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Source / Izvornik: **British Journal of Dermatology, 2018, 179, 30 - 41**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/doi:10.1111/bjd.16138

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:962015

BJD
British Journal of Dermatology

International Bullous Diseases Group: consensus on diagnostic criteria for epidermolysis bullosa acquisita

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Linked Comment: Yamagami. *Br J Dermatol* 2018; **179**:7.

Summary

Background Epidermolysis bullosa acquisita (EBA) is a complex autoimmune bullous disease disease with variable clinical presentations and multiple possible diagnostic tests, making an international consensus on the diagnosis of EBA essential.

Objectives To obtain an international consensus on the clinical and diagnostic criteria for EBA.

Methods The International Bullous Diseases Group (IBDG) met three times to discuss the clinical and diagnostic criteria for EBA. For the final voting exercise, 22 experts from 14 different countries voted on 50 different items. When > 30% disagreed with a proposal, a discussion was held and re-voting carried out.

Results In total, 48 of 50 proposals achieved consensus after discussion. This included nine diagnostic criteria, which are summarized in a flow chart. The IBDG was unable to determine one procedure that would be applicable worldwide. A limitation of the study is that differential diagnosis of bullous systemic lupus erythematosus has not been addressed.

Conclusions This first international consensus conference established generally agreed-upon clinical and laboratory criteria defining the clinical classification of and diagnostic testing for EBA. Holding these voting exercises in person with the possibility of discussion prior to voting has advantages in reaching consensus over Delphi exercises with remote voting.

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Accepted for publication
12 November 2017

Funding sources
Funding was received from the Australasian Blistering Diseases Foundation, and the International Pemphigus & Pemphigoid Foundation.

Conflicts of interest
E.S. and D.Z. have received honoraria for lectures from Roche and Fresenius Medical Care, and funding for research and development projects from Euroimmun (E.S., D.Z.), Fresenius Medical Care (D.Z.), Biotest (D.Z.) and Novartis (E.S.). M.H. has received honoraria for participation on advisory boards from Roche, Janssen, Biogen and Novartis, and grants from Biotest, Fresenius and Topas. D.T.W. holds patents with the University of Southern California Stevens Institute on various forms of human recombinant type VII collagen, has received grants for epidermolysis bullosa

acquisita and type VII collagen research from the National Institutes of Health, Lotus Tissue Repair and Shire Pharmaceuticals, and has been a consultant for Biofusion, Lotus Tissue Repair and Shire Pharmaceuticals. R.J.F. (co-author) receives fees for consulting from Roche. S.Y. (co-author) received Principal Investigator fees from Roche.

A list of the co-authors of the International Bullous Diseases Group is provided in Appendix S1 (see Supporting Information).

DOI 10.1111/bjd.16138

What's already known about this topic?

- Currently, there is a lack of consensus on the diagnosis of epidermolysis bullosa acquisita (EBA).

What does this study add?

- These recommendations, which have been developed by international experts, provide appropriate pathways for EBA diagnosis: the algorithms may be used to distinguish EBA from other blistering diseases, which affect the epithelial basement membrane zone.

The International Bullous Diseases Group (IBDG) was formed in 2005 following a research meeting at the National Institutes of Health for experts in blistering diseases to work towards a consensus for the development and validation of definitions and outcome measures in autoimmune bullous diseases (AIBDs).[1] The IBDG focused its efforts initially on pemphigus, with consensus definitions, development and validation of the Pemphigus Disease Area Index and the Autoimmune Bullous Skin Intensity Score.[1,2] Subsequently, the IBDG published consensus definitions for bullous pemphigoid (BP) and proposed the BP Disease Area Index severity tool,[3] and then consensus definitions for mucous membrane (MM) pemphigoid (MMP) and the MMP Disease Area Index.[4] The current project relates to international consensus definitions on diagnostic criteria for epidermolysis bullosa acquisita (EBA).

Two cases of an adult-onset, acquired blistering disease that was reminiscent of patients with hereditary dystrophic epidermolysis bullosa (EB) were reported in 1895 by Elliott.[5] A landmark paper on the subject was published in 1971 by Roenigk *et al.*, who described three new cases of EBA, reviewed the world literature and proposed the first diagnostic criteria for EBA.[6] These criteria were soon modified by the advent of immunofluorescence (IF) and the finding that all patients with EBA had IgG and sometimes C3 deposits in their dermoepidermal junction (DEJ) and that by immunoelectron microscopy (IEM), these DEJ immune deposits were clearly in a different location than immune deposits observed in BP.[7–10] The IgG autoantibodies (autoAbs) accounting for these DEJ immune deposits were found to be autoAbs directed against a 290-kDa protein called type VII collagen (Col7), the major component of anchoring fibrils (AFs) in the DEJ.[11,12] Since these initial observations, the diagnostic testing for EBA has undergone significant refinement.[13–15]

Methods

The IBDG met three times during 2015: at the annual meetings of the American Academy of Dermatology in San Francisco (21 experts from 11 countries); the European Society of Dermatological Research in Rotterdam (11 experts from six countries); and the European Academy of Dermatology and Venereology (EADV) in Copenhagen (22 experts from 14 countries). Initial presentations and discussions took place at the first two meetings. After further revisions and discussions at the third meeting, consensus voting took place on the definitions and diagnostic techniques. We defined 'consensus' according to Harmonize Outcome Measures for Eczema by having less than one-third of the key opinion leaders disagreeing on a given diagnostic criterion.[16,17] Fifty proposals (Table S1; see Supporting Information) were put forward for discussion and voting related to the diagnostic processes involved in EBA. For each proposal, participants could 'agree', be 'undecided' or 'disagree'. Tables summarizing the literature (Tables S2–S6; see Supporting Information) and examples of cases of EBA (Appendix S2; see Supporting Information) were presented for discussion.

Results

Altogether, 38 experts in EBA from 15 countries took part in this consensus, although not all were able to attend all three international meetings held in San Francisco, Rotterdam and Copenhagen. The actual results of the voting questions (Table S7; see Supporting Information) and the reported anonymized results in the final version of the approved wording or procedures were from 22 blistering disease experts from 14 different countries (Fig. S1; see Supporting Information). Although not all members of the IBDG were present at the final voting meeting, all co-authors were in agreement with the EBA diagnostic criteria detailed herein.

Definition and clinical variants of epidermolysis bullosa acquisita

EBA is defined as a subepithelial AIBD in which patients have tissue-bound autoAbs targeted against Col7 within AFs of the basement membrane zone (BMZ) of the DEJ or chorioepithelial junction (CEJ) in stratified squamous epithelia.

Several forms of EBA exist, with two major types, termed: (i) the classical/mechanobullous form; and (ii) the nonclassical/nonmechanobullous forms, first described by Gammon *et al.* in 1982.[18] The latter includes forms that may clinically resemble BP, MMP, Brunsting-Perry pemphigoid and linear IgA bullous dermatosis.[6,18–35] Table 1 and Figure 1 summarize the descriptions and definitions of these four phenotypes.

The frequency of these subtypes varies in different countries, with the classical type being the most common in European reports and the BP-like form more common in Asia (Table S2).[36–39] It should be recognized that overlapping clinical presentations may also occur (proposal 09, cases 1–5 in Appendix S2). Overall, because EBA can appear clinically, histologically and immunologically similar to BP,[18,20–24] when the initial diagnostic consideration of the dermatologist is BP, EBA should also be considered. Furthermore, if certain clinical clues are somewhat atypical for BP (e.g. lesions that heal with scarring and the formation of milia, head and neck involvement, mucosal involvement, disease onset before 70 years of age),[40] the diagnostic possibility that the patient has EBA, rather than BP, rises considerably. Finally, it is important to classify cases of MM-predominant EBA (MM-EBA) because the severity of mucosal involvement dictates more aggressive, multidisciplinary management.

There has been confusion in the literature as to which forms constitute an 'inflammatory' form of EBA.[13,20,22,23,36–39] The IBDG members agreed that the BP-like form is inflammatory but that both MM-EBA and IgA-EBA may also be inflammatory. However, Brunsting-Perry-like EBA is usually a noninflammatory form of EBA.

