Histopathological features and immunoflorescence patterns in skin lesions

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Abstract

The diagnosis of vesiculo-bullous lesions and many other diseases is fundamentally based on the microscopic study of cells and tissues. This diagnostic method remains the standard, by which all other diagnostic tests are measured. Nevertheless, the era of the pathologist relying entirely on the examination of tissue sections stained by histochemical methods is gradually being replaced by a time
when advanced immunologic and molecular techniques (i.e. analysis of DNA, RNA or protein structure and function) augment the process by which complicated diseases are classified.

Key words
Lichen planus, Pemphigoid, Pemphigus vulgaris, Dermatitis herpetiformis, Erythema multiforme, Immunofluorescence.

Introduction
A diagnosis is a clinical tool that assists in the process of codifying patients into disease groups that tend to share a common outcome, and a common set of responses to therapy. The histological diagnosis in turn is used by clinicians to aid in the management of patients. The most accurate diagnosis is the one that most closely correlates with clinical outcome and helps direct the most appropriate clinical intervention [1].

Thus, there is a close relationship between diagnosis and prognostication by emphasizing certain key observations that have value in identifying particular diseases. Where applicable, ultra structural, immunohistochemical, and molecular aids to diagnosis are discussed. These advances have resulted in increased specificity for many diagnoses. For example, immunofluorescence has long been used to differentiate among vesiculo-bullous disorders [2].

Lichen planus
Lichen planus is a common disorder of the stratified squamous epithelium that affects oral and genital mucous membranes, skin, nails, and scalp. Oral Lichen Planus (OLP) affects middle-aged women and shows distribution patterns and characteristics such as white striations, white plaques or papules, erythema, blisters and erosions, and may be associated with medication and/or dental materials used by the patient. Bullous Lichen Planus it is the most unusual clinical form, exhibiting blisters that increase in size and tend to rupture, leaving the surface ulcerated and painful. The periphery of the lesion is, in general, surrounded by fine keratinized striae [3].

Histopathology diagnosis
Histopathological examination with hematoxylin and eosin stain revealed parakeratosis with hypergranulosis and mild basal cell hyperplasia. Basal cells showed edema, degeneration and early separation with lymphocytic exocytosis. Upper sub-epithelium showed moderate lymphocytic infiltrate. Overall features were consistent with bullous oral lichen planus (Figure - 1) [4].

Figure - 1: Lichen planus - Microphotograph showing Hypergranulosis and basal cell hyperplasia. Subepithelially there are lymphocytes.

Herpes simplex infections
Herpes simplex virus (HSV) infections are common vesicular eruptions of the skin and mucosa. They occur in two forms - systemic or primary - and may be localized or secondary in nature. Both forms are self-limited, but recurrences of the secondary form are common because the virus can be sequestered within ganglionic tissue in a latent state. Control rather than cure is the usual goal of treatment [5].
oral-facial herpetic lesions are due to HSV type 1 (HSV1), although a small percentage may be caused by HSV type 2 (HSV2) as a result of oral-genital contact. Lesions caused by either virus are clinically indistinguishable. HSV2 has a predilection for genital mucosa, with infections having a pathogenesis similar to that of HSV1 infections of the head and neck.

Histopathology diagnosis

Microscopically, intraepithelial vesicles containing exudates, inflammatory cells, and characteristic virus infected epithelial cells are seen (Figure - 2A). The virus-infected keratinocytes contain one or more homogeneous, glassy nuclear inclusions. These cells are also readily found on cytological preparations. HSV1 cannot be differentiated from HSV2 histologically. After several days, herpes-infected keratinocytes cannot be demonstrated in either biopsy or cytological preparations (Figure - 2B).

Figure - 2A: Herpes simplex-induced vesicle.

Figure - 2B: Virus-infected multinucleated keratinocytes in the wall of a vesicle.

Histopathology diagnosis

The classic histological feature seen in PV is acantholysis which is the loss of cell to cell contact in the epithelial cell layers. Development of intercellular edema within the epithelial layers, dissolution of the intercellular bridges and the widening of intercellular spaces, lead to separation between the cells and the formation of blisters just above the basal cell layer. Hence, the split is characteristically suprabasilar and the basal cells remain tightly attached to the basal lamina producing tombstone appearance [6, 7, 8]. Presence of Tzanck cells which are free floating, rounded acantholytic epithelial cells will be found within the vesicle [7]. They can be demonstrated cytologically by Tzanck test. The vesicular fluid and the connective tissue may show scant inflammatory cell infiltration [6]. Spongiosis and acantholysis of the adjacent epithelium can occur (Figure – 3).

