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Comparison of histopathology, immunofluorescence, and serology for the diagnosis of autoimmune bullous disorders: an update

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Introduction

The diagnosis of autoimmune bullous disorders (AIBDs) relies on several different diagnostic methods. These include histopathology, direct immunofluorescence (DIF), indirect immunofluorescence (IIF), enzyme-linked immunosorbent assay (ELISA) and immunoblotting. When faced with a presumptive AIBD, the most widely employed method for diagnosis by dermatologists is a combination of histopathology and DIF. While DIF is still the diagnostic method of choice for linear IgA bullous disease and IgA pemphigus, ELISA is a more accurate, cost-effective and less invasive method of diagnosis for several AIBDs including pemphigus vulgaris and foliaceus, based on currently available evidence [1-3].

The purpose of this article is to review and compare the evidence supporting the use of these diagnostic methods, both traditional and recent, which are available to practitioners. This comparison should provide a practical reference for the evidence-based diagnostic method or combined methods of choice for each AIBD. The techniques and basic science underlying widely available methods are briefly explained. Additionally, newer methods with limited availability but which may become diagnostically relevant in the near future, such as mosaic IIF, are also reviewed.

Overview of methods for the detection of autoantibodies in AIBDs

DIF is the most commonly used technique in practice to diagnose AIBDs. In DIF, the target antigen is the patient's autoantibody. Skin 5 mm from the site of a blister is obtained and placed in a transport medium such as Michel's buffer. Frozen sections of this perilesional skin are prepared, and each section is incubated with a single primary antibody, such as anti-IgG, anti-IgM, or anti-C3. These primary antibodies are linked to fluorescein isothiocyanate (FITC), a fluorophore which is visualized by a pathologist using a fluorescent microscope. The presence or absence of epifluorescence is then reported and can be relatively and subjectively quantified [4].

In IIF, the target antigen is present in a known and readily available substrate. This substrate is incubated with the patient's dilute serum which contains the primary antibody. Secondary antibodies labeled with FITC are then added and bind to the primary antibodies. Epifluorescence is visualized with a fluorescent microscope and can be quantified by successive titration of the patient's serum [4].

In an ELISA, the target antigen of interest (such as the NC16a domain of BP180) is immobilized by physical adsorption or by antibody capture. When antibody capture is utilized, this is referred to as "sandwich ELISA" because the target antigen is bound between the immobilizing antibody and the primary antibody. Primary antibodies are present in the patient's serum. Enzyme-linked secondary antibodies are then added which bind the Fc region of primary antibodies. Substrate is added and converted by the enzyme into a signal. A resulting color change, fluorescence, or electrochemical signal is quantitatively measured and reported [5].

Western blot is synonymous with immunoblot. For this method, cells which express the target antigen or antigens of interest are cultured and then lysed to release proteins. These proteins are denatured and then separated by gel electrophoresis on the basis of their molecular weights, measured in kilodaltons (kDa). Electroblotting then transfers these proteins to a membranous medium. After non-specific sites on the membrane are blocked, it is incubated with the primary antibodies (patient's serum). A secondary antibody linked with a reporter enzyme, frequently horseradish peroxidase, binds to the primary antibody. The enzymatic reaction produces a luminescent signal which is captured by a camera and then quantified [6]. Immunoprecipitation is a variant of immunoblot that can be used to identify, isolate, and concentrate multiple (potential) target antigens. A lysate solution is incubated with a patient's serum, and complexes are formed between target antigens and primary autoantibodies. These antigen-antibody complexes are then immobilized on beads, precipitated out of solution, and then analyzed by standard immunoblotting technique [7].

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Special Issue: Updates in Dermatology

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Pemphigus group

The pemphigus group includes pemphigus vulgaris (PV), pemphigus foliaceus (PF), drug-induced pemphigus (DIP) and paraneoplastic pemphigus (PNP), mediated primarily by IgG autoantibodies directed against desmosomal cadherin proteins involved in intercellular adhesion. Pemphigus vulgaris typically presents in the fifth and sixth decades of life and is characterized by thin-walled, flaccid bullae, which rupture easily to form painful erosions and crusted lesions. PV typically affects mucosal surfaces in addition to keratinized skin, with the majority of cases first presenting in the mouth. Autoantibodies are most often directed against desmoglein 3 (Dsg3), with molecular weight 130 kDa, but may also target desmoglein 1 (Dsg1) in mucocutaneous disease. However, diagnosis is made when anti-Dsg3 autoantibodies are present, regardless of presence or absence of anti-Dsg1. Pemphigus foliaceus is characterized by superficial, flaccid bullae that easily rupture and lead to shallow erosions with adherent scale crust, described as resembling corn flakes (Figure 1). Unlike PV, PF rarely affects mucosal surfaces. Antibodies in PF target desmoglein 1 (Dsg1), with molecular weight 160 kDa [8].

ELISA is the most accurate diagnostic tool for the diagnosis of PV and PF. In a 2012 meta-analysis of 13 studies with a sample size of 1058 patients, ELISA for anti-Dsg3 IgG had a pooled sensitivity of 97% and specificity of 98% [1]. ELISA for anti-Dsg IgG in patients with PF has a sensitivity and specificity of 96% and 99%, respectively [2,3]. One limitation of ELISA is observed at very high antibody titers (equivalent to that of IIF assay titers of 320 or greater), at which readings plateau. In contrast, IIF remains quantitative at high titers [9].