Table 1 Definitions of clinical forms of epidermolysis bullosa acquisita (EBA)

Classical/mechanobullous

One subtype only, characterized by:[6]
- trauma-induced lesions (skin fragility)[a]
- bullous/vesicular lesions or erosions
- encompassed by noninflamed or scarred skin
- scarring[a] and milia formation[a]
- preferably located in trauma-prone sites and the extensor skin surface (dorsal hands,[a] elbows,[a] knees,[a] Achilles tendon, feet[a])
- possible nail dystrophy[a]
- possible scarring alopecia

Nonclassical/nonmechanobullous

BP-like EBA, defined as an eruption:[18,20–24]
- with features characteristic of BP (pruritus, tense bullae on erythematous or urticarial skin, involvement of trunk and folds)
- usually mixed with atypical lesions for a BP (skin fragility, bullae on normal skin, milia, involvement of face or extensor area of the limbs)

MM-EBA, defined as a disease that predominantly affects MM lined by squamous epithelium, i.e. MM of:[20–27]
- mouth
- pharynx
- oesophagus
- epiglottis
- conjunctiva
- genitalia
- anus
- respiratory tract (in malpighian metaplasia)

Brunsting-Perry-type EBA, defined as a chronic recurrent blistering dermatosis confined to the head and neck[28–32]

IgA-EBA, defined as a disease that presents with linear IgA deposits in the BMZ that can be observed by direct IF:[20,33,34]
- it may resemble LABD
- it may be more aggressive with mucosal scarring

BP, bullous pemphigoid; MM, mucous membranes; BMZ, basal membrane zone; IF, immunofluorescence; LABD, linear IgA bullous dermatosis. [a]Criteria of Roenigk.

Laboratory testing for epidermolysis bullosa acquisita

The IBDG reached agreement on the following proposal: routine histopathology, direct IF (DIF) microscopy and indirect IF (IIF) – which are widely available laboratory tests – allow a diagnosis of subepithelial AIBD but are not able to distinguish EBA from another subepidermal AIBD. Routine histopathology on a biopsy obtained from lesional skin or MM of a patient with EBA shows (i) subepidermal or subepithelial cleavage; (ii) great variability in the amount or type of inflammatory infiltrate; and (iii) milia cysts and fibrosis in older lesions. Routine DIF microscopy of perilesional skin or MM shows (i) linear immune deposits along the BMZ of the DEJ or CEJ, and (ii) no labelling of dermal blood vessels. The profile of immune deposits includes IgG and C3 but occasionally IgA or IgM (Table S3, case report 5; see Supporting Information).[41–43] Routine IIF microscopy on monkey, rat or rabbit oesophagus or human skin can detect anti-BMZ autoAbs, but it is often at a low titre.

Currently, a diagnosis of EBA should be confirmed by at least one of the following tests, which are only performed in academic centres and are not widely available to the average dermatologist worldwide:[13,15,35] (i) among tests requiring skin or MM biopsies – electron microscopy (EM) and direct IEM,[44] serration pattern analysis by DIF[41,45,46] and fluorescent overlay antigen mapping (FOAM);[47–49] and (ii) among serological tests for the detection of circulating autoAbs to Col7 – enzyme-linked immunosorbent assay (ELISA), IIF on BIOCHIP™with noncollagenous 1 (NC1) Col7-transfected human cells,[50] immunoblotting (IB), IIF on skin deficient in Col7[51,52] and/or indirect IEM.[44] Obviously, all these serological tests require that the patient with EBA has autoAbs to Col7 circulating in their blood and it must be kept in mind that anti-Col7 autoAbs are also present in bullous systemic lupus erythematosus (BSLE).[53]

Alternative laboratory tests when none of these tests is available include DIF and IIF on salt-split skin (SSS),[54] but they will not absolutely confirm the diagnosis of EBA.[55,56]

Standard transmission electron microscopy and immunoelectron microscopy

In patients with EBA, standard transmission EM shows an electron-dense band immediately below the lamina densa (LD) in the AF zone. Another EM finding suggestive of EBA is that the cleavage occurs below the LD, which remains attached to the roof of the blister. A paucity of AFs also supports a diagnosis of EBA.[44]

