AUTOANTIBODY DIAGNOSTICS IN SKIN-BLISTERING DISEASES

By: Dr Jacqueline Gosink, EUROIMMUN AG, Luebeck, Germany

The analysis of circulating autoantibodies has in recent years become a central tenet of the dermatology workup. The molecular identification of various target antigens in autoimmune bullous dermatoses has paved the way for the development of novel autoantibody tests. Analysis of autoantibodies against bullous pemphigoid (BP) antigens (BP180 and BP230), desmoglein 1 (Dsg1) and 3 (Dsg3), envoplakin and other antigens now contributes greatly to the diagnosis and differentiation of autoimmune skin-blistering diseases. In particular, new technologies for the indirect immunofluorescence assay (IFA) and ELISA have enhanced and refined the laboratory analysis.

OVERVIEW OF AUTOIMMUNE BULLOUS DERMATOSES

Autoimmune bullous dermatoses are organ-specific autoimmune diseases that manifest with blisters and erosions on the skin and mucous membranes. They are characterised by autoantibodies against structural proteins of the skin, which are responsible for cell-to-cell contact within the epidermis and adhesion of the epidermis to the dermis (*see figure 1*). The autoantibodies disrupt the adhesive functions, leading to splitting. Blisters form when tissue fluid flows into the splits.

The umbrella group of autoimmune bullous dermatoses is subdivided into four main disease categories based on the target antigens and the localisation of the blisters. These comprise pemphigoid diseases, pemphigus diseases, acquired epidermolysis bullosa, and dermatitis herpetiformis. In pemphigus diseases the blisters form intraepidermally, whereas in all other autoimmune bullous dermatoses they occur subepidermally. The disease categories and the corresponding target antigens are summarised in *table 1*.

PEMPHIGOID DISEASES

Pemphigoid diseases affect the skin and mucous membranes. Different types include BP, pemphigoid gestationis, mucous membrane pemphigoid and linear IgA dermatosis. With 1.3-4.2 new cases per year per 100,000 inhabitants, BP is the most frequent autoimmune bullous disease in Central Europe and North America. The disease mainly affects elderly people, typically manifesting with tense blisters at the integument. However, it can proceed for weeks or months without blisters. Therefore, BP should always be considered in elderly persons with irritating skin disorders that persist for long periods of time.

Pemphigoid diseases are characterised by autoantibodies against hemidesmosomal proteins, that providing contact between the epidermal cells and the basement membrane. The autoantibodies are mainly directed against two glycoproteins with molecular masses of 180 kDa (BP180) and 230 kDa (BP230). BP230 is localised intracellularly in the hemidesmosomal plaque, while BP180 spans the hemidesmosomal membrane. The immunogenic epitopes of BP180 are clustered in the 16th non-collagenous (NC16A) domain of the protein's ectodomain. Autoantibodies targeting this region are exhibited by the majority of patients with BP.

PEMPHIGUS DISEASES

Pemphigus diseases can manifest in the skin and/or the mucous membranes. They are characterised by autoantibodies against desmosomal proteins, in particular Dsg 1 and Dsg 3, responsible for cell-to-cell adhesion within the epidermis. Different disease types include pemphigus vulgaris (PV), pemphigus foliaceus (PF) and IgA pemphigus. Paraneoplastic pemphigus (PNP) described in the next section also belongs to the group of pemphigus diseases.

PV occurs with an incidence of 1 to 16 new cases per million

inhabitants per year, mainly in middle-aged and elderly persons. It initially always affects the oral mucosa, and this manifestation is characterised by IgG antibodies against Dsg 3. Some patients subsequently develop lesions on other mucous membranes and at the integument, particular on parts of the body that are exposed to pressure and friction. These patients produce IgG antibodies against both Dsg1 and Dsg3.

PF has a reported incidence of between three and six new cases per million inhabitants per year. Blisters rarely found in PF. Instead the disease is characterised by scaly crusts, especially in the seborrheic areas (scalp, face, breast and upper back). The mucosa is never affected, hence this form of disease is only associated with antibodies against Dsg1.

IgA pemphigus is a very rare form of pemphigus. It is characterised by IgA antibodies primarily against desmocollin 1 and secondarily against Dsg1 or Dsg3.