Histology provides important clues, but cannot by itself distinguish between PV and PF given variability in the level of clefting. Generally, acantholysis is suprabasilar in PV and subcorneal or intragranular in PF. However, a 2014 study by Ohata *et al.* found significant histologic overlap in the location of acantholysis between these two diseases with a quarter of all pemphigus cases showing diffuse acantholysis. Additionally, while acantholytic dyskeratotic cells are commonly reported in PV, they were identified in over 60% of casees of both PV and PF. Neutrophils were found as the predominant cell type in the epidermis over eosinophils more often in PF than PV (70.8% versus 54.8%) [10].

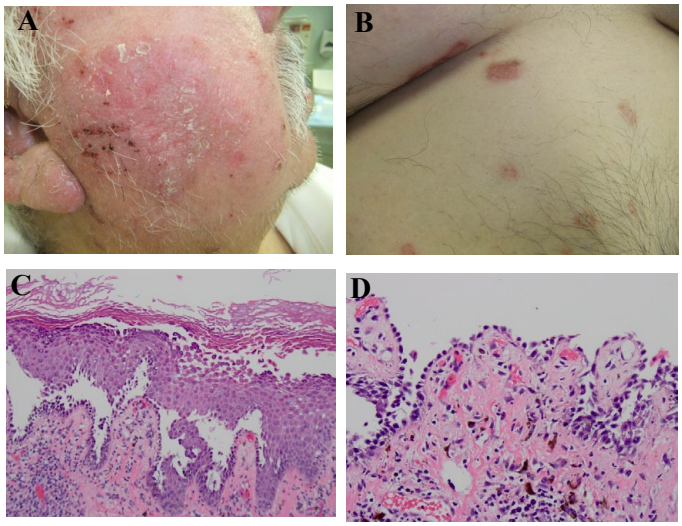

Figure 1. Pemphigus foliaceus. Elderly man with superficially erosive plaques with crusting on the cheek (A) and trunk (B). No mucosal lesions were present. C (100x): histology demonstrated both superficial and suprabasilaracantholysis. D (400x): In some foci, "tombstoning" mimicking pemphigus vulgaris is identified. However, ELISA for anti-Dsg1 demonstrated pathogenic titers while that for anti-Dsg3 did not.

DIF approaches 100% sensitivity, but cannot distinguish PF from PV as both show a similar fluorescence pattern with typical uniform intercellular IgG and complement staining ("swiss cheese" or "chicken wire" pattern) [10-13]. IIF is less sensitive than ELISA and DIF, but can helpful to establish antibody titers. Similar to DIF, IIF cannot distinguish between PV and PF and will demonstrate intercellular staining in both. Sensitivity ranges between 81-95% [13-15]. Of note, the choice of substrate affects the results of IIF. Several studies have compared these monkey and human esophagus. In one study monkey esophagus was superior or equal in sensitivity for antibody detection in either pemphigus subtype while another study found monkey esophagus superior for PV diagnosis and human esophagus superior for PF diagnosis [15,16]. Although not commonly used, immunoblotting differentiates between the different clinical phenotypes of PV (mucocutaneous, purely mucosal or purely cutaneous) with 100% specificity. However, it is only 89% sensitive for the diagnosis of PV and more expensive. For PF, immunoblotting has a sensitivity of 100% and specificity of 95% [17].

In monitoring patient response to therapy, ELISA index values correlate better with disease activity compared to IIF titers [3,18]. In particular, anti-Dsg1 ELISA values correlate with skin lesion severity in both PV and PF, while anti-Dsg3 values do not follow mucosal lesion severity in PV [19]. ELISA is also better than DIF in evaluating immunologic remission of PV. Negative predictive value in one study was 100%, which is important when discontinuing treatment, in order to minimize false negatives [20].

In mucosal PV, the combination of ELISA and Tzanck smear has the best overall sensitivity and specificity, 82% and 98.7% respectively, compared to either test alone or other diagnostic methods [21]. Histologic evaluation alone of affected mucosa can be misleading given frequently nonspecific findings or subtle acantholysis. In a series of 12 cases, two identified initially as mucous membrane pemphigoid (MMP) based on histology alone were ultimately diagnosed as PV [22].

If drug-induced pemphigus (DIP) is suspected, the most specific test is immunohistochemical staining with 32-2B, a monoclonal antibody against Dsg1 and Dsg3. Immunoperoxidase for 32-2B is 84% specific and 70% sensitive for DIP. Normal skin and DIP both show net-like deposits along cytoplasmic membranes in contrast to idiopathic pemphigus, which shows coarse pericytoplasmic granules [23]. Unfortunately, this immunostain is routinely or widely available, and most pathologists are not familiar with its interpretation. Histology, DIF, IIF, ELISA and immunoblotting cannot distinguish DIP from idiopathic pemphigus. Thus, distinction between DIP and idiopathic pemphigus most often depends on clinical correlation.

