CHAPTER I

GENERAL INTRODUCTION

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Pemphigoid diseases are a heterogeneous group of subepidermal autoimmune blistering diseases that are characterized by autoantibodies against various structural proteins of hemidesmosomes in the epidermal basement membrane zone (EBMZ) of skin and mucosa. The group of pemphigoid diseases includes several subtypes with either predominant cutaneous affection or mucous membrane pemphigoid (MMP). Subtypes of pemphigoid comprise bullous pemphigoid (BP), nonbullous pemphigoid, pemphigoid gestationis (PG), anti-p200 pemphigoid, linear IgA disease (LAD), lichen planus pemphigoides (LPP), Brunsting-Perry cicatricial pemphigoid, anti-plectin pemphigoid and epidermolysis bullosa acquisita (EBA). Mucous membrane pemphigoid encompasses subtypes such as ocular MMP, anti-laminin-332 MMP and localized vulvar pemphigoid. Although pemphigoid diseases share clinical characteristics, the subtypes are heterogeneous in clinical presentation, target antigens, prognosis and treatment (Table I). The different subtypes of pemphigoid diseases cannot be distinguished solely on clinical features or histopathology, therefore detection of in-vivo bound autoantibodies in skin or mucosa or circulating autoantibodies in serum are needed for diagnosis.

**BULLOUS PEMPHIGOID**

BP is the most common blistering disease of the skin and mainly affects elderly, the onset of disease is usually after the age of 70 years. Incidences have been estimated ranging 12-43 per million people per year in Europe. Moreover, the incidence rises incrementally with age, up to 300 per million people per year in elderly people above 80 years. The incidence of BP in Europe has more than doubled in the last decade, which might be related to both the increasing age of the general population, multi-drug use, the availability and quality of diagnostic tests and the recognition of atypical clinical variants. BP rarely occurs in infancy and childhood. BP has been associated with a high morbidity and a considerable 1-year mortality rate ranging from 20% to 40%. Most important risk factors for poor outcome are high age, widespread disease, a low Karnofsky score and high doses of oral corticosteroids.