PARANEOPLASTIC PEMPHIGUS

Paraneoplastic pemphigus (PNP) is a life-threatening form of pemphigus that can occur in all age groups. The skin lesions are polymorphic, with the clinical picture being a combination of PV symptoms and skin changes resembling erythema multiforme, usually combined with stomatitis or other mucosal changes. The skin changes are accompanied by malignant diseases, in particular haematological malignant tumours and sarcoma, or benign neoplasms such as Castleman tumours and thymoma. PNP is characterised by autoantibodies against desmosomes (Dsg3, desmoplakin I and II, envoplakin, plectin periplakin) and hemidesmosomes (BP239).

Autoantibodies against envoplakin, in particular, are considered a diagnostic marker for PNP. They are highly specific for PNP and occur in less than 1% of patients with other autoimmune bullous dermatoses. In comparison, anti-periplakin antibodies have a specificity of only about 75% for PNP.

ACQUIRED EPIDERMOLYSIS BULLOSA

Acquired epidermolysis bullosa occurs in three variants, namely mechanobullous, generalised inflammatory and a variant affecting predominantly the mucous membranes. Its typical manifestation is blisters and erosions on mechanically irritated skin. The disease is characterised by autoantibodies against type VII collagen.

DERMATITIS HERPETIFORMIS

Dermatitis herpetiformis (DH) or Duhring's disease is a chronic, strongly itching dermatosis with an episodic course that occurs in around 10% of patients with coeliac disease. The cutaneous condition is one of a multitude of possible coeliac disease manifestations that develop as a result of a genetically caused sensitivity to gluten. Other symptom complexes include intestinal complaints, growth disorders, osteoporosis, neuropathies, carditis, pregnancy problems or lymphoma. Coeliac disease can also occur in asymptomatic, silent, latent and potential forms.

Laboratory parameters for diagnosis of coeliac disease and DH include antibodies against tissue transglutaminase (tTG), endomysium, and deamidated gliadin peptides, as well as genetic analysis of HLA-DQ2/DQ8 alleles.

ADVANCED DIAGNOSTIC METHODS

A host of specialised laboratory tests is available to aid the diagnosis of autoimmune bullous dermatoses. Detection of tissue-bound autoantibodies and/or complement deposits in perilesional skin biopsy by direct immunofluorescence remains the gold standard. However, detection of circulating antibodies in the serum using IFA or ELISA has gained ground as a valuable stand-alone diagnostic tool.

Two state-of-the-art technological approaches have proven particularly useful for analysis of circulating autoantibodies in dermatological diseases. Recombinant-cell indirect immunofluorescence test (RC-IFT) is a cutting-edge technology in which transfected human cells expressing recombinant antigens or optimised fragments thereof are employed as antigenic substrates. Used as miniaturised sections alongside classic tissue sections in IFA BIOCHIP Mosaics (see *figure 2*), these substrates allow parallel characterisation and confirmation of antibody specificities. Autoantibodies can also be detected monospecifically using a range of innovative ELISA systems. Several of these are based on designer target antigens, which have been modified by molecular genetic methods to enhance their diagnostic performance, for example by including multiple copies of the immunoreactive domain or removing regions that cause unspecific reactions. Thus, these test systems provide exceptional sensitivity and specificity, often exceeding the capabilities of assays based on the whole native antigens.

ANTI-BP180/-BP230

Circulating autoantibodies in BP are detected by IFA using the substrates oesophagus and salt-split skin. Autoantibodies against the basement membrane show a fine linear staining between the basal layer and the connective tissue (see *figure 3A*). The specificity of the autoantibodies can be characterised using monospecific tests, such as RC-IFT, IFA with antigen dots, or ELISA based on purified recombinant proteins.

Antibodies against BP180 are detected with highest efficiency using test systems based on the designer antigen BP180-NC16A-4X (see *figure 4*). This recombinant antigen contains only the pathogenetically relevant target structure (NC16A), thus avoiding many unspecific reactions. This structure is, moreover, present as a tetramer (4X) to enhance the immunoreactivity. IFA employing this engineered protein in the form of antigen dots demonstrated a sensitivity for BP of 100% and a specificity of 98% in a clinical study with BP patients (n = 42) and control subjects (n = 330). In a separate study with BP patients (n = 118) pemphigoid gestationis patients (n = 20) and controls (n = 723), the anti-BP180-NC16A-4X ELISA exhibited a sensitivity of 90% for BP and a specificity of 98%. Furthermore, the ELISA detected 100% of patients with gestational pemphigoid. The additional analysis of autoantibodies against BP230 using RC-IFT or ELISA supplements the serodiagnosis of BP by a second, independent parameter, thus cementing the diagnosis.

