Diagnosis of pemphigoid diseases
Meijer, Joost Martien

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LABORATORY DIAGNOSIS AND CLINICAL PROFILE OF ANTI-P200 PEMPHIGOID

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Abstract

Importance
Anti-p200 pemphigoid is a rare subepidermal autoimmune blistering disease characterized by autoantibodies against a 200-kDa protein in the basement membrane zone. Anti-p200 pemphigoid is probably often misdiagnosed because of low availability of diagnostic assays and expertise and classified as bullous pemphigoid or epidermolysis bullosa acquisita.

Objective
To clinically characterize patients with anti-p200 pemphigoid, identified by using indirect immunofluorescence microscopy on skin substrates deficient in type VII collagen and laminin-332 (knockout analysis), to validate this technique by immunoblot with dermal extract, and to incorporate direct immunofluorescence serration pattern analysis in the diagnostic algorithm.

Design, Setting, and Participants
This was a retrospective study performed from January 2014 to June 2015 with biobank patient materials and clinical data for the period 1998 to 2015 from the single national referral center on autoimmune bullous diseases. Patients were selected based on a dermal side binding on 1-mol/L salt (sodium chloride)-split human skin substrate by indirect immunofluorescence microscopy, not diagnosed epidermolysis bullosa acquisita or anti-laminin-332 mucous membrane pemphigoid.

Main Outcomes and Measures
Indirect immunofluorescence microscopy knockout analysis was performed and diagnosis of anti-p200 confirmed by immunoblot with dermal extract. Clinical, histological, and immunological findings were registered. Autoantibodies against laminin γ1 were determined by immunoblot.

Results
Twelve patients with anti-p200 pemphigoid (7 male and 5 female; mean age, 66.6 years) were identified using the indirect immunofluorescence microscopy knockout analysis. Direct immunofluorescence microscopy showed a linear n-serrated IgG deposition pattern along the basement membrane zone in 9 of 11 patients. The diagnosis was confirmed by immunoblot showing autoantibodies against 200-kDa protein in dermal extract in 12 of 12 patients. Autoantibodies against recombinant laminin γ1 were detected by immunoblot in 8 of 12 patients. Remarkable similarities were seen in clinical features with predominantly tense blisters on hands and feet, resembling dyshidrosiform pemphigoid. Mucosal involvement was seen in 6 (50%) of the patients.

Conclusions and Relevance
Predominance of blisters on hands and feet may be a clinical clue to the diagnosis of anti-p200 pemphigoid. Direct immunofluorescence microscopy serration pattern analysis and indirect immunofluorescence microscopy knockout analysis are valuable additional techniques to facilitate the diagnosis of anti-p200 pemphigoid.
KEY POINTS

QUESTION
How is the diagnosis of the rare subepidermal autoimmune bullous disease anti-p200 pemphigoid made and the clinical profile characterized?

FINDINGS
In this retrospective study, 12 patients with anti-p200 pemphigoid were identified using indirect immunofluorescence (IF) microscopy knockout analysis and direct IF microscopy serration pattern analysis. Clinical clues to diagnosis were distribution of blisters on hands and feet and often mucosal involvement.

MEANING
Consider anti-p200 pemphigoid in patients with acral blistering; apart from immunoblotting the diagnosis can also be made using indirect IF microscopy knockout analysis and direct IF microscopy serration pattern analysis.
Anti-p200 pemphigoid is a rare subepidermal autoimmune bullous disease (sAIBD) originally described in 1996 and characterized by autoantibodies against a 200-kDa protein, localized within the lower lamina lucida of the basement membrane zone (BMZ). Anti-p200 pemphigoid is probably often misdiagnosed and classified as bullous pemphigoid (BP) or epidermolysis bullosa acquisita (EBA) because of low availability of diagnostic assays and expertise. Serum samples of most patients with anti-p200 pemphigoid seemed to recognize laminin γ1, and therefore the term anti-laminin γ1 pemphigoid was proposed for the disease. However, until now, ex-vivo and in-vivo studies showed no direct pathogenic role of anti-laminin γ1 antibodies, and the molecular identity of the pathogenic 200-kDa autoantigen still remains unclear.