BP typically presents with severe pruritus, localized or generalized tense blisters (Fig. 1a) and erythema or urticarial plaques (Fig. 1b). Nikolsky’s sign is negative. Predilection sites are the trunk, abdomen and flexural aspects of the extremities. Blister formation may arise on both healthy and erythematous skin, often have a serous exudate and can persist for several days (Fig. 1c). Ruptured blisters leave erosions and crusts, but do not heal with scarring. Mucosal involvement is seen in 10% to 20% of BP patients, mostly the oral mucosa. BP is characterized by the presence of IgG autoantibodies against components of hemidesmosomes in the EBMZ, which maintain the dermo-epidermal integrity. Additionally, deposits of IgA and complement may also be found along the EBMZ. Autoantibodies in BP patients target two hemidesmosomal proteins: BP180 and BP230. Most BP patients have autoantibodies against the extracellular part of the 16th non-collagenous domain (NC16A) of the 180-kDa protein BP180 (immunodominant region). BP230 is a 230-kDa intracellular component of the hemidesmosomal plaque (Fig. 2). However, the pathogenic relevance of autoantibodies against BP230 is not completely understood yet. Binding of autoantibodies to the antigens initiates a complex process of complement activation and an inflammatory response at the EBMZ with various proteases leading to separation of the epidermis and the dermis with subepidermal blister formation. Isoforms of both BP180 and BP230 are also expressed in the central nervous system, which might play a role in the association between BP and neurological diseases. Neurological disorders are present in 30 to 60% of patients with BP, such as dementia, stroke and Parkinson’s disease.
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<td>Bullous pemphigoid</td>
<td>BP180, BP250</td>
<td>Pruritus, urticaria, tense blisters without predominant mucosal involvement</td>
<td>n-serrated IgG ± IgA, C3c epidermal</td>
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<tr>
<td>Nonbullous pemphigoid</td>
<td>BP180, BP250</td>
<td>Pruritus, eczematous lesions, urticarial plaques, erythematous papules or nodules.</td>
<td>n-serrated IgG ± IgA, C3c epidermal</td>
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<tr>
<td>Pemphigoid gestationis</td>
<td>BP180, BP250</td>
<td>In 2nd or 3rd trimester of pregnancy, intense pruritic urticarial rash and tense blisters starting around umbilicus and then spread over the body</td>
<td>n-serrated C3c ± IgG epidermal</td>
</tr>
<tr>
<td>Linear IgA disease</td>
<td>BP180, LAD-1, LABD-97</td>
<td>Tense blisters and erosions in 'string of pearls', without predominant mucosal involvement</td>
<td>n-serrated IgA epidermal</td>
</tr>
<tr>
<td>Epidermolysis bullosa acquisita</td>
<td>Type VII collagen</td>
<td>Mechanobullous variant: acral blistering that heal with scarring and milia. Inflammatory variant: widespread vesicles and blisters, without scarring or milia</td>
<td>u-serrated IgG ± IgA dermal</td>
</tr>
<tr>
<td>Anti-p200 pemphigoid</td>
<td>p200</td>
<td>Pruritus, tense bullae, vesicles, urticarial plaques, predominantly on the extremities and trunk</td>
<td>n-serrated IgG ± C3c dermal</td>
</tr>
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<td>Lichen planus pemphigoides</td>
<td>BP180, BP250</td>
<td>Tense blisters independent of the lichenoid plaques and papules of lichen planus</td>
<td>n-serrated IgG ± C3c epidermal</td>
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<td>Anti-plectin pemphigoid</td>
<td>Plectin</td>
<td>Pruritus, urticaria, tense blisters without predominant mucosal involvement</td>
<td>n-serrated IgG ± C3c epidermal</td>
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<tr>
<td>Brunsting-Perry pemphigoid</td>
<td>BP180, LAD-1</td>
<td>Erosions and blisters confined to the head, face, neck and upper trunk leaving atrophic scars</td>
<td>n-serrated IgG ± C3c epidermal</td>
</tr>
<tr>
<td>Mucous membrane pemphigoid</td>
<td>BP180, BP250</td>
<td>Erosions and blisters of the oral, nasal, eyes, pharyngeal, laryngeal, oesophagus and anogenital mucosa</td>
<td>n-serrated IgG ± IgA, C3c epidermal</td>
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<tr>
<td>Ocular mucous membrane pemphigoid</td>
<td>BP180</td>
<td>Erosions and blisters of the oral, nasal, eyes, pharyngeal, laryngeal, oesophagus and anogenital mucosa</td>
<td>n-serrated IgG ± IgA epidermal</td>
</tr>
<tr>
<td>Localized vulvar pemphigoid</td>
<td>BP180</td>
<td>Erosions and blisters of the oral, nasal, eyes, pharyngeal, laryngeal, oesophagus and anogenital mucosa</td>
<td>n-serrated IgG ± IgA, C3c epidermal</td>
</tr>
<tr>
<td>Anti-laminin-332 mucous membrane pemphigoid</td>
<td>laminin-332</td>
<td>Erosions and blisters of the oral, nasal, eyes, pharyngeal, laryngeal, oesophagus and anogenital mucosa</td>
<td>n-serrated IgG ± C3c dermal</td>
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EBMZ: epidermal basement membrane zone; DIF: direct IF; IIF SSS: indirect IF salt-split-skin; IgG/IgA: immunoglobuline G/A; C3c: complement C3
Fig. 1 Clinical features of bullous pemphigoid: (a) Tense blisters on erythematous skin on predilection sites of BP: the flexural surfaces of the legs and the thighs. Multiple ruptured blisters leave eroded areas. (b) Confluent infiltrated urticarial plaques on the trunk. (c) Tense blisters on inflamed, erythematous skin.
Nonbullous pemphigoid is the subset of patients with immunopathological findings of BP and a pruritic eruption, but no blister development during disease course.\textsuperscript{21,22} Previous studies show approximately 20% of patients do not have skin blistering at time of diagnosis of pemphigoid.\textsuperscript{3,11} Patient with this subtype of pemphigoid are mainly elderly people, presenting with pruritus with nonbullous skin lesions, such as eczematous lesions, urticarial plaques, erythematous papules or nodules, or with scratch marks and no primary skin lesions (Fig. 3).\textsuperscript{22} Pruritus in these patients is frequently misdiagnosed as eczema, xerosis, prurigo nodularis, drug reaction, or scabies, and consequently with a long diagnostic delay.\textsuperscript{22} It is questioned whether patients with nonbullous pemphigoid represent a prodromal phase of bullous pemphigoid, or a distinct nonbullous variant of within the pemphigoid spectrum.\textsuperscript{21,23} In the literature there is no unanimity on how to name the subset of patients with pemphigoid without blistering, such as pruritic nonbullous pemphigoid, pemphigoid nodularis, papular pemphigoid, prurigo-nodularis like pemphigoid, non-bullous BP, prodromal BP, BP incipiens or encompassing cutaneous pemphigoid.\textsuperscript{22,24,25} The exact pathomechanism why these patients do not have skin blistering remains to be elucidated.

