Standardized indirect immunofluorescence-based detection of dermal-binding autoantibodies in pemphigoid diseases: a prospective international multicentre study

https://doi.org/10.1093/bjd/ljaf385

Dear Editor, Pemphigoid diseases are a group of autoimmune subepidermal blistering disorders characterized by autoantibodies targeting structural proteins that anchor epidermis to dermis.[1] The diseases show distinct clinical and immunopathological features, as well as prognosis, and thus require an accurate diagnosis to guide therapy.[2] This particularly applies to patients with anti-laminin 332 mucous membrane pemphigoid (MMP), 25% of whom may experience associated malignancies. Additionally, patients with epidermolysis bullosa acquisita (EBA) usually require more intense therapy than those with bullous pemphigoid (BP) or anti-p200 pemphigoid.[1,3,4]

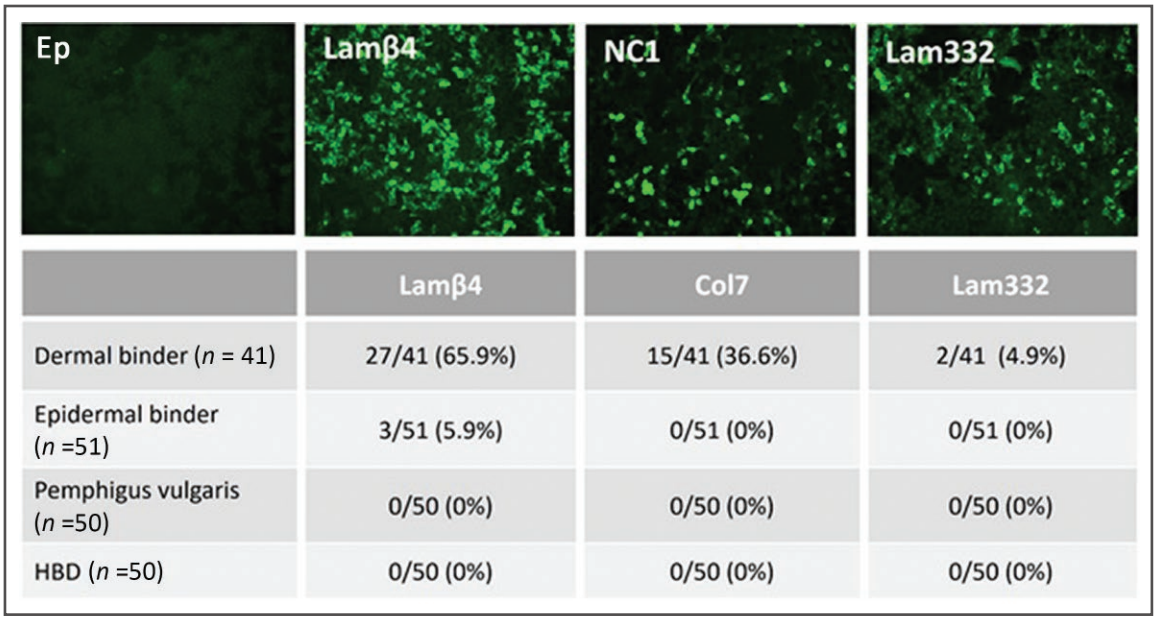
Indirect immunofluorescence (IIF) on human salt-split skin (SSS) is a standard screening method for pemphigoid diseases. Here, autoantibodies bind to the epidermal or dermal side of the artificial split.[2] Autoantibodies against BP180 and BP230 in patients with BP, pemphigoid gestationis, linear IgA disease and MMP label the epidermal side of the split. Dermal binding is seen in EBA with autoantibodies against type VII collagen, anti-laminin 332 MMP with autoantibodies against laminin 332, and anti-p200 pemphigoid with IgG against the laminin β4 and possibly the laminin γ1 subunits.[2] IIF does not allow for the precise identification of the respective target antigen, requiring additional tests such as enzyme-linked immunosorbent assays (ELISA) or immunoblotting.[2] This study aimed to improve the diagnosis of dermal-binding pemphigoid diseases using a Biochip™-based IIF assay. The assay comprised previously developed biochips containing HEK293 cells expressing recombinant NC1 domain of type VII collagen, the laminin 332 heterotrimer and the full-length laminin β4 subunit, the recently identified diagnostically relevant autoantigen of anti-p200 pemphigoid (Figure 1).[3,5,6]

