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Anti-p200 pemphigoid is the most common pemphigoid disease with serum antibodies against the dermal side by indirect immunofluorescence microscopy on human salt-split skin

To the Editor: Pemphigoid diseases are characterized by autoantibodies (AAbs) against structural proteins of the dermoepidermal junction.[1] Indirect immunofluorescence microscopy on salt-split human skin is widely used as a screening test for serum AAbs in the diagnosis of pemphigoid diseases. Binding of AAbs to the dermal side of the artificial split is observed in patients with epidermolysis bullosa acquisita (EBA) (AAbs against type VII collagen [Col7]), anti−laminin 332 (lam332) mucous membrane pemphigoid (MMP), and anti-p200 pemphigoid (AAbs against p200 protein/laminin γ1 [lamγ1]).[1] About 15% of pemphigoid sera are dermal binders (ie, they contain AAbs that label the dermal side by indirect immunofluorescence microscopy on human salt-split skin).[2]

The aim of our cross-sectional study was to determine the relative frequency of pemphigoid disease with dermal binding. A total of 141 consecutive sera that were sent to the routine autoimmune laboratory of the Department of Dermatology between January 2011 and July 2017 revealed dermal binding AAbs. These sera were subjected to Col7-specific enzyme-linked immunosorbent assay (Euroimmun, Lübeck, Germany); Western blotting with extract of human dermis (containing Col7 and p200) and with the recombinant C-terminus of lamγ1; and an indirect immunofluorescence test with HEK cells expressing the recombinant NC1-domain of Col7, lam332, and lamγ1, respectively (Fig 1).[3-5] The study was performed following the principles of the Declaration of Helsinki (12-178). Only sera from senders that send all sera with suspicion of an autoimmune blistering disease to our routine autoimmune laboratory were included, so we estimate that the analyzed cohort mirrors the spectrum of pemphigoid diseases in Central Europe.

Anti-p200/lamγ1 pemphigoid was identified as the most frequent pemphigoid disease in this cohort, followed by EBA and anti-lam332 MMP. In detail, 115 sera (81.6%) showed reactivity against the p200 protein and/or lamγ1. Of these 115 sera, 77 (54.6%) reacted against both the p200 protein and lamγ1; 12 (8.5%) and 13 (9%) had AAbs exclusively against lamγ1 and p200, respectively; 9 revealed additional reactivity against lam332; and 4 revealed additional reactivity against Col7 (Fig 2).

When additional information such as semi-quantitative evaluation of AAb reactivity, clinical information, and direct immunofluorescence results were included, 3 of the 4 sera with dual reactivity against both Col7 and lamγ1 were classified as EBA and 1 was classified as anti-p200/lamγ1 pemphigoid. Of the 9 sera with reactivity against both lam332 and lamγ1/p200, 1 was diagnosed as anti-lam332 MMP and 8 were

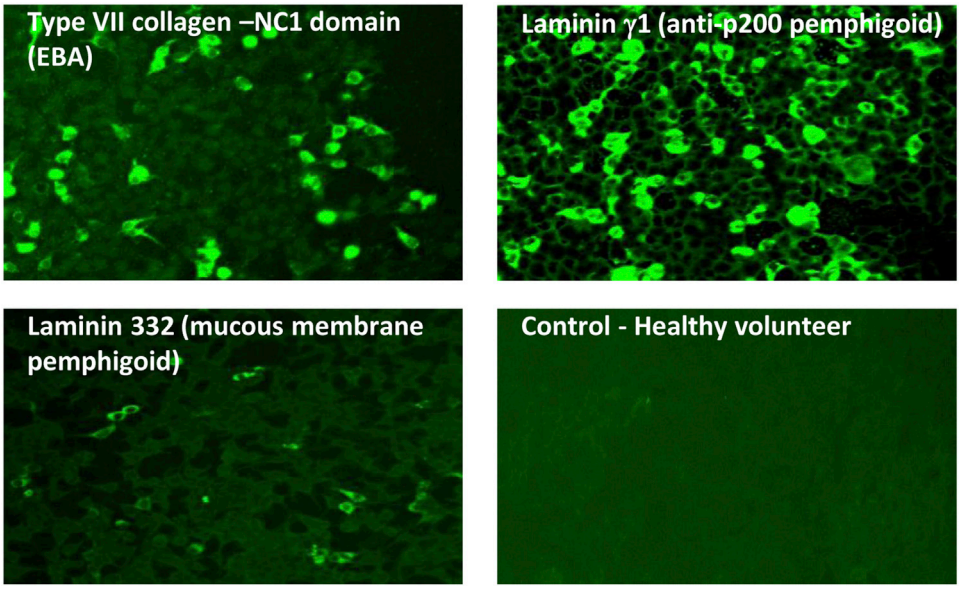

Fig 1. Indirect immunofluorescence test. A Biochip mosaic with the human cell line HEK293 recombinantly expressing the NC1 domain of type VII collagen, laminin 332, and laminin γ1 on the cell surface was used in this study. A compilation of reactivities with positive control antibodies and serum from a healthy volunteer is shown. *EBA*, Epidermolysis bullosa acquisita.