Approximately 100 cases have been published so far, and the incidence is likely higher; the clinical characteristics and disease course are not well known. The heterogeneous clinical presentation of anti-p200 pemphigoid may mimic BP, linear IgA dermatosis and inflammatory EBA. Moreover, the clinical course of anti-p200 pemphigoid is reported to be more benign compared with EBA and even BP.

Diagnosis of anti-p200 pemphigoid is based on a combination of histopathologic analysis, direct immunofluorescence (DIF) microscopy, and immunoserologic testing. Histopathologic analysis alone does not allow differentiating anti-p200 pemphigoid from other sAIBDs, and a linear deposition of IgG and C3c along the BMZ by DIF is found in all IgG mediated subtypes of sAIBD. Dermal side binding on indirect immunofluorescence microscopy on 1-mol/L salt (sodium chloride)-split skin (IIF SSS) enables to differentiate anti-p200 pemphigoid from other sAIBD but not from EBA and anti-laminin-332 mucous membrane pemphigoid (anti-LN-332 MMP). The differentiation is of importance because in anti-LN-332 MMP oncological screening may be indicated and in EBA often a refractory response to treatment is seen.

The laboratory diagnosis of anti-p200 pemphigoid can be made by immunoblot with human dermal extract but is established in only a few specialized laboratories. In addition, IIF microscopy knockout analysis on skin sections from patients with hereditary epidermolysis bullosa completely lacking expression of type VII collagen and laminin-332 has been used prior in experimental studies to provide the evidence that the 200-kDa autoantigen is different from type VII collagen and laminin-332. To further facilitate the laboratory diagnosis of anti-p200 pemphigoid, we incorporated IIF microscopy knockout analysis and DIF serration pattern analysis to the diagnostic algorithm. Furthermore, identification of a series of patients with anti-p200 pemphigoid allowed profiling clinical characteristics, disease course, and immunopathological features.

**METHODS**

**PATIENTS**

Patients were selected from our biobank of the single national referral center on autoimmune bullous diseases in The Netherlands for the period of 1998 to 2015. These patients could be newly diagnosed, referred to our hospital, or referred for laboratory diagnostics. Inclusion criteria were (1) dermal side binding of serum IgG on 1-mol/L SSS human skin substrate by IIF microscopy of patients not diagnosed as having EBA or anti-LN-332 MMP, and (2) linear IgG deposition along the BMZ by DIF microscopy. To distinguish patients with anti-p200 pemphigoid from patients with anti-LN-332 MMP and EBA, DIF serration pattern analysis
was implemented and IIF microscopy knockout analysis was performed with IgG and/or pemphigoid from patients with anti-LN-332 MMP and EBA, DIF serration pattern analysis was implemented and IIF microscopy knockout analysis was performed with IgG and/or IgG4 on skin sections from patients with hereditary epidermolysis bullosa completely lacking expression of type VII collagen (recessive dystrophic epidermolysis bullosa [RDEB], severe generalized) and laminin-332 (junctional epidermolysis bullosa [JEB], severe generalized), as described previously.\textsuperscript{10,12} IgG4 was being used for IIF microscopy knockout analysis in patients with low serum titers of autoantibodies, to reduce background staining on the skin substrate. This retrospective study was performed in line with the guidelines of the University Medical Center Groningen ethics committee; according to the national regulations in The Netherlands, no approval by the ethics committee is needed.

**HISTOPATHOLOGIC FINDINGS**
Lesional skin biopsy specimens were examined by light microscopy of hematoxylin-eosin-stained paraffin sections and the blister level mapped by immunohistochemical analysis using a human type IV collagen monoclonal antibody (Ventana Medical Systems).

