SUMMARY

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Pemphigoid diseases comprise a heterogeneous group of subepidermal autoimmune bullous diseases of the skin and mucosa. These autoimmune bullous diseases are characterized by autoantibodies that target distinct structural proteins of the basement membrane zone (BMZ), which are important for the integrity of the epidermis and dermis. Various subtypes of pemphigoid diseases can be differentiated, including bullous pemphigoid (BP), nonbullous pemphigoid, pemphigoid gestationis, anti-p200 pemphigoid, lichen planus pemphigoides, epidermolysis bullosa acquisita (EBA), linear IgA disease (LAD), mucous membrane pemphigoid (MMP), and anti-laminin-332 MMP (anti-LN-332 MMP). Although the clinical presentations of pemphigoid diseases are heterogeneous, the subtypes differ in clinical symptoms, target antigens, and the treatment and prognosis. This thesis is focused on the diagnosis and clinical presentations of various subtypes of pemphigoid diseases, encompassing laboratory, translational and clinical studies.

In chapter 2 we give an introduction in the history of the disease, with insights from the first theses about bullous diseases from The Netherlands. Initially, the term pemphigus was used to describe a blister, without knowledge of the origin. Since 1953, the disease entity of bullous pemphigoid has been recognized based on histopathology. In the following years, the introduction of the diagnostic technique immunofluorescence microscopy was a breakthrough in diagnostic classification and further subtyping of pemphigoid diseases. Several decades ago, the clinical observations were already made that patients with bullous pemphigoid often had a pruritic prodromal phase before skin blistering occurred. More recent insights show a subgroup of patients does not fulfill the typical clinical picture, but have atypical nonbullous clinical features that may persist without the development of skin blistering. These atypical nonbullous variants are not a rarity, but reported to comprise 20 to 40% of patients. We analyzed the clinical and immunopathological features of the nonbullous variant of pemphigoid by systematic review in chapter 3. Besides pruritus, the most common 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 skin diseases, and therefore clinicians may only be ascertained of misdiagnosis retrospectively when skin blisters appeared. Diagnosis of nonbullous pemphigoid in particular can be challenging; the mean diagnostic delay of reported cases was 22.6 months. It is not known yet why these patients do not develop skin blisters.

Several studies reported detection of circulating autoantibodies against the BP target antigens BP180 or BP230 by immunoblot, ELISA or indirect immunofluorescence in subjects who do not fulfill the available criteria of bullous pemphigoid, such as elderly people, elderly people with pruritus, and also healthy blood donors. In chapter 4 we evaluated the detection of BP-specific autoantibodies in serum of 374 subjects with nonbullous skin disorders who visited our outpatient dermatology clinic, in whom autoimmune bullous diseases were excluded based on negative direct immunofluorescence (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. No association was seen with pruritus, present in most patients due to various skin diseases. These findings address the questions whether these pemphigoid specific autoantibodies are associated with a pre-clinical stage of bullous pemphigoid or could be a risk factor for development of the disease, or represent false-positive results. A minor subgroup of 3.5% of patients showed multiple reactivity in tests of different methodolo-
by both indirect immunofluorescence on salt-split skin (IIF SSS), and ELISA and/or immuno blot. These patients showed clinical characteristics of nonbullous pemphigoid, but did not fulfill the available combined criteria for diagnosis of bullous pemphigoid, lacking positivity by DIF on a skin biopsy. However, no evidence based diagnostic criteria have been established yet.