**Fig. 2** Schematic overview of the hemidesmosome with structural proteins targeted by autoantibodies in patients with pemphigoid diseases. Adapted from M.F. Jonkman.

**NONBULLOUS PEMPHIGOID**

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Epidermolysis bullosa acquisita (EBA) is a subtype of pemphigoid diseases characterized by autoantibodies against type VII collagen, located in the anchoring fibrils in the EBMZ.\(^\text{26,27}\) EBA is a rare subtype with a frequency of approximately 5-6% of pemphigoid diseases, with a mean age of onset of 50 years.\(^\text{28}\) Two major clinical variants have been recognized of this heterogeneous disease; a classical mechanobullous and an inflammatory phenotype, with a ratio of approximately 1:2.\(^\text{29}\) The classic mechanobullous phenotype mimics hereditary dystrophic epidermolysis bullosa with acral blistering and nail dystrophy, explaining the name of ‘acquired’ epidermolysis bullosa. Lesions may heal with scarring, milia and hypo- or hyperpigmentation. The inflammatory phenotype may resemble various subtypes of pemphigoid diseases, such as bullous or nonbullous pemphigoid, MMP or LAD.\(^\text{28}\) In the BP-like phenotype widespread vesicles and bullae on the skin usually heal without scarring, while the phenotype with predominant mucosal involvement (5-10%) resembling MMP leaves scarring on the mucosa with possible strictures.\(^\text{28}\) Early recognition is necessary to avoid these complications and scarring, while EBA often does not respond well to treatment.

Anti-p200 pemphigoid

Originally described in 1996 by Zillikens and Chen as a novel subtype of pemphigoid with autoantibodies against an unknown 200-kDa component of the EBMZ, the disease was consequently termed anti-p200 pemphigoid.\(^\text{30,31}\) Anti-p200 pemphigoid may mimic BP with pruritus and tense bullae, vesicles and erythematous or urticarial plaques that heal without scarring, predominantly on the extremities and trunk. Mucous membranes are involved in a minority of approximately 10% to 20% of patients.\(^\text{32}\) Patients tend to be younger than in BP and the disease

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Fig. 3 Clinical features of nonbullous pemphigoid. An elderly patient with pruritic, excoriated eczematous lesions on the back (a), and in detail (b).
severity is reported to be less severe compared to BP. An association with psoriasis was seen in about 30% of reported cases, mostly in Japanese patients. Serum samples of 90% of anti-p200 patients appeared to recognize the glycoprotein laminin γ1, mainly the C-terminus region. Since then, it was renamed to laminin γ1 pemphigoid as a new entity in pemphigoid diseases. However, ex vivo and in vivo studies were unable to show pathogenic activity of laminin γ1 and the molecular identity of the target antigen remains to be elucidated.

