Epidermolysis bullosa acquisita: diagnostic difficulties

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ABSTRACT

Introduction: Epidermolysis bullosa acquisita is a blistering disease in which the autoimmune response is directed against collagen VII epitopes.

Case report: A 50-year-old patient was admitted to the Department due to erythematous erosions and single blisters. The direct immunofluorescence study from a perilesional biopsy showed *in vivo* bound linear IgG deposits and focal C3 along the dermal-epidermal junction; however, the characteristic u-serrated pattern was not observed. No circulating antibodies were found in the serum. The direct immunofluorescence study performed on salt-split skin revealed immune deposits located along the floor and focally within the roof of the artificial blister. To determine the final diagnosis, type VII collagen was mapped on patient's salt-split skin using double-labelling with anti-collagen VII antibodies and serum from a seropositive epidermolysis bullosa acquisita patient. The overlapping of the identical images was obtained.

Conclusions: Marking the distribution of collagen VII on salt-split skin enabled the diagnosis.

Key words: epidermolysis bullosa acquisita, EBA, diagnostics.

INTRODUCTION

Epidermolysis bullosa acquisita (EBA) is an autoimmune subepidermal blistering disease affecting the skin and mucous membranes [1]. It is a rare disease with a prevalence of 0.2–0.5/1,000,000 individuals [2, 3]. EBA usually develops between 40 and 60 years of age, with no gender predilection [4]. The autoimmune response is mediated by autoantibodies directed against type VII collagen, most often its amino-terminal, non-collagen NC1 domain in the IgG class, less frequently IgA [1]. Type VII collagen is a component of anchoring fibres that connect the lamina densa of the basal membrane zone with the dermis [5]. Destruction of anchoring fibres leads to detachment of the epidermis from the dermis resulting in the formation of blisters which often heal with scarring and milia [6]. The clinical manifestations of EBA are heterogeneous. Two main clinical variants have been described, the mechanobullous and inflammatory [7]. The mechanobullous form of EBA, which represents the majority of cases, has a chronic course, and bullous eruptions occur in the mechanical trauma-prone areas such as the extensor surfaces of the limbs, elbows, knees, hands, feet and buttocks [4, 6]. Skin lesions may be accompanied by scarring alopecia and nail dystrophy [8]. The inflammatory variant is characterised by sudden onset, and skin lesions are not limited to injured areas. EBA also includes bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), linear IgA dermatosis (LABD) and Brunsting-Perry variants. However, two variants of EBA may occur in one patient [7]. Diagnosis of EBA is based on direct immunofluorescence (DIF) with serration pattern analysis and/or detection of serum autoantibodies against type VII collagen (anti-CO7 antibodies) [9]. EBA is mainly treated with high doses of glucocorticosteroids. Other therapeutic options involve dapsone, cyclosporine,

methotrexate, mycophenolate mofetil and cyclophosphamide. In the literature, there are also case reports of EBA treatment with high-dose intravenous immunoglobulin infusions (IVIG), rituximab, plasmapheresis, immunoadsorption and extracorporeal photochemotherapy [10].

OBJECTIVE

To present the case of a patient with seronegative EBA whose diagnosis was confirmed by using anti-collagen VII antibodies and the serum of a seropositive patient with confirmed EBA as a marker for type VII collagen.

CASE REPORT

A 50-year-old patient presented to the Department of Dermatology with tense blisters and erythematous erosions located on the back, palms of the hands and several erosions on the oral mucosa and anogenital region. On the trunk, skin lesions were arranged in the line of excoriations and skin injuries (figs. 1 A–D). He had a history of rheumatoid arthritis for 3 years treated with methotrexate. Laboratory tests showed an increased alanine transaminase (AST, 60 U/l) and accelerated ESR (36 mm/h). The DIF study revealed linear IgG and focal C3 deposits along the dermal-epidermal junction; however, it did not show the characteristic u-serrated pattern. Thus, the DIF on the patient's salt-split skin (SSS) was performed revealing IgG deposits located on the floor of an artificial blister and focally within the roof of an artificial blister; the DIF-SSS result was inconclusive (fig. 2). To determine the proper diagnosis, double labelling was performed using FITC-labelled EBA seropositive patient's serum and Cy-5-labelled IgG anti-collagen VII antibodies to map the antigen – type VII collagen in the previously examined tissue split from the patient's skin. As part of the procedure, the patient's skin was incubated in

