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Case Report

How can immunohistochemistry improve the diagnosis of pemphigus foliaceus?

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ABSTRACT

Purpose: Pemphigus foliaceus (PF) is a rare, autoimmune blistering disorder characterized by the production of autoantibodies against desmoglein 1. The mainstay for diagnosis is the demonstration of immune complex deposition by direct immunofluorescence (DIF) in fresh tissue samples. Immunohistochemistry (IHC) recognizes autoantibodies in formalin-fixed paraffin-embedded specimens, but studies regarding its use in PF are scarce. This study aims to evaluate immunoglobulin and C3 deposition using IHC in patients with confirmed PF by DIF and indirect immunofluorescence (IIF).

Material and methods: Six biopsies obtained from five patients with PF and six healthy individuals were included in this study. Anti-C3c, -IgG, -IgM, and -IgA antibodies were used for DIF and automated IHC. After digitalizing the slides, staining was classified as negative (0) or positive (1 = mild/2 = intense).

Results: DIF revealed intraepidermal intercellular deposition of IgG and C3c (n = 6), without deposits in dermal structures. IHC was positive in the intercellular spaces between keratinocytes with anti-IgG (n = 6) and anti-C3c antibodies (n = 6); no intercellular immune complexes deposition was observed in healthy individuals. In patients with PF, inflammatory cells were tagged by anti-IgG and anti-C3c (n = 6), anti-IgM (n = 1), and anti-IgA (n = 1); and immune complexes at vessel walls were detected with anti-C3c, anti-IgG, anti-IgA (n = 6), and anti-IgM (n = 5) antibodies. Adnexal positivity occurred with anti-C3c and anti-IgG (n = 6), anti-IgM (n = 1), and anti-IgA (n = 3). Healthy individuals also presented positivity in inflammatory cells with anti-IgG and anti-C3c (n = 4), anti-IgM (n = 1), and anti-IgA (n = 3); vessels were stained with anti-IgG and anti-C3c (n = 5), anti-IgM and anti-IgA (n = 4); adnexa were not represented in all samples obtained from healthy individuals.

Conclusion: IHC may serve as a reliable method to assess PF diagnosis. Immune deposits in dermal structures suggest their participation in autoimmune/inflammatory processes in PF. IHC may contribute to evaluate disease mechanisms, prognostic factors, and target-oriented treatment in PF.

1. Introduction

Pemphigus foliaceus (PF) is an autoimmune disease characterized by intraepidermal detachment due to the binding of IgG to desmoglein 1 [1]. Immune complex formation promotes disarray of the desmosomes, resulting in flaccid blisters and erosions predominantly on the face and trunk.

Direct (DIF) and indirect (IIF) immunofluorescence allows the recognition, localization, and titration of *in situ* and circulating autoantibodies, respectively. Immunofluorescence (IF) is a sensitive and specific technique, and is the mainstay diagnostic method for autoimmune blistering diseases (ABD) since 1964 [2–5]. Nevertheless, IF usually requires additional biopsy samples. Immediate tissue processing is recommended if the fragment is kept in saline solution, or preservation in Michel's transport medium is required for further analysis [6]. Preparation of frozen specimen depends upon a cryostat handled by a trained technician, and an epiluminescent microscope is necessary to analyze the slides [7].

Immunohistochemistry (IHC) allows the recognition of antigen expression and antibody bound to the tissue, with the advantage of using formalin-fixed paraffin-embedded (FFPE) specimens [8]. New antibodies and chromogens, automated processing and computerized analysis have increased IHC sensitivity and specificity. This method provides crucial information for the differential diagnosis and prognosis of autoimmune, infectious and neoplastic disorders, and is widely used as a reliable method in several areas of anatomic pathology including

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dermatopathology [9]. However, there is scarce data about the use of IHC for the diagnosis of ABD, especially in comparison to IF assays [10].

In order to evaluate the detection of tissue-bound immunoglobulins and C3c by IHC, we performed immunohistochemical analysis of FFPE skin samples obtained from patients with PF, previously tested by IF. Results were interpreted using a whole slide imaging platform. To the best of our knowledge, this is the first report to analyze biopsies from PF patients using automated IHC in FFPE tissue in a whole slide imaging platform.

2. Material and methods

The Institutional Review Board approved this study. DIF, IIF and immunohistochemical analysis were performed according to previously published protocols in six specimens obtained from five patients with PF. Six skin fragments obtained from healthy individuals were used as normal controls.

2.1. Direct immunofluorescence

For DIF, specimens received in Michel's medium were frozen in tissue-freezing medium (Sakura, Torrance, CA, USA) and 4 μm sections were obtained. After incubation with secondary antibodies (Table 1) for 30 min, slides were washed with Tris-buffered saline twice for 10 min and analyzed under an fluorescent microscope HBO 50w (Zeiss, Marburg, Germany) [11].

2.2. Immunohistochemistry

In total, 4 μm tissue sections of FFPE skin specimens were obtained from the same PF patients. Immunohistochemical analysis was performed using a Ventana Benchmark LT® Automated Platform according to the manufacturer's protocol, which includes deparaffinization, cell conditioning with 0.350–0.475 μL CC1 (Tris-EDTA buffer pH 8.0) for 30 min for antigen retrieval, followed by enzymatic digestion with alkaline protease for 4 min.

