units per milliliter of serum.

STATISTICAL ANALYSIS

Continuous variables are expressed as mean (SD); χ² tests were used for qualitative variables (Fisher exact test was used for small samples), with Bonferroni correction in case of multiple testing and nonparametric Mann-Whitney/Wilcoxon analysis for quantitative values. Statistical significance was set as 2-sided P < .05. Statistical computations were performed using commercial software (SAS, version 8.2; SAS Institute, Inc).

RESULTS

PATIENT CHARACTERISTICS AND ROUTINE IMMUNOLOGIC DATA

One hundred fifty-four patients (101 women and 53 men) with a mean age of 68 (16) years (range, 6-95 years) and a clinical and immunologic diagnosis of MMP were included. Among these, oral lesions were present in 142 patients (92.2%), ocular lesions in 68 patients (44.2%), skin lesions in 67 patients (43.5%), genital lesions in 58 patients (37.7%), and upper aerodigestive tract or esophageal lesions in 32 patients (20.8%). A mean of 2.3 clinical sites (range, 2-6) were involved. The severity of MMP was scored as low in 24 patients (15.6%), intermediate in 51 patients (33.1%), and high in 79 patients (51.3%). Clinical features and antibody profiles of the 18 patients with MMP who had an associated neoplasia are detailed in Table 1. Anti-BMZ autoantibodies were detected by standard indirect IF in 32 patients (20.8%). Anti-BMZ antibodies were detected by SSS-indirect IF in 50 patients (32.5%), with labeling of the roof or floor of the cleavage in 35 and 12 patients, respectively, and a combined dermal/epidermal pattern in 3 patients. Among the 154 patients’ serum samples tested with BP180-NC16A ELISA, 60 samples (39.0%) were positive (ie, titer ≥ 9 U/mL), with a mean score of 49 (43) U/mL. Sixteen of 154 serum samples (10.4%) had positive BP230 ELISA scores, with a mean score of 23 (20) U/mL.

LAM332 ELISA SCORES IN MMP AND CONTROL SERUM

Of the 154 MMP patients’ serum, 31 samples (20.1%) had positive Lam332 ELISA scores (ie, ≥ 9 U/mL), with a mean of 27 (21) U/mL. In contrast, only 1 of 50 BP serum, none of 7 pemphigus serum, and 3 of 32 control serum samples (from hospitalized patients without autoimmune bullous disease) had a positive Lam332 ELISA score (Figure). Clinical features and antibody profiles detected with commercially available BP180 and B230 ELISAs of the 31 patients with MMP and a positive Lam332 ELISA score are reported in Table 2. None of the serum samples from patients whose MMP was graded

<table>
<thead>
<tr>
<th>Sex/Age at Diagnosis of MMP, y</th>
<th>No. of Sites Involved</th>
<th>MMP Severity</th>
<th>Type of Cancer</th>
<th>Interval Between MMP and Cancer, ya</th>
<th>ELISA Score, U/mL</th>
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</thead>
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<tr>
<td>F/63</td>
<td>2</td>
<td>Moderate</td>
<td>Uterus</td>
<td>−13</td>
<td>45 1 14</td>
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<tr>
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<td>B-cell lymphoma</td>
<td>−4 to 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4 1 5</td>
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<tr>
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<td>Breast</td>
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<tr>
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<td>B-cell lymphoma</td>
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<td>1 3 7</td>
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Abbreviations: BP, bullous pemphigoid; ELISA, enzyme-linked immunosorbent assay; Lam332, laminin 332; MMP, mucous membrane pemphigoid.

<sup>a</sup> A negative value indicates that cancer preceded MMP.

<sup>b</sup> Two different cancers occurred at different times.
as low severity showed Lam332 antibodies. Only 2 of these 31 patients with MMP and positive Lam332 ELISA scores had developed cancer 12 and 13 years before MMP was diagnosed (Table 1).

