ORIGINAL ARTICLE

Prospective studies on the routine use of a novel multivariant enzyme-linked immunosorbent assay for the diagnosis of autoimmune bullous diseases

Nina van Beek, MD,[a] Cornelia Dähnrich, PhD,[d] Nora Johannsen, PhD,[d] Susanne Lemcke, PhD,[a,b,c] Stephanie Goletz, PhD,[b] Franziska Hübner, MD,[a] Giovanni Di Zenzo, MD,[e] Marian Dmochowski, MD,[f] Kossara Drenovska, MD, PhD,[g] Shamir Geller, MD,[h] Michael Horn, MD,[i] Cezary Kowalewski, MD,[j] Ljiljana Medenica, MD, PhD,[k] Dedee F. Murrell, MD,[l] Aikaterini Patsatsi, MD,[m] Soner Uzun, MD,[n] Snejina Vassileva, MD, PhD,[g] Detlef Zillikens, MD,[a,c] Wolfgang Schlumberger, PhD,[d] and Enno Schmidt, MD, PhD[a,b]

Lübeck, Germany; Rome, Italy; Poznan, Poland; Sofia, Bulgaria; Tel Aviv, Israel; Bern, Switzerland; Warsaw, Poland; Belgrade, Serbia; Sydney, Australia; Thessaloniki, Greece; and Antalya, Turkey

Background: Serologic diagnosis of autoimmune blistering disease (AIBD) usually follows a sophisticated multistep algorithm.

Objective: We sought validation of a multivariant enzyme-linked immunosorbent assay (ELISA) in the routine diagnosis of AIBD.

Methods: The multivariant ELISA comprising 6 recombinant immunodominant forms of major AIBD target antigens, ie, desmoglein 1, desmoglein 3, envoplakin, BP180, BP230, and type VII collagen was applied in: (1) a cohort of well-characterized AIBD (n = 173) and control sera (n = 130), (2) a prospective multicenter study with 204 sera from patients with newly diagnosed AIBD with positive direct immunofluorescence microscopy, and (3) a prospective monocenter study with 292 consecutive sera from patients with clinical suspicion of AIBD in comparison with the conventional multistep diagnostic algorithm.

Results: Concordant results in the multivariant ELISA compared with direct immunofluorescence microscopy were seen in 94% of patients with pemphigus and 71% of patients with pemphigoid (Cohen κ value, 0.95 and 0.66) and with the conventional multistep diagnostic approach in 91% of patients with pemphigus and 88% of patients with bullous pemphigoid and 93% of autoantibody-negative sera (Cohen κ, 0.95, 0.84, and 0.78).

From the Department of Dermatology,[a] Lübeck Institute of Experimental Dermatology,[b] and Human Immunophenotyping Laboratory,[c] University of Lübeck; Institute of Experimental Immunology, Euroimmun, Lübeck[d]; Molecular and Cell Biology Laboratory, Rome[e]; Autoimmune Blistering Dermatoses Section, Department of Dermatology, Poznan University of Medical Sciences[f]; Department of Dermatology and Venereology, Medical Faculty of Medicine, Sofia[g]; Dermatology Department Tel Aviv, Sourasky Medical Center[h]; University Institute of Clinical Chemistry and Center of Laboratory Medicine, Inselspital, Bern[i]; Dermatology and Immunodermatology, Medical University of Warsaw[j]; Department of Dermatology, University of Belgrade, School of Medicine Pasterova 2[k]; Department of Dermatology St George Hospital, University of New South Wales School of Medicine, Sydney[l]; Second University Dermatology Department, Aristotle University School of Medicine, Papageorgiou General Hospital, Thessaloniki[m]; and Akdeniz University Faculty of Medicine Department of Dermatology, Antalya.[n]

Drs van Beek and Dähnrich contributed equally to this article.

Supported by the Schleswig-Holstein Cluster of Excellence Inflammation at Interfaces (DFG EXC306/2), the Clinical Research Unit 303 *Pemphigoid Diseases* (SCHM 1686/7-1, BE 5866/1-1), and the Australasian Blistering Diseases Foundation (ABDF2013/HYCZNCMKDM/1).

Disclosure: Drs Schmidt and Zillikens have a scientific collaboration with Euroimmun. Drs Dähnrich and Johannsen are employed at Euroimmun. Dr Dähnrich is a shareholder of Euroimmun. Dr Schlumberger is vice-president and shareholder of Euroimmun. Drs van Beek, Lemcke, Goletz, Hübner, Di Zenzo, Dmochowski, Drenovska, Geller, Horn, Kowalewski, Medenica, Murrell, Patsatsi, Uzun, and Vassileva have no conflicts of interest to declare.

