Diagnostics of autoimmune bullous diseases in German dermatology departments

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Characterized by tense blisters and erosions of the skin, mucous membrane pemphigoid by erosion of mucous membranes near the skin surface [4, 5]. Pemphigus vulgaris is always associated with malignancy in 30 % of patients [6–10]. With the description of further target antigens and their significance for prognosis and therapeutic measures, the need for antigen-specific diagnostics will further increase in the future. The aim of the present study was to portray diagnostics of autoimmune bullous dermatoses available in German departments of dermatology.

Introduction
Autoimmune bullous dermatoses represent a broad spectrum of about one dozen different diseases. In Germany, bullous pemphigoid (BP) is by far the most common autoimmune bullous disorder with an incidence of 13.4 new cases/ million yearly. Pemphigoid gestations and mucous membrane pemphigoid are the second most frequent diseases with 2.0/ million/ year [1]. The incidence of pemphigus in Germany is about 1.0/ million/ year [2]. Thus, about 2 500 new cases of autoimmune bullous diseases can be expected in Germany per year. As the incidence of BP increases significantly with age up to 150–190/ million/ year in over 80-year-old patients [1, 3], a growing number of patients and thus an increased need for diagnostics is to be anticipated.

Clinically, the pemphigoid diseases are characterized by tense blisters and erosions of the skin, mucous membrane pemphigoid by erosion of mucous membranes near the skin surface [4, 5]. Pemphigus vulgaris is always associated with malignancy in 30 % of patients and anti-p200/ laminin γ1 pemphigoid is usually easier to treat than BP, while epidermolysis bullosa acquista is distinctly more difficult to influence than BP. In addition to direct IF microscopy, serological diagnostics adapted to the clinical situation is indispensable today [6–10]. With the description of further target antigens and their significance for prognosis and therapeutic measures, the need for antigen-specific diagnostics will further increase in the future. The aim of the present study was to portray diagnostics of autoimmune bullous dermatoses available in German departments of dermatology.

Materials and methods
A standardized questionnaire was sent to all 34 German university departments of dermatology and 39 non-university departments of dermatology and 39 non-university departments.

Data were summarized (<120 words) in [37].
Diagnostics of AIBD in Germany

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The questionnaire included four questions to be answered in a dichotomous manner (yes/no) on diagnostic methods employed for autoimmune bullous dermatoses: (i) direct IF microscopy, (ii) indirect IF microscopy on monkey esophagus, guinea pig esophagus, human salt-split skin, monkey bladder, rat bladder and complement-binding test on human salt-split skin, (iii) commercially available ELISA for detection of autoantibodies against BP180 NC16A, BP230, desmoglein 1, desmoglein 3 and envoplakin and (iv) non-commercial assays including ELISA and immunoblot techniques for autoantibodies against BP180, BP230, the soluble ectodomain of BP180 (LAD-1), epitopes on BP180 outside of the BP180 NC16A domain, laminin 332, type VII collagen, p200 protein, laminin γ1, α6 integrin, β4 integrin, desmplakin I and II, periplakin and envoplakin. The number of annually analyzed samples for each of the studied complexes were to be checked in predetermined categories (< 100, 100–500, 501–1000, and perhaps > 1000/year). A numerical analysis of the survey followed.

Results
All of the 34 (100 %) university departments of dermatology and 31 of the 39 (79 %) of non-university dermatology clinics returned the questionnaire. All returned questionnaires could be evaluated, producing an overall return rate of 89 %. An overview of the 4 recorded diagnostic techniques (direct and indirect IF microscopy, commercial ELISA, non-commercial ELISA/ immunoblots) is depicted in Figure 2.

Direct immunofluorescence
Direct IF microscopy is performed in practically all departments of dermatology (all university departments and 30 of the 31 non-university dermatology clinics) (Figure 3). Direct IF microscopy was not only the most widespread diagnostic method, but was also performed most frequently. Of the clinics 51 % performed 100–500 and 18 % more than 500 analyses yearly (10 of the university departments and 3 of the non-university clinics) (Figure 5a).

