Review

Diagnosis and classification of autoimmune blistering diseases

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ABSTRACT

Blistering skin diseases are a group of autoimmune disorders that are characterized by autoantibodies against structural proteins of the epidermis or the dermal–epidermal junction and clinically by blisters and erosions on skin and/or mucous membranes. Since clinical criteria and histopathological characteristics are not sufficient for diagnosis, direct immunofluorescence microscopy of a biopsy specimen or serological tests are needed for exact diagnosis. The differentiation between the various disorders became more important since prognosis as well as different treatment options are nowadays available for the various diseases. Moreover, some bullous diseases may indicate the presence of an underlying malignancy. The detection of serum autoantibodies have been shown to correlate with disease activity and thus may be helpful in deciding treatment options for these patients.

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1. Introduction

Autoimmune blistering dermatoses comprise a heterogeneous group of diseases that are characterized by autoantibodies directed against adhesion molecules of the skin and adjacent mucous membranes.
According to the skin level at which the blister occurs and by the structural proteins that the autoantibodies target, autoimmune blistering diseases can be categorized into different groups i.e. in pemphigus group, autoantibodies target desmosomal proteins that results in loss of cell adherence between keratinocytes, in pemphigoid diseases, hemidesmosomal proteins of the dermo-epidermal junction are targeted, and in dermatitis herpetiformis, autoantibodies bind to epidermal and tissue transglutaminase (Diagram 1).

The diagnosis of autoimmune blistering dermatoses is based on a constellation of clinical and laboratory findings and due to their rarity and heterogeneous clinical features, they often pose a major diagnostic challenge. Diagnosis cannot be based solely on clinical signs and the histopathological findings and requires the detection of tissue bound and circulating autoantibodies which still remains the diagnostic gold standard in the detection of autoantibodies. Furthermore, sensitive and specific serological assays have been developed to allow the detection of serum antibodies which are used as diagnostic tools as well as for disease activity monitoring.

2. Clinical classification

Autoimmune bullous diseases have a broad spectrum of clinical manifestations and a variety of morphological lesions. Based on the level of the skin blister formation — intraepidermal or subepidermal, they can be classified into pemphigus group and pemphigoid group of diseases respectively. Pemphigus is characterized by flaccid vesicles and erosions of the skin and/or mucous membranes, intraepidermal blistering and the production of IgG autoantibodies against the keratinocytes adhesion molecules. The major subtypes are; pemphigus vulgaris and pemphigus foliaceus, and aless frequent form, IgA pemphigus. Pemphigoid diseases are characterized by autoantibodies directed against structural proteins of the dermal–epidermal junction that clinically can manifest with urticarial lesions, tense blisters and erosions which may involve the mucous membranes. Diseases in this group include bullous pemphigoid, dermatitis herpetiformis, mucous membrane pemphigoi, linear Ig A bullous dermatosis, herpes gestations and epidermolysis bullosa acquisita (Table 1, Diagram 1). Paraneoplastic pemphigus, although classified as a type of pemphigus based on its clinical presentation, shows antibodies against both intraepidermal and subepidermal components.

3. Pemphigus group — diseases of intraepidermal loss of adhesion

3.1. Pemphigus vulgaris (PV)

Pemphigus vulgaris ("pemphigus" stems from the Greek word pemphix for "blister") is the most frequent representative of the group of pemphigus diseases with an incidence of 0.1–0.5/100 000 population [1–3]. The mean onset of PV is usually around middle age with an age peak in the fourth and fifth decade of life and a higher prevalence in patients of Jewish or Mediterranean ancestry[1–4]. PV is a chronic autoimmune intraepithelial blistering disease and potentially life-threatening condition. Despite advances in management, the mortality
rate is currently estimated at 5 to 10% [5]. In the majority of the cases (70–90%) PV presents itself with painful and long-persisting erosions of the mucous membranes, especially of the oral (buccal) mucosa [1,4]. Other sites such as the throat, esophagus, conjunctivae, nasal mucosa, vagina, vulva, penis and anus can also be involved [3]. In general, cutaneous involvement follows oral or genital lesions by three and more months [2,6]. On the skin, PV is characterized by widespread fragile flaccid blisters that arise on normal appearing skin and are easily ruptured to evolve into superficial erosions that become crusted (Fig. 1). Favored sites include the head (mainly the scalp), upper trunk and intertriginous areas [3]. PV can involve only the mucosa (mucosal dominant type) or can cause extensive cutaneous and mucosal lesions (mucocutaneous type). In the active phase of PV, both direct Nikolsky sign (slight rubbing of the perilesional skin results in exfoliation of the outermost layer) and indirect Nikolsky sign (also known as Asboe–Hansen sign or bulla spread sign; in which an intact blister can be shifted laterally and enlarged by digital pressure) can be elicited.

