Diagnostics of autoimmune bullous diseases in German dermatology departments

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Data were summarized (<120 words) in [37].

Keywords
- autoantibody
- bullous pemphigoid
- ELISA
- immunoblot
- immunofluorescence
- pemphigus

Summary
Background: No consistent data are available on the currently employed diagnostic tools for autoimmune bullous diseases in Germany. The aim of this survey was to describe currently performed diagnostic methods for bullous autoimmune diseases in German dermatology departments.
Methods: A standardized questionnaire evaluated the available diagnostic methods i. e. direct immunofluorescence microscopy (IFM), indirect IFM, commercial ELISA systems, and non-commercial serological tests as well as the number of samples per year in all 34 university and 39 non-university dermatology departments.
Results: The overall return rate was 89 %, 100 % and 79 % for the university and non-university departments, respectively. Direct IFM was the most frequently used method and was applied in 98 % of the responding departments. In 74 % of the responding departments, indirect IFM was used mainly on monkey esophagus and human salt-split skin. Commercial ELISA systems were employed in 58 % of the clinics; all of them used anti-desmoglein ELISA, while anti-BP180 and anti-BP230 ELISA were established in 49 % and 48 % of departments, respectively. Non-commercial analytic methods were only performed in 22 % of the departments.
Conclusions: The high return rate of this survey allows a relatively precise description of the current diagnostic methods used in German dermatology departments. Standard diagnostic tests are available nationwide and in bullous pemphigoid and pemphigus, the antigen-specific detection of autoantibodies is routinely performed in half of the departments. Rare disorders may be diagnosed by cooperation with some specialized centers.

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 gestationis 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 mucous membrane lesions, while pemphigus foliaceus typically presents with erosions and scales in seborrheic areas. Clinically, the various entities cannot be definitely differentiated from each other. E.g., the differentiation between pemphigus vulgaris and mucous membrane pemphigoid on the one hand and between BP, anti-p200/ laminin γ1 pemphigoid and epidermolysis bullosa acquisita on the other is often difficult. In addition, premonitory BP is clinically indistinguishable from other puritic skin diseases. For these cases direct immunofluorescence (IF) microscopy of a perilesional skin biopsy is essential. The exact differentiation of the individual entities is both of prognostic and therapeutic significance. Thus, anti-laminin 332 mucous membrane pemphigoid is associated with malignancy in 30 % of patients and anti-p200/ laminin γ1 pemphigoid is usually easier to treat than BP, while epidermolysis bullosa acquisita is distinctly more difficult to influence than BP. In addition to direct IF microscopy, serological diagnostics adapted to the clinical situation is indispensible 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

Questionnaire on the care of patients with autoimmune bullous dermatoses in Germany

Which methods for diagnosing autoimmune bullous dermatoses do you employ in your clinic?

1. **Direct immunofluorescence** Yes ☐ No ☐
   If yes
   < 100 biopsies/year ☐ 100-500 biopsies/year ☐ > 500 biopsies/year ☐
2. **Indirect immunofluorescence**
   a. on monkey esophagus Yes ☐ No ☐
   b. on guinea pig esophagus Yes ☐ No ☐
   c. on human salt-split skin Yes ☐ No ☐
   d. on monkey bladder Yes ☐ No ☐
   e. on rat bladder Yes ☐ No ☐
   f. Complement-binding test (for pemphigus gestationis) Yes ☐ No ☐

   How many sera are examined using indirect immunofluorescence?
   < 100/year ☐ 100-500/year ☐ > 500/year ☐
3. **Commercial ELISA systems**
   a. BP180 NC16A Yes ☐ No ☐
   b. BP230 Yes ☐ No ☐
   c. Desmoglein 1 Yes ☐ No ☐
   d. Desmoglein 3 Yes ☐ No ☐
   e. Envoplakin Yes ☐ No ☐