Indirect immunofluorescence
Tests using indirect IF microscopy were performed in 74 % of all clinics (30 of
the 34 university departments and 18 of the 31 non-university clinics). The most common substrate in these 48 departments of dermatology was monkey esophagus (83 %) and human salt-split skin (65 %) (Figure 4a). Indirect IF microscopy on monkey or rat bladder and the complement-binding test on human salt-split skin was performed predominantly in the university departments (57 % or 27 %, respectively, as opposed to 11 % in non-university clinics) (Figure 4a).

Commercial ELISA

Commercial ELISA were available in 26 of 34 (76 %) university departments of dermatology and in 12 of 31 (38 %) non-university dermatology clinics (on the whole 58 % of clinics). ELISA for anti-desmoglein 1 and 3 antibodies were employed in all 38 clinics, ELISA for anti-BP180 and anti-BP230 antibodies in 84 % or 82 % of these 38 clinics, respectively (Figure 4b). With respect to the

Figure 3: Geographic overview of university (red box) and non-university dermatology departments (black box) that perform direct immunofluorescence microscopy. (Licence: [http://creativecommons.org/licenses/by-sa/3.0/de/ GNU-Lizenz für freie Dokumentation]).
total collective of all surveyed clinics commercial ELISA-systems were used about twice as often in university departments of dermatology in comparison to non-university dermatology clinics (exception: envoplakin ELISA in 2 university and non-university clinics each) (Figure 4b). Non-commercial ELISA and immunoblots Non-commercial techniques for the detection of serum autoantibodies were performed in 21% of the university dermatology clinics (7 of 34) and 23% of the non-university dermatology clinics (7 of 31). Here: particularly methods for detection of autoantibodies against BP180, BP230, laminin 332 and type VII collagen were employed (9%–15%). In only a few clinics was detection of autoantibodies against rarer target antigens such as α6 integrin and plakin possible (Figure 4c).

All diagnostic techniques were used more frequently in university departments of dermatology; the proportion of clinics with more than 500 tests yearly for direct IF microscopy was 4 times higher in university clinics than in non-university clinics (29% vs. 6%) and for indirect IF microscopy about 8 times higher (26% vs. 3%) (Figure 5a, b). More than 500 analyses yearly using commercial and non-commercial ELISA and immunoblot techniques were performed in 26% and 9%, respectively, of the university clinics (0% each for non-university clinics) (Figure 5c, d). The clinics with more than 500 or 1,000 tests using direct IF, indirect IF as well as commercially or non-commercially available ELISA/immunoblot methods are depicted on a geographic overview (Figure 6).

Discussion
This is the first standardized registration of methods of diagnosing autoimmune bullous dermatoses in German dermatology clinics. A similar study for other countries does not exist. The high return rate of 89% allows for a representative portrayal and reflects—just as a recent survey on therapy of autoimmune bullous dermatoses did [11]—the great interest in this disease group. The diagnostic gold standard, direct IF of a perilesional skin (or mucous membrane) biopsy, is performed in practically all surveyed dermatology clinics, in a dozen clinics even more than 500 times yearly. Figure 3 demonstrates that this important method is available in Germany on a nationwide basis. Direct IF is characterized by a high positive predictive value of nearly 100%; only about 10% of samples from pemphigus patients, for example, are false negative [9, 12]. Histopathology belongs to routine diagnostics of autoimmune bullous dermatoses, but nevertheless, does not allow for a secure diagnosis of these diseases [13, 14], and this was therefore not surveyed. Recently, however, a report appeared on the diagnosis of BP via the detection of C3d deposits in formalin-fixed skin [15].

Figure 4: Detailed description of employed serological assays for the diagnosis of autoimmune bullous skin disorders in German dermatology departments. Substrates used for indirect immunofluorescence microscopy (a), autoantigen-specific commercial ELISA systems (b), and autoantigen-specific non-commercial ELISA and immunoblot systems (c).