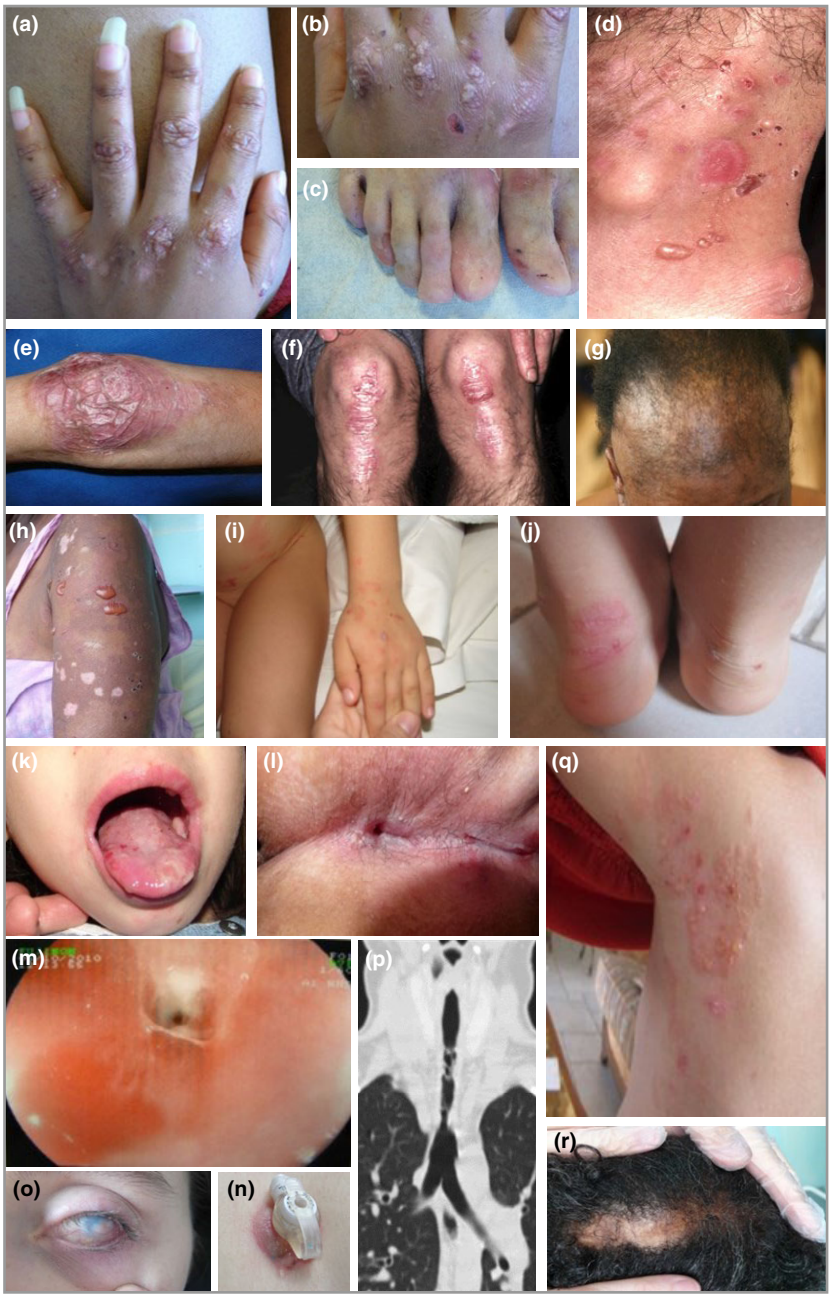

Fig 1. Clinical subtypes of epidermolysis bullosa acquisita (EBA). (a–g) Classical/mechanobullous form of EBA: trauma-induced lesions are usually located on extensor skin surfaces, i.e. (a, b) back of the hands, (c) feet, (d) heel, (e) elbows and (f) knees. (b, d) Bullous/vesicular lesions or erosions are surrounded by noninflamed or scarred skin. Lesions heal with (e, f) scarring and (a, b, f) milia formation. (c) Nail dystrophy and (g) scarring alopecia are possible. (h–j) Bullous pemphigoid (BP)-like form of EBA: tense bullae on (h) erythematous or (i, j) urticarial skin suggestive of BP, in atypical locations for BP at extensor areas of the limbs. (k–p) Mucous membrane (MM)-EBA: all the MM lined by squamous epithelium may be involved in (k) buccal MM (particularly the tongue), (l) anus and (m) oesophagus, leading to strictures and (n) gastrostomy, (o) conjunctival scarring and (p) tracheal stenosis. (q) IgA-EBA with bullous eruption in a 'string of pearls'. (r) Brunsting-Perry-type EBA with recurrent blistering dermatosis confined to the head for 10 years. Images (c), (m) and (o) are reproduced from Figures 61·1(b), 61·1(c) and 61·1(d), and image (h) is reproduced from Fig. 40·2(a) in Prost-Squarcioni C and Caux F. Management of epidermolysis bullosa acquisita. In: *Blistering Disease: Clinical Features, Pathogenesis, Treatment* (Murrell D, ed.). New York: Springer, 2015, with the permission of Springer. Image (g) is reproduced from Supplementary Figure 1(a) in Zumelzu C, Le Roux-Villet C, Loiseau P *et al.* Black patients of African descent and HLA-DRB1*15:03 frequency overrepresented in epidermolysis bullosa acquisita. *J Invest Dermatol* 2011; **131**:2386–93, with the permission of the *Journal of Investigative Dermatology*. Image (p) courtesy of Professor Michel Brauner.

Direct IEM on perilesional skin shows *in vivo* bound immune deposits that are very thick and located in the AF zone, and a cleavage under immune deposits, if EBA is present (Fig. 2).[44] The IBDG agreed on the limitations of direct IEM (proposal 29). Sixteen members of the consensus group attending the EADV round of voting had EM available at their site, but only about half of the group had experience in using EM and only seven were currently using IEM.

Serration pattern analysis

DIF microscopy of a perilesional skin biopsy can distinguish EBA from other subepithelial AIBD by showing a 'u-serrated' linear pattern of immunoglobulin deposits along the BMZ in EBA and BSLE, and an 'n-serrated' pattern of immunoglobulin deposits in BP, antilaminin 332 MMP and anti-p200/laminin γ1 pemphigoid (Fig. 3).[41,45,46,55,56] No agreement was obtained on limitations of the serration pattern analysis (proposal 26). Indeed, it can be performed with routine DIF microscopy (new, unpublished data on the exact requirements can be read in Supplementary Data S2 of Appendix S2). However, to date, this test is not widely available and so far only the teams in Groningen and Lübeck have been able to master the technique (proposal 26bis).

Fluorescent overlay antigen mapping analysis

In EBA, FOAM, using routine IF microscopy with either an image analysis system or laser scanning confocal microscopy,[47–49] shows *in vivo* bound immune deposits below the basal keratinocyte membrane, lamina lucida and LD components (Fig. 4).

Enzyme-linked immunosorbent assay

Commercially available ELISAs using recombinant NC1/noncollagenous 2 (NC2) Col7,[57–60] or NC1 Col7,[50] are widely available. Sensitivities vary depending on the selection criteria. It is very high (79–96·7%) on preselected positive sera by IIF on SSS, with floor labelling (Table S4; see Supporting Information). The sensitivity of ELISA to NC1/NC2 is lower (30–54%) in studies on unselected EBA sera.[39,61]

An ELISA using full-length Col7 is more sensitive than an ELISA using NC1/NC2 Col7 but is not commercially available (Table S5; see Supporting Information).[39,42,62,63]

ELISAs for Col7 are not highly specific as they may be positive in patients with Crohn disease or ulcerative colitis without cutaneous manifestations of EBA,[64] atypical AIBD[65] and patients with recessive dystrophic EB (RDEB) (Tables S5 and S6).[39,63,66,67] Of note, the presence of circulating autoAbs against BP180, laminin 332 and the p200/laminin γ1 chain (detected by IB or ELISA), which may occur as a result of the epitope spreading phenomenon, does not rule out a diagnosis of EBA (supplementary cases 2 and 4 in Appendix S2).[37,68–75]

Novel technique: indirect immunofluorescence using noncollagenous 1 type VII collagen-transfected cells

Patient serum autoAbs could label NC1 Col7-transfected cells on a special slide, a so-called BIOCHIP™ method (Fig. 5). This test is now commercially available and could be used as a substitute for the ELISA outlined above. It is a sensitive, specific and rapid assay for testing preselected positive sera by IIF on SSS with floor labelling.[50] Like serological testing by ELISA, its sensitivity is lower in studies on unselected EBA sera.[39]

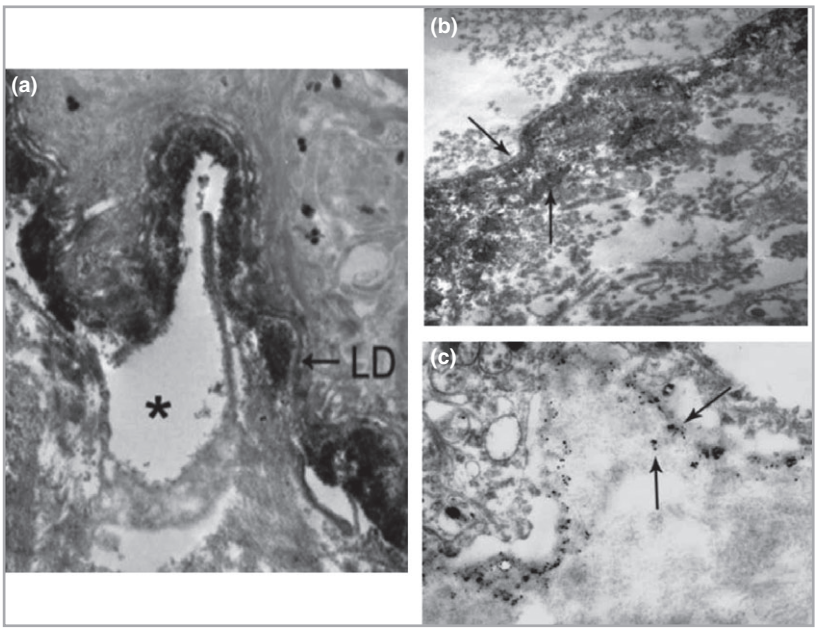

Fig 2. Immunoelectron microscopy (IEM) in epidermolysis bullosa acquisita. (a) Direct IEM using the pre-embedding immunoperoxidase technique. Thick immune deposits are observed in the anchoring fibril zone below the lamina densa (LD) and split (asterisk) under them. (b, c) Indirect IEM using (b) pre-embedding immunoperoxidase and (c) the immunogold technique. Immune deposits (arrows) decorate the ends of anchoring fibrils. Images (a), (b) and (c) are reproduced from Figures 19·10(b), 19·12(a) and 19·12 (b) in Prost-Squarcioni C. Electron microscopy and immunoelectron microscopy. In: *Blistering Disease: Clinical Features, Pathogenesis, Treatment* (Murrell D, ed.). New York: Springer, 2015, with the permission of Springer.