   How many sera are examined using commercial ELISA systems?
   < 100/year ☐ 100-500/year ☐ 501-1000/year ☐ > 1000/year ☐
4. **Non-commercial immunoblot/ELISA systems**
   a. BP180 Yes ☐ No ☐
   b. BP230 Yes ☐ No ☐
   c. LAD-1 (soluble BP 180 ectodomain) Yes ☐ No ☐
   d. Epitopes on BP180 outside of the NC16A domain Yes ☐ No ☐
   e. Laminin 332 (laminin 5) Yes ☐ No ☐
   f. Type VII collagen Yes ☐ No ☐
   g. NC1 domain of type VII collagen Yes ☐ No ☐
   h. p200 protein Yes ☐ No ☐
   i. Laminin γ1 Yes ☐ No ☐
   j. α6 integrin Yes ☐ No ☐
   k. β4 integrin Yes ☐ No ☐
   l. Desmoplakin I/II Yes ☐ No ☐
   m. Periplakin Yes ☐ No ☐
   n. Envoplakin Yes ☐ No ☐

   How many tests using non-commercial test systems are performed annually?
   < 100/year ☐ 100-500/year ☐ 500-1000/year ☐ > 1000/year ☐

Figure 1: Standardized questionnaire about the diagnostic methods at German dermatology departments.

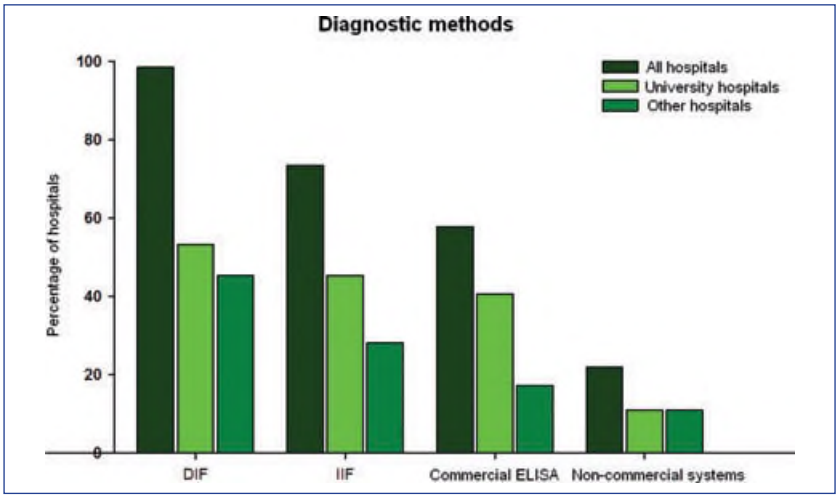

Figure 2: Percentage of German dermatology departments that perform direct immunofluorescence microscopy (DIF), indirect immunofluorescence microscopy (IIF), commercially available ELISA systems, and non-commercial assays for the diagnosis of autoimmune bullous skin diseases. The percentages of university (light green) and non-university hospitals (green) are related to the percentage of investigations performed in all hospitals (dark green).

dermatology hospitals (Figure 1). 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, desmoplakin 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, 501–1 000, and perhaps > 1 000/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

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]).