**IMMUNOFLUORESCENCE MICROSCOPY**
The DIF and IIF microscopy studies were performed using the methods previously described.\textsuperscript{13} Cryosections of 4-µm from perilesional skin biopsy specimens were studied by DIF microscopy for anti-BMZ deposits of IgG, IgA, IgM, and C3c. Routine DIF serration pattern analysis was performed using a Leica DMRA microscope with a standard 40× dry objective and 10× ocular.

**IMMUNOBLOT ANALYSES**
Immunoblot analyses were performed as previously described on 2 types of extracts: (1) human dermal extract,\textsuperscript{1} labeling a 200-kDa protein, and (2) recombinant monomeric C-terminus fragment of human laminin γ1 (hLAMC1-cterm).\textsuperscript{14}

**RESULTS**

**CLINICAL FEATURES**
Using the laboratory tests (vide infra), 12 patients were identified as having anti-p200 pemphigoid. Their mean age was 66.6 years (range, 28-91 years), with a male to female ratio of 1.4:1 (7 [58%] were male, 5 [42%] were female). Patient characteristics are shown in Table 1. The initial clinical presentation of anti-p200 pemphigoid was exclusively cutaneous in 6 of 12 patients (50%), and concomitant mucosal and cutaneous in the 6 other patients (50%). None of the patients had the mucosa exclusively or predominantly affected. All 12 patients showed tense skin blisters, while pruritus was present in 11 of 12 patients. The distribution of skin lesions showed remarkable similarities in 9 of 12 of the patients with tense blisters localized on the hands and feet, resembling dyshidrosiform pemphigoid (Fig. 1a and b). Circumscript monomorphic blisters were observed in patient 5 (Fig. 1d). The bullous eruption was generalized on the skin and mucosa in patients 2 and 6 (Table I). Neither scarring nor milia formation was observed in any patient. Mucosal lesions consisted of gingivitis, blistering on the tongue, and on buccal and/or anogenital mucosa (Fig. 1c). The eyes or larynx were never in
Table I. Clinical characteristics of 12 patients with anti-p200 pemphigoid.

