GENERAL DISCUSSION AND FUTURE PERSPECTIVES

JOOST M. MEIJER

Center for Blistering Diseases, Department of Dermatology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
This thesis is focused on the diagnosis and clinical presentations of various subtypes of pemphigoid diseases, encompassing laboratory, translational and clinical studies. The first proposition “BP or not BP, that is the question” comprises several aspects of this thesis regarding the terminology of pemphigoid, classification of subtypes and the minimal diagnostic criteria of bullous and nonbullous pemphigoid.

Knowledge of the history of a disease contributes materially to its understanding. In chapter 2 we followed the history of bullous pemphigoid from the first descriptions of pemphigus, reported patients with blistering diseases in 19th and 20th century theses from Groningen, to more recent hallmarks and developments. Initially, the term pemphigus was used to describe a blister, without knowledge of the etiology. Thereafter, pemphigus developed into a disease entity of a chronic bullous disease, until further differentiation of other bullous diseases became apparent based on histopathology in 1953. The addition of ‘bullous’ to pemphigoid was introduced to differentiate bullous pemphigoid from benign mucous membrane pemphigoid, and already point of debate at its initiation because of the tautology. The introduction of immunofluorescence microscopy was a breakthrough in diagnostic classification, enabling further unraveling and classification of bullous diseases until today. Although a blister is the most characteristic symptom of the disease, more recent insights show a blister is not a prerequisite for diagnosis of pemphigoid diseases. Looking back, without the sophisticated diagnostic techniques of today the clinical spectrum of bullous and nonbullous pemphigoid could not be foreseen at the initial description of bullous pemphigoid.

The heterogeneous clinical spectrum of bullous and nonbullous pemphigoid is exemplified in the terminology used in previous publications, such as papular pemphigoid, pemphigoid nodularis, pruritic nonbullous pemphigoid, cutaneous pemphigoid, erythrodermic pemphigoid, and polymorphic pemphigoid. In chapter 3 we analyzed the clinical and immuno(pathological) features of reported patients with nonbullous pemphigoid in literature by systematic review. In absence of widespread skin blistering on erythematous skin, these variants are considered to be ‘atypical’, but not a rarity and reported comprising 20 to 40% of patients. The most reported skin lesions in nonbullous pemphigoid were erythematous and urticarial plaques, papules and nodules, or eczematous lesions. These skin lesions are frequently present in patients with other pruritic inflammatory dermatoses. Consequently, the difficult clinical recognition led to a long diagnostic delay, of mean 22.6 months. Clinicians may only be ascertained of misdiagnosis retrospectively when skin blisters appear, and the term prodromal bullous pemphigoid is frequently used. Why these patients with nonbullous pemphigoid do not develop blisters has yet to be elucidated. It could be hypothesized that early recognition and immunosuppressive treatment may alter the disease course of possible skin blistering, as most reported cases did not develop skin blistering in follow-up. Although patients may have had absence of blisters for years before the diagnosis is made, this hypothesis cannot be studied prospectively due to ethical considerations. In contrast, cases have been described of initial bullous pemphigoid with a nonbullous flare-up of the disease.

Diagnosis of pemphigoid can be particularly challenging in patients with nonbullous clinical features. The histopathology of the reported cases of nonbullous pemphigoid often
showed non-specific findings and diagnosis was mainly based on DIF, in less than half of the patients IIF SSS was performed. DIF was a more sensitive test (93%) compared to IIF SSS (90%) in these cases, although DIF was often the only performed immunopathological diagnostic test with a possible overestimation of sensitivity. Of interest, more often circulating autoantibodies against BP230 were reported, opposed to the immunodominant BP180 autoantibodies in bullous pemphigoid. These findings support that nonbullous pemphigoid is not merely a stage of bullous pemphigoid, but a clinical variant within the spectrum of pemphigoid. The clear difference in clinical presentation justifies the naming of the subtype of *nonbullous pemphigoid* next to bullous pemphigoid, to support early detection and further research.