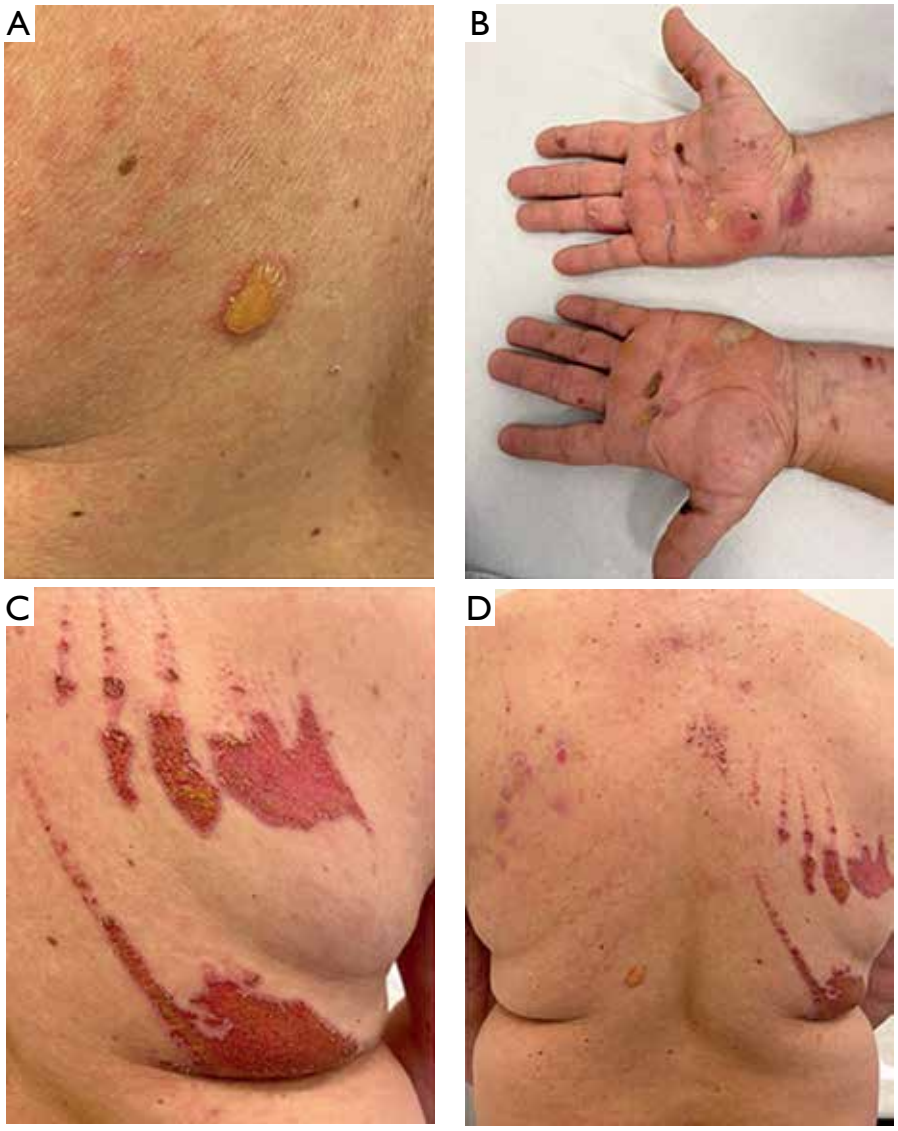

Figure 1. A–D – Blisters and erythematous erosions located on the back and palms

a 1-molar NaCl solution at 4°C for 48 h, which led to the separation of the epidermis from the dermis at the lamina lucida. In the next stage, the patient's tissue was incubated with the serum of EBA seropositive patient diluted at 1 : 10 and anti-collagen VII mouse IgG diluted at 1 : 100 for 30 min. After a triple rinse in phosphate-buffered saline (PBS), the sections were incubated with conjugates: antibodies directed against human IgG labelled with FITC at a dilution of 1 : 100 (green staining) (fig. 3 A) and antibodies directed against mouse IgG labelled with Cyanine 5 – Cy-5 (red staining) (fig. 3 B). After another triple rinse in PBS, the slides were applied in para-phenylenediamine (PPD) staining and evaluated under a fluorescence microscope. The overlapping of images of DIF-SSS and type VII collagen labelled SSS was obtained, which confirmed the diagnosis of EBA. The patient started treatment with prednisone at a dose of 0.6 mg/kg daily, and methotrexate was increased to 20 mg per week orally with topical glucocorticosteroids. Remission was achieved leaving scarring and post-inflammatory hyperpigmentation (figs. 4 A, B). The prednisone dose was gradually decreased until discontinuation. No recurrence of skin lesions was observed throughout the 12-month follow-up period.