**COMPARISON OF LAM332 ELISA RESULTS WITH PATIENTS’ CLINICAL FEATURES AND MMP SEVERITY**

At the time of diagnosis, MMP severity was more frequently scored “high” in patients with a positive Lam332 ELISA score (21 of 31 patients [67.7%] vs 58 of 123 [47.2%]; P = .04) (Table 3). The rate of laryngeal and/or esophageal involvement was slightly higher in patients with MMP and positive Lam332 ELISA scores (10 of 31 patients [32.2%] vs 22 of 123 [17.9%]; P = .08), although this difference did not achieve statistical significance. There was no significant relationship between a positive Lam332 ELISA score and (1) patients’ age (P = .76) or sex (P = .26); (2) the rate of patients with oral (P = .75), ocular (P = .78), skin (P = .54), or genital (P = .58) involvement; (3) the rate of patients with 3 or more involved sites (P = .15); or (4) the proportion of patients with an associated internal cancer (P = .46).

**COMMENT**

To our knowledge, this is the largest study analyzing the prevalence and significance of the detection of anti-Lam332 autoantibodies using a novel ELISA in patients with MMP in terms of clinical presentation and severity of the disease. We found that 20.1% of patients with MMP with active disease at the time of diagnosis had serum anti-Lam332 autoantibodies, which appear to be present only in a subset of patients with severe disease. To date, only 2 prior studies have assessed the autoantibody reactivity of MMP serum against Lam332 by experimental ELISAs. Bekou et al reported the results of the serum detection of Lam332-reactive IgG in a retrospective cohort of 24 MMP, 72 BP, and 51 control serum samples (from patients with pemphigus or healthy individuals) using an ELISA with affinity-purified native human Lam332. They found that IgG autoantibodies against Lam332 were present in 75% of MMP serum samples, and the specificity of this Lam332 ELISA was 84% for MMP when control serum samples were obtained from healthy individuals or patients with pemphigus. However, the investigators also found that 40% of BP serum samples scored positive by this Lam332 ELISA, even though BP is, in clinical practice, the main differential diagnosis of MMP. These results are markedly different from those of our present study, which showed the presence of anti-Lam332 autoantibodies in a minority (20.1%) of MMP serum samples and in only 1 of 50 BP serum samples (2%). These discordant results may be at least partly explained by differences concerning purification of Lam332 (mouse monoclonal antibody 6F12 vs BM165), amount of coated Lam332 (0.5 μg vs 1.5 μg), blocking buffers (phosphate buffer containing 5% bovine serum albumin for 45 minutes at 37°C vs milk buffer for 30 minutes at room temperature), number of washes (5 vs 3), or dilution of tested serum (1:100 vs 1:50), resulting in a better specificity but a lower sensitivity of detection of anti-Lam332 autoantibodies in our present series. More recently, Lazarova et al reported the results of another Lam332 ELISA with purified extracellular matrix proteins of cultured human keratinocytes in 32 selected patients who met strict clinical and immunologic criteria for the diagnosis of anti-Lam332/epiligrin MMP, including circulating anti-Lam332 autoantibodies demonstrated by immunoprecipitation from radiolabeled human keratinocyte extracts. In that study, control participants included 34 patients with BP, 51 with pemphigus, and 87 healthy individuals. Using that IgG4 Lam332 ELISA, they detected anti-Lam332 autoantibodies in 29 of 32 (90%) anti-Lam332/epiligrin MMP serum samples, none of 87 normal human serum samples, none of 52 pemphigus serum samples, and 3 of 34 (10%) BP serum samples. From these results, the diagnostic accuracy of that IgG4 Lam332 ELISA was deemed to be high for anti-Lam332/epiligrin MMP, with a sensitivity of 91% and a specificity of 98%; therefore, the authors concluded that this IgG4 Lam332 ELISA, vs 35 of 123 [28.4%], P = .04; and 7 of 31 [22.6%] vs 9 of 123 [7.3%], P = .02, respectively).

![Figure](https://example.com/figure.png)

**Figure.** Scatterplot representation of enzyme-linked immunosorbent assay (ELISA) laminin 332 (Lam332) results concerning the performance of serum samples from 154 patients with mucous membrane pemphigoid (MMP) and 89 controls, including 32 patients without autoimmune diseases (NC). All serum samples were tested in duplicate; plotted dots represent the mean of the Lam332 ELISA scores obtained for each sample. The horizontal dotted line indicates the upper level of normal for the Lam332 ELISA score. BP indicates bullous pemphigoid.