<table>
<thead>
<tr>
<th>Patient No./Sex/Age Range, y</th>
<th>Skin Symptoms</th>
<th>Distribution of Skin Lesions</th>
<th>Affected Mucous Membranes</th>
<th>Therapy</th>
<th>Outcome (duration until DC/CR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/&gt;90</td>
<td>Bullae, pruritus</td>
<td>Hands, arms</td>
<td>NA</td>
<td>Systemic and topical steroids</td>
<td>CR off therapy (unknown), natural death</td>
</tr>
<tr>
<td>2/F/&gt;50</td>
<td>Bullae, pruritus</td>
<td>Generalized</td>
<td>Oral, anogenital</td>
<td>Systemic and topical steroids, dapsone, doxycycline</td>
<td>CR off therapy (42 mo), 4 relapses</td>
</tr>
<tr>
<td>3/F/&gt;20</td>
<td>Bullae, papules, pruritus</td>
<td>Hands, arms, back</td>
<td>Oral</td>
<td>Topical steroids</td>
<td>CR off therapy (4 mo)</td>
</tr>
<tr>
<td>4/M/&gt;70</td>
<td>Bullae, pruritus</td>
<td>Hands, feet, trunk</td>
<td>NA</td>
<td>Doxycycline, topical steroids</td>
<td>CR on therapy (1 mo), 1 relapse</td>
</tr>
<tr>
<td>5/M/&gt;70</td>
<td>Bullae, vesicles</td>
<td>Hands, arms, legs</td>
<td>Anogenital</td>
<td>Systemic and topical steroids</td>
<td>CR on therapy (4 mo)</td>
</tr>
<tr>
<td>6/F/&gt;30</td>
<td>Bullae, urticarial plaques, pruritus</td>
<td>Generalized</td>
<td>Anogenital</td>
<td>Systemic and topical steroids, azathioprine</td>
<td>CR off therapy (18 mo)</td>
</tr>
<tr>
<td>7/M/&gt;80</td>
<td>Bullae, pruritus</td>
<td>Hands, arms</td>
<td>NA</td>
<td>Systemic and topical steroids, azathioprine, dapsone</td>
<td>CR on therapy (15 mo), 1 relapse</td>
</tr>
<tr>
<td>8/M/&gt;60</td>
<td>Bullae</td>
<td>Hands, arms, legs, feet</td>
<td>NA</td>
<td>Systemic and topical steroids, dapsone</td>
<td>CR off therapy (10 mo)</td>
</tr>
<tr>
<td>9/F/&gt;80</td>
<td>Bullae, urticarial plaques, pruritus</td>
<td>Hands, feet, trunk</td>
<td>Oral</td>
<td>Systemic and topical steroids, dapsone</td>
<td>DC on therapy (2 mo), died from cardiac failure</td>
</tr>
<tr>
<td>10/M/&gt;80</td>
<td>Bullae, papules, pruritus</td>
<td>Hands, arms, legs, trunk</td>
<td>NA</td>
<td>Systemic and topical steroids, azathioprine</td>
<td>CR on therapy (12 mo), 1 relapse, died from cardiac arrest 18 mo after diagnosis</td>
</tr>
<tr>
<td>11/M/&gt;40</td>
<td>Bullae, urticarial plaques, pruritus</td>
<td>Hands, legs, feet, trunk</td>
<td>Anogenital</td>
<td>Systemic and topical steroids</td>
<td>CR on therapy (5 mo)</td>
</tr>
<tr>
<td>12/M/&gt;80</td>
<td>Bullae, pruritus</td>
<td>Hands, feet, legs</td>
<td>NA</td>
<td>Doxycycline, topical steroids</td>
<td>CR on therapy (6 mo), 1 relapse</td>
</tr>
</tbody>
</table>

CR, clinical remission; DC, disease control; NA, not affected.
Fig 1. Clinical manifestations of anti-p200 pemphigoid. a and b, Tense blisters and desquamation of a palm and sole, respectively, resembling dyshidrosiform pemphigoid (patient 9). c, Vesicles on the tongue (patient 9). d, Circumscript monomorphic tense vesicles and blisters on the arm (patient 5).
Psoriasis was not observed in any patient.

All 12 patients received (super)potent topical corticosteroids during the disease course (Table I). One patient (8%) with localized disease (Figure 1d) achieved clinical remission with daily lesional application of clobetasol propionate, 0.05% cream; likewise, 2 of 12 patients (17%) achieved clinical remission with additional doxycycline, 200 mg daily. In 2 of 12 patients, topical whole-body clobetasol propionate, 0.05% cream, application was being used at onset of disease. Nine patients (75%) received systemic oral corticosteroids, mostly prednisolone, 0.5 mg/kg/d, and, in case of severe disease (in patients 2 and 11), up to 1.0 mg/kg/d. Systemic oral corticosteroids were often combined with dapsone (4 of 12 patients) or azathioprine (5 of 12), the adjunctive treatment was continued after achieving clinical remission to prevent relapses. Clinical remission on therapy was achieved in 7 of 12 patients within a mean (SD) duration of 7.2 (5.3) months. Clinical remission off therapy was seen in 4 of 12 patients, with a mean time to remission of 18.5 (16.7) months (Table I). Five patients (42%) experienced relapse during follow-up, and 3 patients (25%) died during follow-up (patients 1, 9, and 10). Detailed information on follow-up of patient 1 was not available; she was in her 90s and died of natural causes. Patient 9 had achieved disease control after 2 months, until she died of cardiac failure during surgery not related to the disease. Patient 10 died from cardiac arrest in his 80s, 18 months after diagnosis.