**SINGLE TEST DETECTION OF CIRCULATING PEMPHIGOID SPECIFIC AUTOANTIBODIES IN ELDERLY SHOULD BE INTERPRETED WITH CARE. (THIS THESIS)**

Several reports have been published of detection of circulating autoantibodies against BP180 or BP230 by immunoblot, ELISA or indirect immunofluorescence on various substrates in subjects who do not fulfill the available combined criteria of bullous pemphigoid: i.e. in elderly people, elderly people with pruritus, and also in healthy blood donors.\(^{12-15}\) In *chapter 4* we assessed the presence of circulating autoantibodies against BP180 and BP230 in 374 subjects with nonbullous skin disorders who visited our outpatient dermatology clinic, in whom autoimmune bullous diseases were excluded based on negative DIF. In 13.6% of subjects at least one positive immunoserological test was found, mainly the BP180 NC16A and BP230 ELISAs and immunoblot with autoantibodies against BP230. Age above 75 years was a predictive factor for presence of a positive immunoserological test compared to an age below 45 years. However, similar to other studies no association was seen between autoantibody detection and pruritus, nor combined age and presence of pruritus. Possibly the latter was obscured because the reason for consultation of the dermatologist and the performed diagnostic tests was pruritus of various etiology. These findings address the questions whether development of pemphigoid specific autoantibodies is associated with the characteristic pruritus or high age of bullous pemphigoid, may consist of a pre-clinical stage of bullous pemphigoid or could be a risk factor for development of the disease.\(^{16}\) A hypothesis for presence of BP180 and BP230 autoantibodies with higher age could be a loss of self-tolerance due to the ageing process of the immune system, and an increased risk to develop autoimmune diseases.\(^{16}\) Alternatively, these findings may be false-positive outcomes of the diagnostic tests intrinsic to nonspecific binding of autoantibodies.\(^{16,17}\) In previous studies, single test positivity by ELISA or immunoblot have been published in a wide range between 7% and 60% of subjects, mainly depending on the study population and the number of used serological tests. The reactivity could not be confirmed by IIF SSS, or replicated by other ELISA test systems.\(^{15}\) Subgroup analysis of our study showed multiple positive reactivity was found in only 3.5%, including ten subjects with positive IIF SSS and multiple reactivity in tests of other methodology. These subjects were of significant higher mean age, and with clinical characteristics similar to nonbullous pemphigoid described in *chapter 3*. Nonetheless, these patients did not fulfill the combined criteria for diagnosis of bullous pemphigoid according to the most recent European expert consensus (2015) having atypical clinical features, lacking the histopathological finding of a subepidermal blister and the required positive DIF.\(^{18}\) In contrast, the German S2k guideline for the diagnosis of bullous pemphigoid describes various combinations of tests for diagnosis, including diagnosis based on typical clinical features and immunoserological tests without a required positive DIF.\(^{19}\) However, no evidence based diagnostic criteria have been established yet for bullous pemphigoid.\(^{20}\)
The serological diagnosis of bullous and nonbullous pemphigoid can be made based on indirect immunofluorescence microscopy on salt-split human skin. (This thesis)