In order to avoid nonspecific binding between the antibodies and tissue structures which could lead to background staining [12], the blocking step was added to the IHC protocol optimized for this study. Each slide was incubated with 100 μL of the primary antibody mixed with the Antibody Dilution Buffer (Ventana, Tucson, AZ) for 32 min at 37 °C (Table 2) and visualized with the chromogen ultraView Universal DAB Detection Kit (Ventana, Tucson, AZ), and counterstained with hematoxylin (4 min) and bluing reagent (4 min). The brown chromogen provided clearer visualization of the positivity with the antibodies utilized in comparison to the red chromogen, which was associated with background staining. Tonsil sections were selected as positive controls for anti-IgA, anti-IgG and anti-IgM, and kidney sections were included as positive controls for anti-C3c. We omitted the primary antibody in the cutaneous tissue sections used as negative controls.

2.3. Immunohistochemical analysis

Analysis was performed after scanning the slides at 40 × magnification with an Aperio ScanScope CS scanner (Leica Biosystems, Nussloch, Germany). Positivity in the epidermis, basement membrane zone (BMZ), inflammatory cells, vessels and adnexa was classified as negative (0) or positive (1 = mild/moderate; 2 = intense).

Table 1
Secondary antibodies utilized for the direct immunofluorescence analysis of samples from patients with pemphigus foliaceus (Dako, Carpenteria, CA, US).

Antibody	Code	Dilution
Rabbit polyclonal anti-human C3c	F0201	1:40
Rabbit polyclonal anti-human IgG	F0202	1:130
Rabbit polyclonal anti-human IgM	F0203	1:20
Rabbit polyclonal anti-human IgA	F0204	1:20

Table 2
Primary antibodies used for the immunohistochemical evaluation of skin specimens from patients with pemphigus foliaceus (Dako, Carpenteria, CA, US).

Antibody	Code	Dilution
Rabbit polyclonal anti-human C3c	A0062	1:5000
Rabbit polyclonal anti-human IgG	A0423	1:20,000
Rabbit polyclonal anti-human IgM	A0425	1:5000
Rabbit polyclonal anti-human IgA	A0262	1:3000

2.4. Patients and results

2.4.1. Patient 1

A 19-year-old white male was referred to our clinic with a 20-day history of diffuse erythema and desquamation. During the first examination the patient was erythrodermic (Fig. 1A, B), and was hospitalized for clinical support and diagnostic investigation. A skin biopsy of the right forearm was obtained and histopathology revealed a subcorneal detachment with acantholysis. DIF showed moderate, intercellular, intraepidermal deposition of C3c and IgG (Fig. 1C), and IIF with anti-IgG was positive (1:2560), confirming the diagnosis of PF. The patient received prednisone 100 mg/day (1.48 mg/kg/day) and vancomycin 2 g/day due to secondary bacterial infection. After 21 days the patient was discharged with improvement of skin lesions.

IHC analysis showed moderate intercellular deposition of C3c, IgG and IgA in the epidermis, BMZ, inflammatory cells and vessels. IgM stain was mildly positive in the vessels. Adnexal structures exhibited moderate C3c and mild IgG and IgA deposition (Fig. 1D, E).

2.4.2. Patient 2

A 79-year-old white female patient had a 10-year history of PF. She was in remission without treatment, when small pruriginous vesicles and blisters with herpetiform distribution appeared on the trunk with no evident triggering factor (Fig. 2A, B). Skin biopsy on the dorsum was performed to rule out pemphigus herpetiformis. Histopathology showed eosinophilic and neutrophilic spongiosis. Intercellular deposits of C3c and IgG were observed in the epidermis by DIF (Fig. 2C). Nuclear fluorescence of keratinocytes was also observed with C3c. IIF (anti-IgG) titers were 1:320. Lesions healed with topical corticosteroid and the patient achieved complete remission.

IHC revealed C3c (moderate), IgG (mild) and IgA (mild) deposits within the intercellular spaces of keratinocytes. Moreover, mild IgA deposits at the BMZ and vessel walls were seen. Intraepidermal intercellular C3c and IgG staining, as well as IgG and C3c deposits were observed at vessel walls and adnexa (Fig. 2D, E).

2.4.3. Patient 3

A 19-year-old white female patient was referred to our clinic with exfoliative erythroderma. Histopathology of a skin specimen (dorsum) exhibited a superficial intraepidermal blister with acantholysis (Fig. 3A, B). DIF revealed the presence of a moderate intercellular IgG deposition in the epidermis, mild intercellular C3c deposition within the epidermis and BMZ, and moderate IgM deposition at the BMZ (Fig. 3C, E). IIF titer (anti-IgG) was 1:1280. After systemic prednisone 90 mg/kg/day (1.5 mg/kg/day) and cephalexin 4 g/day for 14 days, the skin lesions completely healed, and the patient was discharged after 17 days.

IHC analysis revealed epidermal intercellular staining with all conjugates. Anti-C3c and -IgM stained the BMZ. Immune complexes with IgG, C3c and IgM were observed in the inflammatory cells and adnexa. Deposits at vessel walls were present with all antibodies (Fig. 3D, F).