Accessible Diagnostic Methods to Differentiate between Epidermolysis Bullosa Acquisita and Other Subepidermal Autoimmune Bullous Diseases

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Indian J Dermatol 2018;63(5):445-8

Epidermolysis bullosa acquisita (EBA) is an infrequent subepidermal autoimmune bullous disease (SAIBD) associated with autoantibodies against the N-terminal collagenous domain (NC1) of type VII collagen. The autoantibodies in most of EBA patients are of immunoglobulin G (IgG) subtype. However, immunoglobulin A (IgA) anti-type VII collagen autoantibodies may be observed either as the only immunoglobulin class or in combination with IgG autoantibodies. The classical variant of EBA is the mechanobullous variant resembling dystrophic epidermolysis bullosa (DEB). Another phenotype of EBA is the inflammatory “bullous pemphigoid (BP)-like” variant, which manifests with the urticarial plaques commonly seen in BP. Lesions frequently heal with scarring and milia may develop. Severe forms of EBA may result in severe complications of the mucous membranes, including blindness as well as esophageal and anal strictures. Early recognition and appropriate treatment are essential to evade these permanent complications. Interestingly, the predominant pathogenic immunoglobulin class in ocular EBA is apparently IgA. The presence of exclusive linear IgA deposits at the basement membrane zone (BMZ) may complicate the differentiation from linear IgA bullous dermatosis, as type VII collagen can also be targeted in this disease.

Due to the heterogeneous clinical presentation, diagnosing EBA based on the clinical presentation alone is difficult. The differentiation between EBA and other SAIBDs is challenging both clinically and immunopathologically. Although several tests are available for this purpose, most of which are not available in routine pathology laboratories. Histopathology

The routine biopsy reveals a subepidermal blister that enables distinguishing from intraepidermal bullous diseases. In the classical mechanobullous variant, a scarce or no inflammatory infiltrate in the dermis can be detected. Fibrosis may be present and correspond to the cicatrical changes encountered clinically. In the inflammatory variant, infiltration of neutrophils with variable numbers of eosinophils, monocytes, and lymphocytes is found in the upper dermis. Histopathology alone cannot differentiate between the different SAIBDs.

Direct Immunofluorescence

A perilesional biopsy for routine direct immunofluorescence (DIF) microscopy shows linear fluorescence with IgG, but often IgA and C3 are also positive. Similarly, the routine DIF microscopy does not allow to distinguish EBA from other SAIBDs.

Indirect Immunofluorescence

When EBA sera are tested by the routine indirect immunofluorescence (IIF) microscopy on monkey esophagus substrate, linear staining at BMZ is obtained, making distinguishing from other SAIBDs not feasible. By the use of human salt-split skin as a substrate, EBA sera label the dermal side of the artificial split and can thus be differentiated from bullous pemphigoid, pemphigoid gestationis, mucous membrane pemphigoid (MMP; excluding anti-laminin 332 subtype), and lichen planus pemphigoides. Sera from patients with anti-p200 pemphigoid and anti-laminin 332 MMP demonstrate the same binding pattern as EBA. The sensitivity of IIF microscopy on human salt-split skin for the detection of anti-BMZ autoantibodies in EBA ranged between 27% and 100% in several studies, with a pooled measure of 75.9% in 426 EBA sera reported so far.

Following the aforementioned routine assays available in most immunopathological laboratories, the challenge is to differentiate between EBA, anti-p200 pemphigoid, and anti-laminin 332 MMP, because in all of these diseases, autoantibodies deposit on the floor of the artificial blister when IIF microscopy is performed on salt-split human skin. The differentiation is of importance because in anti-laminin 332 MMP oncological screening may be indicated, and EBA is often characterized by a refractory response to treatment, whereas anti-p200 pemphigoid has a favorable and rapid response to immunosuppressants. Here, we present the currently acceptable methods to differentiate EBA from the latter two diseases, with methods 1 to 3 being inexpensive and accessible in most immunopathological laboratories worldwide, whereas methods 4 and 5 are restricted to few specialized laboratories [Figure 1].