3.2. Pemphigus foliaceus (PF)

Pemphigus foliaceus (from the Latin word folium meaning “leaf”) is considered a superficial form of pemphigus. Lesions usually begin with multiple, pruritic, crusted, circumscribed patches in seborrheic areas such as the scalp as well as the face and trunk. In PF, blisters are more superfi cial than PV and erosions are more prominent than blisters. Untreated lesions do not heal and slowly develop into hyperkeratotic scales. Lesions can become confluent and can resemble exfoliative erythroderma. PF, in contrast to PV, does not affect the oral mucosa and has a more favorable prognosis than PV [5]. There are two clinical variants of superficial pemphigus: pemphigus foliaceus (including the endemic form known as fogo selvagem) and pemphigus erythematosus. Fogo selvagem, an endemic variant of PF, predominantly affects young women in endemic regions in Brazil and in North Africa. Fogo selvagem shares the same clinical, histological and immunological profile of classic PF [3].

3.3. Pemphigus erythematosus (PE)

Pemphigus erythematosus (PE) is a localized form of PF, affecting more commonly the elderly, is characterized by superficial erosions, erythema and crusts of the malar area of the face and seborrheic regions. Clinical features may resemble cutaneous lupus erythematosus and in about 80% of cases antinuclear antibodies (ANA) can be detected, without the presence of anti-ds-DNA antibodies.

3.4. IgA pemphigus

IgA pemphigus is an intraepidermal blistering disease, a rare entity among the pemphigus diseases. Clinically it manifests with fragile clear fluid-filled blisters that transform into pustules, owing to the accumulation of neutrophils [7]. Lesions have a distinct tendency to coalesce and there is an associated pruritus. Frequently affected sites for IgA pemphigus are distal trunk and proximal limbs as well as intertriginous sites [7]. Mucosal involvement is rare.

3.5. Paraneoplastic pemphigus (PNP)

Paraneoplastic pemphigus is a rare autoimmune blistering disease that typically manifests with hemorrhagic stomatitis and extensive mucous membrane erosions, associated with obvious or occult neoplasia. The oral mucosa is most severely affected but other mucous membranes such as the nose, pharynx, larynx, esophagus, conjunctivae, and genitals may also be affected [8]. Oral lesions in PNP characteristically involve the vermilion border of the lips [3]. Cutaneous manifestations present after the appearance of the mucous membrane lesions [8–10], and have heterogeneity in their presentation including; polymorphic lesions such as blisters, lichen-planus like skin changes [8] as well as lichenoid palmoplantar exanthema and erythema multiforme-like lesions resembling toxic epidermal necrolysis [1]. Less commonly, psoriasiform and
pustular variants as well as palmoplantar hyperkeratosis have been reported [11,12]. The most frequent neoplasms associated with PNP in two-thirds of the cases are non-Hodgkin's lymphoma and chronic lymphocytic leukemia, followed by Castleman's disease, thymoma, Waldenström macroglobulinemia, sarcoma and carcinomas of the pancreas, colon and prostate [8,12–15]. PNP may precede the manifestation of the malignancy hence, when paraneoplastic pemphigus is suspected clinically, a comprehensive work-up is mandatory. If there is remission of neoplasm, improvement of the lesions is possible.

4. Pemphigoid diseases — diseases of subepidermal loss of adhesion

4.1. Bullous pemphigoid (BP)

Bullous pemphigoid is the most common acquired bullous autoimmune dermatosis with an estimated annual incidence of 6 to 14 new cases per 1 million population and a peak manifestation at the age of 70 years [1,2,16,17,29]. It is primarily a disease of the elderly with an equal incidence in men and women. It is the only autoimmune disease in which incidence increases with age [2,18].