PNP has a wide range of clinical manifestations. Mucosal lesions may resemble Stevens-Johnson syndrome with hemorrhagic crusting of the lips and widespread mucosal erosions. Cutaneous lesions may appear lichen planus-like, erythema multiforme-like, bullous pemphigoid-like or with erosions typical of pemphigus. The implicated target antigens are also more varied and include desmoplakin 1 (250 kDa), envoplakin (210 kDa), periplakin (190 kDa) and bullous pemphigoid antigen-1 (BPAg1, 230 kDa), in addition to Dsg1 and Dsg3. Recently, alpha-2-macroglobulin-like protein 1 (A2ML1) has also been characterized as a target in PNP [8].

Immunoblotting for envoplakin and/or periplakin approaches

100% sensitivity and is considered the gold standard diagnostic method. However, specificity is lowered by false positives to 82-91% [24-26]. IIF on rat bladder is the best confirmatory test; although sensitivity ranges from 67-95%, specificity is close to 100% [24,26,27]. The histology of PNP is nonspecific and is lichenoid as often as it is acantholytic (Figure 2); both reaction patterns may be observed together in up to 60% of cases. Of note, keratinocyte necrosis is associated with a poorer prognosis. DIF is considered diagnostic (specificity up to 97%) when staining for IgG and/or C3 is observed both within intercellular spaces as well at the dermoepidermal junction (DEJ). However, less than half of PNP cases show both intercellular and junctional immunofluorescence, making this unique finding insensitive [24,26,28]. DIF is 100% sensitive when considering either intercellular or junctional staining, but only 40% specific [27].

Currently, ELISA is considered a complementary test only for PNP. A 2015 study by Ohzono *et al.* evaluated several ELISAs for PNP diagnosis. ELISA for anti-Dsg1 and anti-Dsg3 IgG was 86% sensitive, but these assays cannot distinguish PNP from PF or PV. ELISA for IgG against desmocollins (Dsc) 1, 2, and 3 demonstrated a combined sensitivity of 72%. ELISA for IgG against A2ML1 has a sensitivity of 60% [27]. Separate studies have evaluated ELISA arrays for envoplakin, with variable sensitivity (30-100%) but high specificity (90-100%) [24,25,29]. A study by Huang *et al.* of 16 PNP cases found positive IgG autoantibodies against envoplakin and periplakin by ELISA in all 16 cases of PNP evaluated [30].

IgA pemphigus presents with flaccid vesicles or pustules in annular or circinate configuration with central crusting (Figure 3). The axilla and groin are most commonly involved, but it also found on the trunk and extremities. Two distinct subtypes of this disease have been identified. The target antigen in the subcorneal pustular dermatosis (SPD) type is Dsc1. The intraepidermal neutrophilic (IEN) type has an unknown target antigen [31].

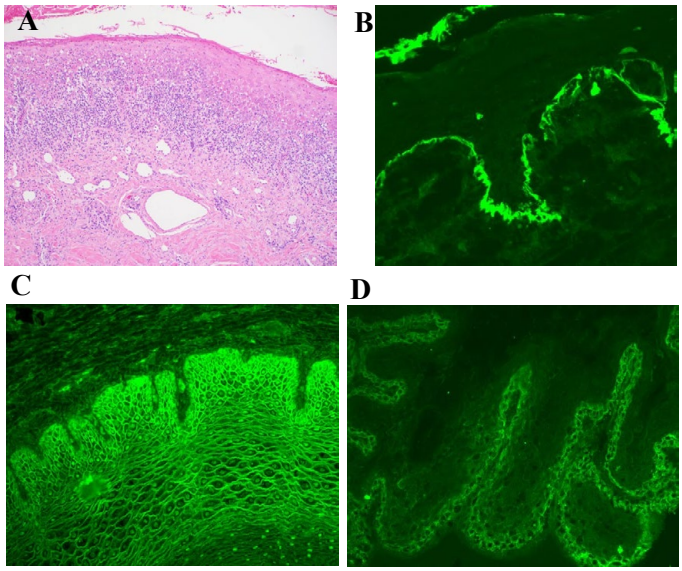

Figure 2. Paraneoplastic pemphigus. A (100x): Histology demonstrates a lichenoidmucositis. Acantholysis is absent. B (200x): DIF demonstrates linear, "shaggy" fibrinogen at the dermoepidermal junction, mimicking lichen planus. C (200x): IIF on monkey esophagus is strongly positive. Taken with the results of the DIF, this indicates autoimmunity to a target antigen other than Dsg1 or Dsg3. ELISA for anti-Dsg3 was negative. D (200x): IIF on rat bladder, which expresses plakins but not desmogleins, is strongly positive. The patient was an elderly woman with intractable stomatitis and a history of thymoma. Courtesy of Travis Vandergriff, MD.