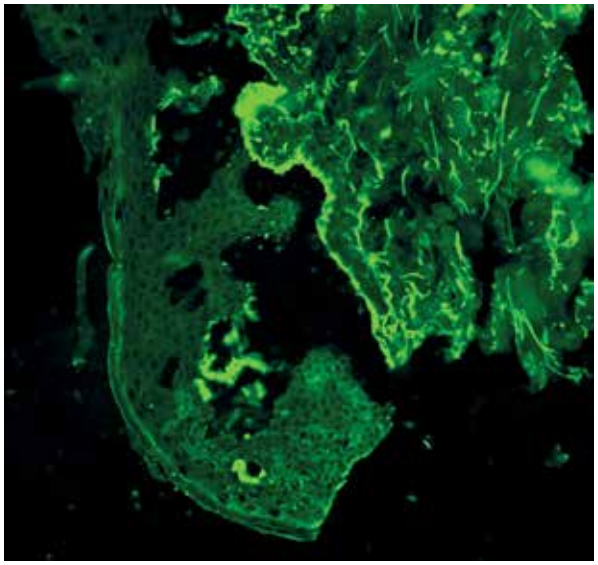

Figure 2. The direct immunofluorescence study on patient's salt-split skin. Visible immune deposits attached to the blister floor and focally within the epidermal roof

DISCUSSION

EBA may pose difficulties due to the similarity of clinical, histopathological and immunological features to other subepidermal autoimmune bullous diseases (AIBD) and the variability of the clinical presentation depending on the EBA variant. Thus, diagnosis of EBA requires a combination of laboratory investigations, including DIF and serological tests, to differentiate EBA from BP, MMP, LABD and anti-p200 pemphigoid. Histopathology can only be used to diagnose AIBD and exclude intraepidermal autoimmune diseases (like pemphigus). In the inflammatory form of EBA, neutrophilic infiltrates are observed, including variable numbers of eosinophils, monocytes and lymphocytes in the dermis, and skin fibrosis like scarring, and milia [9]. There are several important clinical factors to consider when differentiating between EBA and the most common AIBD, such as BP. Both diseases manifest as the formation of tense blisters on the skin however, in the mechanobullous variant of EBA, skin lesions occur mainly on trauma-prone areas such as elbows, knees, hands and feet [11]. Moreover, BP affects mainly elderly individuals during the 8th decade of life, whereas EBA usually develops around the age of 50ties [4, 12]. In the presented case, the diagnosis was determined at the age of 50, which is much earlier than the reported average age of onset for BP. Many studies have shown that EBA is more commonly associated with autoimmune diseases such as rheumatoid arthritis (RA), diabetes, cryoglobulinemia, psoriasis, and inflammatory

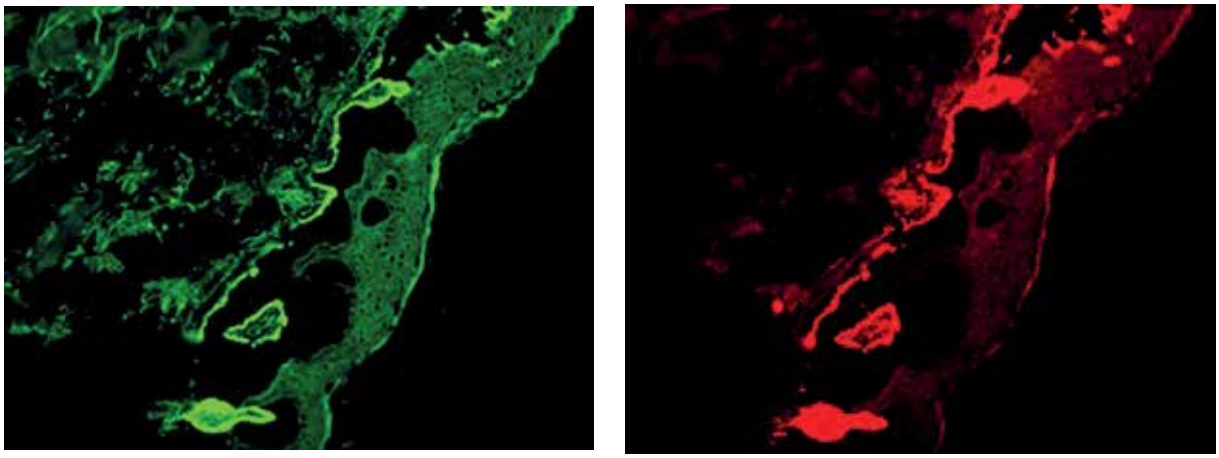

Figure 3. Images of DIF-SSS study. **A** – Fluorescence by using FITC-labelled epidermolysis bullosa acquisita seropositive patient's serum. **B** – Fluorescence by using Cy-5-labelled anti-collagen VII antibodies