To assess the optimal diagnostic strategy and minimal diagnostic criteria of bullous and nonbullous pemphigoid, in chapter 5 we compared several pairwise performed diagnostic tests in a large cohort of 1125 subjects with suspected pemphigoid. Opposed to single test research, to reflect the sequential, multivariable diagnostic process in daily practice, we used multivariable logistic regression analysis to assess the additional diagnostic value of tests. One in four patients initially presented as nonbullous pemphigoid. DIF was confirmed to be the most robust diagnostic test for bullous and nonbullous pemphigoid and positive in 88% of patients, with a perilesional biopsy site superior to lesional or healthy skin. DIF was less sensitive in the subgroup of nonbullous pemphigoid, in which definitions of biopsy sites are ambiguous. Based on our findings the optimal biopsy site for DIF can be defined as erythematous nonbullous skin. The approximately 11% with negative DIF could mostly be identified by positivity in IIF SSS and confirmed with positive reactivity using other immunoserological tests of different methodology. The combined performance of DIF and IIF reached a sensitivity of 98.8%, therefore these combined tests should be considered the minimal required laboratory tests for diagnosis. Our results confirmed the previously reported high diagnostic value of IIF SSS, with a very high specificity and diagnostic odds ratio. These findings show that positive epidermal side staining of IgG by IIF SSS allows serological diagnosis of bullous and nonbullous pemphigoid. The lower sensitivity of 77% indicates that to confirm diagnosis in the remaining number of patients with a high clinical suspicion, a biopsy for DIF is also required. In contrast, the commonly used BP180 NC16A and BP230 ELISAs were not sufficiently specific for initial diagnosis, with a high false-positivity rate. The BP180 NC16A ELISA did not have added diagnostic value in addition to DIF and IIF SSS in multivariable logistic regression analysis. Compared to other studies we found a significantly lower sensitivity and specificity, explained by the differences in methodology and the populations. Hence, sensitivity and specificity are not intrinsic to the test, but to the population of use. Comparing confirmed positive patients to healthy controls in previous studies may have given an overestimation of the diagnostic accuracy. Of interest, false-positive results were more often found in nonbullous subjects than subjects with blisters.

In the subgroup analysis of subjects with bullous and nonbullous pemphigoid remarkable associations were seen. The subjects with bullous pemphigoid showed a higher mean serum autoantibody titer against BP180, which was associated with a positive biopsy for DIF. In contrast the subjects with nonbullous pemphigoid had significantly more often solely autoantibodies against BP230, similar to the findings in chapter 3. Moreover, lower serum titers or absence of autoantibodies against BP180 were observed. The BP250 autoantibodies contributed mainly to positivity by IIF SSS in serum, in contrast BP180 autoantibodies were associated with positivity by DIF in a skin biopsy. The latter has been reported previously in literature and could be an explanation why false-negative DIF biopsies may occur, by missing the intracellular target of the BP230 protein. Autoantibodies against BP230 may not be visualized properly by direct immunofluorescence on an intact skin biopsy, while the saline induced tissue damage of an artificial split in the lamina lucida of the EBMZ exposes the BP230 antigen. Autoantibodies against BP230 were recognized in bullous pemphigoid four decades ago, prior to the identification of BP180. The pathogenicity of autoantibodies against BP180 and the role in blister formation has been studied well, while the direct pathogenic role in blister formation
has been studied well, while the direct pathogenic role of autoantibodies against BP230 has been debated since.

The availability of full-length ELISAs may improve the sensitivity, by detection of autoantibodies outside the immunodominant NC16A domain. Differences in phenotype have been reported in patients with autoantibodies targeting epitopes outside the NC16A domain of the BP180 protein, with less inflammation and erythema. The non-NC16A autoantibodies did not differ significantly between nonbullous and bullous subjects in our study, only representing a small minority of patients. Our study reveals the ELISA could be of better use as add-on test for relative disease monitoring in confirmed patients, without the need of a positivity cut-off value for individual patients. Similar findings were recently reported, suggesting a higher positivity cut-off to improve the specificity, at cost of sensitivity. More comparative studies are needed in the population of intended use for other more recently introduced diagnostic test systems, such as the IIF Biochip with BP180 and BP230 and IIF on primate SSS. In contrast to IIF on human salt-split skin for detection of anti-EBMZ antibodies, the multivariate single-specific antigen testing systems require the various antigens of pemphigoid diseases to improve sensitivity and cover patients with more rare subtypes.