The serum level of anti-BP180 antibodies correlates with the disease activity of BP, and the serum level of anti-BP230 antibodies with the duration of the disease. Thus, these parameters are also suitable for monitoring responses to therapy and for assessing the length of the illness.

ANTI-DSG 1/-DSG 3

Autoantibodies against Dsg1 and Dsg3 can be successfully detected by IFA with the substrate oesophagus. A clear staining of the desmosomes is observed (see *figure 3B*). However, this substrate does not allow ➔

▼TABLE 1: Target antigens in autoimmune bullous dermatoses

Autoimmune dermatoses	Blister location	Target antigens
Pemphigoid diseases	Subepidermal	BP180, BP230
Pemphigus diseases	Intraepidermal	Desmoglein 1 (Dsg1), desmoglein 3 (Dsg3), various plakins, e.g. envoplakin
Acquired epidermolysis bullosa	Subepidermal	Type VII collagen
Dermatitis herpetiformis	Subepidermal	Tissue transglutaminase/endomysium, deamidated gliadin peptides

differentiation between anti-Dsg1 and anti-Dsg3. The antibody specificities to Dsg1 and Dsg3 can be reliably characterised using RC-IFT or ELISA based on purified recombinant proteins, as demonstrated by numerous clinical studies.

In RC-IFT over 98% of patients with PV (n = 65) showed a reaction with the recombinant anti-Dsg3 cells and 52% with the anti-Dsg1 cells. The anti-Dsg1 substrate further detected 90% of individuals with PF (n = 50). Comparable results were obtained with the corresponding ELISAs. Anti-Dsg3 antibodies were detected in 100% of PV test subjects (n = 71), with 46% also demonstrating anti-Dsg1 antibodies. Anti-Dsg1 antibodies were detected in 96% of PF patients (n = 50). The anti-desmoglein RC-IFT and ELISA systems all showed specificities of over 99% with respect to healthy subjects and patients with other skin diseases.

These data demonstrate the usefulness of autoantibody parameters for diagnosing and discriminating pemphigus diseases. Moreover, the levels of anti-Dsg1 and anti-Dsg3 in the serum generally correlate with the severity and activity of the disease and the responses to therapy. Thus, these test parameters are also suitable for long-term monitoring of patients with pemphigus.

ANTI-ENVOPLAKIN

IFA with bladder sections as the substrate is widely used to search for circulating autoantibodies in PNP, in particular against envoplakin and periplakin. For the specific detection of anti-envoplakin antibodies a novel ELISA based on a recombinant N-terminal fragment of envoplakin has been developed. The sensitivity of the test for PNP amounts to 86% with a specificity of 98%. The titer of anti-envoplakin antibodies correlates with the extent of PNP symptoms, with the titer dropping significantly after successful conservative or operative tumour therapy.

ANTI-TYPE VII COLLAGEN

Autoantibodies against type VII collagen can be determined by RC-IFT using transfected cells expressing the type VII collagen NC1 domain. This test provides a sensitivity of 88% and a specificity of 100% for acquired epidermolysis bullosa. An ELISA based on this recombinant antigen is currently under development.

ANTI-TTG/GLIADIN

Autoantibodies against tissue transglutaminase are determined by ELISA (anti-tTG) or IFA (anti-endomysium). IFA is the reference method for this application, but ELISA is usually preferred due to its simplicity, cost-effectiveness and automatability. Both methods provide high sensitivity and specificity. Antibodies against deamidated gliadin peptides have a high relevance when determined using test systems based on a designer trimeric fusion protein GAF-3X (*see figure 5*). In a published study, the analysis of anti-GAF-3X IgA and IgG antibodies identified 91% of DH patients (n = 45). Combined testing of anti-GAF-3X IgA and IgG together with anti-tTG IgA provided the highest serological detection rate in this patient panel.