**HISTOPATHOLOGIC FINDINGS**

Histopathologic Findings In 11 of 12 patients with anti-p200 pemphigoid, lesional skin biopsies were available (Table II). In all biopsy specimens, subepidermal blistering was observed along with mild to dense inflammatory infiltrates in the upper dermis. The inflammatory infiltrate was exclusively composed of neutrophils in 5 patients (42%), whereas a mixed infiltrate of neutrophils and eosinophils was seen in 5 other patients (Table II). In 2 patients, papillary microabscesses were observed combined with an exclusively neutrophilic infiltrate (patient 7) and neutrophilic spongiosis (patient 9), respectively. In patient 11, only eosinophilic spongiosis was seen. Staining of type IV collagen was performed in 10 of 11 lesional biopsies. In all patients, type IV collagen was mapped to the dermal side (the floor) of the blister (Table II).

**LABORATORY DIAGNOSTICS**

Direct immunofluorescence microscopy of perilesional skin biopsy specimens showed linear depositions of IgG and C3c along the BMZ in all 12 patients (Table III). In addition, depositions of IgA and/or IgM along the BMZ were present in 7 patients (58%) and 2 patients (17%), respectively. An n-serrated immunodeposition pattern along the BMZ was identified in 9 of 11 available biopsy specimens (82%). In the remaining 2 patients, the serration pattern of the linear deposition was considered undetermined by 2 observers (J.M.M. and G.F.H.D.). The sera of all anti-p200 patients showed binding of IgG to the dermal side of the split in IIF SSS, and additional binding of IgA was found in 4 of 12 patients (33%). Knockout analysis by IIF microscopy of all 12 sera showed a positive reactivity on skin substrates deficient in type VII collagen and laminin-332, and on control healthy human skin (Table III, Fig. 2). Immunoblot analysis with human dermal extract demonstrated reactivity to the 200-kDa protein in all 12 patients. Reactivity to the recombinant C-terminus of laminin γ1 was seen in 8 of 12 patients (67%) (Table III).
Table II. Histopathological and immunohistochemical findings in 12 patients with anti-p200 pemphigoid.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Subepidermal blistering</th>
<th>Dermal infiltrate other findings</th>
<th>Type IV collagen mapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>Moderate mixed infiltrate neutrophils/eosinophils</td>
<td>Dermal</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>Moderate infiltrate neutrophils</td>
<td>Dermal</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>Moderate infiltrate neutrophils</td>
<td>Dermal</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>Moderate mixed infiltrate neutrophils/eosinophils</td>
<td>Dermal</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>Moderate infiltrate neutrophils</td>
<td>Dermal</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>Dense mixed infiltrate neutrophils/eosinophils</td>
<td>Dermal</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>Moderate infiltrate neutrophils, papillary microabcesses</td>
<td>Dermal</td>
</tr>
<tr>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>Moderate infiltrate neutrophils, neutrophilic spongiosis, papillary microabcesses</td>
<td>Dermal</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>Moderate mixed infiltrate eosinophils/neutrophils</td>
<td>Dermal</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>Moderate eosinophilic spongiosis</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>Mild mixed infiltrate eosinophils/neutrophils</td>
<td>Dermal</td>
</tr>
</tbody>
</table>

CR, clinical remission; DC, disease control; NA, not affected.