**The proposed minimal diagnostic criteria of pemphigoid consist of at least two positive out of three criteria:**

1. pruritus and/or predominant cutaneous blisters,
2. positive linear IgG and/or complement C3 depositions (in n-serrated pattern) along the BMZ by DIF on a skin biopsy,
3. positive epidermal side staining of IgG by IIF SSS on serum. (this thesis)

The minimal diagnostic criteria encompass the complete clinical spectrum of bullous and non-bullous pemphigoid, and differentiate it from other subtypes. The criteria thus contradict that presence of blisters or histopathology is a prerequisite for diagnosis of pemphigoid. To distinguish bullous and nonbullous pemphigoid from other subtypes, the predominance of cutaneous lesions opposes mucous membrane pemphigoid. Summarized in the diagnostic algorithm in Fig. 1, the finding of positive DIF with linear IgG depositions along the BMZ does not always imply a definitive diagnosis. The required performance of IIF SSS excludes the subtypes of pemphigoid diseases with dermal side binding of autoantibodies: anti-p200/laminin γ1 pemphigoid, EBA/bSLE, and also anti-laminin-332 mucous membrane pemphigoid (MMP). LAD is excluded by detection of solely autoantibodies of IgA isotype, and pemphigoid gestationis by the distinct patient population. Subtyping in seronegative patients requires DIF serration pattern analysis to identify the linear n-serrated pattern in pemphigoid opposing to the linear u-serrated pattern in EBA, described in chapters 7 to 9.
clinical suspicion

**direct immunofluorescence**

- u-serrated
  - epidermolysis bullosa acquisita
  - bullous SLE

- n-serrated
  - all other pemphigoid diseases

**indirect immunofluorescence salt-split skin substrate**

- dermal side
  - epidermolysis bullosa acquisita
  - bullous SLE

- epidermal side
  - anti-p200 / anti-laminin γ1 pemphigoid
  - anti-laminin-332 MMP

- bullous / nonbullous pemphigoid
- pemphigoid gestationis
- linear IgA dermatosis
- lichen planus pemphigoides
- mucous membrane pemphigoid

**additional diagnostic tests for subtyping**

- Immunoblot
- ELISA col7
- NC1/NC2
- IIF knockout
- FOAM
- Direct immunoe-EM

- Immunoblot
- Dermal/epidermal extract
- Immunoblot/ELISA laminin γ1
- Immuno-precipitation
- IIF knockout
- FOAM
- Laminin-332 footprint assay

- IIF monkey/rabbit oesophagus
- IIF biochip
- Immunoblot
- ELISA BP180 NC16A/BP180
- ELISA BP230

Fig.1 Diagnostic algorithm of pemphigoid diseases.
Nonbullous pemphigoid is not a white raven in the high-risk population of nursing home residents, and should routinely be screened in those patients with chronic severe pruritus. (This thesis)

Although epidemiological data show pemphigoid is not a rare disease above 80 years, studies are often limited to the outpatient clinic or hospitalized patients. With ageing also comes itch, and when no clear diagnosis can be made often senile pruritus is the diagnosis by exclusion. We hypothesized nonbullous pemphigoid might be an unrecognized cause of pruritus, and nursing home residents a very high-risk population for pemphigoid with a high age on average and presence of associated neurodegenerative disease and polypharmacy. In chapter 6 we evaluated the prevalence of pruritus and pemphigoid in nursing home residents in the prospective SSENIOR study. The prevalence of 47% of pruritus confirmed the common presence of the burdensome complaint, often of chronic duration. One other study analyzed how often skin blistering in an elderly population of nursing home residents was caused by bullous pemphigoid, and found a substantial higher incidence rate of nearly 5% compared to reports in the general population. The SSENIOR study was the first study using serological screening for pemphigoid in nursing home residents, and assessment of nonbullous pemphigoid. Our findings showed a high prevalence of pemphigoid of 6%, a two hundredfold higher prevalence compared to the general population (0.026%). All cases had concomitant neurodegenerative disease, mainly dementia. Remarkably, more often patients with unrecognized nonbullous pemphigoid were diagnosed than bullous pemphigoid. This raises the question whether the typical clinical features of bullous pemphigoid indeed are typical, or possibly the atypical nonbullous clinical features are more frequent, but unrecognized within the spectrum of pemphigoid. Diagnosis of the cases of nonbullous pemphigoid was made using IIF SSS, with negative DIF in all cases. The serological diagnosis was confirmed with positive IIF MO, immunoblot or ELISA. These findings implicate serological screening for pemphigoid using IIF SSS should be included in the diagnostic work-up of elderly people with moderate to severe pruritus, with or without skin blistering. In cases of negative IIF SSS and a high clinical suspicion of pemphigoid, a skin biopsy for DIF is recommended.