The clinical presentation includes tense blisters with serous or rarely hemorrhagic content which may arise on an erythematous base or on normal skin. The degree of itchiness varies but severe pruritus is often present. Blisters are often preceded over a period of weeks to months, by an intractable pruritic eczema-like or urticarial eruption, so-called nonbullous phase (Fig. 2). Lesions may be localized or generalized with a predilection for the abdomen and flexural aspects of the limbs. Scarring is usually not observed. The indirect Nikolsky sign is always positive whereas the direct Nikolsky sign can be elicited only on perilesional skin [16]. In 10–30% of the cases there is an involvement of the oral mucous membranes, usually in the form of erosions, more rarely as blisters [2,16,18]. Atypical and localized forms of BP have been described with a variety of different denominations, such as palmoplantar pemphigoid, prurigo nodularis-like, prurigo-like, erythrokeratoma-like, echyma gangrenosum-like, intertrigo-like, and toxic epidermal necrolysis-like lesions [19–21].

4.2. Mucous membrane or cicatricial pemphigoid (MMP)

MMP is a chronic inflammatory subepithelial blistering disease with a predilection for the elderly population and an estimated incidence of 1.6 per million population [1,2,16,17,29]. MMP is characterized by recurrent blistering of the mucous membrane but also of the skin. Lesions tend to heal with deforming scars. MMP primarily involves the oral and ocular mucosa, but the nasopharynx, esophagus, larynx, and anogenital mucosa may also be involved [4]. Initially, involvement of the oral mucous membrane presents with desquamative gingivitis, followed by recurrent blistering that heals with scarring. Ocular involvement usually starts unilaterally with conjunctivitis, entropion and trichiasis. The consequences of the disease to the mucous membranes can be severe and devastating; potentially MMP can lead to blindness, airway constriction, dysphagia and urinary and sexual dysfunction [1,4]. Cutaneous findings occur in 20–30% of cases with lesions clinically resembling bullous pemphigoid with generalized blistering [1,4]. MMP can also be localized presenting itself on the scalp and upper trunk which can lead to scarring alopecia and atrophic scars respectively. This is known as the Brunsting-Perry variant of localized BP.

4.3. Linear IgA bullous dermatosis (LAD)

Linear IgA dermatosis is one of the rarer subepidermal blistering diseases, with an estimated incidence of only 0.5 per million in western Europe [25]. LAD has two peaks of onset, in childhood and in adulthood. It represents the most common autoimmune bullous disorder of childhood with age peak of 4–5 years [1,2]. In adulthood it usually manifests between the 40th and 60th year of life [1]. Typical lesions present as pearl necklace-like grouped, centrifugally arranged tense blisters at the edge of inflammatory erythema (Fig. 3). Extensive pruritus usually exists. Annular or multiforme-like lesions can also occur [2]. In adults, LAD lesions comprise of urticarial plaques and papules with vesicles and blisters; the string-of-pearls grouping of blisters is less common [25]. Predilection sites of LAD are the trunk and limbs. Iliosacral location or large body folds can also be affected [1,25]. Mucosal involvement is common (up to 50%) especially the oral mucosa, with erosions and ulcers [1,25]. If there is involvement of the conjunctiva healing can result with scarring formation. Hoarseness may indicate pharyngeal involvement. In most cases LAD starts spontaneously but drug-induced cases are recognized, with vancomycin frequently being the culprit drug [11]. In individual cases an association with lymphoproliferative disorders was found even after remission of the skin disease [12,26–28]. The therapeutic response to diaminodiphenylsulfone (dapsone) is good, in contrast to systemic corticosteroids which is poor.

4.4. Epidermolysis bullosa acquisita (EBA)

EBA is a rare autoimmune subepidermal bullous disease that involves the skin and mucous membranes. EBA can manifest at any age, with a peak incidence between 40 and 60 years of age and an estimated incidence of 0.2 and 0.5 new cases per million inhabitants per year [11,17]. The disease presents with four clinical variants: classical, inflammatory, cicatricial pemphigoid-like, and Brunsting-Perry pemphigoid-like forms [30]. The classical variant is the mecanobullous, noninflammatory form, in which slight trauma elicits blistering and erosions of the skin. Sites of predilection are mechanically irritated acral locations such as the hands and feet, but also the elbows and knees. In contrast to BP, healing of the lesions results with atrophy, milia, scars and pigmentation but distinction can be made with immunofluorescence studies [1,30]. Severe cases are characterized by fibrosis of the hands and feet, scarring alopecia and nail dystrophy even leading to acquired anonychia. The generalized inflammatory form of EBA, clinically resembling BP, is characterized by tense blisters usually on an erythematous, urticarial base. The cicatricial (localized) variant usually presents itself on the mucous membranes and clinically resembles MMP. The fourth variant presents with head and neck involvement, scarring and minimal mucosal disease resembling the Brunsting-Perry variant of cicatricial pemphigoid.