Direct immunofluorescence (DIF) demonstrates homogenous epithelial cell surface staining particularly in the intercellular spaces, with IgG and possibly also IgA, IgM, C3, C1 properdin and properdin factor B. Indirect Immunofluorescence (IIF) demonstrates the circulating antibodies. The quantification of serum titres with IIF is useful to observe disease progression.

Pemphigus

Pemphigus includes a group of autoimmune blistering diseases of the skin and mucous membrane. It is characterized histologically by intradermal blisters and immunologically by circulating autoantibodies directed against the cell surface of keratinocytes.
over time and to evaluate therapeutic interventions (Figure - 4, 5) [6, 8].

**Figure - 3:** Acantholysis in pemphigus vulgaris.

**Figure - 4:** Pemphigus vulgaris: Direct immunofluorescence pattern.

**Figure - 5:** Indirect immunofluorescence showing intercellular deposits of IgG in pemphigus vulgaris.

**Bullous pemphigoid**

Bullous pemphigoid (BP) is a subepidermal blistering skin disease that usually occurs in the elderly population and is characterized by large tense blisters with immunopathological findings of linear deposits of C3 and IgG at the basement membrane zone [9]. It primarily affects elderly individuals in the fifth to seventh decade of life, with average age of onset being 65 years. BP in childhood has been reported from various countries including India [10]. There is no known ethnic, racial, or sexual predilection.

The diagnosis of BP is confirmed by histological and immunopathological investigations. Histopathology from lesional skin demonstrates a sub-epidermal blister. The inflammatory infiltrate is typically polymorphous, with an eosinophilic predominance (Figure - 6). Mast cells and basophils may be prominent early in the disease course. Tzanck smear shows only inflammatory cells.

**Figure – 6:** Bullous-Pemphigoid-histopathology.

DIF studies on normal appearing peri-lesional skin within 2 cm of a lesion demonstrate *in vivo* deposits of IgG antibodies in 90-95% of cases and C3 in 100% cases at the basement membrane zone. IgG deposits are rarely present in the absence of C3, but presence of IgA, IgM and IgE has been described [11]. This pattern of immunoreactants is not specific to BP and may be seen in
cicatricial pemphigoid and epidermolysis bullosa acquisita. BP can be differentiated from these conditions by the salt-split technique in which patient's skin biopsy sample is incubated in 1 mol/l salt solution prior to performing DIF. This process induces cleavage through the lamina lucida. DIF on salt-split skin reveals IgG on the blister roof (epidermal side of split skin) in BP. In a study from India, Satyapal, et al. [12] evaluated salt-split technique of immunofluorescence in BP. Thirty-two cases of BP were subjected to DIF and IIF using normal and salt-split skin. DIF positivity of 100% was noted with both routine and salt-split methods. Additional immunoreactants were also noted with DIF on salt-split skin in five cases; patterns of fluorescence with salt-split skin were roof (40.6%), floor (9.4%) and combined roof and floor (50%).

On IIF, positivity was almost doubled with salt-split technique (68%) as compared to routine method (36%). IIF studies document IgG (subclass IgG4) circulating autoantibodies in the patient's serum that target the skin basement membrane component. Circulating autoantibodies are detected in 60-80% of cases on IIF [13]. The antibody titre (detected by IIF) does not correlate with disease course. Danesh Pazhooh, et al. [14] compared the antibody titres of blister fluid and serum in patients with subepidermal immunobullous diseases and concluded that IIF sensitivity on blister fluid is no more than that on serum, but the performance of this test on blister fluid in addition to serum may reduce the number of false-negative results of IIF found using either of these two substrates alone. Zhou, et al. [15] concluded that blister fluid can be used as an alternative to serum for IIF in subepidermal immuno-bullous diseases.

**Bullous systemic lupus erythematosus**

Bullous systemic lupus erythematosus (BSLE) is a rare variant of systemic lupus erythematosus (SLE) which histologically resembles dermatitis herpetiformis (DH) and responds dramatically to dapsone. BSLE is an autoantibody mediated subepidermal blistering disease that occurs in patients with SLE. Histologically, bullous lupus erythematosus is characterized by neutrophil-rich, subepidermal bulla. There are often neutrophils along the dermal-epidermal junction and papillary microabcess formation. (Figure - 7).

![Figure - 7: Microphotograph of Bullous Lupus Erythematosus.](image)

Immunohistologically, it resembles LE with immunoglobulins and complement components deposited in a linear-granular pattern along the dermal-epidermal junction. Bullous lupus erythematosus is often associated with autoimmunity to type VII collagen [16].