OTHER PEMPHIGOID DISEASES

Pemphigoid gestationis (PG), previously termed herpes gestationis, is a pregnancy-associated subtype of pemphigoid which manifests in the 2nd or 3rd trimester of pregnancy. PG presents with pruritic urticarial plaques, vesicles and tense blisters often starting around the umbilicus, followed by expansion over the trunk and the distal extremities. In a small percentage PG persists and converts into BP, a transient mild form of BP may be seen in neonates. Recurrence of PG occurs in more than 90% of the additional pregnancies. PG is characterized by autoreactivity to the NC16A domain of BP180. A hypothesis is that PG is caused by an immune response with the formation of autoantibodies against placental BP180, after which a cross-reaction occurs in the skin with BP180.

Linear IgA disease (LAD) is a subtype of pemphigoid diseases characterized by autoantibodies exclusively from the IgA class, targeting different antigens of the EBMZ. LAD affects primarily young children at age of four to five years old and adults in their fifties. Typical clinical features are tense circinate vesicles and blisters on urticarial plaques on the trunk and limbs, configured in a ring as a “crown of jewels” or more serpiginous in a “string of pearls”. Mucous membrane involvement occurs in up to 80% of cases. The main antigen is BP180, with reactivity to the LAD-1 antigen (120-kDa cleaved ectodomain of BP180), and a 97-kDa LAD antigen 1 (LABD97). The sublamina densa-type LAD is also termed IgA EBA, with exclusively IgA autoantibodies against type VII collagen. A rare case of IgA anti-p200 pemphigoid has been described. Vancomycine is the most reported trigger factor of LAD.

Lichen planus pemphigoides is a combination of clinical, histological and immunological features of both lichen planus and BP. In the bullous form of lichen planus blistering is restricted to lichen planus lesions, while in LPP blisters also appear on normal appearing skin. Histopathology shows typical findings of both lichen planus and subepidermal blistering in biopsies of bullous lesions, with compatible findings by DIF. LPP is associated with an autoimmune response directed against the NC16A domain of BP180. A suggested theory is that lichen planus lesions damage the basal keratinocytes and expose the BP180 antigen, leading to a secondary autoimmune response to autoantibodies in the EBMZ.

Anti-plectin pemphigoid is a very rare subtype of pemphigoid diseases characterized by autoantibodies against plectin, a member of the plakin family in the hemidesmosome. All reported patients with anti-plectin autoantibodies demonstrated concomitant antibodies against other pemphigoid antigens, most patients show clinical manifestations resembling BP. Most often reactivity is also seen against BP180, BP230, and/or LAD-1. Because of this reactivity against multiple antigens, the direct pathogenicity and clinical manifestations of anti-plectin antibodies cannot be determined. A possible explanation could be the epitope spreading phenomenon, a secondary autoimmune response to other antigens in hemidesmosomes during a chronic autoimmune process in the EBMZ.

Brunsting-Perry cicatricial pemphigoid is a rare form of localized pemphigoid that clinically presents with erosions and blisters on the skin of the head, neck and shoulder area that
heal with scarring and milia. Mucosal involvement is rarely seen. DIF is necessary for correct diagnosis, the targeted C-terminal domain of BP180 that is located in the lamina densa and might be responsible for the scarring phenotype.