4.5. Dermatitis herpetiformis (DH)

Dermatitis herpetiformis (Duhring's disease) is a chronic, polymorphic, and very pruritic skin disease that can manifest at any age, but often the onset is between the age of 25 and 55 years [1,31,32]. Men are affected almost double as frequently as women. The annual
incidence was 0.4 to 2.6 cases per 100,000 [32–34]. Typically, DH lesions are grouped, 1- to 3-mm large papules, vesicles, (hence the name “herpetiform”), erosions and excoriations. Only rarely blisters are seen. Lesions often heal with lichenification, hypopigmentation or hyperpigmentation. DH is a polymorphic skin disease and usually presents with symmetrical skin involvement at sites of predilection such as the extensor surfaces of the extremities with accentuation of the elbows and knees as well as the gluteal region and back. The oral mucosa is rarely involved. DH is closely related to gluten-sensitive enteropathy and clinically manifest in 5–10% of patients with celiac disease [1,2,31]. Although most of the patients are asymptomatic in 90% endoscopic examination can reveal minimal duodenal involvement and submucosal endoscopic biopsies can reveal lymphocyte infiltration and jejunal atrophy [1,2]. The rapid improvement of pruritus and inflammatory skin lesions after administration of gluten free diet and dapsone are characteristic and dapsone is considered the first line treatment for DH. Unlike, celiac disease that does not respond to dapsone [31].

5. Pemphigoid gestationis (PG)

PG is a rare autoimmune subepidermal bullous dermatosis of pregnancy. This disease shares some similarities with BP on clinical, histological and pathophysiological aspects with most of the patients developing antibodies against hemidesmosomal proteins, BP180 and less frequently BP230 [1,2]. The trigger for the development of autoantibodies remains unknown. The placenta is known to be the main source of paternal antibodies and can thus present an immunologic target during gestation. Incidence is estimated to be 0.5 cases per million people per year. PG typically manifests during late pregnancy, with an abrupt onset of extremely pruritic urticarial papules and blisters on the abdomen and trunk. No increase in fetal or maternal mortality has been demonstrated but a greater prevalence of premature and small-for-gestational-age (SGA) babies was reported with PG. 5–10% of infants may present with transient cutaneous involvement that resolves quickly.

6. Histology

Histopathologically examination is used to interpret the level of adhesion loss i.e. intraepidermal or subepidermal, but also for characterization of the cutaneous inflammatory infiltrate. Tissue biopsy should be obtained from the edge of an active lesion and include some adjacent healthy skin so that the level of the blister can be accurately identified (Table 1, Diagram 1).

7. Pemphigus group — diseases of intraepidermal loss of adhesion

In PV, as in all forms of pemphigus, the basic abnormality is the loss of keratinocyte adhesion, known as acantholysis. In PV there is intraepidermal, suprabasal acantholysis. This process leads to the formation of a cleft within the epidermis, which then enlarges into a bulla [3] (Fig. 4a). The presence of residual basal keratinocytes on the dermo-epidermal junction zone, at the site of the blister’s floor is known as “tombstone effect”. In older blisters, neutrophilic and eosinophilic granulocytes are seen with scant perivascular infiltrate. In early lesions eosinophilic spongiosis may be present, even as the sole histological manifestation.