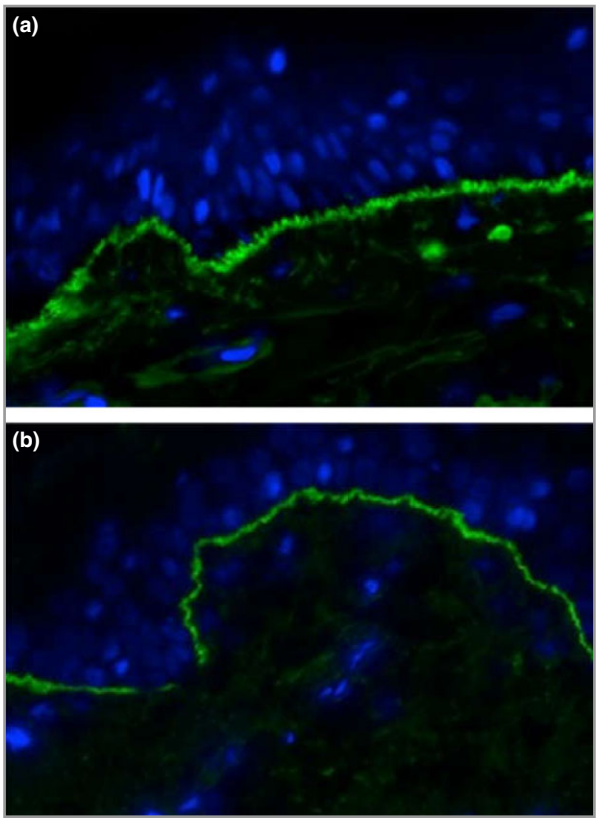

Fig 3. Serration pattern analysis by direct immunofluorescence. The u-serrated pattern is characterized by closed arches at the bottom appearing like 'growing grass', whereas the n-serrated pattern shows closed arches at the top. (a) Direct immunofluorescence (DIF) of epidermolysis bullosa acquisita skin showing linear IgG deposition along the epidermal BMZ in a u-serrated pattern (× 400). (b) DIF of bullous pemphigoid skin showing linear IgG deposition along the epidermal basement membrane zone in an n-serrated pattern (× 400). Images courtesy of Dr Gilles Diercks. [Colour figure can be viewed at wileyonlinelibrary.com]

Immunoblotting

IB is a serological technique performed on extracts of tissues or cells or recombinant Col7 proteins (Fig. 6).[13,15,35] IB is effective for diagnosing EBA by detecting autoAbs in patient sera that label the Col7 α-chain. IB can substitute for the

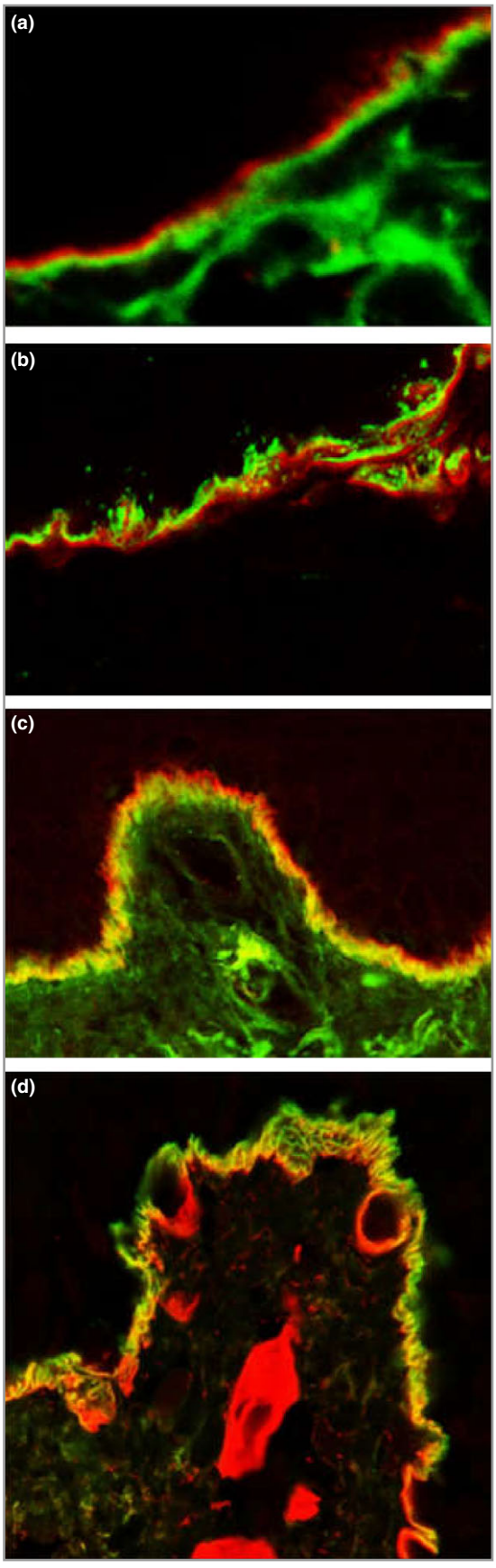

Fig 4. Fluorescence overlay antigen mapping (FOAM) in skin biopsies. In epidermolysis bullosa acquisita (EBA), *in vivo* bound immune deposits are below the α6β4 integrin of the basal keratinocyte membrane and below the components of the lamina lucida and the lamina densa (laminin 332 and type IV collagen). (a) In a patient with EBA, *in vivo* bound IgG (green) is below type IV collagen (red). (b) In a patient with a bullous pemphigoid, *in vivo* bound IgG (green) is above laminin 332 (red). (c) In a patient with mucous membrane pemphigoid (MMP), *in vivo* bound IgG (green) is below or co-localized (yellow) with laminin 332 (red). (d) In a patient with MMP, *in vivo* bound IgG (green) is above or co-localized (yellow) with type IV collagen (red). Images courtesy of Dr Katarzyna Wozdniak.

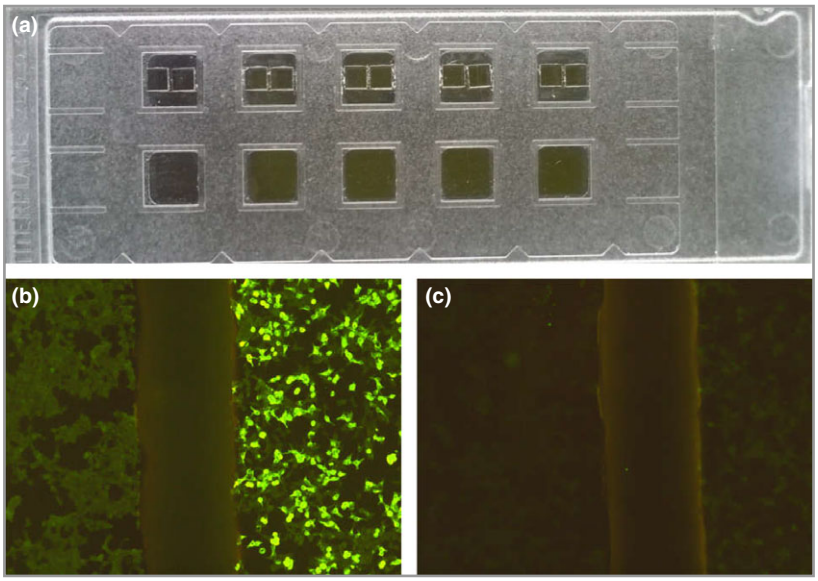

Fig 5. Indirect immunofluorescence microscopy with noncollagenous 1 type VII collagen (NC1 Col7)-expressing human cells by BIOCHIP™ technology. Patient serum autoantibodies could label molecularly engineered epidermal cells that express human NC1 Col7 on a special slide. (a) On a standard-sized slide, there are five incubation fields each with two different BIOCHIPs: one with human embryonic kidney (HEK)293 cells transfected with pTriEx-1, which serve as negative control, and one with human HEK293 cells transfected with NC1 Col7. (b) Autoantibodies in the serum of a patient with epidermolysis bullosa acquisita labelled NC1 Col7-expressing HEK293 cells (right) but not non-NC1 Col7-expressing cells (left). (c) No reactivity of either NC1 Col7-expressing or non-NC1-Col7-expressing cells is seen with normal human serum. Images courtesy of Dr Aucouturier, Paris.

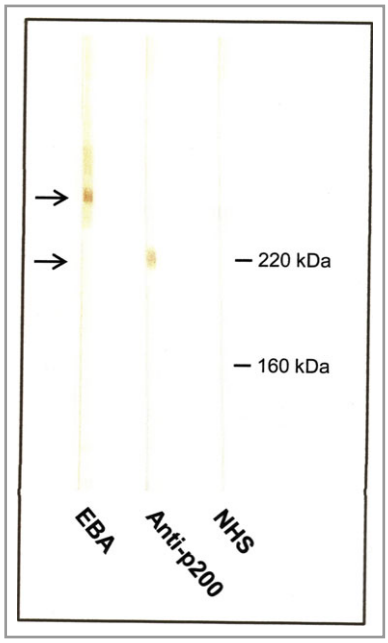

Fig 6. Immunoblotting in epidermolysis bullosa acquisita (EBA). By immunoblotting with dermal extract, EBA serum recognized a band at 290 kDa, which is the alpha chain of type VII collagen (Col7), different from laminin γ1/p200 protein. A second band at 145 kDa, which is the amino terminal noncollagenous 1 domain of the alpha chain of Col7, could be seen. NHS, normal human serum.

commercially available ELISA. The IBDG agreed on the limitations of IB (proposal 33).