Fig 2. Indirect immunofluorescence (IIF) microscopy knockout analysis on skin substrate of a patient with epidermolysis bullosa completely deficient of type VII collagen (RDEB) or laminin-332 (JEB). a and b, Test results for anti-laminin-332 mucous membrane pemphigoid (MMP). In absence of the target antigen, serum of a patient with anti–laminin-332 MMP is negative (neg) on laminin-332 deficient skin (a). c and d, Test results for epidermolysis bullosa acquisita (EBA). Similarly, serum of a patient with EBA is negative on type VII collagen deficient skin (d). e and f, Test results for anti-p200 pemphigoid. Serum of a patient with anti-p200 pemphigoid is positive (pos) on both skin substrates, therefore excluding autoantibodies to solely laminin-332 and type VII collagen.
Table III. Laboratory diagnostics of 12 patients with anti-p200 pemphigoid.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>DIF</th>
<th>Serration pattern</th>
<th>IIF</th>
<th>Knockout</th>
<th>IB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-BMZ</td>
<td>MO</td>
<td>SSS dermal staining</td>
<td>Laminin-332</td>
<td>Type VII collagen</td>
</tr>
<tr>
<td>1</td>
<td>IgG3+ C5c2+</td>
<td>N-serrated</td>
<td>+/-</td>
<td>IgG2+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>IgG3+ C5c3+</td>
<td>NA</td>
<td>+/-</td>
<td>IgG2+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>IgG2+ C5c3+</td>
<td>N-serrated</td>
<td>+</td>
<td>IgG2+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>IgG+ C5c2+</td>
<td>N-serrated</td>
<td>+/-</td>
<td>IgG3+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>IgG2+ C5c3+</td>
<td>N-serrated</td>
<td>+</td>
<td>IgG+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>IgG3+ IgA+ C5c2+</td>
<td>N-serrated</td>
<td>+/-</td>
<td>IgG3, IgA+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>IgG3+ IgA+ C5c3+</td>
<td>Undetermined</td>
<td>+/-</td>
<td>IgG2+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>IgG3+ IgA+ C5c2+</td>
<td>N-serrated</td>
<td>+/-</td>
<td>IgG+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>IgG3+ IgA+ C5c3+</td>
<td>N-serrated</td>
<td>+/-</td>
<td>IgG2, IgA+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>IgG+ IgA+ IgM+ C5c3+</td>
<td>N-serrated</td>
<td>NA</td>
<td>IgG+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>IgG3+ IgA2+ C5c3+</td>
<td>N-serrated</td>
<td>+/-</td>
<td>IgG3, IgA+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>IgG3+ IgA2+ IgM+ C5c3+</td>
<td>Undetermined</td>
<td>+/-</td>
<td>IgG3, IgA+</td>
<td>+</td>
</tr>
</tbody>
</table>

BMZ, basement membrane zone; DIF, direct immunofluorescence microscopy; IB laminin γ1, immunoblot recombinant C-terminus laminin γ1; IB p200, immunoblot human dermal extract; IIF, indirect immunofluorescence microscopy; MO, monkey oesophagus; NA, not available; SSS, salt (sodium chloride)-split skin; +, feature is present; −, feature is absent.
Discussion

Laboratory diagnosis of anti-p200 pemphigoid was feasible by using IIF microscopy knockout analysis in our series of 12 patients. The clinical profile of this subtype of pemphigoid had a remarkable acral distribution of blisters on hands and feet, resembling dyshidrosiform pemphigoid. Moreover, mucosal involvement was seen in half of the patients. The disease course of these 12 patients with anti-p200 pemphigoid was more severe than so far reported in the literature; treatment with systemic corticosteroids was needed in three-quarters of patients.