Autoantibodies in the diagnosed patients with pemphigoid mainly targeted BP230. Studies to the relationship between anti-BP230 autoantibodies and disease activity in pemphigoid patients have been contradictory, with no clear association. Various mouse models did not show consistent skin blistering, but skin fragility was observed with development of neurological defects with sensory neuron degeneration. Therefore, BP230 might contribute more than a skin phenotype in patients with pemphigoid. Possibly, studies with a focus on skin blistering may have overlooked features of a different phenotype of the disease, of nonbullous pemphigoid. In addition to the skin, recent studies have reported expression of the BP autoantigens BP180 and BP230 in the central nervous system. Presence of dementia gives a twofold increased risk of bullous pemphigoid, whereas neurodegenerative diseases often precede the development of pemphigoid. It is hypothesized the strong association with neurodegenerative diseases could be explained a process of neurodegeneration or neuroinflammation and with a subsequent cross-reactive immune response between neuronal and cutaneous autoantigens, driven by an age related loss of immunotolerance. Ageing of the immune system may impair the control of autoreactive immune cells, leading to development of autoreactive antibodies due to a loss of self-tolerance. Immunological tolerance could be described as a state of indifference (non-reactivity) towards substances that would normally not excite an immunological response. The loss of self-tolerance is also demonstrated in an increased seroprevalence.
of anti-nuclear antibodies (ANA) and rheumatoid factors in elderly. Overall, these insights suggest pemphigoid might be considered a cutaneous autoimmune disease of the elderly, that is more than skin deep.

U, NU! (Joost van den Vondel, 1620)

DIF on a skin biopsy has been the standard for diagnosis of pemphigoid diseases for fifty years, the detection of a linear deposition of immunoglobulins along the BMZ does not allow differentiation of subtypes of pemphigoid diseases. Subtyping in pemphigoid diseases is of importance because of differences in treatment, prognosis and complications of the disease, such as a scarring phenotype and recalcitrant disease course in EBA and associated malignancy in anti-laminin-332 MMP.\(^{40,41}\) DIF serration pattern analysis has been used for more than a decade in Groningen to differentiate EBA from other pemphigoid diseases based on a skin biopsy, however with limited use in other laboratories due to perceived technical hurdles.\(^{42}\) In chapter 7 we addressed these technical aspects of serration pattern analysis, and provide a protocol for implementation of the technique in the routine diagnostics for pemphigoid diseases. Clinical features and histopathology are not sufficient for subtyping of pemphigoid diseases and therefore direct immunofluorescence and immunoserology are necessary for differentiation.\(^{43,44}\) For EBA various serological assays are available for identification of the specific autoantibodies against type VII collagen, depending on availability and expertise.\(^{45}\) In EBA, however, approximately 50% of patients do not have detectable circulating autoantibodies by IIF SSS or type VII collagen ELISA (NC1, NC1/2).\(^{46,47}\) Moreover, flaws in study design may have led to an overestimation of sensitivity of serological assays in EBA, using an inadequate reference standard for diagnosis of EBA and testing only confirmed patients.\(^{48,49}\) According to the recent consensus on diagnostic criteria for EBA (2017), the diagnosis is based on detection of skin bound autoantibodies by either DIF using serration pattern analysis or direct immuno-electron microscopy (DIEM), and additionally combined with serological assays.\(^{50}\) DIEM has limited availability nowadays and is less applicable as routine diagnostic technique. Consequently, a large number of patients with EBA may currently be misdiagnosed as BP or MMP.