Indirect immunofluorescence on type VII collagen-deficient human skin and indirect immunoelectron microscopy

A definitive diagnosis of EBA can be demonstrated by IIF when presumptive EBA sera label the DEJ of normal human skin but do not label skin from generalized severe RDEB with no Col7 (Fig. 7).[51,52] Obviously, this is not offered in commercial laboratories and is limited by access to skin specimens from patients with this exceedingly rare disease.

A definitive diagnosis of EBA can also be demonstrated when presumptive EBA sera label AFs by indirect IEM (Fig. 2).[44]

Direct and indirect immunofluorescence microscopy on salt-split skin

DIF and IIF on SSS are alternative laboratory tests that only give a probable diagnosis of EBA. DIF is performed after splitting the skin biopsy of the patient using NaCl 1 mol L^{-1} (Supplementary Data S3 in Appendix S2).[54,76] It is not possible with MM biopsies. IIF may be performed on either normal human SSS using a similar procedure,[19,77–79] or monkey SSS, which is commercially available. Immune deposits in patients with EBA remain on the dermal floor of the separation, whereas BP immune deposits remain with the epidermal roof

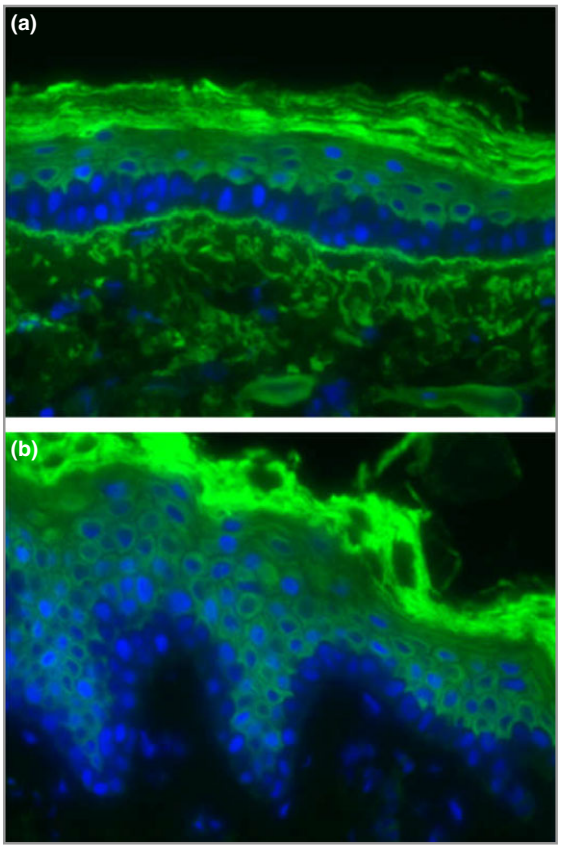

Fig 7. Indirect immunofluorescence (IIF) microscopy on type VII collagen (Col7)-deficient skin vs. normal skin. (a) IIF microscopy with epidermolysis bullosa acquisita (EBA) serum on Col7-containing normal human skin showing positive IgG binding along the epidermal basement membrane zone (BMZ) (× 400). (b) IIF microscopy with EBA serum on Col7 knockout skin from a patient with severe generalized recessive dystrophic epidermolysis bullosa showing negative IgG binding along the epidermal BMZ (× 400). Images courtesy of Dr Hendri Pas, Groningen, the Netherlands.

(Fig. 8). However, this dermal labelling is not specific to EBA. It is also seen in antilaminin 332 MMP and anti 1-p200/ laminin γ1 pemphigoid (Fig. 4). Additional tests are necessary to exclude reactivity against these molecules and finally diagnose EBA. Overall, IIF on SSS is more sensitive than IIF on monkey or rat oesophagus or unsplit human skin for detecting anti-BMZ autoAbs.[77,79]

Lastly, the IBDG updated consensus criteria for EBA diagnosis (2015) include combinations of the following tests: (1) a bullous disorder within the defined clinical spectrum; (2) histopathology revealing a subepidermal or subepithelial blister; (3) a positive DIF microscopy of perilesional skin or MM with linear IgG, C3, IgA and/or IgM deposits within the epithelial BMZ; (4) detection of circulating autoAbs against Col7 by IB, ELISA and/or IIF microscopy on Col7-expressing human cells; (5) labelling AFs by indirect IEM or negative IIF microscopy on Col7-deficient skin; (6) a 'u-serration' pattern by DIF microscopy; (7) direct IEM of perilesional skin demonstrating immune deposits within AFs zone ± the lower LD; (8) *in vivo* bound immune deposits below type IV collagen by FOAM; (9) alternatively to items (4)–(8), dermal labelling by DIF and/or IIF on SSS.

The IBDG did reach agreement that the ideal scenario is for a patient with putative EBA disease (criterium 1) to exhibit a subepidermal bulla by histology (criterium 2 optional), a positive DIF microscopy (criterium 3) and an ELISA (or another serological test) showing that the patient's serum autoAbs target Col7 [criterium (4) or (5)]. In this scenario, a highly probable diagnosis of EBA is made and no further tests need to be done for confirmation. Unlike this ideal scenario for the diagnosis of EBA, the problem that often arises is when a patient with EBA lacks circulating autoAbs and therefore IIF, SSS IIF and ELISA are negative.[39,61] Then the diagnosis of EBA could be considered definitive if criteria (1) and (3) and at least one of criteria (6)–(8) are present

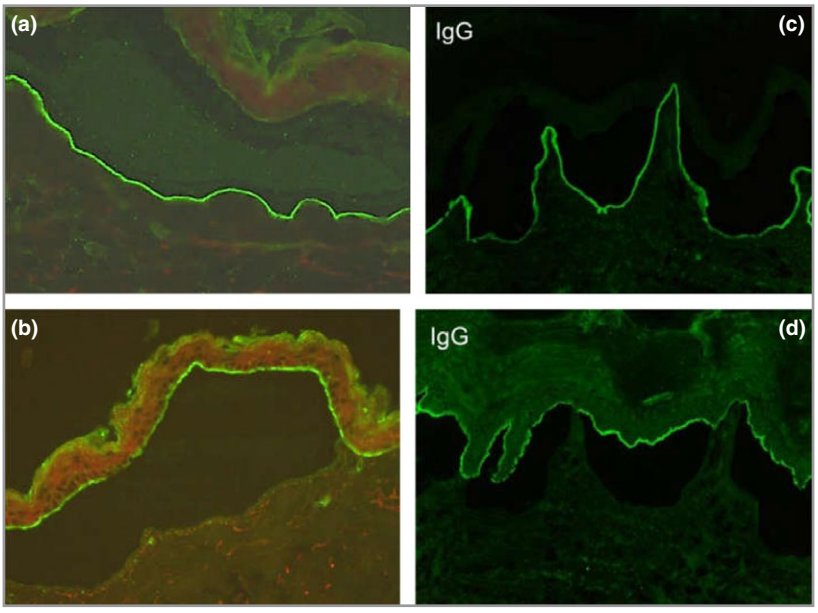

Fig 8. Indirect immunofluorescence microscopy on salt-split skin. An artificial cleavage is induced by NaCl 1 mol L^{-1} in (a, b) monkey skin (counterstained with Evans blue; images Courtesy of Dr Aucouturier, Paris) or (c, d) normal human skin. (a, c) Labelling of the floor of the cleavage by the serum of a patient with EBA. (b, d) Labelling of the roof of the cleavage by the serum of a patient with bullous pemphigoid.