Direct IF: IgG and C3 are deposited at the epidermal basement membrane zone. The pattern is linear, but sometimes may be ‘shaggy’ or ‘granular band-like’. A linear rather than granular pattern along the BMZ is associated with the presence of higher titer of circulating auto antibodies. IgM and IgA are present in approximately 50 to 60% of cases respectively. In general, granular patterns (60%) represent deposition of circulating immune complexes in situ or in situ binding of antigen and antibody in compartmentalized zones. Similar deposition of IgG, IgM or complement in the normal skin is known as positive lupus band test. Bullous SLE is associated with a higher incidence of IgA
deposition (76%) than other forms of SLE (17%) and this may also correlate with renal involvement. C3 is usually deposited in lesional skin [17].

**Varicella-zoster infections**

Primary varicella-zoster virus (VZV) infections in sero-negative individuals are known as varicella or chickenpox; secondary or reactivated disease is known as herpes zoster or shingles. Structurally, VZV is very similar to HSV, with a DNA core, a protein capsid, and a lipid envelope.

The morphology of the VZV and the inflammatory response to its presence in both varicella and herpes zoster are essentially the same as those with HSV. Microscopically, virus-infected epithelial cells show homogeneous nuclei, representing viral products, with margination of chromatin along the nuclear membrane. Multinucleation of infected cells is also typical. Acantholytic vesicles eventually break down and ulcerate. In uncomplicated cases epithelium regenerates from the ulcer margins with little or no scar (Figure 8) [5].

**Figure - 8: Microphotograph of varicella zoster.**

Erythema multiforme

Erythema multiforme (EM) is an acute mucocutaneous hypersensitivity reaction characterized by a skin eruption, with or without oral or other mucous membrane lesions. Occasionally EM may involve the mouth alone. EM has been classified into a number of different variants based on the degree of mucosal involvement and the nature and distribution of the skin lesions. EM minor typically affects no more than one mucosa, is the most common form and may be associated with symmetrical target lesions on the extremities. EM major is more severe, typically involving two or more mucous membranes with more variable skin involvement – which is used to distinguish it from Stevens - Johnson syndrome (SJS), where there is extensive skin involvement, and significant morbidity and a mortality rate of 5-15%.

The microscopic pattern of EM consists of epithelial hyperplasia and spongiosis. Basal and parabasal apoptotic keratinocytes are also usually seen. Vesicles occur at the epithelium-connective tissue interface, although intraepithelial vesiculation may be seen. Epithelial necrosis is a frequent finding. Connective tissue changes usually appear as infiltrates of lymphocytes and macrophages in perivascular spaces and in connective tissue papillae (Figure - 9) [2].

**Figure - 9: Microphotograph of Erythema Multiforme**

Immuno-pathologic studies are nonspecific for EM. The epithelium shows negative staining for immunoglobulins. Vessels have been shown, however, to have IgM, complement, and fibrin deposits in their walls. This latter finding has
been used to support an immune complex vasculitis cause for EM. Autoantibodies to desmoplakins 1 and 2 have been identified in a subset of EM major-affected patients, suggesting that both cell-mediated and humoral immune systems may contribute to the pathogenesis of EM (Figure - 10).

**Figure – 10:** C3 Immunofluorescence in Erythema Multiforme.

**Measles**

Measles is a highly contagious viral infection caused by a member of the paramyxovirus family of viruses. The virus, known simply as measles virus, is a DNA virus and is related structurally and biologically to viruses of the orthomyxovirus family, which cause mumps and influenza.

**Histopathology diagnosis**

Infected epithelial cells, which eventually become necrotic, overlie an inflamed connective tissue that contains dilated vascular channels and a focal inflammatory response. Lymphocytes are found in a perivascular distribution. In lymphoid tissues, large characteristic multinucleated macrophages, known as Warthin-Finkeldey giant cells, are seen [18].

**Mucous membrane pemphigoid**

Mucous membrane pemphigoid (MMP) is a chronic blistering or vesiculo-bullous disease that affects predominantly oral and ocular mucous membranes. It is also known as cicatricial pemphigoid, benign mucous membrane pemphigoid, ocular pemphigus, childhood.

Pemphigoid, and mucosal pemphigoid; when it affects gingiva exclusively, it is referred to clinically as gingivosis or desquamative gingivitis [5].

MMP is a sub-epithelial clefting disorder, and there is no acantholysis. In early stages few lymphocytes are seen, but with time, the infiltrate becomes more dense and mixed (Figure - 11).

**Figure - 11:** Mucous membrane pemphigoid showing characteristic subepithelial separation.

**Histopathology diagnosis**

The MMP is histologically characterised by junctional separation at the level of the basement...
membrane giving rise to a sub-basilar split as in other forms of pemphigoid.

