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Review Article

Direct immunofluorescence in bullous disorders: A mini-review

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ABSTRACT

Background: Immunofluorescence microscopy is an invaluable tool for different lesions in dermatopathology. These lesions include bullous lesions, vasculitis, and connective tissue disorders/ systemic lupus erythematosus. In this review, we take a look at bullous disorders.

Some of these diagnoses are not possible on routine histopathology alone and require immunofluorescence for confirmation. Different diseases can be diagnosed according to the immunoglobulins/complements, sites of deposition (dermo-epidermal / intercellular spaces / dermal), and their patterns (linear, granular). Knowing the exact type of lesion can help guide treatment accordingly.

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INTRODUCTION

Autoimmunity plays an important role in the pathogenesis of bullous disorders. These bullous lesions are difficult to diagnose because they mimic each other clinically; in addition, there is a lot of overlap in microscopic appearance. Direct immunofluorescence (DIF) is quite helpful in differentiating these lesions and is considered the gold standard for the diagnosis of vesico-bullous disorders. DIF also has a role in monitoring response to treatment and predicting relapse.[1-4] The beginnings of immunofluorescence started in 1942 when Albert Coons et al demonstrated the labeling

of anti-pneumococcal antibodies in lung tissue with the help of fluorescein.[5] Immunofluorescence was first started in dermatology in the 1960s by Beutner and Jordon while studying vesiculobullous lesions.[6] In the procedure for immunofluorescence, an antibody is conjugated to a fluorochrome. These labeled antibodies bind to their corresponding antigens in tissue which allows their detection after visualization in an immunofluorescence microscope. A fluorochrome is a dye that absorbs radiation and causes excitation of the molecule, leading to electron redistribution and emission of a different wavelength of light. Antibodies that are chemically conjugated to the fluorochrome bind to their respective antigens and can be visualized through fluorescence.[7]

Non-specific staining and auto-fluorescence should not be confused with positive staining while reporting these slides. Autofluorescence refers to the fluorescence that is intrinsic to the tissue, which is usually of a slightly different color, most commonly yellow or orange rather than the typical green color seen. Non-specific staining refers to staining by fluorescein isothiocyanite(FITC) proteins, which are more commonly found in thicker sections; hence, care must be taken during frozen-section of the tissue to be examined.[8]

The principle of immunofluorescence is based on the visualization of fluorochrome-tagged antigen-antibody complexes under ultraviolet light. These fluorochromes contain electrons that become irradiated with the appropriate wavelength of light. This leads to an unstable higher energy state. These fluorochromes will finally release light and return to their previous state.[9]

A fluorophore or fluorochrome is a substance that can emit light after excitation by light. Fluorescein along with its active amine isothiocyanate derivative has been the most commonly used fluorophore and it stains green or yellow. A semiquantitative scoring system for immunofluorescence is usually done, on a scale of + to ++++.[10]

SITE OF BIOPSY

Two biopsies should be taken, one from the site of the lesion for histopathology and one from the peri-lesional area for DIF. In the blistered skin, deposits may be degraded due to inflammation leading to negative DIF findings.[11]

In most bullous lesions, a skin biopsy is taken from peri-lesional skin near a fresh blister since areas within the blister may have negative DIF due to the removal of antibodies by inflammation. In the case of lupus erythematous, it is recommended that both lesional areas and sun-exposed normal skin should be sampled. In contrast, in the case of discoid lupus, the biopsy should be only taken from the lesional skin since the normal skin is typically negative for DIF. In pemphigus vulgaris, DIF positivity correlates with clinical activity but it may persist even in inactive lesions.[12]

PATTERNS OF STAINING

Histopathology of bullous lesions typically shows the site of bulla and the inflammatory infiltrate and may have overlapping features so a differential diagnosis has to be made. Immunological and molecular methods are required for accurate diagnosis. The disease can be diagnosed according to the site of deposition, the type of immunoglobulin, and the pattern of deposits. Different patterns of staining of different antibodies need to be assessed for diagnosis. Some examples are given in Figure 1.