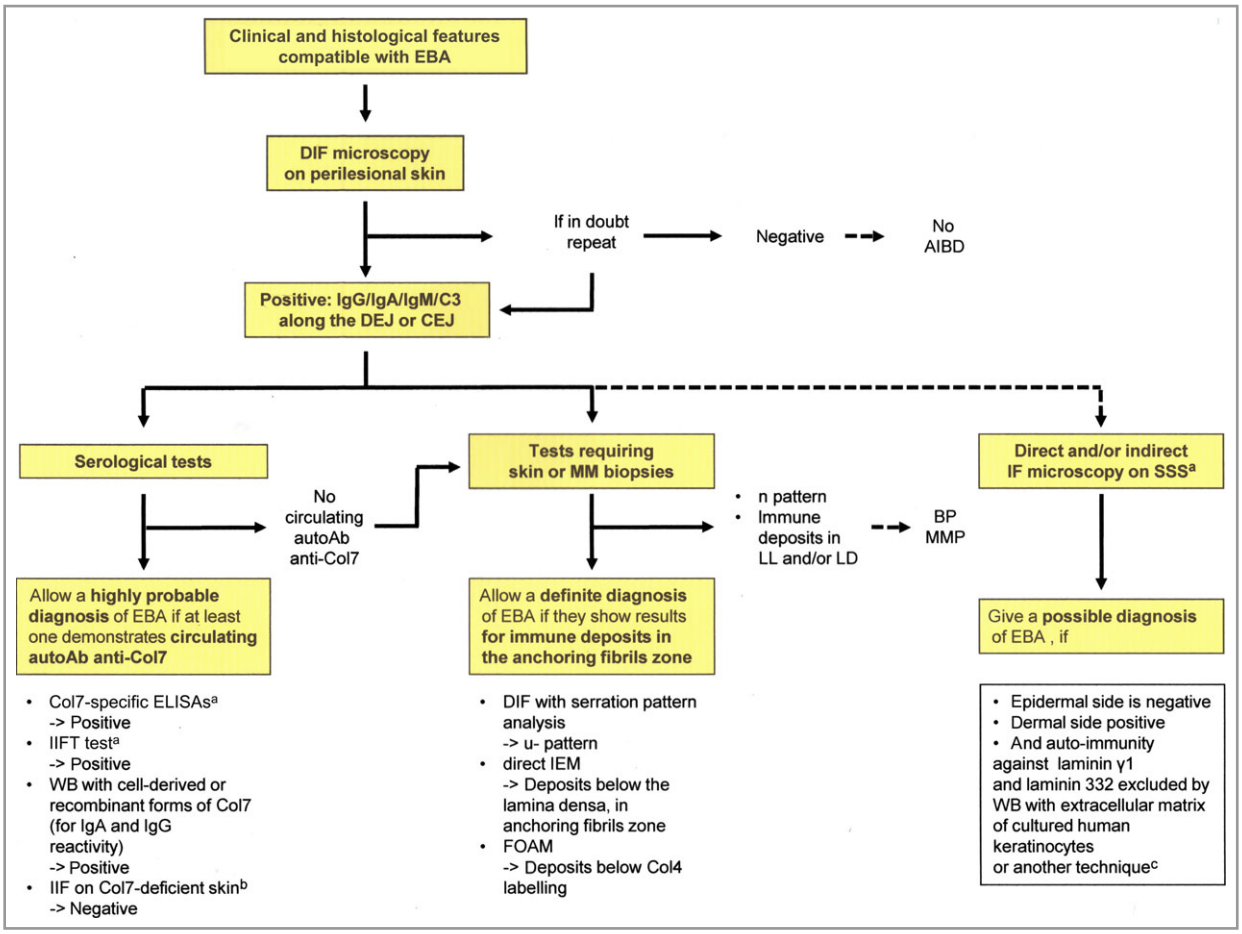

Fig 9. Flow chart for diagnosis of epidermolysis bullosa acquisita (EBA). Type VII collagen (Col7)-specific enzyme-linked immunosorbent assays (ELISAs) include noncollagenous (NC)1, NC1/NC2 or full-length Col7 ELISAs. BIOCHIP™ technology uses NC1 Col7-transfected human embryonic kidney (HEK)293 cells as a substrate for indirect immunofluorescence (IIF) microscopy (IIFT test). Col7-deficient skin is obtained from patients with generalized severe recessive dystrophic epidermolysis bullosa. DIF, direct immunofluorescence; DEJ, dermoepidermal junction; CEJ, chorioepithelial junction; AIBD, autoimmune blistering disease; autoAb, autoantibody; Col7, type VII collagen; WB, Western blotting; MM, mucous membranes; IEM, immunoelectron microscopy; FOAM, fluorescence overlay antigen mapping; Col4, type IV collagen; LL, lamina lucida; LD, lamina densa; BP, bullous pemphigoid; MMP, mucous membrane pemphigoid; SSS, salt-split skin (artificial cleavage obtained by incubation of normal monkey or human skin with NaCl 1 mol L^{-1}). [a]NC1 ELISA, NC1/NC2 ELISA, NC1 Col7-transfected HEK293 cells and monkey SSS are commercially available; [b]negativity on Col7-deficient skin is significant if IIF microscopy on normal human skin is positive; [c]include WB with dermal extract or recombinant C-terminus laminin γ1 or IIF on laminin 332-deficient skin.

[criterium (2) is optional]. Lastly, if tests (6)–(8) cannot be done, a diagnosis of EBA is possible if items criteria (1), (3) and (9) are present; then diagnosis has to be confirmed by exclusion of autoimmunity against laminin 332 or the p200/laminin γ1 chain. The consensus conference was not able to determine one procedure that would be applicable worldwide. There was an animated discussion about what should be considered routine (Proposal 18). Which test(s) that the practitioner chooses will likely be determined by the clinical presentation of the patient (classical/mechanobullous type or not), the geography of the practitioner and which test is most logistically accessible. Figure 9 summarizes the different diagnostic investigative pathways.

In conclusion, this consensus of the criteria for the diagnosis of EBA provides a general framework for establishing a diagnosis of EBA and takes into account the clinical presentation and available laboratory testing. These criteria should help prevent clinicians from misdiagnosing other AIBDs and missing the diagnosis of EBA. They will also be useful for future studies designed to define the natural history and therapeutic outcomes of EBA.

Acknowledgments

We thank Dr Jean-Jacques Raynaud, Dr Hendri Pas, Dr Gilles Diercks, Dr Françoise Aucouturier, Dr Katarzyna Wozniak and Professor Michel Brauner for providing the original images used. We thank the Australasian Blistering Diseases Foundation and the International Pemphigus & Pemphigoid Foundation for funding use of the meeting rooms and facilities.