The clinical presentation of anti-p200 pemphigoid has been described as highly variable mimicking BP, linear IgA dermatosis, and the inflammatory variant of EBA. The findings in these 12 patients showed a common denominator in initial clinical presentation with acral distribution of tense blisters on hands and feet. Although not exclusive to anti-p200 pemphigoid, the acral blisters may give a clinical clue to diagnosis. In contrast to mechanobullous EBA, no scarring or milia formation was observed. In most patients, skin blistering was limited to the extremities; only 2 patients presented with a generalized bullous eruption. Involvement of oral and/or anogenital mucosa was observed in 50% of patients, which was much higher than previously estimated in literature in about 20% of reported cases. However, the mucosal lesions were not a dominant feature in the patients and less severe (no ocular or laryngeal involvement) than in patients with anti–LN-332 MMP. Although a rare disease, owing to a relatively low number of patients (12), these clinical findings do not allow us to draw conclusions on general characteristics of anti-p200 pemphigoid. Patients with anti-p200 pemphigoid tend to be slightly younger compared with those with BP, which mainly affects patients older than 70 years. Based on routinely performed IIF SSS in the diagnostic algorithm for sAIBD, we estimate anti-p200 pemphigoid to constitute approximately 1.5% of seropositive patients with sAIBD in our clinic (unpublished data). For the differential diagnosis of sAIBD with dermal side binding by IIF SSS, the ratio of anti-p200 pemphigoid, anti–LN-332 MMP and EBA was 23.5% (total sAIBD, 1.3%), 21.6% (total sAIBD, 1.2%), and 54.9% (total sAIBD, 3.0%), respectively. Diagnosis of anti-p200 pemphigoid and anti-LN-332 MMP can be confirmed only based on serologic test results. Consequently, seronegative patients diagnosed as having EBA based on the linear u-serrated anti-BMZ immunodeposition by DIF are not included in this calculation. Summarizing, 1 in 4 patients with dermal side binding by IIF SSS was diagnosed as having anti-p200 pemphigoid.

No treatment recommendations are known for anti-p200 pemphigoid; therapy usually corresponds with standard treatment for BP. The clinical course of anti-p200 pemphigoid has previously been described as variable, but usually more benign compared with that of EBA, and even BP. In contrast, in our study population, most (75%) received systemic corticosteroids and often adjuvant immunosuppressive drugs. Nevertheless, 5 patients (42%) experienced relapse during follow-up. Although they had a more severe disease course than was expected, a number of patients with localized disease showed a prompt response to immunosuppressive treatment.

Currently the diagnosis of anti-p200 pemphigoid is based on DIF microscopy showing linear depositions of IgG and C3c along the BMZ and dermal side binding by IIF on 1-mol/L SSS. Using the latter technique, anti-p200 pemphigoid can be differentiated from BP but not from EBA and anti–LN-332 MMP. For the first time, to our knowledge, routine DIF serration pattern analysis was included in the diagnostic algorithm of anti-p200 pemphigoid (Figure 3). To perform DIF serration pattern analysis, it is sufficient to use a conventional IF microscope.
Dermal side binding of IgG autoantibodies

<table>
<thead>
<tr>
<th>Serum IIf SSS</th>
<th>Biopsy by direct IF</th>
<th>Differential diagnosis</th>
<th>Confirmatory serological test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear n-serrated anti-BMZ IgG</td>
<td>Anti-p200 pemphigoid</td>
<td>Immunoblot dermal extract</td>
<td>p200 (200 kDa) or Immunoblot Recombinant C-terminus laminin γ1</td>
</tr>
<tr>
<td>Linear u-serrated anti-BMZ IgG</td>
<td>Anti-laminin-332 MMP</td>
<td>Immunoblot ECM cultured keratinocytes</td>
<td>Laminin-332 (α3, β3, and γ2 chains) or IIF knockout analysis Type VII collagen-deficient skin - or Laminin-332-deficient skin +</td>
</tr>
</tbody>
</table>

Immunoprecipitation Laminin-332 (α3, β3, and γ2 chains)

ELISA Laminin-332

Immunoblot dermal extract Type VII collagen (290 kDa)

IIF knockout analysis Type VII collagen-deficient skin - or Laminin-332-deficient skin +

ELISA Type VII collagen

Fluorescence overlay antigen mapping

EBA

**Fig. 3.** Algorithm for diagnosis of anti-p200 pemphigoid in patients with subepidermal autoimmune bullous diseases (sAIBDs) and IgG autoantibodies binding to the dermal side on SSS by (IIF) microscopy.