Supplemental material is available at http://www.jaad.org.

Accepted for publication November 3, 2016.

Reprint requests: Enno Schmidt, MD, PhD, Lübeck Institute of Experimental Dermatology, University of Lübeck, Ratzeburger Allee 160, D-23538, Lübeck, Germany. E-mail: enno.schmidt@uksh.de.

Published online December 28, 2016.

0190-9622/$36.00

http://dx.doi.org/10.1016/j.jaad.2016.11.002

Limitations: IgA autoantibodies and less common target antigens were not analyzed.

Conclusions: The multivariant ELISA is a practical, highly standardized, and widely available novel diagnostic tool for the routine diagnosis of AIBD. (J Am Acad Dermatol http://dx.doi.org/10.1016/j.jaad.2016.11.002.)

Key words: autoantibody; blistering; bullous pemphigoid; epidermolysis bullosa acquisita; pemphigus.

CAPSULE SUMMARY

- Serologic diagnosis of autoimmune blistering diseases usually follows a sophisticated multistep algorithm.
- A novel multivariant enzyme-linked immunosorbent assay shows concordant results to the conventional multistep algorithm in the routine diagnosis of autoimmune blistering diseases.
- The multivariant enzyme-linked immunosorbent assay is a practical, highly standardized, and widely available novel diagnostic tool for the routine diagnosis of autoimmune blistering diseases.

Autoimmune blistering diseases (AIBDs) are clinically characterized by blisters and erosions on the skin and surface-close mucosal epithelia. Most patients cannot be differentiated based on the clinical picture alone and a large variety of clinical variations has been described.[1,2] Diagnosis of AIBDs relies on the clinical picture and the detection of skin-/mucous membrane-bound and circulating autoantibodies. Although the visualization of skin-/mucous membrane-bound autoantibodies by direct immunofluorescence (IF) microscopy is still the diagnostic gold standard,[1,2] serologic diagnosis using standardized widely available enzyme-linked immunosorbent assay (ELISA) based on the recombinant target antigens is increasingly applied.[3-12]

Conventional serologic diagnosis of AIBDs is usually performed as multistep procedure and includes an initial screening step by indirect IF microscopy on frozen tissue sections, eg, monkey esophagus (for pemphigus and dermatitis herpetiformis) and human salt-split skin (for pemphigoid diseases).[1-4] The test systems used for the subsequent target antigen identification differ between laboratories. The above-mentioned ELISA systems are most widely used.

Here, to facilitate and further standardize the serologic diagnosis of AIBDs, a multivariant ELISA was developed using the recombinant immunodominant forms of 6 major target antigens of pemphigus (desmoglein 1, desmoglein 3, envoplakin) and pemphigoid diseases (BP180, BP230, type VII collagen).

METHODS

Human sera

The multivariant ELISA was evaluated using sera from 3 different cohorts. The studies were approved by the ethics committee of the University of Lübeck (12-178, 11-078) and the corresponding local ethics committees of the study centers. All sera were stored at −20°C until used.

Validation cohort

In this cohort, sera (n = 173) from patients with bullous pemphigoid (n = 20), pemphigus foliaceus (n = 20), pemphigus vulgaris (n = 20), linear IgA disease (n = 15), paraneoplastic pemphigus (n = 28), and epidermolysis bullosa acquisita (n = 70) were included. All patients had clinical disease compatible with the respective AIBD, were positive by direct IF microscopy of a perilesional biopsy specimen, and had circulating autoantibodies as detected by indirect IF microscopy on monkey esophagus, salt-split human skin, and/or rat/monkey bladder, respectively, as described previously.[5,13,14] Moreover, sera from patients with noninflammatory skin diseases (n = 30) and healthy blood donors (n = 100) were included.

Multicenter cohort

Sera from patients with newly diagnosed AIBD and positive direct IF microscopy (n = 204) were randomly selected from a cohort prospectively assembled in a multicenter approach. Sera were taken at the time of diagnosis before treatment was initiated.

Monocenter cohort

Consecutive sera were prospectively collected from patients with clinical suspicion of AIBD (n = 292). Sera were sent to the autoimmune routine laboratory of the Department of Dermatology, University of Lübeck, for evaluation.

Multivariant ELISA

The novel multivariant ELISA comprises 6 different antigens, BP180, BP230, type VII collagen, desmoglein 1, desmoglein 3, and envoplakin. Except the type VII collagen ELISA, all individual ELISA