Serration pattern analysis of direct immunofluorescence microscopy

In 2004, Vodegel et al. described serration pattern analysis by routine DIF showing linear n-serration
or linear u-serration immunodepositions along the BMZ. The u-serration pattern confirms the diagnosis of EBA and reflects immunoglobulin depositions in upstanding arms of the sublamina densa zone between the rootlets of basal keratinocytes. In all other SAIBDs, the autoantigens are located in the lamina lucida or above, and thus, the immunodeposits follow the rootlets of the basal keratinocytes showing the n-serration pattern [Figure 2].

**Type IV collagen immunostaining**

Immunohistochemical stain for type IV collagen on paraffin-embedded lesional biopsy is a simple technique that enables to differentiate EBA from other SAIBDs. Type IV collagen forms the fibrous two-dimensional network of the lamina densa. The dermoepidermal separation in EBA occurs most often in the sublamina densa, and thus, type IV collagen is present above blister cavity, whereas it is seen below the cleft in all other SAIBDs.\(^6\)

Examining 11 skin biopsies of patients with DEB, Petronius *et al.*\(^7\) demonstrated that type IV collagen was demonstrated in the epidermal roof of the blister whenever this immunohistochemical stain was reactive. Since the level of separation is identical in both EBA and DEB, it is conceivable that similar pattern will be observed in EBA. In canine EBA, the sensitivity and

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**Figure 1:** Graphical summary of diagnostic algorithm for epidermolysis bullosa acquisita. Abbreviations: IIF- Indirect immunofluorescence, DIF- Direct immunofluorescence, MMP- Mucous membrane pemphigoid, EBA- Epidermolysis bullosa acquisita, and DEB- Dystrophic epidermolysis bullosa

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**Figure 2:** Graphical representation of the diagnostic algorithm for EBA.
specificity of this technique were estimated at 71% and 90%, respectively.\(^6\) The diagnostics of this technique was not investigated in humans. However, the sensitivity and specificity observed in canine EBA are comparable with that of some of the expensive and inaccessible serological immunoassays performed only in specialized laboratories.

**Immunomapping (indirect immunofluorescence knockout analysis)**

Immunomapping utilizing a panel of skin deficient in specific BMZ constituents is a relatively simple technique that can be performed in all laboratories with the facility of immunofluorescence microscopy. Anti-type VII collagen antibodies cannot be detected by IIF microscopy by the use of type VII collagen-deficient skin from patients with DEB. Incubation of EBA sera with type VII collagen-deficient substrate will not demonstrate any labeling of the BMZ, while on normal/salt-split human skin, as well as on laminin 332-deficient skin, linear fluorescence is seen at the BMZ.\(^6\)

**Immunoserological detection of anti-type VII collagen antibodies**

Serological diagnosis in EBA is hampered by the relatively low detection rate of circulating autoantibodies, which in some studies does not exceed 60% of sera.\(^7\) Three detection systems for serum anti-type VII collagen antibodies are available, two enzyme-linked immunosorbent assay and an IIF microscopy-based tests. All of them are based on the recombinant expression of the immunodominant NC1 or a combination of the NC1 and NC2 domains of type VII collagen. Alternative immunological tests available in specialized laboratories include immunoblotting, with the dermal extract containing the full-length 290 kDa type VII collagen protein and the recombinant NC1 domain.\(^8\) In a recent multicenter retrospective serological study on 95 sera from EBA patients, Schmidt et al. concluded that type VII collagen combined NC1/NC2 domains ELISA is superior to ELISA based on type VII collagen NC1 domain only, immunoblotting, and IIF on salt-split skin, with a sensitivity of 97.9%.\(^8\) All of the immunoassays above are restricted to several specialized laboratories across the world.

**More sophisticated diagnostic approaches**

In those patients with inconclusive serration pattern and nonreactive type IV collagen immunostaining, who do not show serum reactivity against type VII collagen, the diagnosis of EBA may be made by fluorescence overlay antigen mapping technique or direct immunogold electron microscopy considered as the gold standard of diagnosis.\(^9\) However, these technologies are only available in few laboratories worldwide.

Taken together, Figure 1 demonstrates our proposed algorithm for the diagnosis of EBA starting with simple and inexpensive methods available in most immunopathological laboratories, progressing to more sophisticated methods performed in specialized laboratories only when the formers are not suggestive.

**Acknowledgment**

This figure was taken from the publication of Prof. Ludwig RJ after he has kindly granted his permission. The legend accompanying the illustration has also been included because it conveys the needed message and do not require any change.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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