Light microscopy of lesional biopsies in pemphigus foliaceus reveals subcorneal acantholysis in the upper stratum spinosum or granulosum with slight inflammatory infiltration in the upper dermis. Acantholytic cells can also be seen in other bullous conditions that need to be differentiated, especially chronic impetigo that clinical and histological can resemble PF [3]. In impetigo, lesions are usually fewer, asymmetrically distributed and generally, improve rapidly with antibiotic therapy; circulating or tissue-fixed intercellular antibodies are absent. Histologically in paraneoplastic pemphigus there is suprabasal acantholysis and interface dermatitis with vacuolar degeneration of basal keratinocytes and keratinocyte necrosis (dyskeratotic cells) [3,18]. Often there is a dense lichenoid lymphocytic inflammatory infiltrate.
in the vicinity of the dermoepidermal junction zone. IgA pemphigus is characterized histopathologically by slight acantholysis and an intraepidermal or subcorneal infiltration of neutrophils. Acantholysis in IgA pemphigus is much milder and may be absent. If present, clefts can be localized in the subcorneal region, in subcorneal pustular dermatosis-type IgA pemphigus, whereas in intraepidermal neutrophilic dermatosis-type IgA pemphigus they can be present in the entire or midepidermis. Neutrophilic spongiosis, i.e. epidermal spongiosis with neutrophils in the epidermis can be the only histological finding in IgA pemphigus.

8. Pemphigoid Diseases — diseases of subepidermal loss of adhesion

Histopathologically, hematoxylin and eosin staining of an early bulla in bullous pemphigoid reveals subepidermal blistering with dense inflammatory infiltrate consisting predominantly of eosinophils, but also lymphocytes and neutrophils. Eosinophils are found within the blister as well as in the edematous papillary dermis [32]. In the early nonbullous phase, subepidermal clefts and eosinophilic spongiosis (epidermal spongiosis with eosinophils within the epidermis) can be found [32] (Fig. 4b). A cell poor variant also exists. Mucous membrane pemphigoid is characterized by noninflammatory subepidermal blistering in early lesion. Later, the inflammatory infiltration of the upper dermis similar to that in BP can appear [32]. Linear IgA dermatosis has less characteristic histologic features. There is subepidermal blistering with a variable infiltrate of lymphocytes and neutrophils and even sometimes intrapapillary microabscesses are present [25,32]. Skin biopsy in epidermolysis bullosa acquisita demonstrates subepidermal blisters with dermal inflammatory infiltration of the papillary dermis consisting primarily of neutrophils and mononuclear cells. Bullous pemphigoid-like features can often be found [30]. Dermatitis herpetiformis in the blistering stage is characterized by pathognomonic subepidermal cleft and papillary abscesses consisting predominantly of neutrophils at the tips of dermal papillae accompanied by a perivascular mixed inflammatory infiltrate [32,35] (Fig. 4c).

9. Direct immunofluorescence microscopy

The diagnostic gold standard of autoimmune bullous diseases is the detection of autoantibodies in patients’ epidermis or mucosal epithelium. Autoantibodies production in bullous diseases results as a pathogenic immune response against structural proteins of keratinocytes or of the dermoepidermal basement membrane zone. Tissue-bound autoantibodies are detected by direct immunofluorescence microscopy (DIF) (Table 1, Diagram 1).

In both PV and PF DIF of perilesional skin show intercellular sustenance deposition in a typical net-like pattern (honey comb like) of IgG and/or C3 [36]. In PV an accentuation is seen in the suprabasal epidermis while in PF the accentuation is subcorneal [32]. In about 90% of patients with pemphigus tissue fixed intercellular antibodies are present and are considered uncommon finding in individuals without pemphigus, therefore DIF can be used as an important tool in the diagnosis [3,37]. PE has also granular deposits along BM. In PNP, in addition to the above findings, an abnormal band-like deposits of immunoglobulin and/or complement are detected in the dermal–epidermal junction [32].

In IgA pemphigus depositions of IgA predominate in the cell–cell contact region ([17,32]. DIF in pemphigoid diseases with subepidermal loss of adhesion, such as BP, MMP, PG and EBA, in most cases is found as linear staining of IgG and/or C3 seen at the dermo-epidermal junction zone [32,36]. To further distinguish between these subepidermal bullous diseases, the salt split DIF technique is employed [38]. In general, in this technique, the punch biopsy sample is incubated in a solution of NaCl (1 mol/L) at 4 °C for 24 h, followed by a gentle manual separation of the epidermis from the dermis. On this specimen the DIF is performed. The level of the IgG/C3 deposit at the blister formed serves to distinguish between the diseases: In BP deposits are seen at the blister roof, in EBA at its floor and in MMP in either roof or floor depending on the location of the targeting antigen [39]. In patients with DH, DIF shows granular IgA and C3 deposits along the dermoepidermal junction, and especially in the tips of the papillary dermis (pathognomonic for DH) as well as along dermal blood vessels [34,38,39]. In the perilesional skin of LAD, DIF shows linear deposition of IgA along the basement membrane zone and, in some instances IgG, Ig M, or C3 are also seen [25].