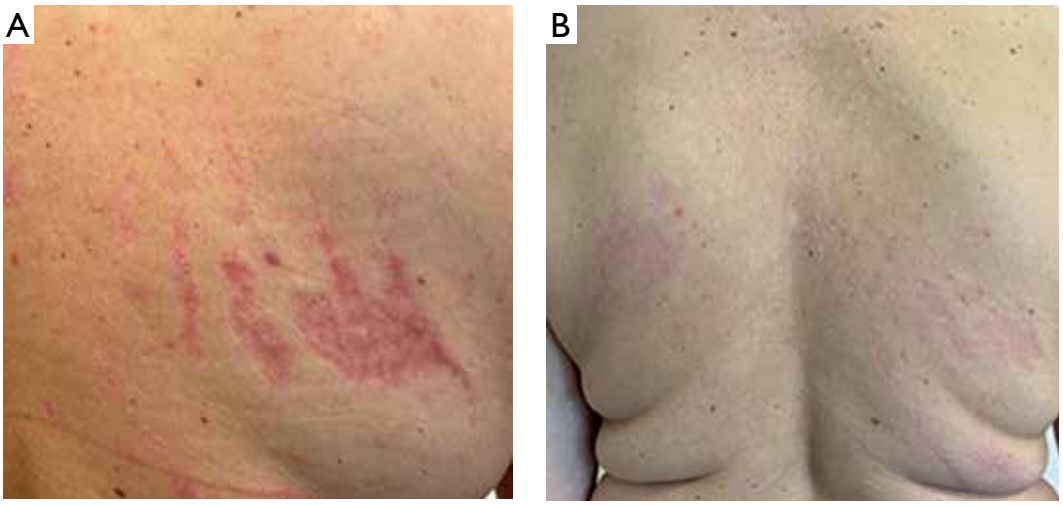

Figure 4. A, B – Remission of skin lesions with scarring and post-inflammatory hyperpigmentation

bowel diseases, while BP is more commonly associated with neurological and cardiovascular conditions [8, 11, 13]. Our patient was diagnosed with RA 5 years prior to EBA.

The current gold standard to diagnose EBA is the DIF study from the perilesional skin biopsy with serration pattern analysis. Importantly, in EBA linear IgG and/or IgA deposits give a characteristic u-serrated pattern along the dermal-epidermal junction at 400–600× microscopic magnification. Observing the u-serrated pattern is sufficient to determine the diagnosis, although the same fluorescence pattern can be present in bullous systemic lupus erythematosus [6]. In contrast to EBA, in other autoimmune bullous diseases such as BP, MMP, LABD and anti-p200 pemphigoid, the n-serrated fluorescence pattern can be visualised [14]. In the presented case, the DIF study showed IgG deposits along the dermal-epidermal junction. However, the fluorescence u-serrated pattern was not explicit.

According to the recommendations, the detection of circulating antibodies is the primary diagnostic approach in EBA if the serration pattern is not observed. However, it should be stressed that the circulating antibodies in EBA are found in about 60% of patients, depending on the method, such as IIF on salt-split skin (IIF-SSS), ELISA, BIOCHIP mosaics or immunoblot [7]. Diagnostic difficulties arise when a patient with EBA has no detectable circulating autoantibodies, as in the presented case. Therefore, the DIF on salt-split skin (DIF-SSS) from perilesional biopsy is recommended, typically presenting immunological deposits along the floor of an artificial blister in EBA and along the roof of an artificial blister in BP, MMP and LABD. Of note, antibodies in rare types of pemphigoid directed against laminin 332 or p200/laminin γ-1 chains are also bound to the floor of an artificial blister; thus, it is impossible to rule out these diseases from EBA [9]. In our patient, the result of DIF-SSS on the skin taken from the perilesional region was ambiguous and did not allow us to establish a clear-cut diagnosis. In such a situation, the type VII collagen can be labelled using fluorescence overlay antigen mapping (FOAM). The literature indicates that type VII collagen and β4 integrin are the most suitable molecules as topographic reference markers to differentiate EBA from BP [15]. However, the FOAM method requires a rarely used and highly specialised laser. In the presented case, we proposed double labelling using FITC-labelled EBA seropositive patient's serum and Cy-5-labelled anti-collagen VII IgG antibodies to map collagen VII in the previously examined tissue split from the patient's skin. The images of DIF-SSS and type VII collagen labelled SSS were identical indicating that the focal fluorescence in the roof of an artificial blister was associated with improper separation of the patient's skin in SSS bellow type VII collagen. Other AIBDs were excluded. We did not find the literature describing the potential use of serum from a patient with confirmed EBA as a marker for collagen VII in the diagnosis of EBA.