We analyzed various technical aspects of DIF serration pattern analysis, by comparing the procedures and performance in two laboratories in Groningen and Lübeck, Germany. Technical factors such as transport medium, thickness of cryosections, microtome blade or observer did not significantly affect the recognition of the serration patterns. The serration pattern can be well identified, shown by the high inter-rater conformity of 95.7% and the fact that no erroneous serration patterns were classified. DIF serration pattern analysis is the most informative and cost-effective diagnostic test for pemphigoid diseases. Recognizing the pathognomonic u-serrated pattern may increase the number of diagnosed cases of EBA substantially, especially seronegative cases. Therefore, we conclude technical requirements are not an obstacle to use serration pattern analysis as a routine diagnostic technique for differentiating pemphigoid diseases.

We further evaluated the ability to learn recognition of serrated patterns by testing observers with levels of expertise in chapter 8. Practice makes perfect in case of serration pattern analysis. However, the use of the rather simple technique as routine diagnostic method is still limited, possibly by a lack of experience with the distinctive patterns. The image-based online n-versus-u test and instruction video was created to stimulate practicing serration pattern analysis, to test the recognition rate in various observers, and to spread the knowledge about the tech-
nique. Improvement in recognition rate was seen after a short instruction indepen-
dent of the level of expertise of the participant, with a mean recognition rate of nearly 80%. The training 
images and instruction video remain available at www.nversusu.umcg.nl. The u-serrated pat-
ttern was better recognized than the n-serrated pattern.

THE U-SERRATED PATTERN IS A RECOGNIZABLE FINGERPRINT OF EPIDERMOLY-
SIS BULLOSA ACQUISITA. (THIS THESIS) 
The spikes or ridge-endings of the u-serrated patterns are distinctive recognizable features and 
represent the fingerprint of EBA. This distinctive recognizable feature of ridge-endings of the 
U-serrated pattern was used for computer aided pattern recognition in DIF images in chapter 9. We developed an automatic technique that identifies the BMZ and detect the distinct 
U-serrated pattern with trainable (COSFIRE) filters that are only selective for ridge-endings. 
Finally, a score and region of interest is provided that indicates the reliability of pattern recog-
nition. We achieved an average recognition rate of 82.2% in 180 DIF images, which equals or 
exceeds the manual recognition rate. The technique of ridge-endings detector can be used for 
other applications, such as vessel-endings in retinal fundus images, and minutiae detection in 
fingerprints.