**MUCOUS MEMBRANE PEMPHIGOID**

Mucous membrane pemphigoid (MMP) is the subgroup of pemphigoid diseases which predominantly affects mucous membranes (Table I).48 Previously, the term cicatricial pemphigoid was used synonymously for MMP. At present, the term refers to the rare clinical phenotype of Brunsting-Perry cicatricial pemphigoid.48 The incidence of MMP as a group has been estimated at 1.5-2.0 per million per year in France and Germany. MMP often occurs earlier in life than BP, with age of onset between 60-70 years. Several subtypes are classified based on clinical symptoms and target antigens, such as ocular mucous membrane pemphigoid (OMMP), localized vulvar pemphigoid (LVP) and anti-laminin 332 MMP (anti-LN-332 MMP).1 Autoantibodies are mainly directed against BP180 and often recognize the C-terminal epitopes of BP180.49,50 Various mucosa can be affected, mostly erosions and blistering of the oral mucosa (85%), conjunctiva (65%), and less frequently, the nose (20-40%), oesophagus (5-15%), pharynx (20%), larynx (5-10%) and genitals (20%)(Fig. 4).48 Clinical severity is highly variable in the different subtypes of MMP. Oral complaints in MMP may be limited to gingival lesions resulting in the desquamative gingivitis. Mucosal blisters often rupture rather quickly as a result of mechanical injury. When MMP is limited to the conjunctivae (approximately 20%), the term ocular MMP is used.51 Ocular MMP usually starts unilaterally and can proceed bilaterally, with recurrent inflammation resulting in clinical features of burning, dryness, conjunctivitis and proceeding to scar formation with fornix shortening, symblepharon, and finally blindness.51 Localized vulvar pemphigoid is a rare subtype of pemphigoid with solitary lesions of the skin of vulva and perineum.

**ANTI-LAMININ-332 MUCOUS MEMBRANE PEMPHIGOID**

Anti-laminin 332 MMP (anti-LN-332 MMP) is a rare subtype of MMP that is difficult to distinguish from other forms of MMP with similar clinical features.48,52 However, progressive scar formation is a severe complication of anti-LN-332 MMP, resulting in blindness or upper airway obstruction.55 Therefore, early diagnosis is important due to the aggressive clinical course. Patients have an increased relative risk for malignancy, especially adenocarcinoma.53,54 Anti-LN-332 MMP was previously termed anti-epiligrin cicatricial pemphigoid, or later anti-laminin-5 MMP. In most patients the IgG autoantibodies predominantly target the laminin α3 subunit, although IgG autoantibodies targeting the β3 or γ2 subunits have also been described.48,52

**DIAGNOSIS OF PEMPHIGOID DISEASES**

Subtypes of pemphigoid diseases cannot be differentiated based on clinical features alone due to the heterogeneous presentations.1 Clinical criteria have been established to differentiate typical cases of BP with positive DIF from other subtypes, when three of four clinical criteria are present: age greater than 70 years, absence of atrophic scars, absence of mucosal involvement, and absence of predominant bullous lesions on the neck and head.55,56 However, the criteria
are not applicable for the complete spectrum of pemphigoid, such as nonbullous pemphigoid. Histopathology of a lesional biopsy of a bulla can be helpful in guiding to a diagnosis of BP with typical findings of a subepidermal bulla and characteristic eosinophilic infiltrate. However, histopathology is not sufficient for diagnosis of pemphigoid diseases and may be non-specific in nonbullous pemphigoid, such as the presence of only a superficial eosinophilic infiltrate.\textsuperscript{57} Therefore, diagnosis of pemphigoid diseases is based on a combination of clinical features, and detection of skin bound autoantibodies using direct immunofluorescence microscopy (DIF) and immunoserological tests for detection and subtyping of serum autoantibodies. Immunofluorescence microscopy has become the cornerstone of diagnosis of autoimmune bullous diseases, the technique of visualization of autoantibodies can be applied to both a biopsy specimen and serum.

Fig. 4 Clinical features of mucous membrane pemphigoid. (a) blistering on the oral mucosa. (b) MMP limited to the oral mucosa with desquamative gingivitis. (c) ocular MMP with conjunctivitis and fornix shortening.
 DIRECT IMMUNOFLUORESCENCE MICROSCOPY