**Figure - 12:** Mucous membrane pemphigoid; basement membrane immunofluorescent staining.

Classical histopathological features include a subepithelial split with a chronic inflammatory infiltrate containing eosinophils, lymphocytes, and neutrophils as well, in the lamina propria. However, routine biopsy of a patient suspected of having MMP is often not enough to fully differentiate the disease from other mucocutaneous disorders [19].

**Direct immunofluorescence (DIF)**

The DIF is often helpful in making the broad diagnosis of pemphigoid if immunostaining shows deposits of IgG and C3 in a homogeneous linear manner in the BMZ along the dermoeipidermal junction. This procedure involves performing an incisional biopsy from a peri-lesional site adjacent to a new vesicle or bulla. The specimen should be transferred in a specific transport media (Michel’s solution) or snap-frozen on liquid nitrogen, and must be processed in a timely manner. The tissue is then incubated with fluoresceinated antibodies against IgG, complement and fibrinogen, and examined under a fluorescent microscope. In some cases, additional biopsies may be necessary to demonstrate the presence of immune deposits in the BMZ. The following recommendations by the Consensus Statement may enhance positive results [20].

- In patients with single-site mucosal involvement, a biopsy specimen should be obtained from tissue next to the areas of inflammation.
- When patients present with multiple-site involvement, the biopsy should be taken from tissue adjacent to an inflamed non-ocular site.
- Patients who present with both skin and mucosal involvement should have a skin biopsy taken from an inflamed lesion.
- For patients with ocular involvement requiring a biopsy, the procedure should be performed cautiously both to minimise injury and additional scarring.

Essentially all patients with MMP and CP have, on DIF, in vivo bound IgG, IgA or C3, presenting as a homogeneous line in the BMZ of lesionsal and perilesional mucosa. Deposition of C3 in the BMZ is detected in almost all patients, sometimes is the sole immunologic reactant, and is considered diagnostically significant.

The DIF analysis of biopsy specimens of MMP where the epithelium is separated from the underlying connective tissue may show IgG deposits on the basal pole of the epithelial cells in an interrupted linear pattern [19].

DIF is thus useful in several ways: first, a positive result confirms the diagnosis of IMSEBD. Second, DIF differentiates IgG-mediated diseases (BP, MMP, HG and acquired epidermolysis bullosa (EBA) from IgA mediated diseases (dermatitis herpetiformis and linear IgA disease).

**Hand-foot-and-mouth disease**

Hand-foot-and-mouth disease is caused by a coxsackievirus, an Enterovirus. In most instances coxsackievirus type A16 has been isolated; only
rarely has another type, such as A5 or A9, been found [21]. Hand-foot-and-mouth disease occurs in small epidemics, affecting mainly children and having a mild course that usually lasts less than a week. Transmission is mainly via fecal -oral contact and less commonly by respiratory droplets [22].

**Histopathology diagnosis**

Early vesicles are intraepidermal, whereas old vesicles may be subepidermal in location. There is pronounced reticular degeneration of the epidermis, resulting in multilocular vesiculation. In the deep layers of the epidermis, some ballooning degeneration may be found. Neither inclusion bodies nor multinucleated giant cells are present (Figure - 13).

**Figure - 13:** Histopathology of Hand Foot Mouth Disease.

**Neoplasia induced pemphigus**

Paraneoplastic pemphigus is a recently described. Rare vesiculo-bullous disorder that affects patients who have a neoplasm, usually lymphoma or chronic lymphocytic leukemia. It is thought that cross- reactivity develops between antibodies produced in response to the tumour and antigens associated with the desmosomal complex and the basement membrane zone of the epithelium. A variety of different antibodies that attack these epithelial adherence structures are produced, resulting in an array of clinical features [5].

**Histopathology diagnosis**

The features of Paraneoplastic pemphigus on light microscopic examination may be as diverse as the clinical features. In most cases, a lichenoid mucositis is seen, usually with subepithelial clefting (like pemphigoid) or intraepithelial clefting (like pemphigus).

Direct immunofluorescence studies may show a weakly positive deposition of immunoreactants (IgG and complement) in the intercellular zones of the epithelium and/or a linear deposition of immunoreactants at the basement membrane zone [23]. Indirect immunofluorescence should be conducted using a transitional type of epithelium (such as rat urinary bladder mucosa) as the substrate. This shows a fairly specific pattern of antibody localization to the intercellular areas of the epithelium. Immunoprecipitation studies remain the gold standard for the diagnosis of Paraneoplastic pemphigus. However, because the various antibodies that characterize this condition can be identified with a considerable degree of specificity. Antibodies directed against desmoplakin I and II. Major bullous pemphigoid antigens envoplakin and periplakin, in addition to desmoglein 1 and 3 are typically detected (Figure – 14).