In chapter 5, we evaluated the optimal diagnostic strategy for bullous and nonbullous pemphigoid and aimed to develop much-needed minimal diagnostic criteria. We compared several pairwise performed diagnostic tests in a large cohort of 1125 subjects with suspected pemphigoid. To reflect the diagnostic process in daily practice and opposed to single test comparison, we used multivariable logistic regression analysis to assess the additional diagnostic value of various tests. One in four patients initially presented as having nonbullous pemphigoid. DIF was confirmed to be the most robust diagnostic test and was positive in 88% of patients, although it was less sensitive in the subgroup of nonbullous pemphigoid (81%). The remaining approximately 11% of patients with negative DIF could mostly be identified by positivity in IIF SSS and confirmed with positivity in other immunoserological tests. The combined performance of DIF and IIF identified 98.8% of patients; therefore these combined tests should be considered the minimal required laboratory tests for diagnosis to identify the vast majority of patients. The BP180 NC16A ELISA did not have added diagnostic value in addition to DIF and IIF SSS, with a considerable false-positivity rate. The subjects with nonbullous pemphigoid had significantly more often solely autoantibodies against BP230 and lower serum titers or absence of autoantibodies against BP180. The BP230 autoantibodies contributed mainly to positivity by IIF SSS in serum. 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. Based on our findings, 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.

With ageing also comes itch. We hypothesized nonbullous pemphigoid might be an unrecognized cause of pruritus, and that nursing home residents are 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. Our findings showed a high prevalence of pemphigoid of 6%, a two-hundredfold higher prevalence compared to the general population (0.026%). Remarkably, more often patients with unrecognized nonbullous pemphigoid were diagnosed than with 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. These findings implicate serological screening for pemphigoid using IIF SSS is recommended in the diagnostic work-up of elderly people with moderate to severe pruritus, with or without skin blistering. 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 auto-
antigens, driven by an age related loss of immunotolerance. Overall, these insights suggest pemphigoid might be considered a cutaneous autoimmune disease of the elderly, that is more than skin deep.

In chapter 7 we described differentiation of subtypes of pemphigoid diseases based on serration pattern analysis by DIF on a skin biopsy. 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. DIF serration pattern analysis has been used for more than a decade in Groningen to differentiate EBA with a u-serrated pattern from other pemphigoid diseases with an n-serrated pattern. We analyzed various technical aspects for implementation of the technique in routine diagnostics, 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 in seronegative cases.

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 technique. Improvement in recognition rate was seen after a short instruction independent 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 pattern was better recognized than the n-serrated pattern.

In chapter 9 we explored a novel automated technique of computer aided serration pattern recognition based on the fingerprint of EBA, the u-serrated pattern. The spikes or ridge-endings of the u-serrated patterns are distinctive recognizable features. We developed an automatic technique that identifies the basement membrane zone 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 recognition. 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.

In chapter 10 we used the combined diagnostic techniques of IIF knockout analysis and serration pattern analysis to identify 12 patients with the rare subtype of anti-p200 pemphigoid. The subtype is probably under-recognized and misdiagnosed, because sophisticated serological techniques are needed for definitive diagnosis. The clinical features were heterogeneous and the acral distribution of blisters might be a clue for diagnosis. In contrast to Japanese patients,
no association with psoriasis was found and the diseases severity was higher than previously reported. The circulating autoantibodies of patients with anti-p200 pemphigoid show dermal side binding of IgG by IIF SSS and serves as a first step for identification of anti-p200 pemphigoid. While autoantibodies against laminin γ1 were found in two-thirds of patients with anti-p200 pemphigoid, the significance of anti-laminin γ1 antibodies and molecular identity of the target antigen remain unclear.

Finally, in chapter 11 we evaluated the treatment approach for bullous pemphigoid among dermatologists and residents in dermatology in The Netherlands (n=175), and made a comparison with the treatment approach of British dermatologists (n=375). 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. The dermatologists and residents in dermatology from The Netherlands showed a high adherence to the treatment recommendation in The Netherlands of transcutaneous systemic clobetasol therapy, used by more than half of the participants of the survey. Treatment 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. Systemic anti-inflammatory antibiotics were commonly used in both The Netherlands (73%) and UK (79%) as alternative or adjunctive treatment, mainly doxycycline. Half of the participants considered oral corticosteroids as first-line treatment, the majority (58%) with adjuvant immunosuppressive therapy. The survey should be repeated in the upcoming five years to evaluate whether dermatologists follow recent guidelines and implicate new results of clinical trials for treatment of bullous pemphigoid.