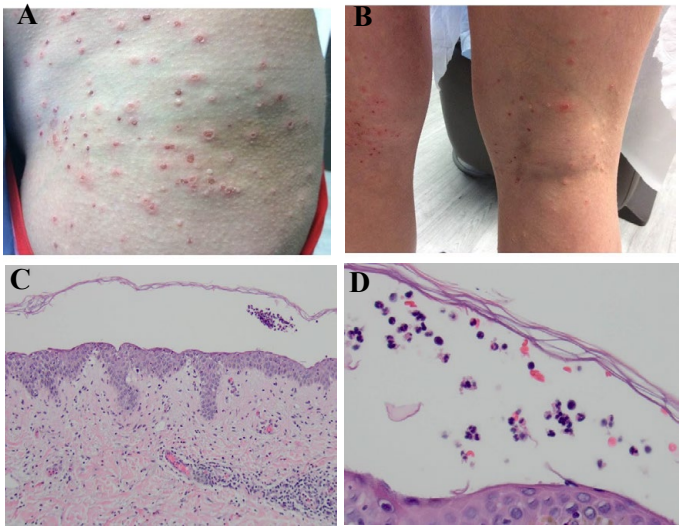

Figure 3. IgA pemphigus. A and B: Pustules distributed in an annular configuration and involving the flexures. C (100x) and D (400x): subcorneal pustules with rare superficial acantholytic keratinocytes. DIF demonstrated IgA staining within the upper one-third of the epidermis.

DIF is the most sensitive and specific test for IgA pemphigus, demonstrating intercellular deposition of IgA in all cases. In the SPD type, IgA deposits are concentrated in the upper epidermis, while in the IEN type, IgA is observed throughout the entire epidermis [32,33]. DIF should be performed to distinguish cases of subcorneal pustular dermatosis (Sneddon-Wilkinson disease) from the SPD type of IgA pemphigus. Sneddon-Wilkinson disease demonstrates a negative DIF but is associated with monoclonal gammopathy, while the SPD type of IgA pemphigus demonstrates a positive DIF but is not associated with hematologic abnormalities [34]. IIF has shown variable sensitivity for the diagnosis of IgA pemphigus, ranging from 50-100% and is often negative in the IEN type [33,35]. Histology is highly nonspecific with a broad differential diagnosis, and unlike classic pemphigus, acantholysis is typically absent. ELISA is approximately 20% sensitive for the diagnosis of IgA pemphigus based on detection of IgG or IgA against Dsg1 or Dsg3, but may be useful in excluding classic pemphigus [33,36].

Pemphigoid group

Bullous pemphigoid (BP) is the most common AIBD and is characterized by the typical clinical presentation of pruritic urticarial lesions that evolve into tense blisters. Typical histologic features are generally nonspecific, and include eosinophil-rich or cell-poor subepidermal blisters. Urticarial lesions demonstrate numerous eosinophils at the DEJ (Figure 4). In BP, autoantibodies target two hemidesmosomal proteins at the DEJ: BP antigen 1 (BPAG1 or BP230), a 230-kDa intracellular plakin-family protein, and BP antigen 2 (BPAG2 or BP180), a 180-kDa transmembrane collagen-family protein comprised of a central domain separating two N- and C- terminal domains [37-39]. Due to the greater prevalence of BP180 autoimmunity and the ability of BP180-directed antibodies to reproduce disease in animal models, BP180 is thought to be the major antigenic target in BP. However, a small proportion of patients with BP only develop antibodies against BP230 [40-42].

DIF is the most sensitive test for the diagnosis of BP and serves as a reference to evaluate to sensitivities of other methods. DIF most commonly shows linear deposition of IgG and C3, although additional

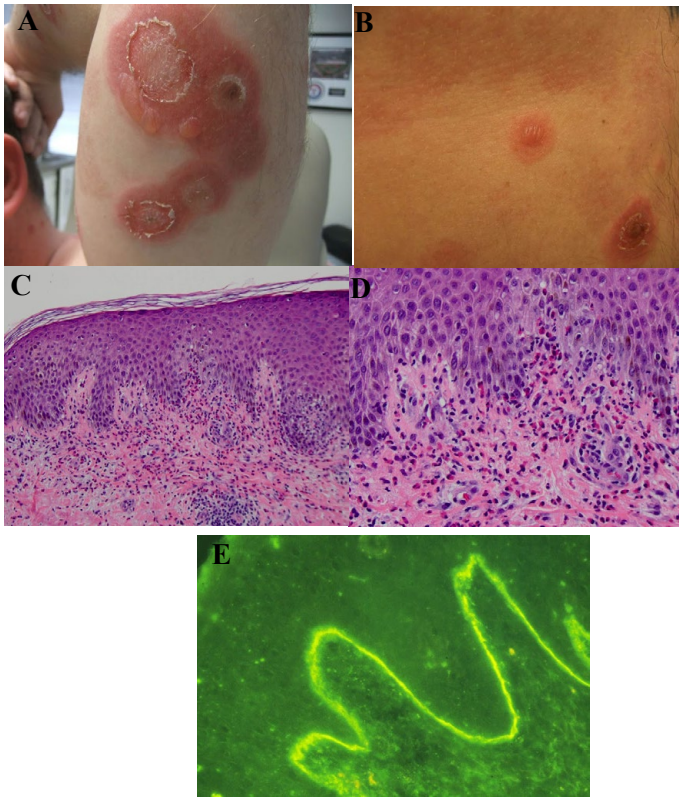

Figure 4. Bullous pemphigoid.A and B: Tense bullae overlying urticarial plaques on the trunk and extremities. C (200x) and D (400x): Biopsy of the urticarial plaques demonstrates eosinophilicspongiosis with numerous eosinophils lining the dermoepidermal junction. Eosinophilic exocytosis is also present. E (400x): DIF demonstrates linear IgG; linear C3 was also present. ELISA for IgG against BP180 was also confirmatory in this case.

immunoreactants including IgM, IgA, and/or IgE may also be seen. Given its role as a reference test, it is difficult to assess the true sensitivity of DIF, but numbers ranging from 82-96% have been reported [43-45]. Given that DIF does not identify antigenic targets, this test does not differentiate between BP and other AIBDs with linear staining at the DEJ such as mucous membrane pemphigoid (MMP) or epidermolysis bullosa acquisita (EBA).