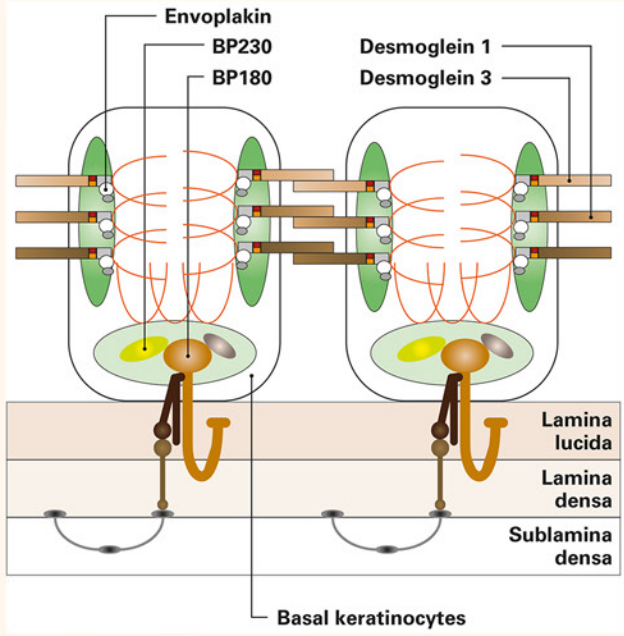
CONCLUSIONS

Accurate diagnosis and discrimination of the different forms of autoimmune bullous dermatosis is crucial for deciding on the most suitable therapeutic regime and for making a prognosis. It is estimated that the highly sensitive and specific assays available today allow a serological diagnosis in around 90% of patients. Given that the incidence of immunobullous disorders is on the increase due to the ageing population and improved diagnostics, for example doubling in Germany over the last ten years, these novel tests will continue to play a crucial role in the dermatology clinic.

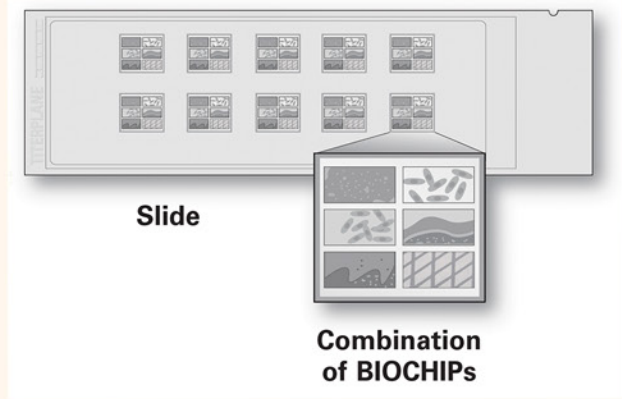
REFERENCES

References available on request (magazine@informa.com)

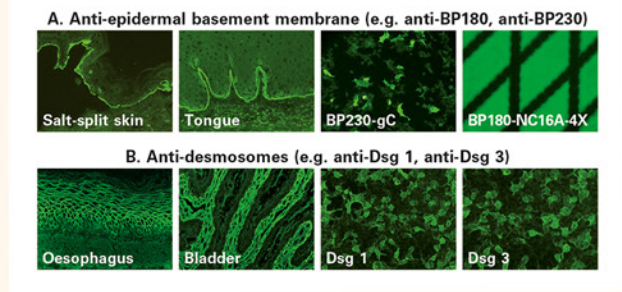
▼FIGURE 1: Structure of the skin



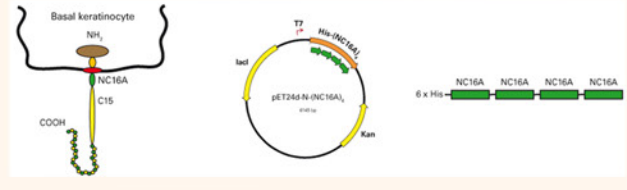
▼FIGURE 2: BIOCHIP Dermatology Mosaic



▼FIGURE 3: IFA patterns in **(A)** BP and **(B)** pemphigus diseases



▼FIGURE 4: BP180-NC16A-4X construct



▼FIGURE 5: GAF-3X construct