10. Indirect immunofluorescence microscopy

Indirect immunofluorescence microscopy (IIF) serves to detect and quantitate circulating autoantibodies in patients’ serum and in most cases the patterns observed in IIF resemble those in DIF but with lower sensitivity (Table 1). All forms of pemphigus are associated with the presence of circulating autoantibodies against keratinocyte cell-surface antigens (Diagram 1). A variety of substrates are used according to the subtypes. In most cases guinea pig esophagus or monkey esophagus are employed as substrate.
as they are the most sensitive in detecting circulating intraepidermal autoantibodies of PV and PF. Rat bladder epithelium is the most appropriate substrate for screening for antibodies to PNP due to its high expression of plakins [18,32]. In pemphigus diseases IIF reveals circulating IgG autoantibodies in the serum of the patients in a lace-like pattern of fluorescence within the epidermis [13,38]. Circulating intraepidermal antibodies are present in about 80% of patients with active pemphigus disease, and their titre usually correlates with disease activity. [3,38]. Low titres can also be found in patients with antibodies to ABO blood group antigens or with burns, fungal infections, or allergic drug reactions. In IgA pemphigus cultured skin is used as a substrate and circulating intercellular IgA autoantibodies can be detected in only about 50% of cases [32].

In autoimmune bullous dermatoses with subepidermal loss of adhesion, up to 70% of patients present with circulating IgG-anti-BMZ autoantibodies (Diagram 1). Similar to DIF, IIF on salt-split human split-thickness skin (SSST)–induced by incubation in one molar sodium chloride solution–has been identified as the optimal substrate and is used to differentiate between serum autoantibodies that bind to the roof and those that stain the floor of the artificial split, reflecting the different autoantibody specificities [36]. This split at the level of the lamina lucida of the dermo-epidermal junction zone allows the differentiation between different pemphigoid diseases i.e. in BP there is linear fluorescence on the epidermal side of the split, while in EBA linear fluorescence is found on the dermal side. In around 80% to 85% of patients with BP, IIF microscopy reveals circulating IgG autoantibodies [16].

In MMP and LAD mixed fluorescence both on the epidermal as well as on the dermal side has been reported [32]. Low titres of IIF are found in LAD and are more frequently encountered in children (74%) rather than in adults (30%) [25]. Unlike pemphigus diseases, no correlation exists between the autoantibody titer and disease activity [38]. Nowadays further research includes visualization on electron microscopic examination. This procedure is termed direct and indirect immunoelectron microscopy which ultrastructurally localizes in vivo bound IgG autoantibodies (direct immunoEM) or the binding site of circulating IgG autoantibodies (indirect immunoEM) at the basement membrane.

For DH, ELISA-based assays are the preferred tests, but IIF may also be used to demonstrate IgA reactivity directed against the endomysium and are visualized on substrates containing smooth muscle, such as monkey esophagus [32,36,38,39]. The titer of circulating autoantibodies reflects the disease activity [38,39] but the method is expensive, time consuming and not routinely available in all laboratories.

11. Target antigen-specific serological confirmatory tests by ELISA and Western blotting

Enzyme-Linked Immuno Sorbent Assay (ELISA), immunoblotting or immunoprecipitation are recently developed, highly sensitive and specific detection systems of autoantibodies utilizing recombinant autoantigens or keratinocyte extracts from healthy human skin. Unlike immunoblotting, immunoprecipitation is performed with native, rather than denatured, protein and is more sensitive. The latter assays, in addition to serving as serological confirmatory tests (in many cases used as a definitive diagnostic step for autoimmune bullous dermatoses) are used as a valuable tool for immunoserological follow-up. These above named tests are able to detect IgG or IgA autoantibodies against desmoglein 1, desmoglein 3, BP180, BP230, tissue transglutaminase as well as against envoplakin and are available commercially. Immunoprecipitation is more difficult to perform and is generally less available than immunoblotting (Diagram 1). Due to the increasing availability of the assays, the diagnostic spectrum of autoimmune bullous skin diseases has been expanded considerably in recent years and has gained both in sensitivity as well as specificity (Table 1).

