Immunodermatology Guidelines

Fleming Dermatopathology, 2010

Bullous pemphigoid

Direct immunofluorescence

Pattern, Sensitivity: Linear IgG at the BMZ in 70-90% of cases; linear C3 in 90-100%.

Specificity: EBA and cicatricial pemphigoid produce identical findings.

Substrate: Normal perilesional skin

Indirect immunofluorescence

Conjugate: IgG

Pattern, Substrate: Linear staining on epidermal side of salt-split human skin

Sensitivity: 70%

Specificity: Identical findings in cicatricial pemphigoid and EBA

ELISA

Sensitivity: 90%

Specificity: 98%

Cicatricial pemphigoid (mucous membrane pemphigoid)

Direct immunofluorescence

Pattern, Sensitivity: Linear C3 at the BMZ in 50% of cases; linear IgG in 25%; linear IgA in 20%.

Specificity: Identical findings in BP and EBA

Substrate: Normal perilesional skin or mucosa

Indirect immunofluorescence

Conjugate: IgG

Pattern, Substrate: The roof of salt-split skin is stained if caused by anti-BPAg2 Ab and the floor if caused by anti-laminin-5 Ab

Sensitivity: Only 20%

Specificity: BP produces the same findings in this assay as mucous membrane pemphigoid caused by anti-BPAg2 Ab, and EBA produces the same findings as mucous membrane pemphigoid caused by anti-laminin-5 Ab. Other diseases that produce the latter pattern (linear band on floor of salt-split skin) include bullous SLE, p105 pemphigoid, and p200 pemphigoid.

ELISA

Sensitivity: The antibodies to BPAg2 that occur in this disease are usually directed against an epitope different from the immunodominant epitope in BP (NC16A). Commercially available pemphigoid ELISA's use a NC16A target, and therefore should be negative in CP. However, sometimes they are positive. There seems to be no literature addressing the frequency of this occurrence.

Specificity: As mentioned, pemphigoid ELISA's were designed for BP.

Western Blot

Abs against BPAg2 (180kd), BPAg1 (230kd), epiligrin (part of laminin-5), and/or $\alpha 6\beta 4$ integrin subunits.

Pemphigoid gestationis

Direct immunofluorescence

Pattern, Sensitivity: Linear C3 at BMZ, in approximately 100% of cases; linear IgG in 25-30%.

Specificity: Similar findings in other forms of pemphigoid

Substrate: Perilesional skin

Indirect immunofluorescence (complement fixing assay)

Conjugate: C3

Pattern, Substrate: Salt-split normal human skin

Sensitivity: 93%

Specificity: Other forms of pemphigoid produce similar results

ELISA

Sensitivity: About 90%. This disease is caused by antibodies against the same NC16A epitope on BPAg2 that is immunodominant in BP, and therefore the BPAg2 ELISA performs similarly in pemphigoid gestationis and BP.

Specificity: 98%

Pemphigus vulgaris

Direct immunofluorescence

Pattern, Sensitivity: Staining of epidermal intercellular spaces with C3, IgG, and sometimes IgM and IgA. Overall sensitivity is 90-100%.

Specificity: All forms of pemphigus produce the same pattern, though there is some tendency for staining to be more superficial within the epidermis in PF than in PV.

Substrate: Intact perilesional skin

Indirect immunofluorescence

Conjugate: IgG

Pattern, Substrate: Staining of intercellular spaces in monkey esophagus

Sensitivity: About 80%

Specificity: All forms of pemphigus produce the same pattern

ELISA

Sensitivity: In untreated PV, the sensitivity of Dsg3 and Dsg1 ELISA's are approximately 100% and 85%, respectively. Anti-Dsg3 titers are higher than anti-Dsg1 titers in PV. Presence of Dsg1 correlates with mucosal disease. Anti-Dsg titers correlate with disease activity. For unexplained reasons, Dsg1 titers correlate much better with disease activity than Dsg3 titers.