References

1 Murrell DF, Dick S, Ahmed AR *et al.* Consensus statement on definitions of disease, end points, and therapeutic response for pemphigus. *J Am Acad Dermatol* 2008; **58**:1043–6.
2 Rosenbach M, Murrell DF, Bystryn JC *et al.* Reliability and convergent validity of two outcome instruments for pemphigus. *J Invest Dermatol* 2009; **129**:2404–10.
3 Murrell DF, Daniel BS, Joly P *et al.* Definitions and outcome measures for bullous pemphigoid: recommendations by an international panel of experts. *J Am Acad Dermatol* 2012; **66**:479–85.
4 Murrell DF, Marinovic B, Caux F *et al.* Definitions and outcome measures for mucous membrane pemphigoid: recommendations of an international panel of experts. *J Am Acad Dermatol* 2015; **72**:168–74.
5 Elliot GT. Two cases of epidermolysis bulllosa. *J Cutan Genitourin Dis* 1895; **13**:10.
6 Roenigk HH Jr, Ryan JG, Bergfeld WF. Epidermolysis bullosa acquisita. Report of three cases and review of all published cases. *Arch Dermatol* 1971; **103**:1–10.
7 Kushnirik W. The immunopathology of epidermolysis bullosa acquisita. *Can Med Assoc J* 1973; **108**:1143.
8 Gibbs RB, Minus HR. Epidermolysis bullosa with electron microscopical studies. *Arch Dermatol* 1975; **111**:215.
9 Nieboer C, Boorsma DM, Woerdeman MJ, Kalsbeek GL. Epidermolysis bullosa acquisita: immunofluorescence, electron microscopic and immunoelectron microscopic studies in four patients. *Br J Dermatol* 1980; **102**:383.
10 Yaoita H, Briggaman RA, Lawley TJ *et al.* Epidermolysis bullosa acquisita: ultrastructural and immunological studies. *J Invest Dermatol* 1981; **76**:288–92.
11 Woodley DT, Briggaman RA, O'Keefe EJ *et al.* Identification of the skin basement-membrane autoantigen in epidermolysis bullosa acquisita. *N Engl J Med* 1984; **310**:1007–13.
12 Woodley DT, Burgeson RE, Lunstrum G *et al.* Epidermolysis bullosa acquisita antigen is the globular carboxyl terminus of type VII procollagen. *J Clin Invest* 1988; **81**:683–7.
13 Ludwig RJ. Clinical presentation, pathogenesis, diagnosis, and treatment of epidermolysis bullosa acquisita. *ISRN Dermatol* 2013; **2013**:812029.
14 Schmidt E, Zillikens D. Pemphigoid diseases. *Lancet* 2013; **381**:320–32.
15 Vorobyev A, Ludwig RJ, Schmidt E. Clinical features and diagnosis of epidermolysis bullosa acquisita. *Expert Rev Clin Immunol* 2017; **13**:157–69.
16 Schmitt J, Spuls P, Boers M *et al.* Towards global consensus on outcome measures for atopic eczema research: results of the HOME II meeting. *Allergy* 2012; **67**:1111–17.
17 Chalmers JR, Schmitt J, Apfelbacher C *et al.* Report from the third international consensus meeting to harmonise core outcome measures for atopic eczema/dermatitis clinical trials (HOME). *Br J Dermatol* 2014; **171**:1318–25.
18 Gammon WR, Briggaman RA, Wheeler CE Jr. Epidermolysis bullosa acquisita presenting as an inflammatory bullous disease. *J Am Acad Dermatol* 1982; **7**:382–7.
19 Gammon WR, Briggaman RA, Woodley DT *et al.* Epidermolysis bullosa acquisita – a pemphigoid-like disease. *J Am Acad Dermatol* 1984; **11**:820–32.
20 Briggaman RA, Gammon WR, Woodley DT. Epidermolysis bullosa acquisita of the immunopathological type (dermolytic pemphigoid). *J Invest Dermatol* 1985; **85**:79s–84s.
21 Gammon WR. Epidermolysis bullosa acquisita. *Semin Dermatol* 1988; **7**:218–24.
22 Woodley DT. Epidermolysis bullosa acquisita. *Prog Dermatol* 1988; **22**:1–13.
23 Woodley DT, Briggaman R, Gammon WR. Review and update of epidermolysis bullosa acquisita. *Semin Dermatol* 1988; **7**:111–22.
24 Dahl MG. Epidermolysis bullosa acquisita – a sign of cicatricial pemphigoid? *Br J Dermatol* 1979; **101**:475–84.
25 Schattenkirchner S, Lemann M, Prost C *et al.* Localized epidermolysis bullosa acquisita of the esophagus in a patient with Crohn's disease. *Am J Gastroenterol* 1996; **91**:1657–9.
26 Luke MC, Darling TN, Hsu R *et al.* Mucosal morbidity in patients with epidermolysis bullosa acquisita. *Arch Dermatol* 1999; **135**:954–9.
27 Alexandre M, Brette MD, Pascal F *et al.* A prospective study of upper aerodigestive tract manifestations of mucous membrane pemphigoid. *Medicine (Baltimore)* 2006; **85**:239–52.
28 Kurzhals G, Stolz W, Meurer M *et al.* Acquired epidermolysis bullosa with the clinical feature of Brunsting-Perry cicatricial bullous pemphigoid. *Arch Dermatol* 1991; **127**:391–5.
29 Lee CW, Jun KM. Epidermolysis bullosa acquisita presenting with localized facial blisters. *Clin Exp Dermatol* 1992; **17**:363–5.
30 Joly P, Ruto F, Thomine E *et al.* Brunsting-Perry cicatricial bullous pemphigoid: a clinical variant of localized acquired epidermolysis bullosa? *J Am Acad Dermatol* 1993; **28**:89–92.
31 Choi GS, Lee ES, Kim SC *et al.* Epidermolysis bullosa acquisita localized to the face. *J Dermatol* 1998; **25**:19–22.
32 Woodley DT, Chen M. Epidermolysis bullosa acquisita. In: *Fitzpatricks' Dermatology in General Medicine* (Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K, eds). New York: McGraw Hill, 2003; 609–16.
33 Vodegel RM, de Jong MC, Pas HH *et al.* IgA-mediated epidermolysis bullosa acquisita: two cases and review of the literature. *J Am Acad Dermatol* 2002; **47**:919–25.
34 Lally A, Chamberlain A, Allen J *et al.* Dermal-binding linear IgA disease: an uncommon subset of a rare immunobullous disease. *Clin Exp Dermatol* 2007; **32**:493–8.
35 Groves R, Schmidt E. Immunobullous diseases. In: *Rook's Textbook of Dermatology* (Griffiths C, Barker J, Bleiker T, Chalmers R, Creamer D, eds). Chichester: Wiley-Blackwell, 2016; 50.01–50.56.
36 Kim JH, Kim SC. Epidermolysis bullosa acquisita. A retrospective clinical analysis of 30 cases. *Acta Derm Venereol* 2011; **91**:307–12.
37 Buijsrogge JJA, Dieercks GFH, Pas HH, Jonkman MF. The many faces of epidermolysis bullosa acquisita after serration pattern analysis by direct immunofluorescence microscopy. *Br J Dermatol* 2011; **165**:92–8.
38 Iranzo P, Herrero-Gonzales , Mascarro-Galy JM *et al.* Epidermolysis bullosa acquisita: a retrospective analysis of 12 patients evaluated in four tertiary hospitals in Spain. *Br J Dermatol* 2014; **171**:1022–30.
39 Seta V, Aucouturier F, Bonnefoy J *et al.* Comparison of three collagen VII assays for serological diagnosis of epidermolysis bullosa acquisita. *J Am Acad Dermatol* 2016; **74**:1166–72.
40 Vaillant L, Bernard P, Joly P *et al.* Evaluation of clinical criteria for diagnosis of bullous pemphigoid. French Bullous Study Group. *Arch Dermatol* 1998; **134**:1075–80.
41 Vodegel RM, Jonkman MF, Pas HH *et al.* U-serrated immunodeposition pattern differentiates type VII collagen targeting bullous diseases from other subepidermal bullous autoimmune diseases. *Br J Dermatol* 2004; **151**:112–18.
42 Tsuchisaka A, Ohara K, Ishii N *et al.* Type VII collagen is the major autoantigen for sublamina densa-type linear IgA bullous dermatosis. *J Invest Dermatol* 2015; **135**:626–9.
43 Suchniak JM, Diaz LA, Lin MS *et al.* IgM-mediated epidermolysis bullosa acquisita. *Arch Dermatol* 2002; **138**:1385–6.