The differential diagnosis includes anti-p200 pemphigoid, anti-laminin-332 mucous membrane pemphigoid (MMP) and epidermolysis bullosa acquista (EBA). To distinguish between these sAIBDs, final diagnosis can be confirmed by using I or a combination of available laboratory techniques: immunoblotting with appropriate substrates, immunoprecipitation, enzyme-linked immunosorbent assay (ELISA), fluorescence overlay antigen mapping or IIF knockout analysis on type VII collagen and laminin-332-deficient skin. Reactivity to laminin γ1 is seen in 70% to 90% of patients with anti-p200 pemphigoid and can be used as a diagnostic marker.

ECM, extracellular matrix; IF, immunofluorescence; IIF, indirect immunofluorescence; SSS, salt (sodium chloride)-split skin. *Diagnosis of EBA can be made by direct immunofluorescence (DIF) microscopy alone, based on a linear u-serrated deposition pattern of IgG along the basement membrane zone (BMZ).
with 40× dry objective and 10× ocular. The linear n-serrated pattern is found in all sAIBD with immunodeposits located in hemidesmosomes, lamina lucida, or lamina densa. In contrast, in EBA and in bullous systemic lupus erythematosus, a linear u-serrated pattern is seen corresponding with the ultralocalization of type VII collagen in the sublamina densa zone. Described previously by Vodegel et al. in 2 patients, our findings confirm that anti-p200 pemphigoid is characterized by a linear n-serrated pattern by DIF microscopy. This finding corresponds with the observed ultrastructural localization of anti-p200 immunodeposits along the lower lamina lucida and upper lamina densa of the BMZ.

The histopathological features do not allow to distinguish anti-p200 from other sAIBD. Nevertheless, the mainly neutrophilic inflammatory dermal infiltrate is in contrast to a typically eosinophilic infiltrate in BP and may prompt a histopathologist to consider the diagnosis of anti-p200 pemphigoid and perform DIF microscopy and serological studies to define or exclude the diagnosis. Type IV collagen in anti-p200 pemphigoid locates to the blister floor and may be helpful to differentiate it from EBA, where in type IV collagen is found on the blister roof.

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Previously, Dainichi et al. described the presence of antibodies against laminin γ1 in 90% of anti-p200 pemphigoid sera, confirmed by enzyme-linked immunosorbent assay (ELISA) using the recombinant C-terminus of laminin γ1. In our study, 8 of 12 patients with anti-p200 pemphigoid (66.7%) showed reactivity against hLAMC1-cterm by immunoblot. Differences in patient population may play a role; for example, an association with psoriasis has been reported in up to 30% of cases, mostly in Asian patients. In contrast, coexistence of psoriasis was not observed in any of our cases. Moreover, this difference might be explained by reactivity of autoantibodies to epitopes of laminin γ1 outside of hLAMC1-cterm or with another 200-kDa dermal protein. Although relapses occurred only in patients with reactivity to laminin γ1, no correlations were seen with disease area and severity. Reactivity to laminin γ1 by immunoblot or ELISA may be useful as a diagnostic marker for anti-p200 pemphigoid.

Laboratory techniques, such as immunoblot with dermal extract or recombinant laminin γ1 and IIF microscopy knockout analysis, may not be available in most medical centers. In contrast, DIF serration pattern analysis can be performed in routine laboratories and allows identification of patients with EBA. In patients with dermal side binding of autoantibodies on IIF SSS suspected for anti-p200 pemphigoid, it is recommended to specify the diagnosis of anti-p200 pemphigoid in referral centers for autoimmune blistering disease.

CONCLUSIONS

Laboratory diagnosis of anti-p200 pemphigoid is possible not only by immunoblot with human dermal extract, but also by IIF microscopy knockout analysis on skin substrates deficient in type VII collagen and laminin-332. We add DIF serration pattern analysis to the diagnostic algorithm to differentiate anti-p200 pemphigoid (n-serrated) from EBA (u-serrated). Clinical clues of anti-p200 pemphigoid in our series of 12 patients with anti-p200 pemphigoid include acral distribution of blisters without scarring or milia, mild mucosal involvement in half of them, and a slightly younger age of onset of disease compared with patients with BP.
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