IIF on salt split skin (SSS) allows improved localization of the target antigen and may be used to confirm the diagnosis of BP. IIF demonstrates localization of autoantibodies to the epidermal side of the salt split, although some cases demonstrate immunofluorescence on the dermal side as well. Sensitivities are comparable to those of ELISA and slightly lower than those reported for DIF, ranging from 81-96%. (39, 41) IIF positivity has been found to correlate more strongly with ELISA for anti-BP230 compared to that for anti-BP180 [41,43,46]. This may explain why IIF titers do not correlate well with disease activity (see below) [40].

Commercial availability of ELISA for IgG autoantibodies against BP180 and BP230 has popularized the use of this assay for the diagnosis of BP and has allowed for a standardized comparison of sensitivities and specificities. ELISA for anti-BP180 evaluates for the presence of antibodies against the NC16a region of BP180. Reported sensitivity ranges from 79-100% with a specificity above 95% [1,39,41,46,47]. Of note, reported sensitivities may be inflated by the requirement of DIF positivity for inclusion in comparison studies. Studies including DIF-negative cases of BP have reported sensitivities near 72% [43]. Additionally, the sensitivity of ELISA for anti-BP180 may be limited by exclusion of targets outside the NC16a domain of the target antigen. Fairley *et al.* found that 4 of 51 patients with BP had sera that reacted with areas of BP180 outside of the NC16a domain by immunoblot [48]. However, immunoblot is not recommended for the diagnosis of BP given that it frequently detects nonpathogenic autoantibodies directed against antigens outside of the NC16a domain.(49) ELISA titers for anti-BP180 correlate with disease response [39,50-55]. and, when high initially, predict relapse following treatment cessation [56].

ELISA for IgG against BP230 demonstrates lower sensitivities for diagnosis, ranging from 57-61% [41,43,46]. When combined with ELISA for anti-BP180, it does offer a modest increase in sensitivity (8-10%) for the diagnosis of BP [41,42,46]. Due to a high rate of false negatives, ELISA for anti-BP230 is not preferred as an initial diagnostic screening test. However, following the use of ELISA for anti-BP180 as a diagnostic screen, ELISA for anti-BP230 may be evaluated in patients seronegative for antibodies against BP180. Additionally, ELISA for anti-BP230 may also be selected in patients with mucosal disease. Unlike ELISA titers for IgG against BP180, titers for IgG against BP230 do not correlate with disease course [39,50-55].

MMP (also referred to as cicatricial pemphigoid, CP) is an AIBD characterized by autoantibodies against BP230, BP180, laminin-332 (laminin 5), integrin β4, or type VII collagen (COL7). Clinical manifestations include mucosal erosions with scarring, with or without cutaneous lesions [57,58]. The oral and ocular mucosae are most commonly involved; other sites include the nasopharynx, oropharynx, larynx and anogenital regions [57,59,60]. Histology is non-specific, revealing a subepidermal blister with fibrosis, pauci-inflammatory or with chronic inflammation [57]. Sera from patients with MMP may target one or several autoantigens [57,58]. The most commonly identified autoantigens are BP180 and laminin 5 [58,60].

Linear DIF for IgG, IgA, IgM, and/or C3 at the DEJ in the appropriate clinical context is the most sensitive method for diagnosis, but is not specific [57,58,61]. Examination at high power for the n-serration pattern can distinguish MMP from possible epidermolysis bullosa acquisita (EBA) or bullous systemic lupus erythematosus (BSLE) [59,62]. ELISA may be useful to identify the specific autoantigen(s), confirm the diagnosis, or subtype the disease. ELISA for identification of anti-laminin 5 MMP (antiepiligrin CP, AECP) is important due to the increased risk of visceral malignancy and has been demonstrated to be both sensitive and specific for this diagnosis [63-67]. Reactivity to the C-terminal domain of BP180 is more common in MMP than other AIBDs and may help distinguish MMP from BP [60,68-71]. Yasukochi *et al.* demonstrated that ELISA for IgG against the C-terminal of BP180 has a high specificity but low sensitivity: 39% positive in non-anti-laminin 5 MMP, 17% positive in BP, and 9% positive in anti-laminin 5 MMP [60].

IIF has minimal utility in diagnosing MMP as it is neither sensitive nor specific [57,58,67]. When positive, sera from patients with MMP may label the epidermal side, dermal side, or both sides of the salt split, reflecting the locations of the varied target antigens [57,58].

Gestational pemphigoid (GP) is similar in pathogenesis to BP, with autoantibodies against BP180, particularly the NC16a region. GP presents with pruritic urticarial plaques that evolve into subepidermal blisters [72,73]. GP occurs in the post-partum period or the second or third trimesters of pregnancy [74]. Histopathology classically demonstrates a subepidermal blister with eosinophils, but these findings are highly nonspecific [75].