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

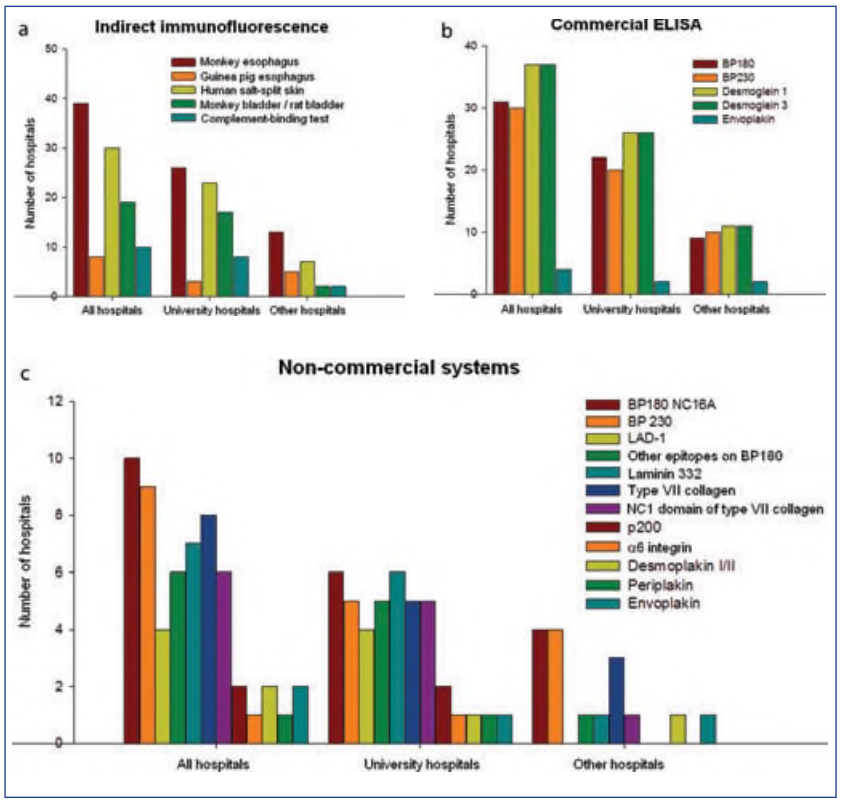

Figure 4: Detailed description of employed serological assays for the diagnosis of autoimmune bullous skin disorders in German dermatology departments. Substrates used for indirect immunofluorescence microscopy (a), autoantigen-specific commercial ELISA systems (b), and autoantigen-specific non-commercial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

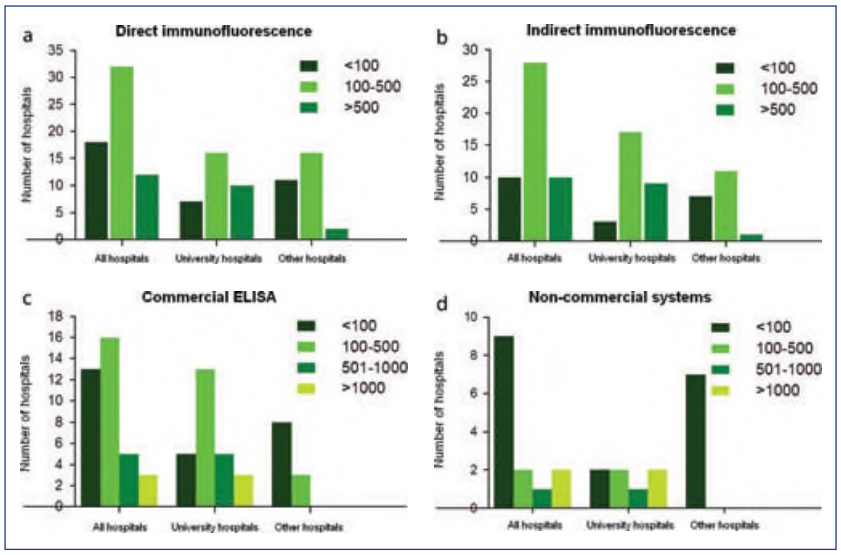

Figure 5: Frequency of diagnostic methods for autoimmune bullous skin diseases in German dermatology departments (a–d).

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 6: Geographic overview of the dermatology departments with the highest number of diagnostic assays: (green box) > 500 direct IF microscopy/year, (blue box) > 500 indirect IF microscopy/year, (yellow box) > 1 000 commercial ELISA/years, (red box) > 1 000 non-commercial serological assays/year. (License: [http://creativecommons.org/licenses/by-sa/3.0/de/ GNU-Lizenz für freie Dokumentation]).

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.

Through the identification and molecular characterization of target antigens 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 pemphigus) [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 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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