Direct immunofluorescence (DIF) microscopy is an important diagnostic tool for detection of autoantibodies deposition in patient skin or mucosa. Pemphigoid diseases are characterized by a linear immunodeposition of mainly IgG, and additional IgA and/or complement C3 along the EBMZ. A perilesional biopsy is recommended, with a reduced background staining when transported in saline. Sensitivities have been reported ranging 82-96% with a very high specificity, although the sensitivity is difficult to assess because of the role as reference standard for diagnosis. The nature of the immunodeposition by DIF can differentiate LAD or IgA EBA with only IgA depositions from other pemphigoid diseases. In the majority of cases the linear immunodeposition along the EBMZ can further be classified by the serration pattern, which enables to differentiate between subtypes of pemphigoid diseases (Table I, Fig. 4). The serrated pattern is based on the localization of the autoantigen in the EBMZ. The u-serrated pattern is pathognomic for pemphigoid disease with autoantibodies against type VII collagen: most commonly EBA and also in bullous systemic lupus erythematosus (SLE). The u-serrated pattern can be explained by the localization of immunodepositions in the upstanding arms of the sublamina densa zone between the rootlets of the basal keratinocytes. In contrast, the n-serrated pattern is found in all other pemphigoid diseases with antibodies against hemidesmosomal components above the lamina densa; most commonly bullous and nonbullous pemphigoid, PG, anti-p200 pemphigoid, MMP, anti-laminin-332 MMP and LAD. With an n-serrated pattern, the immunodepositions are localized above the lamina densa and follow the plasma membrane in the basal cell rootlets. The serration pattern may not be observed in mucosal biopsies, and a additional skin biopsy or thinner cryosections may be needed.

 INDIRECT IMMUNOFLUORESCENCE MICROSCOPY

Indirect immunofluorescence microscopy is used to detect circulating antibodies in serum, by incubating a substrate tissue with patient serum and visualize present autoantibodies. The most commonly used substrate is monkey oesophagus, in pemphigoid diseases a linear deposition along the EBMZ can be observed. Sensitivities have been reported for BP ranging between 60-80% with a high specificity. Using human salt-split skin as a substrate for indirect immunofluorescence improves the sensitivity for the diagnosis of pemphigoid diseases ranging between 70-80%, and specificity above 95%. Incubating normal human skin for 48-72 hours in 1.0M sodium chloride leads to a reproducible split in the lamina lucida. By separating epidermal and dermal located antigens, the technique enables differentiation of subtypes of pemphigoid diseases. Antigens located in the roof of salt-split skin are BP180 and BP230. Consequently, the subtypes of bullous and nonbullous pemphigoid, MMP, PG, and LPP show staining of IgG on the epidermal (roof) side of the split. In contrast, the antigens laminin 332, p200 and type VII collagen are located in the floor of the split, leading to staining on the dermal side in anti-laminin 332 MMP, anti-p200 pemphigoid, EBA, and bSLE. Specificity of salt-split skin is high for all subtypes of pemphigoid diseases, and ranges between 97-100%. In EBA and MMP much lower sensitivities of indirect immunofluorescence have been observed, probably due to lower titers of circulating autoantibodies. Presence of IgA in addition to IgG can be demonstrated and solely IgA deposition can be seen in LAD and IgA EBA, although false-positive results for IgA are more commonly seen.
Immuno-assays

The main serological techniques for diagnosis of pemphigoid diseases are immunoblotting, immunoprecipitation and enzyme-linked immunosorbent assay (ELISA). The techniques are based on the ability to detect and visualize circulating autoreactive IgG that bind to the specific target antigen.

Immunoblotting, also called Western blotting, is a qualitative technique performed with patient serum incubated on a membrane filter containing cultured keratinocyte protein extracts. The skin proteins are separated using gel electrophoresis and fractionized according to molecular mass. The bound IgG is stained and antigens can be identified by the molecular weight. During the process protein complexes are dissolved, such as the hemidesmosomes containing the pemphigoid antigens. Therefore, the advantage of immunoblot is the possibility to test various antigens and the full length molecule is available. A disadvantage is that the native conformation and structure of epitopes are destroyed during this process and may not be recognizable by immunoblotting. The epitope is the part of the antigen that is recognized by the antibody. Epitopes can be either a linear epitope with a continuous stretch of amino acids, or a conformational epitope with stretches of amino acids that are located closely together in the native conformation of the protein, and are contingent upon the structure. For diagnosis of anti-p200 pemphigoid immunoblotting can be performed with a dermal extract, which requires a sophisticated extraction procedure and is only performed in few specialized laboratories.