Although there is no consensus on the diagnostic criteria for EBA, a general framework for establishing the diagnosis of EBA has been developed, taking into account the clinical presentation and available laboratory tests. In seronegative patients, the diagnosis can be established based on the clinical appearance of subepidermal blisters, a characteristic fluorescence in DIF, and positivity of one of the additional skin tests, such as DIF-SSS, after exclusion of autoimmunity to laminin 332 or p200/laminin γ-1 chains [9].

CONCLUSIONS

In the presented case, the diagnosis of EBA posed difficulties due to seronegativity and inconclusive DIF-SSS study. However, using anti-collagen VII antibodies and the serum of a seropositive patient with EBA to mark the distribution of collagen VII on the patient's split skin enabled the diagnosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

References

1. **Ludwig R.J.:** Clinical presentation, pathogenesis, diagnosis, and treatment of epidermolysis bullosa acquisita. ISRN Dermatol 2013, 812029.
2. **Bernard P., Vaillant L., Labeille B., Bedane C., Arbeille B., Denoeux J.P., et al.:** Incidence and distribution of subepidermal autoimmune bullous skin diseases in three French regions. Bullous Diseases French Study Group. Arch Dermatol 1995, 131, 48-52.
3. **Bertram F., Bröcker E.B., Zillikens D., Schmidt E.:** Prospective analysis of the incidence of autoimmune bullous disorders in Lower Franconia, Germany. J Dtsch Dermatol Ges 2009, **7**, 434-440.
4. **Kim J.H., Kim Y.H., Kim S.C.:** Epidermolysis bullosa acquisita: a retrospective clinical analysis of 30 cases. Acta Derm Venereol 2011, 91, 307-312.
5. **Sakai L.Y., Keene D.R., Morris N.P., Burgeson R.E.:** Type VII collagen is a major structural component of anchoring fibrils. J Cell Biol 1986, 103, 1577-1586.
6. **Buijsrogge J.J., Diercks G.F., Pas H.H., Jonkman M.F.:** The many faces of epidermolysis bullosa acquisita after serration pattern analysis by direct immunofluorescence microscopy. Br J Dermatol 2011, 165, 92-98.
7. **Vorobyev A., Ludwig R.J., Schmidt E.:** Clinical features and diagnosis of epidermolysis bullosa acquisita. Expert Rev Clin Immunol 2017, 13, 157-169.
8. **Chen M., Kim G.H., Prakash L., Woodley D.T.:** Epidermolysis bullosa acquisita: autoimmunity to anchoring fibril collagen. Autoimmunity 2012, 45, 91-101.
9. **Prost-Squarcioni C., Caux F., Schmidt E., Jonkman M.F., Vassileva S., Kim S.C., et al.:** International Bullous Diseases Group: consensus on diagnostic criteria for epidermolysis bullosa acquisita. Br J Dermatol 2018, 179, 30-41.
10. **Iwata H., Vorobyev A., Koga H., Recke A., Zillikens D., Prost-Squarcioni C., et al.:** Meta-analysis of the clinical and immunopathological characteristics and treatment outcomes in epidermolysis bullosa acquisita patients. Orphanet J Rare Dis 2018, 13, 153.
11. **Gammon W.R., Briggaman R.A., Woodley D.T., Heald P.W., Wheeler C.E. Jr.:** Epidermolysis bullosa acquisita--a pemphigoid-like disease. J Am Acad Dermatol 1984, 11, 820-832.
12. **Miyamoto D., Santi C.G., Aoki V., Maruta C.W.:** Bullous pemphigoid. An Bras Dermatol 2019, 94, 133-146.
13. **Kalińska-Bienias A., Kowalczyk E., Jagielski P., Bienias P., Kowalewski C., Woźniak K.:** The association between neurological diseases, malignancies and cardiovascular comorbidities among patients with bullous pemphigoid: case-control study in a specialized Polish center. Adv Clin Exp Med 2019, 28, 637-642.
14. **Terra J.B., Meijer J.M., Jonkman M.F., Diercks G.F.:** 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, 100-105.
15. **Bruins S., De Jong M.C., Heeres K., Wilkinson M.H., Jonkman M.F., Van der Meer J.B.:** Fluorescence overlay antigen mapping of the epidermal basement membrane zone: III. Topographic staining and effective resolution. J Histochem Cytochem 1995, 43, 649-656.

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