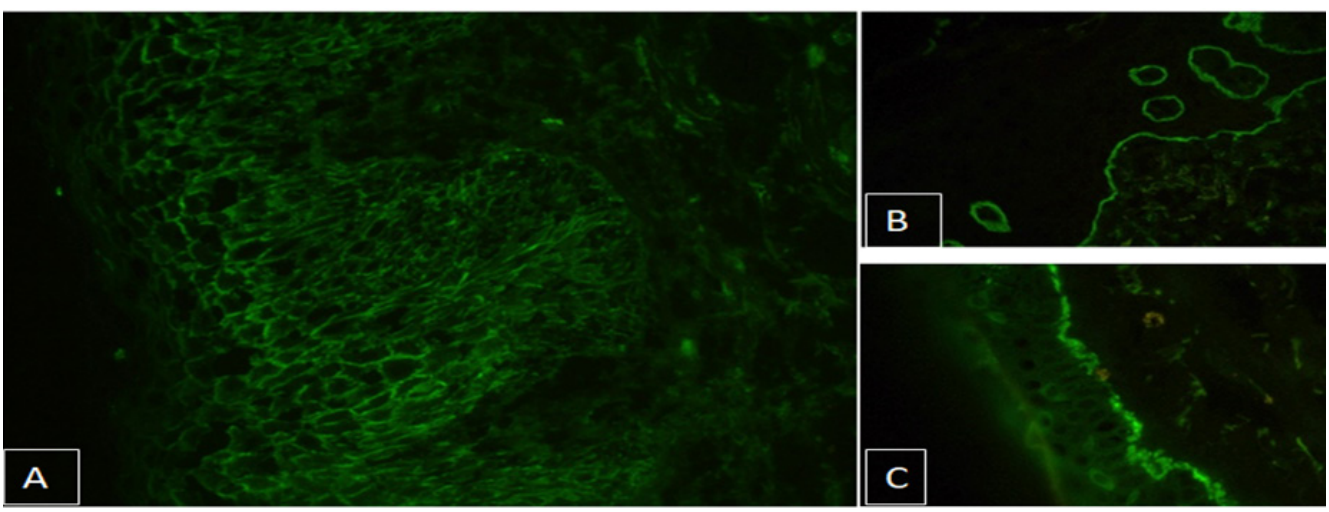

Figure 1: *DIF slides showing positivity for different diseases. (A) Pemphigus vulgaris: Fish-net pattern staining of IgG in epidermis. (B) Linear IgA disease: Linear staining of IgA in the dermo-epidermal junction (C) Bullous pemphigoid: Linear c3 staining in dermo-epidermal junction.*

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In a study done in India by Khandpur et al, IgG was present in 51 out of 58 cases of pemphigus vulgaris in a fishnet pattern along with 7 cases also showing c3.[13] Basement membrane zone staining with both IgG and c3 seen in 18 out of 25 cases of bullous pemphigoid. One case of linear IgA disease was seen in which there was linear basement membrane deposition of IgA. Similarly, two cases of dermatitis herpetiformis showed granular deposition of IgA in the basement membrane zone. Pemphigus vulgaris was the most common disease in the study. Bullous pemphigoid typically occurs in elderly patients in the fifth to seventh decade of life. It occurs in both sexes with variable sex ratios.[13-15] IgG, IgA or only c3 deposition may be seen. In bullous pemphigoid, c3 deposition in the basement membrane zone is seen in 100% of cases and IgG is seen in 65%-95% of cases. Flexural areas are more suitable for biopsy as there is a higher presence of bullous pemphigoid antigens. Pemphigoid gestationalis/herpes gestationalis may have similar findings except IgG is less commonly expressed.[17] Bullous systemic lupus erythematous may also show similar basement membrane zone staining as bullous pemphigoid and needs to be differentiated clinically and serologically. Lupus erythematosus shows deposition of IgG, IgM, IgA, and C3 along the basement membrane zone. In addition, antinuclear antibodies can also be seen in the epithelial cell nuclei. The granular basement membrane zone is typically seen in systemic lupus erythematosus also known as the lupus band test. Staining is seen in the lesional as well as non-lesional skin. Epidermolysis bullosa acquisita and bullous systemic erythematosus may show positivity for all immunoglobulins IgG, IgA, IgM, and C3. Almost all cases show positivity for IgG and C3.[17] Similarly, basement membrane zone staining of IgA is seen in linear IgA disease.