Specificity: Approximately 98%

Pemphigus foliaceous

Direct immunofluorescence

Pattern, Sensitivity: Similar to PV, though with a tendency for more superficial staining

Specificity: Similar to PV

Substrate: Similar to PV

Indirect immunofluorescence

Conjugate: IgG

Pattern, Substrate: ICS staining on monkey esophagus

Sensitivity: 70%

Specificity: Same in other forms of pemphigus

ELISA

Sensitivity: In untreated patients, the sensitivity of anti-Dsg1 ELISA is approximately 100%. Anti-Dsg3 is usually negative in PF.

Specificity: Approximtely 98%

Paraneoplastic pemphigus

Direct immunofluorescence

Pattern, Sensitivity: IgG and C3 in the epidermal intercellular spaces in almost 100% of cases. Occasionally ICS staining is observed only in adnexae. Linear C3 and/or IgG along the BMZ in about 40% of cases.

Specificity: ICS staining occurs in other forms of pemphigus, but the combination of ICS and BMZ staining is specific for PNP.

Substrate: Intact perilesional skin

Indirect immunofluorescence

Conjugate: IgG

ICS staining on rat bladder is relatively specific for PNP (because it is caused by Abs to plakins), and has a sensitivity of 80% and a specificity of about 83%.

ICS staining on monkey esophagus has a sensitivity of about 85%. Because this is caused by Ab to desmogleins, it does not distinguish PNP from other forms of pemphigus.

Roof of salt-split skin is stained if Abs to BPAg2 are present. This pattern overlaps with pemphigoid.

ELISA

Sensitivity: The Dsg1 and Dsg3 ELISAs designed for PV can be positive in PNP. However, there does not appear to be any published information on the frequency with which this occurs. Abs to Dsg1 and, especially, Dsg3 do occur in PNP (along with Abs to the plakins), but the immunodominant epitopes are different in PV and PNP.

Specificity: Antibodies to desmogleins occur in other forms of pemphigus. Recently, an ELISA was developed that detects antibodies to plakins. This is specific for PNP, but it is not commercially available.

Western Blot

Antibodies against desmoplakin I and II, BPAg1, envoplakin, periplakin, plectin, and an unidentified 170-kd protein

Dermatitis herpetiformis

Direct immunofluorescence

Pattern: Granular (occasionally fibrillar) IgA and C3 in the tips of dermal papillae

Sensitivity: 90%. Dapsone therapy does not affect DIF results, but the immunoreactants will gradually disappear in patients on a gluten-free diet.

Specificity: Very high, but probably somewhat operator dependent, as in our experience staining artifacts can occur with IgA conjugate.

Substrate: Intact perilesional skin

Indirect immunofluorescence

Conjugate: IgA

Substrate: Endomysium

Sensitivity: 80%

Specificity: Positive in celiac disease as well as dermatitis herpetiformis

ELISA

Detects anti-transglutaminase Abs

Sensitivity: 90%

Specificity: Lower than IIF; apparently can be positive in other autoimmune disease as well as celiac disease.

Linear IgA disease

Direct immunofluorescence

Pattern, Sensitivity: Linear IgA at the basement membrane zone in 100% cases (since that is how the disease is defined). Weaker staining for IgG, IgM, and/or C3 is also found in about 20% of cases.

Specificity: Some cases of EBA are caused by IgA anti-collagen VII Ab, and these produce a similar pattern on DIF. Linear IgA at the BMZ occurs in 20% of mucous membrane pemphigoid cases. In these diseases the IgA is often accompanied by other immunoreactants at the BMZ, but this can also occur in linear IgA disease, and there is some overlap among the three conditions. Linear or granular IgA can be observed at the BMZ in lupus.

Substrate: Intact perilesional or uninvolved skin

Indirect immunofluorescence

Conjugate: IgA

Pattern, Substrate: Linear staining on roof or floor of salt-split human skin

Sensitivity: 50%

Specificity: Some overlap with pemphigoid, especially mucous membrane pemphigoid, and IgA EBA

ELISA

Not available

Epidermolysis bullosa acquisita

Direct immunofluorescence

Pattern, Sensitivity: Linear IgG at the BMZ in almost 100% of cases; linear C3 in somewhat more than half of cases. IgM and IgA can also sometimes be observed. In IgA-mediated EBA, there is only IgA, not IgG.

Specificity: Linear IgG is observed in the pemphigoids and sometimes in lupus. However, a "u-serrated" pattern of staining has been described as specific for EBA, and different from the "n-serrated" pattern that occurs in other subepidermal blistering diseases with linear BMZ staining.