Immunoprecipitation is a more labor intensive technique than immunoblotting, and therefore not much used for routine diagnostics. The technique enables to identify unknown antigens and more epitopes on the autoantigen than immunoblotting because conformational epitopes remain intact. Nowadays non-radioactive immunoprecipitation is most commonly performed, which is a combination of immunoprecipitation and immunoblotting.

ELISA is a quantitative technique that enables to monitor disease activity based on the antibody titer. In contrast to immunoblotting, ELISA measures the autoantibody response of a single autoantigen. A plastic well plate is coated with recombinantly produced molecules

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**Fig. 5** Direct immunofluorescence microscopy serration pattern analysis. Left: linear u-serrated immunodepositions of IgG along the BMZ, found in epidermolysis bullosa acquisita. Right: linear n-serrated immunodepositions of IgG along the BMZ, found in all other subtypes of pemphigoid diseases.
consisting of parts or the full-length antigen, and containing both linear and conformational epitopes. The coated well is incubated with patients’ serum and when IgG to the specific antigen is present, it will bind and can be visualized with a colorimetric reaction. The color intensity is an indication of the amount of specific IgG in the serum of the patient, which is used for serological disease monitoring. The BP180 NC16A ELISA titers correspond with disease activity, in contrast to BP230 ELISA titers.\(^{70-72}\) ELISAs are commercially available for the pemphigoid antigens BP180, BP230 and type VII collagen. The BP180 ELISA contains a recombinant fragment of NC16A, the immunodominant region of the extracellular domain of BP180. Sensitivities have been reported ranging 70-100% with very high specificities above 95%, depending on the selected patient populations.\(^{63}\) A meta-analysis of 17 studies using various BP180 ELISAs reported an overall sensitivity of 87%.\(^{73}\) The sensitivity of the BP230 ELISA has been reported ranging 59-73%, with also high specificities.\(^{63}\) Detection of IgG autoantibodies against BP180 and BP230 by ELISA or immunoblot has been reported in elderly and elderly with pruritic dermatoses, most commonly anti-BP230 antibodies.\(^{74-77}\) Whether this might be representing a preclinical stage of pemphigoid or non-specific binding of autoantibodies is unclear.\(^{23}\) The previously reported high sensitivity of the ELISA to type VII collagen was found to have a much lower sensitivity of 30-54% in an unselected study population, because approximately half of the patients had a very low undetectable serum titers.\(^{78,79}\) The ELISA values of antibodies against type VII collagen correspond well with disease activity.

**OTHER LABORATORY TECHNIQUES**

In addition to routine diagnostic techniques, more advanced immunofluorescence techniques can be used to identify or further characterize autoantibodies in patients’ skin or serum. In cases in which the serration pattern by DIF in a skin biopsy is unrecognizable, the level of the immunodeposition can be determined by fluorescent overlay antigen mapping (FOAM).\(^{80}\) Using a double staining for the deposited IgG and a topographic reference marker (antibody) of a known antigen, such as BP180 or type VII collagen, overlapping staining patterns can be observed indicating co-localisation. FOAM can be performed using a standard immunofluorescence microscope and software for overlay imaging.

Another indirect immunofluorescence technique is using knock-out skin as a substrate. Using skin of patients with hereditary forms of epidermolysis bullosa completely deficient of type VII collagen (recessive dystrophic epidermolysis bullosa [RDEB], severe generalized) or laminin-332 (junctional epidermolysis bullosa [JEB], severe generalized), enables to differentiate between EBA and anti-laminin-332 MMP.\(^{67}\) EBA with antibodies against type VII collagen will show no staining on type VII collagen-deficient skin, but a contrasting positive linear staining on laminin-332-deficient skin. In anti-laminin-332 MMP the opposite results will be found. Positive staining on both deficient-skin substrates suggest a diagnosis of anti-p200 pemphigoid, which shows similar dermal side staining on salt-split skin.\(^{81}\) The disadvantage of this technique is the required presence of sufficient concentration of circulating autoantibodies, which might be rather low in EBA and MMP and a skin biopsy is needed for serration pattern analysis or FOAM.
TREATMENT AND FOLLOW-UP