Several studies have supported the use of ELISA for IgG against BP180 (NC16a) as a sensitive and specific means to diagnose GP [76,77]. Powell *et al.* reported a sensitivity and specificity of 96% [77]. In a small study comparing sensitivity of IIF and ELISA, 4 of 4 patients with GP tested positive by ELISA while only 2 of 4 were seropositive by IIF [76]. Sitaru *et al.* also demonstrated a high sensitivity and specificity of this assay: 84% and 99%, respectively [78]. Even with a higher threshold for seropositivity, Tani *et al.* reported a sensitivity of 92% [75]. Given its accuracy, a benefit of ELISA is its ability to noninvasively differentiate GP from polymorphic eruption of pregnancy (PEP, previously known as pruritic urticarial plaques and papules of pregnancy), which are often considered in the same clinical differential diagnosis.

The target antigen in EBA is COL7, an important structural component of the sublamina densa [79]. Clinical manifestations include skin fragility, blisters, erosions, scars, milia and nail loss. Two major clinical subtypes of EBA have been described, including a mechanobullous type which presents with skin fragility and scarring over extensor surfaces and sites of trauma (Figure 5), and an inflammatory type that presents with blisters over non-traumatized areas [80,81]. As the clinical presentation of the inflammatory subtype overlaps with a range of immunobullous disorders including BP, MMP, and LABD, immunofluorescence testing is necessary for accurate diagnosis [82].

DIF has been shown be highly sensitive and specific for EBA when evaluated for the pattern of immunodeposition. A characteristic u-serrated fluorescence pattern has been described that is both sensitive and specific for EBA and BSLE, both of which feature autoimmunity towards COL7A [62,83]. Using this technique, Vodegel *et al.* were able to detect 26 of 26 patients with EBA [62]. These findings have been reproduced with a sensitivity of 89% and a specificity over 97% reported [84].

ELISA for anti-COL7 has been shown to be a highly specific (above 95%) but variably sensitive test for the diagnosis of EBA. Detection of autoantibodies by ELISA has been correlated with detection by IIF in several studies, with a reported sensitivity of over 80% in IIF-positive cases. However, in IIF-negative cases, ELISA was only positive in 23% of cases [85,86]. Other studies have provided corroborating evidence for the diagnostic utility of ELISA for anti-COL7, with sensitivities above 90% and strong correlation with disease course and severity [87-91]. Of note, low sensitivities, close to 20%, have also been reported [92]. Immunoblot appears to be the gold standard for diagnosis, with sensitivity and specificity approaching 100% [86,92].

IIF is utilized to differentiate EBA from BP since DIF and histology are usually indistinguishable in these two entities. IIF localizes autoantibodies to the dermal side of SSS in EBA. However, up to 50% of cases are negative for autoantibodies by IIF [85]. The sensitivities reported by most studies range from 50-100%[13,86,87,91,92].

IGA-mediated bullous dermatoses

Dermatitis herpetiformis (DH) presents with symmetric, small, clustered vesicles located on the elbows, knees and buttocks. Due to intense pruritus, intact vesicles are encoutered less often than small erosions and excoriations. As a cutaneous manifestation of a gluten-sensitive enteropathy, target antigens are epidermal transglutaminase (eTG) and tissue transglutaminase (tTG).

DIF and ELISA for anti-eTG IgA autoantibodies are the most accurate tests for diagnosis. Granular deposition of IgA in the dermal papillae as observed by DIF (Figure 6) is 92-100% sensitive for DH, but is less specific than ELISA for anti-eTG given the variability of immmunofluorescence patterns [93]. Continuous granular IgA along the dermoepiermal junction has been identified as the predominant staining pattern in up to 60% of patients [94,95]. ELISA for anti-eTG is 90-100% sensitive and nears 100% specificity if the patient undergoing evaluation is consuming gluten [96]. However, sensitivity drops to 50% if a patient's diet is gluten-free. ELISA for anti-tTG is 79-100% sensitive, but not specific for DH given that patients with Celiac disease also produce this autoantibody [97]. Similar to eTG, the sensitivity decreases, to approximately 20%, if a patient's diet is gluten-free [98]. A study of nine DH patients found a significant correlation between both anti-eTG and anti-tTG titers and the degree of enteropathy [99]. IIF on monkey esophagus for circulating anti-endomysial

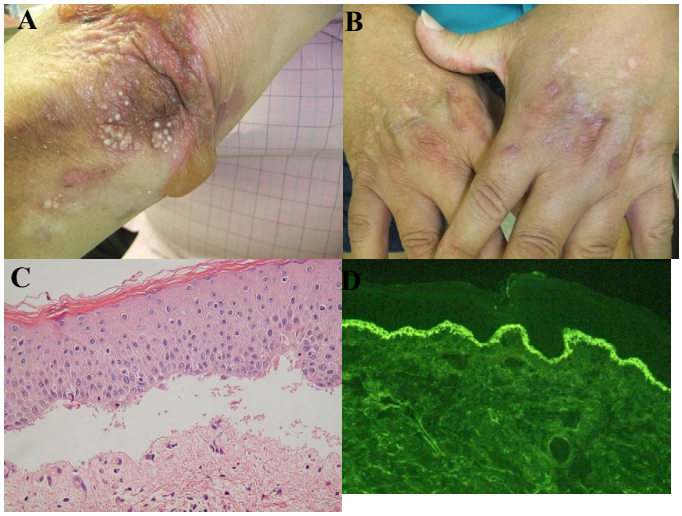

Figure 5. Epidermolysisbullosaacquisita. A and B: Bullae with scarring and milia distributed over the elbows and dorsal hands, sites prone to trauma, are characteristic. C (400x): A cell-poor subepidermal blister is present. D (100x): DIF demonstrates linear IgG, indistinguishable from that of bullous pemphigoid.