Participants were recruited from a prospective international multicentre study involving consecutive patients with autoimmune blistering diseases across 19 centres in 14 countries. The study included 41 patients [12 women, 28 men and 1 of unknown sex; mean (SD) age 65.7 (16.6) years (median 68.5)] with linear IgG binding at the (muco) cutaneous basement membrane zone by direct immunofluorescence (DIF) and dermal IgG binding on human SSS. Control patients included participants in a prospective international multicentre study with (i) the same DIF pattern and epidermal IIF binding on SSS [$n=51$ (32 women, 19 men); mean (SD) age 72.2 (19.1) years (median 75.5)]; (ii) pemphigus vulgaris (PV; $n=50$) with compatible DIF and serum IgG against desmoglein 3; and (iii) healthy blood donors ($n=50$). Sera were stored at −80 °C until used. Sera were diluted 1 : 10 and tested according to the manufacturer's instructions (Euroimmun, Lübeck, Germany).

All 41 dermal binders showed reactivity with at least one of the target antigens presented on the Biochip mosaic. More specifically, 27 sera (66%) reacted with the laminin β4 subunit, 2 (5%) with laminin 332 and 15 (37%) with the NC1 domain of type VII collagen (Figure 1). Post hoc, routine reference assays (ELISA and immunoblots) were performed. Twenty-four of 27 laminin β4-positive samples recognized p200 by immunoblot on dermal extract, 19 reacted with recombinant laminin γ1 and 3 were negative in both assays.[7] Three other sera showed dual reactivity with laminin β4 and type VII collagen, which may be explained by epitope spreading.[8] In sera from patients with PV or healthy blood donors, no reactivity was observed with any of the three substrates. Three sera with epidermal binding recognized laminin β4-expressing cells (Figure 1). Faint dermal binding in addition to epidermal reactivity was shown by retesting these sera by IIF on SSS, as well as reactivity with recombinant laminin β4, recombinant laminin γ1 and the p200 antigen in human dermal extract by immunoblotting (data not shown). These data show that the 'dermal binder' Biochip mosaic is a highly sensitive tool for detecting serum IgG autoantibodies in patients who show dermal binding of IgG by IIF on SSS.

A limitation of this proof-of-concept study is the small sample size, which may not reflect true disease incidences. Reference testing was performed post hoc using routine methods. A prospective multicentre study is currently being performed to describe the characteristics of the Biochip mosaic test independently in larger cohorts in comparison with respective conventional in-house assays.

The 'dermal binder' Biochip mosaic seems to be a valuable and useful test for the parallel detection of autoantibodies against laminin β4, type VII collagen and laminin 332. The high laminin β4 reactivity highlights anti-p200 pemphigoid as an underdiagnosed pemphigoid disorder and justifies the



	Lamβ4	Col7	Lam332
Dermal binder (*n* = 41)	27/41 (65.9%)	15/41 (36.6%)	2/41 (4.9%)
Epidermal binder (*n* =51)	3/51 (5.9%)	0/51 (0%)	0/51 (0%)
Pemphigus vulgaris (*n* =50)	0/50 (0%)	0/50 (0%)	0/50 (0%)
HBD (*n* =50)	0/50 (0%)	0/50 (0%)	0/50 (0%)

Figure 1 Reactivities of pemphigoid sera on the 'dermal binder' Biochip™ mosaic. (Top) Representative images of sera reacting with laminin β4 (Lamβ4), laminin 332 (Lam322) and the NC1 domain of type VII collagen (Col7). A dermal binder serum showed no reactivity with HEK293 cells transfected with the empty plasmid (EP). Magnification × 200. (Bottom) Serum reactivities detected with the 'dermal binder' Biochip in this study. In a post hoc analysis, faint dermal binding was seen in addition to the epidermal binding on salt-split skin. HBD, healthy blood donor.

importance of including this antigen in routine testing. This composite diagnostic approach may considerably improve the diagnostic workflow for pemphigoid diseases.

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Acknowledgements: We thank Vanessa Krull and Dr Stephanie Freyher (Lübeck) for their excellent technical assistance.

Funding sources: This work was supported by structural funding from the Schleswig-Holstein Excellence Cluster 'Precision Medicine in Chronic Inflammation' (German Research Foundation [DFG] EXC 2167/1) and the Collaborative Research Center (CRC) 1526 'Pathomechanisms of Antibody-mediated Autoimmunity (PANTAU) – Insights from Pemphigoid Diseases' (SFB1526/01).