BP can have a clinical course that may last from several months to years. The high age of BP patients and the possible presence of co-morbidities can make the treatment management more difficult. Recommended first-line therapy for mild, moderate and severe disease is superpotent topical steroids (clobetasol propionate) 30 to 40 g/day applied daily over the whole body, including blisters, erosions and healthy skin, but sparing the face. Whole body application of superpotent topical corticosteroids is considered to be effective and save and has a lower cumulative dose of corticosteroids and less side-effects compared to oral corticosteroids.

Patients with localized BP can be treated with superpotent topical corticosteroids applied to lesional skin only. Oral corticosteroids prednisone in a dosage of 0.5mg/kg/day are often used in treatment of moderate to severe BP and may be accompanied by adjunctive superpotent topical corticosteroids and/or immunosuppressive agents, such as azathioprine, mycophenolate mofetil, mycophenolic acid or methotrexate. Systemic anti-inflammatory antibiotics (tetracyclines) are commonly used as alternative or adjunctive treatment. In refractory cases of pemphigoid diseases, anti-CD20 monoclonal antibody (rituximab) or alternatively intravenous immunoglobulin may be considered.

BP can last for several years and has the tendency to relapse. Determination of anti-BP180 NC16A IgG antibodies by ELISA appeared to follow disease activity and severity and can also be used to identify patients with a high risk of relapse. Current evidence suggests to continue the initial topical treatment until 15 days after disease control, when no new lesions arise and lesions begin to heal. Treatment should be reduced by a tapering schedule of four months. Tapering of oral corticosteroids is based on clinical course, and when available on serum levels of anti-BP180 NC16A IgG. Doses of oral corticosteroids should be tapered gradually after disease control, based on clinical symptoms.

OUTLINE AND AIM OF THE THESIS

The aim of this thesis was to further elucidate the relatively unknown nonbullous pemphigoid and to facilitate early diagnosis, by investigating the optimal diagnostic strategy of pemphigoid and provide minimal diagnostic criteria. Secondly, we aimed to optimize and support serration pattern analysis for diagnosis of various pemphigoid diseases.

In chapter 2, we revive historical research to bullous diseases from in The Netherlands, to contribute to its understanding today. Chapter 5 provides a review of the literature of cases of nonbullous pemphigoid to further characterize the nonbullous spectrum of the disease, with a focus on the clinical symptoms, the diagnostic process and disease course. In chapter 4, we analyzed the detection of circulating pemphigoid autoantibodies in patients who do not have the disease, or do they? In chapter 5, we compared the diagnostic accuracy of direct and indirect immunofluorescence and of various immuno-assays for diagnosis of pemphigoid in the largest cohort up to now. We aimed to evaluate the optimal diagnostic strategy and compose minimal diagnostic criteria. In chapter 6, we investigated the prevalence of pruritus and pemphigoid in a high-risk population of nursing home residents, to assess whether nonbullous pemphigoid could be identified as an unrecognized cause of pruritus. Chapter 7 describes an image-based online test for serration pattern analysis, to assess the learnability of pattern recognition and
to encourage the use of this diagnostic technique in daily practice for dermatologists and pathologists. In chapter 8, we compared the technical procedures for serration pattern analysis in two laboratories and analyzed the conformity among observers. We sought to determine the technical requirements for implementation in routine diagnostics, and provide a protocol. In chapter 9, we describe the technological development of automated serration pattern analysis and identification of the digital fingerprint of the u-serrated pattern. In chapter 10, we report the use of serration pattern analysis and indirect immunofluorescence knock-out analysis to facilitate the diagnosis of anti-p200 pemphigoid, and describe clinical features in a cases series study of twelve patients. Chapter 11 provides an insight in the current treatment approach of bullous pemphigoid in The Netherlands and United Kingdom.
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