**Table 3**

<table>
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<tr>
<th>Lam332 ELISA Score, U/mL</th>
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<th>20</th>
<th>30</th>
<th>40</th>
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<th>70</th>
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**Table 4**

<table>
<thead>
<tr>
<th>COMPARISON OF LAM332 ELISA RESULTS WITH INDIRECT IF AND BP180 AND BP230 ELISAS</th>
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<tr>
<td>In MMP serum samples, a positive Lam332 ELISA score was not associated with BP180 autoantibodies detection using BP180-NC16A ELISA (P = .94) or with the presence of serum anti-BMZ autoantibodies as detected by standard indirect IF (P = .62) (Table 4). Serum anti-BMZ autoantibodies as detected by indirect IF on SSS and anti-BP230 autoantibodies as detected by ELISA were more frequently detected in MMP serum samples with a positive Lam332 ELISA score (15 of 31 patients [48.4%] vs 35 of 123 [28.4%], P = .04; and 7 of 31 [22.6%] vs 9 of 123 [7.3%], P = .02, respectively).</td>
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like indirect IF studies on SSS, could be used to identify patients who deserve rigorous and specialized testing (e.g., immunoblot, immunoprecipitation, or both). Likewise, in our present study, Lam332 autoantibodies were barely detected in controls, including patients with BP or pemphigus and 32 healthy donors. However, using an experimental ELISA of high specificity, we estimated the prevalence of Lam332 autoantibody to be only 20.1% within a large population of unselected patients with MMP. Nevertheless, our prevalence estimate (20.1%) of anti-Lam332 MMP is a minimal figure, since our ELISA detects only IgG autoantibody to Lam332. Indeed, IgA and IgE autoantibodies against Lam332 also may be detectable by immunoblotting in a small subset of patients.
with MMP. Further studies are needed to determine the frequency of IgA and IgE anti-Lam332 autoantibodies using ELISA and whether they play a pathogenic role in MMP.

To date, estimates of the prevalence of anti-Lam332 autoantibodies among MMP patients were made mainly on the basis of indirect IF on SSS, immunoprecipitation or immunoblot data from small series of patients with MMP diagnosed using direct IF and clinical findings. In agreement with our study, anti-Lam332 MMP rates ranged from 18% to 30% among patients with the MMP clinical phenotype; however, these studies were performed on limited series of patients. For instance, in a French retrospective study on 23 cicatricial pemphigoid serum samples, 5 samples (22%) showed dermal staining and another 5 samples (22%) showed combined epidermal/dermal staining by indirect IF on SSS. In a German study on 16 cicatricial pemphigoid serum samples, 5 samples (31%) showed dermal staining and 2 samples (13%) showed combined staining by indirect IF on SSS. In the latter study, all 5 cicatricial pemphigoid serum samples with dermal binding immunoprecipitated Lam332 from extracts and culture media. Conversely, none of 13 MMP serum samples and only 3 of 124 MMP samples showed autoantibodies to Lam332 by immunoblotting in the series of Yeh et al and Oyama et al, respectively. Here, we found that almost half of MMP serum with positive Lam332 ELISA scores had negative results of indirect IF on the dermal side of split skin (Table 4), which might be partly related to a lack of sensitivity of our indirect IF on SSS technique, which was routinely performed on a commercially available substrate. Conversely, binding of the epidermal side of the split skin in some MMP serum with positive Lam332 ELISA scores may be explained both by the low sensitivity of our indirect IF on SSS technique and by the rather frequent detection of anti-BP180 and anti-BP230 autoantibodies (Table 4). Mucous membrane pemphigoid is a clinical phenotype that encompasses several subsets, with a wide spectrum of clinical presentation, clinical course, and prognosis. In our study, determination of patients’ autoantibody profiles using commercial ELISAs allowed us to confirm that BP180 is a major autoantigen in MMP, as previously demonstrated by many studies performed in the past 2 decades and based on immunoblot analysis using human epidermal, amniotic membrane, or fusion proteins. These studies showed that circulating IgG autoantibodies from patients with MMP can recognize several intracellular and extracellular BP180 epitopes, sometimes distinct from the NC16A domain. Using a panel of cell-derived and recombinant proteins covering the entire BP180 molecule, Schmidt et al found that 19 of 26 MMP serum samples recognized BP180, including 6 (32%) that showed only IgA reactivity to this autoantigen. By immunoblotting using human epidermal, dermal, and placental amnion proteins of a large series of MMP, Oyama et al similarly showed that most patients with MMP (75%) had IgG to BP180, including its soluble ectodomains. In our study, autoantibodies against the NC16A domain of BP180 were detected by ELISA in almost 40% of MMP serum samples. Other antigens targeted predominantly by IgG autoantibodies in MMP included BP230. Other autoantibodies against BP230 are detected only occasionally in MMP serum, as shown in the present study. Interestingly, serum autoantibodies directed against BP230 are more frequently detected in the serum of patients with MMP when anti-Lam332 autoantibodies are also present. This association is possibly a secondary immune phenomenon related to epitope spreading. At some stage of the pathologic progression of MMP, damage to basal epithelial cells and BMZ might result in abnormal exposure or release of BP230, which is intracellularly situated. The same phenomenon of epitope spreading possibly exists for the NC16A domain of BP180, which is highly immunogenic. Hence, the immune system of patients with MMP may produce autoantibodies against BP180 and BP230, although autoantibodies to BP180-NC16A or to BP230 did not correlate with MMP severity in our study (data not shown). This is in keeping with the fact that their specific role in the pathogenesis of the disease is still undetermined. Finally, it remains unclear whether the development of multiple target antigens is of pathogenic importance or a secondary phenomenon occurring within the setting of more severe or long-standing MMP.