Diseases of the pemphigus group are characterized by the presence of autoantibodies against desmosomal structure proteins (Diagram 1). Desmoglein 3 autoantibodies can be detected in PV with exclusive mucosal involvement whereas in the mucocutaneous variant of pemphigus vulgaris both desmoglein 3 and desmoglein 1 autoantibodies can be found [37]. Autoantibody titre usually correlates with disease activity [2,3,16,33] and even though they are not widely available in routine clinical practice, are appropriate for follow-up monitoring. It has been shown that autoantibodies of the IgG4 as well as IgG1 class are indicative of active rather than remittent disease [2]. Western blotting analyses are not useful in the diagnosis of pemphigus based on the fact that most of the epitopes on desmoglein 1 and 3, are conformational. Furthermore, acetylcholine receptors have been reported as autoantigens in pemphigus vulgaris but their pathogenetic relevance is unclear.

PF with its two subsets of endemic (Fogollevaghem) and non-endemic forms is characterized by exclusive involvement of desmoglein 1 autoantibodies, but not against desmoglein 3 or against plakins (Diagram 1). In PE antinuclear antibodies are present [36]. In most cases of PNP IgG autoantibodies against desmoglein 3 and 1 are found but PNP is characterized by the detection of autoantibodies against desmosomal proteins of the plakin family. Envoplakin and periplakin are the most specific target antigens followed by desmoplakin 1 and 2, plectin, and a 170 kDa [17,32] (Diagram 1). IgA pemphigus is characterized by the detection of IgA autoantibodies against desmoglein 1 and desmoglein 3 in the IEN type and against desmocollin 1 in the SPD type [17,32] (Diagram 1). Bullous pemphigoid is characterized by autoantibodies that target 2 hemidesmosomal structure proteins, BP180 and BP230. BP180 is a transmembrane glycoprotein of which the extracellular portion–16th non-collageneous (NC16A)–was identified as the immunodominant region in patients with BP (Diagram 1). Serum levels of anti-BP180 NC16A antibodies can be detected using ELISA and it has been shown that antibodies levels correlate with disease activity in BP patients [40,41]. In several reports, ELISA has been demonstrated to be highly sensitive and specific. It should be taken into consideration that a considerable proportion of older patients with polymorphic pruritic skin diseases also show reactivity of IgG autoantibodies against BP230 and BP180. Hence, the diagnosis of BP is not based solely on the detection of IgG against BP230 and/or BP180 but also on the identification of tissue-bound autoantibodies in DIF. ELISA test systems for the detection of IgG against BP230 are available commercially. ELISAs based on recombinant proteins encoded by BP230 and BP180 have been developed but are not commercially available and offer an investigational tools. In MMP, IgG autoantibodies are found against BP180; as well as, autoantibodies against laminin 332 (＝ laminin 5), α6 and γ4- integrin and occasionally against BP230 (Diagram 1). Autoantibodies can be detected using Western blotting, immunoprecipitation and ELISA. It has been reported that autoantibodies against laminin 332 can be associated with malignancy whereas autoantibodies against α6-integrin with oral involvement and against γ4-integrin with ocular involvement [41]. The autoantigen of linear IgA dermatosis is a 120 kDa large extracellular product ectodomain of the BP180, named LAD–1, while in only 20% of patients sera recognize BP180 NC16A [42,43] (Diagram 1).

EBA is characterized by autoantibodies directed against type VII collagen (Diagram 1). Collagen VII is the main component of the anchoring fibrils of the dermoeipidermal junction zone. Autoantibodies can be detected by immunoblotting with extract of human dermis and in almost all patients IgG reactivity against the NCI domain of type VII collagen is detected [44]. Besides EBA collagen type VII autoantibodies can be detected in bullous systemic lupus erythematosus.

Epidermal transglutaminase has been identified as the autoantigen of DH (Diagram 1). In gluten-sensitive persons after oral gluten exposure, IgA autoantibodies develop in the epithelium of the small intestine against a complex consisting of gluten and tissue transglutaminase. The autoantibodies cross-react with epidermal transglutaminase, which participates as an isoenzyme in keratinocyte differentiation. Immune complexes of IgA and epidermal transglutaminase are found as diagnostic markers in the papillary dermis. ELISA systems are available for detecting IgA reactivity against tissue transglutaminase in DH [45]. Furthermore, ELISA employing gliadin-analogous multimeric fusion proteins are available.