Substrate: Intact perilesional skin

Indirect immunofluorescence

Conjugate: IgG

Pattern, Substrate: Linear band on the floor of salt-split skin

Sensitivity: 50%

Specificity: The same pattern occurs in antiepiligrin cicatricial pemphigoid, bullous SLE, p105 pemphigoid, and p200 pemphigoid.

ELISA

An ELISA to detect anti-collagen VII Ab has been developed but is not commercially available,

Lupus erythematosus

Direct immunofluorescence

In lupus erythematosus, immunoreactants are deposited as a granular or linear band at the basement membrane zone. The most common immunoreactant is IgM, but any combination of IgM, IgG, IgA, and C3 may occur.

It appears that this pattern results from Abs to multiple BMZ components. One of them is collagen VII, the target Ag in EBA. High titer Abs to collagen VII produce the clinical phenotype of bullous SLE.

When evaluated on nonlesional sun-exposed skin, the "lupus band test" is positive in SLE, and should be negative in purely cutaneous disease. It is positive in about 80% of SLE cases. However, the sensitivity and specificity depend on the how the lupus band test is defined, ie how many immunoreactants must be present for the test to be declared positive. Usually, two are required, and the test defined in this way has a specificity of 60%. Specificity increases with the number of immunoreactants, reaching 99% for five.

When evaluated on nonlesional sun-protected skin, the "lupus band test" is positive in a smaller proportion of cases (about 50-60%), but correlates with markers of disease activity, such as titers of anti-dsDNA Abs and the presence of renal disease. Also, patients with multiple immunoreactants on nonlesional sun-protected skin have more severe disease than those with only IgM.

A lupus band is found in lesional, sun-exposed skin in up to 70-90% of cases, including DLE, SLE, and SCLE.

About 20% of normal, healthy people will have a positive lupus band on sun-exposed skin. However, in this context almost always only a single immunoreactant is observed, and staining is often dim.

Indirect immunofluorescence

Conjugate: IgG

Pattern, Substrate: Linear band on the floor of salt-split skin

Sensitivity: This is positive only in bullous SLE, where it results from Ab to type VII collagen, the same target as in EBA.

Specificity: See EBA, above

Lichen planus

Direct immunofluorescence

Pattern, Sensitivity: Heavy, "shaggy" deposits of fibrin are observed at the BMZ, especially in oral lesions of lichen planus. IgM, and sometimes other immunoreactants, bind nonspecifically to colloid bodies in the upper dermis. Sensitivity is not reported.

Specificity: Colloid bodies occur in other lichenoid and interface dermatitides, and occasionally in small numbers in normal skin. The "shaggy" BMZ staining is more specific, but of course BMZ staining occurs in many other diseases.

Substrate: Lesional skin

Porphyria cutanea tarda

Direct immunofluorescence

Pattern, Sensitivity: Multiple immunoreactants, especially IgG, C3, and fibrin, are observed in the thickened vessels of the papillary dermis. Multiple immunoreactants, especially IgG, can be observed at the BMZ. These are nonspecific findings, caused by adsorption of immunoreactants to thickened basement membranes.

Specificity: Vascular staining occurs in vasculitis, in various other inflammatory conditions, and occasionally in diabetes and liver disease. BMZ staining occurs in many other diseases.

Substrate: Lesional skin

Vasculitis

Direct immunofluorescence

Pattern, Sensitivity: Studies in the 1970's suggested that DIF is positive only in early lesions (<18 - 24 hours old). However, more recent work shows high sensitivity (92%) in all stages of the disease. In early lesions, there is vascular staining for IgM, C3, and fibrin; IgG and fibrin are found in fully developed lesions; and C3 and fibrin are observed in resolving lesions. In Henoch-Schonlein purpura, IgA is the predominant immunoreactant.

Specificity: The specificity of vascular immunoreactant deposition in the diagnosis of vasculitis has never been formally assessed. However, vascular staining is characteristic of porphyria, has been reported in diabetes and liver disease, and in our experience occurs in various other inflammatory conditions, presumably as a result of nonspecific vascular injury.

Substrate: Lesional skin