44 Prost-Squarcioni C. Electron microscopy and immunoelectron microscopy. In: *Blistering Diseases: Clinical Features, Pathogenesis, Treatment* (Murrell D, ed.). New York: Springer, 2015; 213–37.
45 Terra JB, Pas HH, Hertl M *et al.* Immunofluorescence serration pattern analysis as a diagnostic criterion in antilaminin-332 mucous membrane pemphigoid: immunopathological findings and clinical experience in 10 Dutch patients. *Br J Dermatol* 2011; **165**:815–22.
46 Terra JB, Meijer JM, Jonkman MF *et al.* The n- vs. u-serration is a learnable criterion to differentiate pemphigoid from epidermolysis bullosa acquisita in direct immunofluorescence serration pattern analysis. *Br J Dermatol* 2013; **169**:100–5.
47 De Jong MC, Bruins S, Heeres K *et al.* Bullous pemphigoid and epidermolysis bullosa acquisita. Differentiation by fluorescence overlay antigen mapping. *Arch Dermatol* 1996; **132**:151–7.
48 Wozniak K, Kazama T, Kowalewski C. A practical technique for differentiation of subepidermal bullous diseases: localization of in vivo-bound IgG by laser scanning confocal microscopy. *Arch Dermatol* 2003; **139**:1007–11.
49 Wozniak K, Hashimoto T, Ishii N *et al.* Fluorescence overlay antigen mapping using laser scanning confocal microscopy differentiate linear IgA bullous dermatosis from epidermolysis bullosa acquisita mediated by IgA. *Br J Dermatol* 2013; **168**:634–8.
50 Komorowski L, Müller 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.
51 Vodegel RM, Kiss M, Cjm De Jong M *et al.* The use of skin substrates deficient in basement membrane molecules for the diagnosis of subepidermal autoimmune bullous disease. *Eur J Dermatol* 1998; **8**:83–5.
52 Harman KE, Bhogal BS, Eady RA *et al.* Defining target antigens in linear IgA disease using skin from subjects with inherited epidermolysis bullosa as a substrate for indirect immunofluorescence microscopy. *Br J Dermatol* 1999; **141**:475–80.
53 Camisa C, Sharma HM. Vesiculobullous systemic lupus erythematosus. Report of two cases and a review of the literature. *J Am Acad Dermatol* 1983; **9**:924–33.
54 Gammon WR, Kowalewski C, Chorzelski TP *et al.* Direct immunofluorescence studies of sodium chloride-separated skin in the differential diagnosis of bullous pemphigoid and epidermolysis bullosa acquisita. *J Am Acad Dermatol* 1990; **22**:664–70.
55 Goletz S, Hashimoto T, Zillikens D, Schmidt E. Anti-p200 pemphigoid. *J Am Acad Dermatol* 2014; **71**:185–91.
56 Meijer JM, Diercks GF, Schmidt E *et al.* Laboratory diagnosis and clinical profile of anti-p200 pemphigoid. *JAMA Dermatol* 2016; **152**:897–904.
57 Saleh MA, Ishii K, Kim Y-J *et al.* Development of NC1 and NC2 domains of type VII collagen ELISA for the diagnosis and analysis of the time course of epidermolysis bullosa acquisita patients. *J Dermatol Sci* 2011; **62**:169–75.
58 Kim JH, Kim YH, Kim S *et al.* Serum levels of anti-type VII collagen antibodies detected by enzyme-linked immunosorbent assay in patients with epidermolysis bullosa acquisita are correlated with the severity of skin lesions. *J Eur Acad Dermatol Venereol* 2013; **27**: e224–30.
59 Marzano AV, Cozzani E, Fanoni D *et al.* Diagnosis and disease severity assessment of epidermolysis bullosa acquisita by ELISA for anti-type VII collagen autoantibodies: an Italian multicentre study. *Br J Dermatol* 2013; **168**:80–4.
60 Calabresi V, Sinistro A, Cozzani E *et al.* Sensitivity of different assays for the serological diagnosis of epidermolysis bullosa acquisita: analysis of a cohort of 24 Italian patients. *J Eur Acad Dermatol Venereol* 2014; **28**:483–90.
61 Terra JB, Jonkman MF, Diercks GFH *et al.* Low sensitivity of type VII collagen enzyme-linked immunosorbent assay in epidermolysis bullosa acquisita: serration pattern analysis on skin biopsy is required for diagnosis. *Br J Dermatol* 2013; **169**:164–7.
62 Pendaries V, Gasc G, Titeux M *et al.* Immune reactivity to type VII collagen: implications for gene therapy of recessive dystrophic epidermolysis bullosa. *Gene Ther* 2010; **17**:930–7.
63 Woodley DT, Cogan J, Wang X *et al.* *De novo* anti-type VII collagen antibodies in patients with recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 2014; **134**:1138–40.
64 Chen M, O'Toole EA, Sanghavi J *et al.* The epidermolysis bullosa acquisita antigen (type VII collagen) is present in human colon and patients with Crohn's disease have autoantibodies to type VII collagen. *J Invest Dermatol* 2002; **118**:1059–64.
65 Muller R, Dahler C, Möbs C *et al.* T and B cell responses targeting distinct regions of the noncollagenous domain (NC1) of collagen VII correlate with the clinical activity of EBA. *J Invest Dermatol* 2007; **127**:S67.
66 Tampoia M, Bonamonte D, Filoni A *et al.* Prevalence of specific anti-skin autoantibodies in a cohort of patients with inherited epidermolysis bullosa. *Orphanet J Rare Dis* 2013; **8**:132.
67 Venugopal SS, Yan W, Frew JW *et al.* A phase II randomized vehicle-controlled trial of intradermal allogeneic fibroblasts for recessive dystrophic epidermolysis bullosa. *J Am Acad Dermatol* 2013; **69**:898–908.
68 Kawachi Y, Ikegami M, Hashimoto T *et al.* Autoantibodies to bullous pemphigoid and epidermolysis bullosa acquisita antigens in an infant. *Br J Dermatol* 1996; **135**:443–7.
69 Tanaka H, Ishida-Yamamoto A, Hashimoto T *et al.* A novel variant of acquired epidermolysis bullosa with autoantibodies against the central triple-helical domain of type VII collagen. *Lab Invest* 1997; **77**:623–32.
70 Wakelin SH, Bhogal B, Black MM *et al.* Epidermolysis bullosa acquisita associated with epidermal-binding circulating antibodies. *Br J Dermatol* 1997; **136**:604–9.
71 Jonkman MF, Schuur J, Dijk F *et al.* Inflammatory variant of epidermolysis bullosa acquisita with IgG autoantibodies against type VII collagen and laminin alpha3. *Arch Dermatol* 2000; **136**:227–31.
72 Fairley JA, Woodley DT, Chen M *et al.* A patient with both bullous pemphigoid and epidermolysis bullosa acquisita: an example of intermolecular epitope spreading. *J Am Acad Dermatol* 2004; **51**:118–22.
73 Furukawa H, Miura T, Takahashi M *et al.* A case of anti-p200 pemphigoid with autoantibodies against both a novel 200-kD dermal antigen and the 290-kD epidermolysis bullosa acquisita antigen. *Dermatology* 2004; **209**:145–8.
74 Yamada T, Suzuki M, Koike Y *et al.* A case of epidermolysis bullosa acquisita with autoantibody to anti-p200 pemphigoid antigen and exfoliative esophagitis. *Dermatology* 2006; **212**:381–4.
75 Pastar Z, Rados J, Lipozencic J *et al.* Case of concurrent epidermolysis bullosa acquisita and anti-p200 pemphigoid – how to treat it? *Int J Dermatol* 2007; **46**:295–8.
76 Domloge-Hultsch N, Bisalbutra P, Gammon WR *et al.* Direct immunofluorescence microscopy of 1 mol/L sodium chloride-treated patient skin. *J Am Acad Dermatol* 1991; **24**:946–51.
77 Gammon WR, Briggaman RA, Inman AO III *et al.* Differentiating anti-lamina lucida and anti-sublamina densa anti-BMZ antibodies by indirect immunofluorescence on 1.0 M sodium chloride-separated skin. *J Invest Dermatol* 1984; **82**:139–44.
78 Logan RA, Bhogal B, Das AK *et al.* Localization of bullous pemphigoid antibody – an indirect immunofluorescence study of 228 cases using a split-skin technique. *Br J Dermatol* 1987; **117**:471–8.

79 Lazarova Z, Yancey KB. Reactivity of autoantibodies from patients with defined subepidermal bullous diseases against 1 mol/L salt-split skin. Specificity, sensitivity, and practical considerations. *J Am Acad Dermatol* 1996; **35**:398–403.

Supporting Information

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

Appendix S1 Co-authors of the International Bullous Diseases Group.

Appendix S2 Supplementary data.

Fig S1 Countries of origin of the voters.

Table S1 List of the proposals.

Table S2 Frequency of clinical subtypes of epidermolysis bullosa acquisita in the literature.

Table S3 Direct immunofluorescence in the case series of the literature.

Table S4 Enzyme-linked immunosorbent assay data of noncollagenous (NC)1/NC2 Col7 and of NC1-Col7 in the literature.

Table S5 Comparison of enzyme-linked immunosorbent assays (ELISAs) of noncollagenous (NC)1/NC2 Col7 and of full-length Col7 in the literature.

Table S6 False positives in inflammatory bowel disease and atypical autoimmune bullous disease.

Table S7 Results of vote on proposals P01–P50.