SERRATION PATTERN ANALYSIS AND INDIRECT IMMUNOFLUORESCENCE 
MICROSCOPY KNOCKOUT ANALYSIS ARE VALUABLE ADDITIONAL TECHNIQUES 
TO FACILITATE THE DIAGNOSIS OF ANTI-P200 PEMPHIGOID. (THIS THESIS) 
The rare subtype of anti-p200 pemphigoid is probably more common than currently estimated, 
because of under-recognition and the need of sophisticated serological techniques for definitive 
diagnosis. Clinical features are heterogeneous and the acral distribution of blisters, described in 
the cases series in chapter 10, may give a clue for diagnosis. Similarly, histopathology with 
a predominant neutrophilic than eosinophilic infiltrate could be an indication for additional 
testing for anti-p200 pemphigoid. Serration pattern analysis enables to distinguish anti-p200 
pemphigoid (n-serrated) from EBA (u-serrated), but is not confirmative for diagnosis of anti-
p200 pemphigoid. Consequently, these patients are often misdiagnosed as bullous pem-
phigoid or EBA and additional serological assays are necessary for diagnosis. This emphasizes 
the required routinely performance of IIF SSS in pemphigoid diseases, as a first step for iden-
tification of anti-p200 pemphigoid. The circulating autoantibodies of patients with anti-p200 
pemphigoid show dermal side binding of IgG by IIF SSS. The autoantibodies label a 200-kDa 
deprotein by immunoblotting with dermal extract, corresponding with γ1 chain of laminin 311 
and 511. Subsequently, the denomination to anti-laminin γ1 pemphigoid has been suggested 
since. However, the significance of anti-laminin γ1 antibodies remain unclear as ex-vivo and 
in-vivo studies failed to show a direct pathogenic role. We reported detection of autoanti-
bodies against laminin γ1 in two-thirds of patients with anti-p200 pemphigoid, which could 
be used as a diagnostic marker. Other serological assays have been used to facilitate diagnosis 
of anti-p200 pemphigoid, such as ELISA using a recombinant c-terminus fragment of laminin 
γ1, immunoblot with epidermal abstract or immunoblot studies with recombinant laminin 
trimers as diagnostic marker. A small number of patients show circulating autoantibodies 
against BP180 and BP230. These patients revealed a mirroring staining pattern of both 
epidermal and dermal side binding by IIF SSS. It is questioned whether this is associated with 
intra-molecular epitope spreading, leading to detection of autoantibodies against other pem-
phigoid antigens, or vice versa with development of autoantibodies against the p200 antigen.
A MAJORITY OF DUTCH DERMATOLOGISTS AND RESIDENTS IN DERMATOLOGY SHOW HIGH ADHERENCE TO TREATMENT RECOMMENDATIONS FOR BULLOUS PEMPHIGOID BY USING TRANSCUTANEOUS SYSTEMIC CLOBETASOL THERAPY. (THIS THESIS)

Bullous and nonbullous pemphigoid have a high burden of disease due to the intense pruritus and/or blistering. Because of the potential chronic relapsing disease course, an effective treatment approach is needed. In chapter 11 we evaluated the treatment approach for bullous pemphigoid among dermatologists and residents in dermatology in The Netherlands, and made a comparison with the treatment approach of British dermatologists. Clinical studies to the optimal treatment of pemphigoid are limited, due to the relative rarity and distinct patient population with high age and comorbidities. Current recommended first-line therapy for mild, moderate and severe disease in The Netherlands is systemic transcutaneous clobetasol therapy (clobetasol propionate) 30-40 grams applied daily over the whole body, including blisters, erosions and healthy skin, but sparing the face. The dermatologists and residents in dermatology from The Netherlands showed a high adherence to this treatment recommendation, used by more than half of the participants of the survey. Treatment was was often continued after clinical remission to prevent relapses. In contrast, dermatologists from the United Kingdom preferred a different treatment strategy with lesional application of topical corticosteroids and discontinuation after remission. This difference can be explained by variation in national guidelines and health service models, with practical factors regarding topical therapy.

The European consensus on management of bullous pemphigoid recommends equivalent first-line treatment with oral corticosteroids (prednisone 0.5-0.75mg/kg/day) for moderate to extensive disease. Dosage <0.5mg/kg/day and >0.75mg/kg/day have not been validated and seem ineffective or associated with higher mortality and side-effects. Half of the participants used oral corticosteroids as first-line treatment, the majority (58%) with adjuvant immunosuppressive therapy, most commonly azathioprine. A current clinical study evaluates the efficacy and safety of initial treatment of bullous pemphigoid with prednisone 0.5mg/kg/day, which appears to be insufficient in moderate to extensive disease. Systemic anti-inflammatory antibiotics were commonly used for in both The Netherlands (73%) and UK (79%) as alternative or adjunctive treatment, mainly doxycycline. The recently published BLISTER trial intended to provide an evidence base for treatment of bullous pemphigoid with doxycycline. The study tested whether a strategy of starting treatment with doxycycline may give acceptable short-term blister control and possible long-term safety advantages over starting treatment with oral corticosteroids. The authors concluded a treatment strategy starting with doxycycline is non-inferior compared to standard treatment with oral prednisolone for short-term blister control in bullous pemphigoid and safer in the long-term. Doxycycline is clearly safer than oral corticosteroids, and clearly has a reduced success rate. Although the evidence for non-inferiority is subjectively based on the definition of the non-inferiority margin, the trial gives an indication doxycycline is at least partly effective. Participants were allowed to use (lesional) topical corticosteroids for symptomatic relief, which could serve as an initial treatment strategy in mild disease. Alternatively, oral corticosteroids could be used as an induction therapy to achieve clinical remission and reduce treatment related morbidity and mortality, with maintenance therapy of continued use of doxycycline to prevent relapses. More studies are being reported evaluating treatment of pemphigoid diseases with anti-CD20 monoclonal antibody (rituximab). Although less effective than in pemphigus, rituximab may demonstrate efficacy in treatment of especially MMP and refractory cases of bullous pemphigoid.
To evaluate whether dermatologists follow recent guidelines or implicate new results of clinical trials for treatment approach, the survey should be repeated in the upcoming five years.