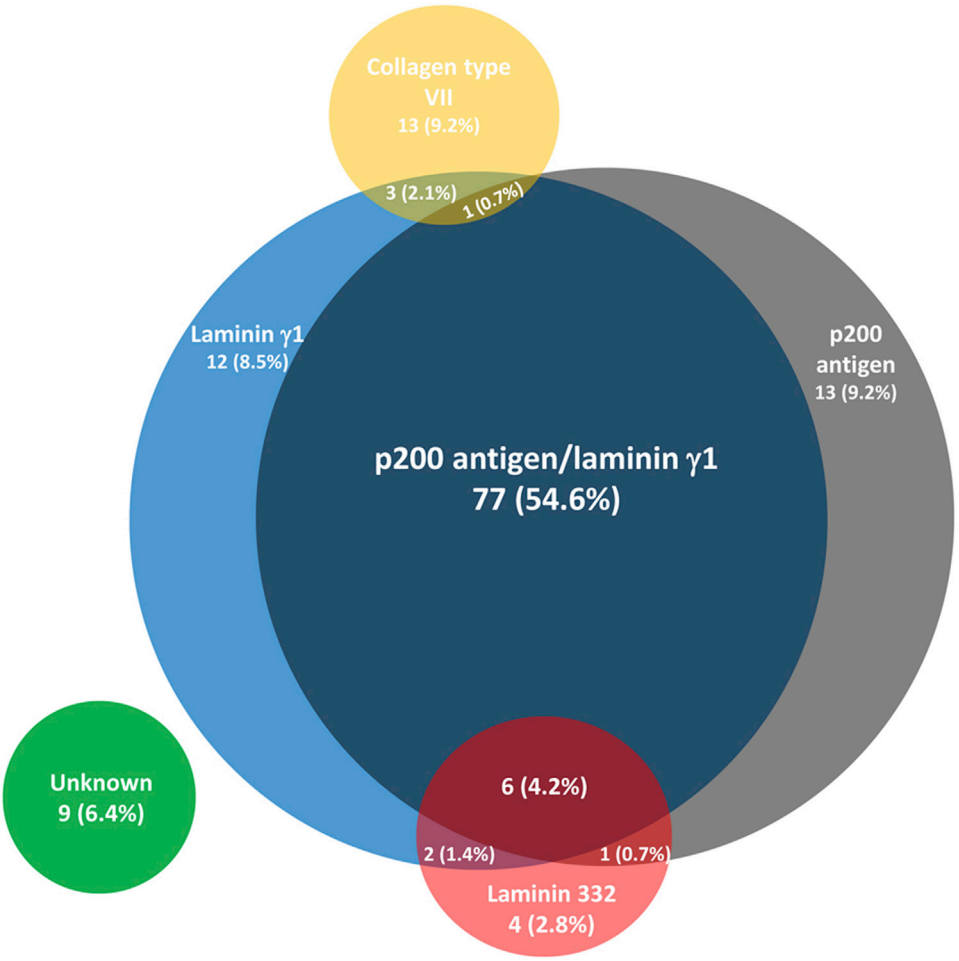

Fig 2. Target antigens of serum autoantibodies. A Venn diagram is used to show the targets of serum autoantibodies in a cohort of 141 consecutive sera with dermal binding by indirect immunofluorescence microscopy on human salt-split skin.

diagnosed as anti-p200/lamγ1 pemphigoid. In addition, 13 sera (9.2%) contained AAbs exclusively reactive with Col7, and 4 (2.8%) contained AAbs exclusively reactive with lam332. In 9 of the cohort of 141 sera (6.4%), no target antigen was identified (Fig 2).

In summary, anti-p200/lamγ1 pemphigoid was by far the most frequent pemphigoid disease, with 78.7% of the 141 patients having dermal binding AAbs, followed by EBA in 11.4% of patients and anti-lam332 MMP in 3.5% of patients.

Dual reactivity with different antigens may be explained either by cross-reactive AAbs or by epitope spreading, which is a phenomenon that describes the generation of AAbs with different antigen specificities in the same patient.[1] Our data suggest that epitope spreading may occur more frequently in anti-p200/lamγ1 pemphigoid than in EBA and anti-lam332 MMP.

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Methodologic gaps and risk of bias in randomized controlled trials of local anogenital wart treatments

To the Editor: The latest guidelines for first-line treatment of anogenital warts (AGWs) in immunocompetent adults failed to establish a therapeutic hierarchy[1,2] because of methodologic gaps in research and insufficient randomized controlled trial (RCT) evidence. This section of our systematic review (Prospero no. CRD42015025827) addresses these insufficiencies and provides recommendations for future RCTs of AGW treatments.

A search was conducted through 12 databases from their inception to August 1, 2018 (supplemental material available at https://www.mendeley.com/community/journal-of-the-american-academy-of-dermatology/). RCTs were included when a provider- or patient-administered treatment was reported in ≥1 parallel treatment group; the other inclusion criteria were reported in a previous paper.[3] The primary outcomes were percentage of clearance and percentage of recurrence, and the Cochrane Collaboration risk of bias tool and its methodology[4] were used.

In total, 70 unique RCTs involving 9931 individual patients (mean 142 participants/study) fulfilled the inclusion criteria (Appendix S2). The overwhelming majority of included RCTs (66/70) were found to be of poor quality (Appendix S3). A risk of performance bias due to knowledge of the allocated intervention by participants and personnel (excluding outcome assessor) was detected in 31 of 70 RCTs (row 3, Appendix S3). The risk of detection bias due to knowledge of the allocated intervention by outcome assessors was high in 10 of 70 and unclear in 35 of 70 RCTs (row 4, Appendix S3). The risk of selection bias due to inadequate generation of a randomized sequence was high in 7 of 70 and unclear in 38 of 70 RCTs (row 1, Appendix S3). Other biases corresponded mainly to pharmaceutical funding or to conflicts of interest; the risk was high in 25 of 70 RCTs and unclear in 35 of 70 RCTs (row 7,