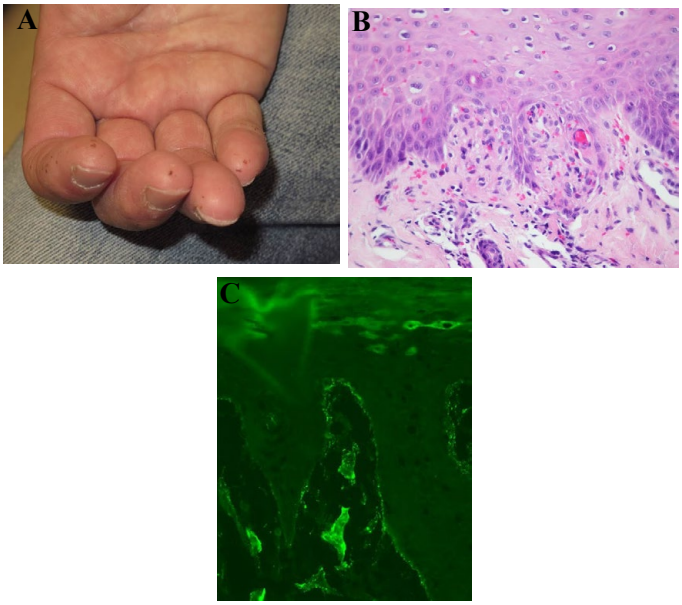

Figure 6. Dermatitis herpetiformis. A: A petechial eruption on volar surfaces is an uncommon and unique finding. B (400x): Biopsy demonstrates rare neutrophils and subtle karryorhexiswithin the dermal papillae. C (200x): DIF demonstrates granular IgA deposits. ELISAs for anti-tTG and anti-eTG IgA autoantibodies were also positive.

antibodies is between 60-90% sensitive, but is not specific for DH given that these autoantibodies are also present in Celiac disease. IIF for IgA autoantibodies against basement mebrane zone antigens is always negative in DH [93,97]. Common histologic findings in DH are small subepidermal vesicles with neutrophilic infiltrate forming microabscesses within dermal papillae. Eosinophils may be rare or numerous. However, these classic findings are relatively insensitive given that 25-54% of cases demonstrate nonclassic findings such as predominantly perivascular lymphocytic infiltrates, minimal dermal papillary inflammation, and absence of neutrophils. Although H&E has poor sensitivity, the specificity of classic findings is 95% [95,100,101].

Linear IgA bullous disease (LABD) presents with tense blisters in a linearly or annular arrangement ("string of pearls," Figure 7). There are two subgroups of LABD, lamina lucida-type and sublamina densa-type. The target antigens in the lamina lucida-type are the 97 kDA ectodomain of BP180 (LABD97) or the 120 kDa antigen termed LAD-1, also located on BP180 [102,103]. The target antigen of the sublamina densa-type has been reported to be COL7 by several small studies [104].

DIF is the best method for the diagnosis of LABD, showing linear IgA deposition at the DEJ in 100% of patients. Approximately 50% of cases will also demonstrate linear junctional staining for other reactants including IgG, IgM, C3 and fibrinogen [105]. IIF in an insensitive (15-36%) diagnostic test [13,105]. Csorba *et al.* developed an ELISA for the detection of IgA autoantibodies against BP180. Among 30 patients with LABD, the study authors found a sensitivity of 83% and specificity of 100% with this assay [106]. In a 2015 study Tsuchisaka, *et al.* developed an ELISA for IgA against COL7 and found that 8 of 12 sera from patients with sublamina-densa type of LABD reacted while all 16 control sera were negative [104].