**FUTURE PERSPECTIVES**

What makes the blister, remains a key question in bullous pemphigoid and topic of current studies. Or alternatively, why do patients with nonbullous pemphigoid do not make a blister. Various possible differences in the underlying pathophysiology for blister formation have been suggested, such as the serum autoantibody concentration and the targeted autoantigen or epitopes within the antigen, complement activation, eosinophilic infiltration, or BP-specific IgE autoantibodies. Considerable lower concentrations of BP-specific IgE compared to IgG complicates diagnostic testing and require validated test systems. Although IgE staining along the BMZ has been reported incidentally, no replication of these findings have been reported in literature yet and focus changes to BP-specific IgE on mast cells. Current developments of IgE ELISA may enhance the research to the role of IgE in pemphigoid and explain possible differences between bullous and nonbullous pemphigoid. Since the recognition of patients with nonbullous pemphigoid has increased in the last years, more comparative studies are needed to address these possible differences. The epidemiology of nonbullous pemphigoid has not been studied extensively, and possibly treatment options and prognosis of nonbullous pemphigoid may differ from bullous pemphigoid. It is of interest whether the nonbullous phenotype could be considered a milder disease variant, or has similar treatment response and prognosis as bullous pemphigoid.

For future applications machine learning could be suitable for detection of serration patterns using a deep learning convolutional neural network (CNN), capable of learning from images and teaching itself to improve the performance of pattern recognition. Recent studies showed a CNN for detection of melanoma based on dermoscopy images out-performed expert physicians with a higher sensitivity and less misdiagnosis. Instead of man versus machine, artificial intelligence could serve as an aid for physicians and increase the diagnostic probability, or limit the need for additional diagnostic tests and expenses.

Despite recent advantages in diagnostics and subtyping of pemphigoid diseases, the molecular identity of the pathogenic 200-kDa autoantigen still remains unsolved. The IIF knockout technique is a diagnostic method by exclusion of other known antigens of pemphigoid diseases. Immunoprecipitation studies of sera of patients with anti-p200 pemphigoid might give new insights in the targeted antigen. Another approach could be similar to the used diagnostic technique of IIF knockout analysis in our case series. Instead of using skin substrate of patients with hereditary epidermolysis bullosa deficient of type VII collagen or laminin-332, the technique could be used conversely. With the existence of forms of epidermolysis bullosa with deficiency of the various pemphigoid antigens, current unsolved cases with unknown mutations could serve as a substrate for yet to be found p200 epidermolysis bullosa and future experiments.
The SSENIOR study, described in chapter 6, gave a first sign of the frequent diagnosis of unrecognized nonbullous pemphigoid in a high-risk population of nursing home residents. Further studies are needed to confirm a higher prevalence of nonbullous pemphigoid than bullous pemphigoid in this population, which often remained out of sight of the researchers. These insights may change the concept of pemphigoid, being a cutaneous autoimmune disease presenting in elderly people, with or without skin blistering.
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