**Figure - 14:** Direct immunofluorescence showing IgG deposition in the intercellular spaces of the Epidermis.
Dermatitis herpetiformis
Dermatitis herpetiformis is a cutaneous vesiculo-bullous disease characterized by intense pruritus. The disease is associated with granular IgA deposits in the papillary dermis that precipitate with an epidermal transglutaminase, an enzyme not normally present in the papillary region of normal skin. Serum IgA in patients with dermatitis herpetiformis also binds epidermal transglutaminase. Dermatitis herpetiformis is frequently associated with the gluten-sensitive enteropathy, celiac disease, which is characterized by IgA-type autoantibodies to a closely related enzyme, tissue transglutaminase.

Histopathology and immunopathology
Collections of neutrophils, eosinophils, and fibrin are seen at the papillary tips of the dermis. Subsequent exudation at this location contributes to epidermal separation. A lymphophagocytic infiltrate is seen in perivascular spaces (Figure - 15).

Figure - 15: Microphotograph showing histological features of Dermatitis Herpetiformis.

The immunologic finding of granular IgA deposits at the tips of the connective tissue papillae is specific for dermatitis herpetiformis. In addition, it is possible to localize the third component of complement (C3) in lesional and perilesional tissue in a distribution similar to that of IgA (Figure - 16) [24].

Linear IgA disease
Linear IgA disease is a chronic autoimmune disease of the skin that commonly affects mucous membranes, including gingiva. Unlike dermatitis herpetiformis, it is not associated with gluten-sensitive enteropathy (and may not be responsive to dapsone therapy or dietary gluten restrictions). Skin lesions may be urticarial, annular, targetoid, or bullous. Oral lesions, present in a majority of cases, are ulcerative (preceded by bullae). Ocular lesions, also seen in a majority of cases, are in the form of ulcers. Patients respond to sulfones or corticosteroids.

The biological basis of linear IgA disease is not well understood. Central to the disease are autoantibodies to BP180 (collagen XVII), which normally functions as a cell-matrix adhesion molecule through stabilization of the hemidesmosome complex and whose extracellular portion is constitutively shed from the cell surface by ADAMs (proteinases that contain adhesive and metalloprotease domains). Similar to MMP, in vivo and in vitro studies provide experimental evidence for a central pathogenic role of BP180 but that the serum level and epitope specificity of these antibodies influence phenotype and disease severity.

Microscopically, separation at the basement membrane is seen. Neutrophils and eosinophils
often fill the separation. With DIF, linear deposits of IgA are found at the epithelium-connective tissue interface (Figure - 17) [25].

**Figure - 17:** Linear IgA Bullous Dermatosis.

Linear IgA disease is managed in a similar manner to MMP with topical corticosteroids as the initial therapy. Systemic corticosteroids or other immunosuppressive agents (azathioprine, cyclophosphamide, and cyclosporine) may be used in more severe or refractory cases (Figure - 18) [5].

**Figure - 18:** Linear IgA deposits along basement membrane zone on direct immunofluorescence.

Toxic epidermal necrolysis and stevens-johnson syndrome
Toxic epidermal necrolysis (TEN) and Stevens Johnson Syndrome (SJS) are severe adverse cutaneous drug reactions that predominantly involve the skin and mucous membranes. Both are rare, with TEN and SJS affecting approximately 1 or 2/1,000,000 annually, and are considered medical emergencies as they are potentially fatal. They are characterized by mucocutaneous tenderness and typically hemorrhagic erosions, erythema and more or less severe epidermal detachment presenting as blisters and areas of denuded skin. Currently, TEN and SJS are considered to be two ends of a spectrum of severe epidermolytic adverse cutaneous drug reactions, differing only by their extent of skin detachment.

**Histopathology diagnosis**
Microscopically, showing full thickness epidermal necrosis with a basket weave-like stratum corneum and separation of the dermis and epidermis (Figure – 19) [26].

**Figure - 19:** Histopathology of TEN.

**Conclusion**
Histopathology, sometimes also referred to as cellular pathology, is a complex and crucial area of study in modern medicine. It is an extremely detailed branch of science that focuses solely on the anatomical changes that occur in diseased tissue at a microscopic level. This science is so specific that a histopathologist not only has to perform tests, conduct analysis and gather data, but is also responsible for interpreting the information gathered and making the final diagnosis so a patient's doctor can then move forward to manage the treatment for the specific disease.
References