Conflicts of interest: C.P. and S.M. are employees of Euroimmun. L.K. is a member of the Executive Board of Euroimmun. E. Schmidt has a scientific cooperation with Euroimmun and is an inventor on patents with Euroimmun. S.G. is an inventor on a patent with Euroimmun. E. Sprecher is Section Editor of the Translational Research section of the *BJD*. The other authors declare no conflicts of interest.

Data availability: The data underlying this article will be shared on reasonable request to the corresponding author.

Ethics statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Lübeck (11-078).

Patient consent: Written patient consent for publication was obtained.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

References

1 Amber KT, Murrell DF, Schmidt E *et al.* Autoimmune subepidermal bullous diseases of the skin and mucosae: clinical features, diagnosis, and management. *Clin Rev Allergy Immunol* 2018; **54**:26–51.
2 van Beek N, Holtsche MM, Atefi I *et al.* State-of-the-art diagnosis of autoimmune blistering diseases. *Front Immunol* 2024; **15**:1363032.
3 Goletz S, Probst C, Komorowski L *et al.* A sensitive and specific assay for the serological diagnosis of antilaminin 332 mucous membrane pemphigoid. *Br J Dermatol* 2019; **180**:149–56.

4 Patzelt S. Schmidt E. Autoimmunity against laminin 332. *Front Immunol* 2023; **14**:1250115.
5 Goletz S, Probst C, Komorowski L *et al*. Sensitive and specific assay for the serological diagnosis of anti-p200 pemphigoid based on the recombinant laminin beta4 subunit. *Br J Dermatol* 2024; **191**:140–1.
6 Komorowski L, Muller R, Vorobyev A *et al*. Sensitive and specific assays for routine serological diagnosis of epidermolysis bullosa acquisita. *J Am Acad Dermatol* 2013; **68**:e89–95.
7 Dainichi T, Kurono S, Ohyama B *et al*. Anti-laminin gamma-1 pemphigoid. *Proc Natl Acad Sci U S A* 2009; **106**:2800–5.
8 Holtsche MM, Goletz S, von Georg A *et al*. Serologic characterization of anti-p200 pemphigoid: epitope spreading as a common phenomenon. *J Am Acad Dermatol* 2021; **84**:1155–7.

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Co-primary endpoints PASI 90 and IGA 0/1 at Week 16 were met.**Secondary endpoints. †N= mNRI, missing data were imputed with mNRI (patients with missing data following treatment discontinuation due to lack of efficacy or a TRAE were counted as non-responders; multiple imputation methodology was used for other missing data). ‡43.9% (n=189/431), and 43.4% (n=116/267) of biologic-naïve and TNFi-IR PsA patients achieved the primary endpoint of ACR 50 at Week 16 in BE OPTIMAL and BE COMPLETE, respectively (vs 10.0% [n=28/281] and 6.8% [n=9/133] placebo, p<0.0001); 54.5% (n=235/431) and 51.7% (n=138/267) maintained it at Week 52 (NRI).[4–6]

ACR 50, ≥50% response in the American College of Rheumatology criteria; **AS**, ankylosing spondylitis; **CRP**, C-reactive protein; **DMARD**, disease-modifying antirheumatic drug; **HS**, hidradenitis suppurativa; **IGA**, Investigator's Global Assessment; **(m)NRI**, (modified) non-responder imputation; **MRI**, magnetic resonance imaging; **nr-axSpA**, non-radiographic axial spondyloarthritis; **NSAID**, non-steroidal anti-inflammatory drug; **PASI 75/90/100**, ≥75/90/100% improvement from baseline in Psoriasis Area and Severity Index; **PsA**, psoriatic arthritis; **PsD**, psoriatic disease; **PsO**, psoriasis; **TNFi-IR**, tumour necrosis factor-α inhibitor – inadequate responder; **TRAE**, treatment-related adverse event.

References: 1. Gordon KB, et al. Lancet. 2021;397(10273):475–486. 2. Blauvelt. 2025. AAD Presentation 62275. 3. Mease PJ, et al. Rheumatol Ther. 2024;11(5):1363–1382. 4. BIMZELX SmPC. 5. Ritchlin CT, et al. Ann Rheum Dis. 2023;82(11):1404–1414. 6. Coates LC, et al. RMD Open. 2024;10(1):e003855. 7. Strober B, et al. AAD 2024;oral presentation.

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