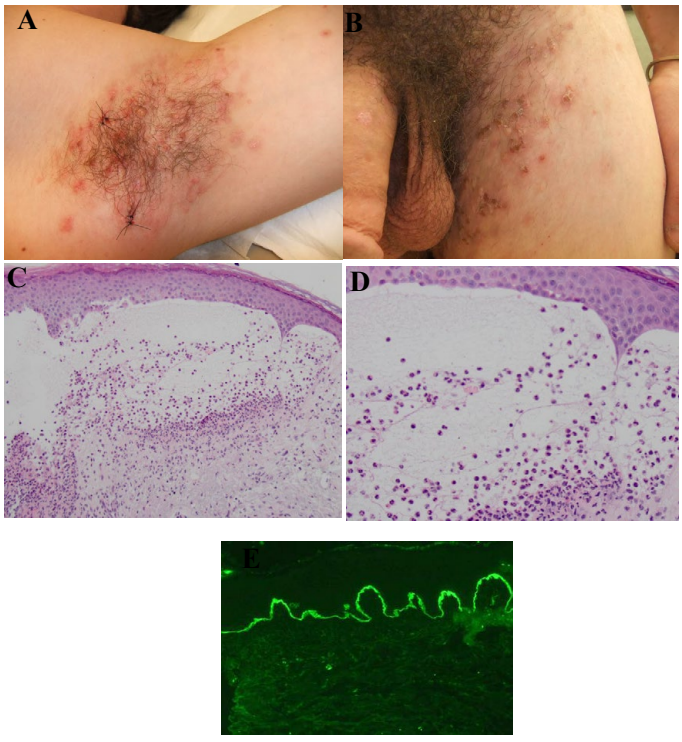

Figure 7. Linear IgA bullous dermatosis. A and B: Teenager with blisters in the axillae and genitocrural area. Perioral lesions were also present. C (100x) and D (400x): Subepidermal blister with abundant neutrophils, a nonspecific pattern. E (40x): DIF demonstrates linear IgA at the dermoepidermal junction.

Newer methods

Several new diagnostic modalities are now available allowing for efficient and accurate testing. These new immunoasssays include the Biochip mosaic-based IIF, automated DIF, and DNA microarray scanner. The Biochip mosaic-based IIF consolidates a multiple step diagnostic pathway into a single incubation, allowing for screening and testing of multiple target antigen-specific substrates of BP, PF and PV at once. Six test substrates (monkey esophagus, primate salt-split skin, antigen dots of tetrameric BP180, and BP230, Dsg1 and Dsg3-transfected HEK293 cells) are plated onto a glass slide. Following incubation with the patient serum and detection antibody, the case is ready for review in one hour. The sensitivity and specificity for the diagnosis of PV, PF, and BP are above 90% with this method. Sensitivity for individual IgG autoantibodies are 90% for anti-Dsg1, 99% for anti-Dsg3, 100% for anti-BP180 and 54% for anti-BP230 [107,108].

Automated DIF allows for quicker and less labor-intensive staining than conventional manual DIF. Compared to the standard manual procedure, automated DIF creates more intense IF signals and less background staining [109]. Euroimmun, based in New Jersey, USA, manufactures the Biochip mosaic IIF and automated DIF tests.

A DNA microarray scanner can be used in place of standard epifluorescent microscopy as a digital fluorescence microscope to evaluate multiple antibodies at one time. It is more sensitive and efficient than standard fluorescence microscopy, but more expensive. Frozen sections of skin biopsies are incubated with cyanine-labeled antibodies and then scanned using a machine originally created to analyze gene expression, allowing for review of the entire specimen at once [110].

Conclusion and summary

Although DIF, in conjunction with routine histology, is most frequently used for the diagnosis of AIBDs, several more recently applied methods such as ELISA are useful and in some cases, more accurate. ELISA for anti-Dsg3 and anti-Dsg1 is the most accurate diagnostic method for PV and PF, and anti-Dsg1 titers correlate with the course of cutaneous disease [1-3]. For the diagnosis of mucosal-only PV, ELISA for anti-dsg3, in combination with Tzanck smear to identify acantholysis, is highly accurate and noninvasive [21]. Distinction of DIP from idiopathic PV or PF most often requires clinical correlation; immunohistochemistry for 32-2B is useful but not widely available [23]. The diagnostic methods of choice for PNP are immunoblot (western blot) for IgG against envoplakin and/or periplakin and IIF on rat bladder epithelium [24-26]. DIF is the most accurate method for the diagnosis of IgA pemphigus and allows distinction from Sneddon-Wilkinson disease [32-34].

For bullous pemphigoid, the most sensitive diagnostic test is still DIF [43-45]. However, ELISA for IgG against BP180 is cost-effective, noninvasive, and highly accurate, making it useful as a screening test [1,6,39,41,46,47]. If negative, ELISA for IgG against BP230 should be evaluated [41,42,46]. Additionally, ELISA for anti-BP180 is the best test for assessing disease course in BP [39,50-55]. For CP, DIF is the best test currently available for diagnosis, but is still highly nonspecific [57,58,61]. Clinicopathologic correlation is required for accurate diagnosis of CP, and ELISA for anti-laminin 5 should be evaluated to exclude the diagnosis of AECP [63-67]. Similar to BP, DIF and ELISA for anti-BP180 are the most accurate diagnostic tests for GP, and ELISA serves as a cost-effective and noninvasive screen in patients for whom the clinical differential diagnosis includes PEP [75-78]. Immunoblot

for IgG against COL7 is the gold standard for EBA [86,92]; ELISA for anti-COL7 correlates well with disease activity but is variably sensitive for diagnosis [85-92]. While DIF alone is nonspecific, evaluation for the u-serration pattern is both sensitive and specific for EBA [62,83,84].

In DH, DIF and ELISA for IgA against eTG are the most accurate tests; if either or both of these tests are negative in a patient who consumes gluten, the diagnosis of DH can be confidently excluded [93,96]. For LABD, DIF is still the best diagnostic test [105].

Among newer methods, the Biochip mosaic IIF is a highly accurate method to screen for multiple AIBDs at once, is less expensive and quicker than ELISA, and is available to practitioners. Results can be confirmed by ELISA to complete a cost-effective and noninvasive multistep diagnostic algorithm [107,108].

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