Clinically, the importance of identifying anti-Lam332 MMP within the spectrum of mucosal-
dominant autoimmune bullous diseases has been underlined by its association with internal malignant neoplasms. In addition to several case reports, this concept mainly relies on a cohort of 35 patients with anti-Lam332 autoantibodies detected by immunoprecipitation, among whom 10 patients (28.6%) developed a solitary cancer.\textsuperscript{25} From these results, an increased risk of solid cancers compared with the general population was calculated (relative risk, 6.8; 95% CI, 3.3-12.5). This increase in risk for anti-Lam332 MMP appears close to that of cancer among adults with dermatomyositis, with a relative risk ranging from 3.0 to 6.2 in population-based studies.\textsuperscript{46-48} The risk of cancer was highest during the first year of anti-Lam332 MMP,\textsuperscript{22} and there was typically a short interval between the onset of MMP and a cancer diagnosis.\textsuperscript{23,48} Unexpectedly, our findings did not confirm these previous results. Conversely, the proportion of patients with MMP and associated cancer, including solid tumors and lymphomas, was not higher in patients with positive anti-Lam332 autoantibodies (Table 3). Furthermore, most cancers occurred many years before the onset and diagnosis of MMP, including in the 2 MMP patients with positive anti-Lam332 autoantibodies as well as associated cancer. In addition, the prevalence rate of cancer in our population of patients with MMP (11.7%) is not different from that of the general population in the same age range in France.\textsuperscript{49} In our series, a potential ascertainment bias seems unlikely because dermatologists of our reference centers have been aware of the possible association between MMP and cancer since 2001.\textsuperscript{22} Therefore, most patients in our series, the majority of whom were followed up in these tertiary care medical centers, were also monitored for cancer. What is more, the size of our series and the follow-up duration were sufficient to detect or observe the occurrence of subsequent cancer. To date, there have been only 17 cases of anti-Lam332 MMP reported in association with cancer\textsuperscript{48}; prospective case-control studies are required to demonstrate the reality of the association.

The main result of our study is that anti-Lam332 autoantibodies are more likely to be present when MMP is severe. This result, obtained from a large series of patients with MMP, completes and extends prior observations suggesting that levels of anti-Lam332 autoantibodies seem to relate to the extent of the overall clinical activity in MMP.\textsuperscript{36} Apart from Lam332 reactivity, some studies\textsuperscript{14,20} have suggested that more severe clinical features were associated with antibody reactivity to multiple BMZ antigens, including the BP180 component, and that a dual BP180 reactivity with IgG and IgA was associated with a more severe phenotype. In conclusion, this novel Lam332 ELISA assay appears to be a relevant diagnostic and prognostic tool allowing the identification of a subset of patients with more severe MMP. In these patients, the more frequent association with anti-BP230 autoantibodies, possibly originating from an epitope-spreading phenomenon, could relate a chronic and severe autoimmune bullous condition. Longitudinal studies including further testing of autoantibodies against autoantigens or epitopes relevant for MMP (ie, B, integrin and C-terminus domain of BP180) will be important to determine the prognostic value of the epitope-spreading phenomenon in this polymorphic disease.

\section*{REFERENCES}