In pemphigus vulgaris, IgG autoantibodies can be found in skin and oral squamous mucosa to desmoglein 1 or 3. IgG deposits are seen in the lower levels, with 100% of active cases showing mucosal involvement. Pemphigus vulgaris shows 100% positivity in cases of pemphigus vulgaris in a fishnet or chicken wire pattern in the epidermis. Staining is more prominent in the suprabasal part of the epidermis in Pemphigus vulgaris, whereas it is more common in the superficial layers in Pemphigus folliaceous. In pemphigus vulgaris, DIF positivity correlates fairly well with clinical activity but it may persist despite clinical inactivity.[11]IgG intercellular pattern is seen in all types of pemphigus, other than IgA pemphigus. C3 deposition will also show a similar staining pattern but usually stains less intensely. In pemphigus vulgaris, autoantibodies are directed against desmoglein 1 and 3, which are required for cell-to-cell adhesion in the epidermis. Cases with pemphigus foliaceous have IgG autoantibodies target desmoglein 1 which is the main antigen. Paraneoplastic pemphigus can be associated with neoplasms and also affects mucus membranes. It has a similar pattern of immunofluorescence as Pemphigus vulgaris but may also show homogenous basement membrane staining with IgG and C3.[16]

IgA pemphigus is a neutrophilic acantholytic dermatosis. Typically, it has inter-cellular intraepidermal IgA deposits. IgA pemphigus is of two forms, namely sub-corneal pustular dermatoses and intraepidermal neutrophilic type in which IgA epidermal inter-cellular staining is seen in the upper epidermal layers and throughout/lower layers respectively.[16] Dermatitis herpetiformis occurs commonly between 25-55 years of age as reported by Nicolas et al. Granular deposits at dermal papillae are seen. False negative cases may be seen in up to 20% of cases due to various technical errors. These include an absence of epidermis in sectioned tissue, exposure to light, improper site of biopsy, and longer duration of sample in normal saline.[16]

The salt-split technique can also be used for direct immunofluorescence. Artificial splitting of the skin is done with incubation in 1 M solution of sodium chloride for 24 hours. Basement membrane zone staining may be present in the blister roof (bullous pemphigoid) the floor (epidermolysis bullosa acquisita) or combined (bullous pemphigoid). Epidermolysis bullosa acquisita also shows the presence of antibodies against type VII collagen.[18]

Limitations of immunofluorescence include photobleaching in which there is destruction of fluorophore due to exposure to excitation light. Autofluorescence may be seen in tissue which may also hamper the interpretation.[19]

Non-specific staining and autofluorescence should be ruled out while reporting. Autofluorescence refers to the fluorescence that is intrinsic to the tissue, which is of a slightly different color, usually yellow or orange. Non-specific staining refers to staining by FITC proteins, which may be found in thicker sections. [19] Further pitfalls of immunofluorescence microscopy include false positive and negative results. False positive reactions may be because of non-specific staining in the epidermis or basement membrane region. A Pemphigus-like staining pattern may be seen due to the crushing and freezing of the tissue. False negatives include technical errors including contamination with formalin, delay in sample processing, and drying of the biopsy. Lack of representative epithelium is another major cause of false negativity, so communication with the dermatologist is crucial. [17-20]

CONCLUSIONS

Autoimmune blistering diseases are potentially fatal diseases that affect the skin and mucosal membranes. Direct immunofluorescence has a very important role in identifying the lesions, which may show similar findings in histopathology. This will help the patient receive a more specific treatment. Timely and accurate diagnosis and treatment can help to improve the quality of life of these patients.

Conflict of Interest: None

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