Subepidermal type VII collagen speckles as an additional clue for diagnosing epidermolysis bullosa acquisita by salt-split skin serum analysis

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Further studies are required to examine the dose–response relationship and elucidate the underlying mechanism.

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**Conflicts of interest**
The authors have no conflict of interest to declare.

**Data availability statement**
The data that support the findings of this study are available from the corresponding author upon reasonable request.

**References**


**Subepidermal type VII collagen speckles as an additional clue for diagnosing epidermolysis bullosa acquisita by salt-split skin serum analysis**

*Dear Editor,*

Epidermolysis bullosa acquisita (EBA) is an autoimmune disorder in which anti-type VII collagen IgG and/or IgA autoantibodies cause subepidermal blistering and scarring. Two main forms can be distinguished, the classical mechanobullous form and the inflammatory form that mimics other subepidermal blistering diseases as bullous pemphigoid, mucous membrane pemphigoid, Brunsting–Perry pemphigoid or linear IgA dermatosis. Therefore, laboratory diagnostics is essential for a correct diagnosis. It is best done on a biopsy by analysing the serration pattern of the IgG deposition at the epidermal basement membrane zone (BMZ) by direct immunofluorescence (DIF) microscopy, as EBA is the only form of pemphigoid that has a u-shaped deposition pattern. Alternatively, also immunoelectron microscopy will demonstrate EBA by the unique localization of the IgG deposits. If only serum is available, indirect immunofluorescence on salt-split skin (IIF-SSS) substrate will narrow down the diagnosis to either EBA, anti-laminin-332 mucous membrane pemphigoid (anti-LN332 MMP) or anti-p200 pemphigoid by demonstrating the binding to the floor of the artificial blister. The final diagnosis of EBA relies on the demonstration of anti-type VII collagen antibodies by either enzyme-linked immunosorbent assay (ELISA), immunoblotting or immunofluorescence knock-out analysis. Recently, Hayakawa et al. reported that in addition to the linear BMZ, type VII collagen was also present in small subepidermal speckles localized 2–8 µm below the BMZ. Double staining revealed that type IV collagen and elastic colocalized, but the function or deposition mechanism of these speckles remain completely unknown. In addition, EBA patient serum IgG bound to these speckles on normal human skin substrate. Therefore, here, we investigated if the speckles could be potentially useful as an additional diagnostic clue for EBA on IIF-SSS. We performed IIF-SSS staining of 53 serum samples, 15 of patients with EBA of which 10 had the inflammatory type and five had the mechanobullous type, 15 from patients with anti-LN332 MMP, 13 of anti-p200 pemphigoid patients and 10 normal human control sera. All patient samples demonstrated dermal binding by IF-SSS, and were extensively diagnosed by keratinocyte footprint assay for anti-LN332 MMP and by knock-out analysis on both type VII collagen and LN-332 negative skin and immunoblot for anti-p200. A total of 14 EBA sera were ELISA-positive for anti-type VII collagen IgG. As SSS substrate, we used a human skin biopsy which after storage overnight in physiological salt was incubated another night in 1 mol/L NaCl in PBS pH 7.2, containing 1 mmol/L EDTA. After freezing, 4 µm thick sections were cut and incubated for 30 min with sera diluted 1:8 in PBS. However, secondary antibody served an anti-human IgG4-FITC conjugate. An anti-total IgG conjugate was not possible to use due to excessive dermal background IgG. Of the 43 selected sera, 37 demonstrated IgG4 binding in IIF-SSS, 4 EBA and 2 anti-LN332 MMP sera were negative. We found that all 11 EBA sera, of nine patients with the inflammatory type and two with the mechanobullous type, demonstrated binding of IgG4 to the type VII collagen speckles.

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(73.3% sensitivity) while all anti-LN332 MMP, anti-p200 pemphigoid and normal human control sera did not show any binding (100% specificity) (Fig. 1). In case of doubt due to weaker IgG4 staining a double staining with a monoclonal antibody to type VII collagen will ascertain if IgG does colocalize with the speckles.

The speckles are unevenly distributed but are best found at the top of the dermal space between the rete ridges. Surprisingly, by staining with monoclonal antibodies to all three chains of laminin-332 we found that the speckles also contained a small amount of laminin-332. However, this amount is apparently too low to be picked up by anti-LN332 MMP sera as all 13 anti-LN332 MMP sera IgG4 bound linear along the BMZ but did not colocalize with the speckles.

Briefly, binding of patient serum IgG4 to subepidermal type VII collagen speckles in immunofluorescence salt-split skin analysis is a simple additional tool for diagnosing EBA.

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Conflicts of interest
None to declare.

Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figure 1 Double staining of patient serum and anti-type VII collagen monoclonal LH7.2 on salt-split skin. (a) Double staining with EBA serum, in the overlay LH.72 in red and IgG4 in green. The type VII collagen speckles colocalize with serum IgG4. (b) Double staining with anti-LN-332 MMP patient serum. No overlap is seen of IgG4 and type VII collagen speckles. (c) Double staining with anti-p200 pemphigoid patient serum IgG4 and type VII collagen. No overlap is seen of IgG4 and type VII collagen speckles. White bar is 10 µm.
A fallen-snow pattern of hair in trichoteiromania

Dear Editor,

A 10-year-old boy presented with a bald patch that had persisted for over 1 month. Physical examination revealed a 3 × 3 cm square nonscarring alopecia patch located in the right temporal/parietal scalp, with regular and clear borders. Close inspection revealed white spots on the end of the broken hairs (Figure 1). The hair-pull test was negative, and no obvious abnormality was found in the remaining examination. Dermoscopic examination showed monotonous and numerous short broken hairs with a dandelion-like appearance at the ends, collectively presented as the ‘fallen-snow-like pattern’ (Figure 2). Occasional scratching marks and crust were also shown. Black or yellow dots were not observed. The parents complained of hyperactivity of the boy. We did not perform a scalp biopsy. Instead, we introduced the clinical manifestation of compulsive hair disorders in detail to the patients and parents. A further inquiry revealed that the patient had inadvertently rubbed his scalp when studying. Hair pulling or rubbing was not reported. Although the child complained of itching of the hairless area, there was no history of seborrheic dermatitis, psoriasis or atopic dermatitis. He was diagnosed with trichoteiromania. The boy was referred to a psychologist. Previously, the family is