Figure 5: Frequency of diagnostic methods for autoimmune bullous skin diseases in German dermatology departments (a–d).
Indirect IF is a recognized screening method for detection of serum autoantibodies in autoimmune bullous dermatoses. Most sensitive substrates reported were monkey esophagus (for pemphigus vulgaris and dermatitis herpetiformis), guinea pig esophagus (for pemphigus foliaceus), human salt-split (with 1 M NaCl solution) skin (for pemphigoid diseases and epidermolysis bullosa acquisita as well as – after preincubation with a complement source – for pemphigoid gestationis) as well as monkey and rat bladder (for paraneoplastic pemphigus) [16–22]. The most important screening tests for pemphigus and pemphigoid diseases, indirect IF on monkey esophagus and human salt-split skin are performed in about one-half of the clinics, to be precise, in about two-thirds of university departments of dermatology and one-third of non-university clinics. In 10 clinics over 1,000 indirect IF analyses are performed annually, so that an important instrument for the serological diagnosis of autoimmune bullous dermatoses is available on a widespread basis in German dermatology clinics and is employed frequently.
Diagnostics of AIBD in Germany

The development of sensitive and specific ELISA for the detection of circulating autoantibodies has become possible. In the meantime commercial ELISA are available for antibodies against desmoglein 3 (pemphigus vulgaris), desmoglein 1 (pemphigus foliaceus and pemphigus vulgaris), BP180 (bullous pemphigoid, pemphigus gestationis, mucous membrane pemphigoid, lichen planus pemphigoides), BP230 (bullous pemphigoid) and envoplakin (paraneoplastic pemphigoid) [23–27]. With the exception of envoplakin ELISA, these are employed in about three-quarters of university and in about one-third of non-university dermatology clinics, which demonstrates the important role that these relatively new techniques already play. In comparison to indirect IF these methods are highly standardized and allow for simple monitoring of the disease course, as the levels of serum antibodies against desmoglein 1 and BP180 and usually also against desmoglein 3 correlate with the disease activity of the respective disease [25, 28–30]. Already in the survey by Hoffmann et al. these instruments were being used in about one-half of the 32 surveyed clinics for therapy monitoring in BP and pemphigus [11]. Besides these five ELISA various test systems for further target antigens have been established in German dermatology clinics in recent years, among others, for epidermolysis bullosa acquisita), laminin 332 (mucous membrane pemphigoid, linear IgA dermatosis), NC16 domain in BP (mucous membrane pemphigoid in female patients). ELISA for the detection of circulating antigen-specific antibodies allows for the detection of antibodies against desmoglein 3 (pemphigus vulgaris), desmoglein 1 and BP180 and usually also against desmoglein 3 correlate with the disease activity of the respective disease [25, 28–30]. Already in the survey by Hoffmann et al. these instruments were being used in about one-half of the 32 surveyed clinics for therapy monitoring in BP and pemphigus [11]. Besides these five ELISA various test systems for further target antigens have been established in German dermatology clinics in recent years, among others, for epitopes outside the immunodominant NC16 domain in BP (mucous membrane pemphigoid, linear IgA dermatosis), laminin 332 (mucous membrane pemphigoid), type VII collagen (epidermolysis bullosa acquisita), laminin γ1 (anti-p200 pemphigoid), desmocollin (pemphigus) as well as periplakin and desmoplakin I/II (paraneoplastic pemphigus) [31–36]. These methods allow for differentiation between the individual pemphigoid diseases, which is of prognostic and increasingly of therapeutic significance, on the one hand, and on the other it was shown that their combination allows for the detection of autoantibodies in practically all patients with BP and mucous membrane pemphigoid [19, 34]. The non-commercial assays are employed in about one-fifth of the clinics. Exhaustive further diagnostics of these – in the end effect – rare diseases appears widespread, while they – at least in large numbers – are limited to a few specialized centers (Figure 6). The aim of this paper was to register the health care situation of patients with autoimmune bullous dermatoses with respect to available diagnostics in German dermatology clinics; the methods mentioned therefore do not necessarily have to be performed in the dermatology clinics themselves. Nonetheless, it would be desirable that direct IF as a specialty-specific test continues to be performed in the departments of dermatology to guarantee optimal clinico-pathologic correlation. A challenge for all clinics involved in serological autoimmune diagnostics will surely be future accreditation of the laboratory. It can be expected that the commercialization of further serological test systems will advance the distribution of antigen-specific analyses and thus allow for the continual optimization of the diagnostics of autoimmune bullous dermatoses in Germany. In summary, diagnostics of autoimmune bullous dermatoses in Germany appears advanced both with respect to availability as well as to the differentiation of the individual diseases. Diagnostics of rare entities is possible via cooperation with several specialized centers.

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Conflicts of interest
None.

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