

Serological Biomarkers and Their Detection in Autoimmune Bullous Skin Diseases

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ABSTRACT Autoimmune bullous diseases (AIBDs) are a group of skin-related disorders that involve damage to structures maintaining cell-cell adhesion, such as desmosomes and hemidesmosomes. Key AIBDs include pemphigus related diseases, pemphigoid related conditions, acquired epidermolysis bullosa (EBA), and dermatitis herpetiformis (DH). Each group of conditions exhibits characteristic clinical lesion patterns and is associated with specific autoantibodies targeting epidermal and dermal structures involved in cell-cell adhesion and skin integrity. Pemphigus diseases primarily target desmoglein (Dsg) 3 and Dsg1 proteins but several non-Dsg autoantibodies have also been linked to pemphigus.

Pemphigoid diseases typically target bullous pemphigoid (BP)180 and BP230; EBA is associated with antibodies directed against anti-type VII collagen and DH by IgA autoantibodies against tissue transglutaminase and deaminated gliadin. Investigation into the serological biomarkers found in AIBDs have allowed the development of diagnostic assessments (i.e. tissue antibody detection and serological testing) based on the unique autoantibody profiles of a particular disease group. The methods for the detection and quantification of disease-associated autoantibodies continue to evolve and improve.

Introduction

The skin is the largest organ of the body, protecting us from the environment, regulating body temperature and permitting the sensation of touch. It is divided into 3 main layers: the outermost epidermis layer, the middle dermis layer and the lower hypodermis layer [1]. The epidermis is mainly made up of keratin producing cells called keratinocytes. The

protective barrier, regulation of epidermal temperature and nutrients and other functions of the epidermis are dependent upon the maintenance of stable connections between keratinocytes and other epidermal structures primarily mediated by adhesive desmosomal and hemidesmosomal proteins [2].

Desmosomes are specialized tight junctions critical to cellular adhesion (Figure 1). They are arranged on adjacent sides of plasma membranes and can be seen in tissues

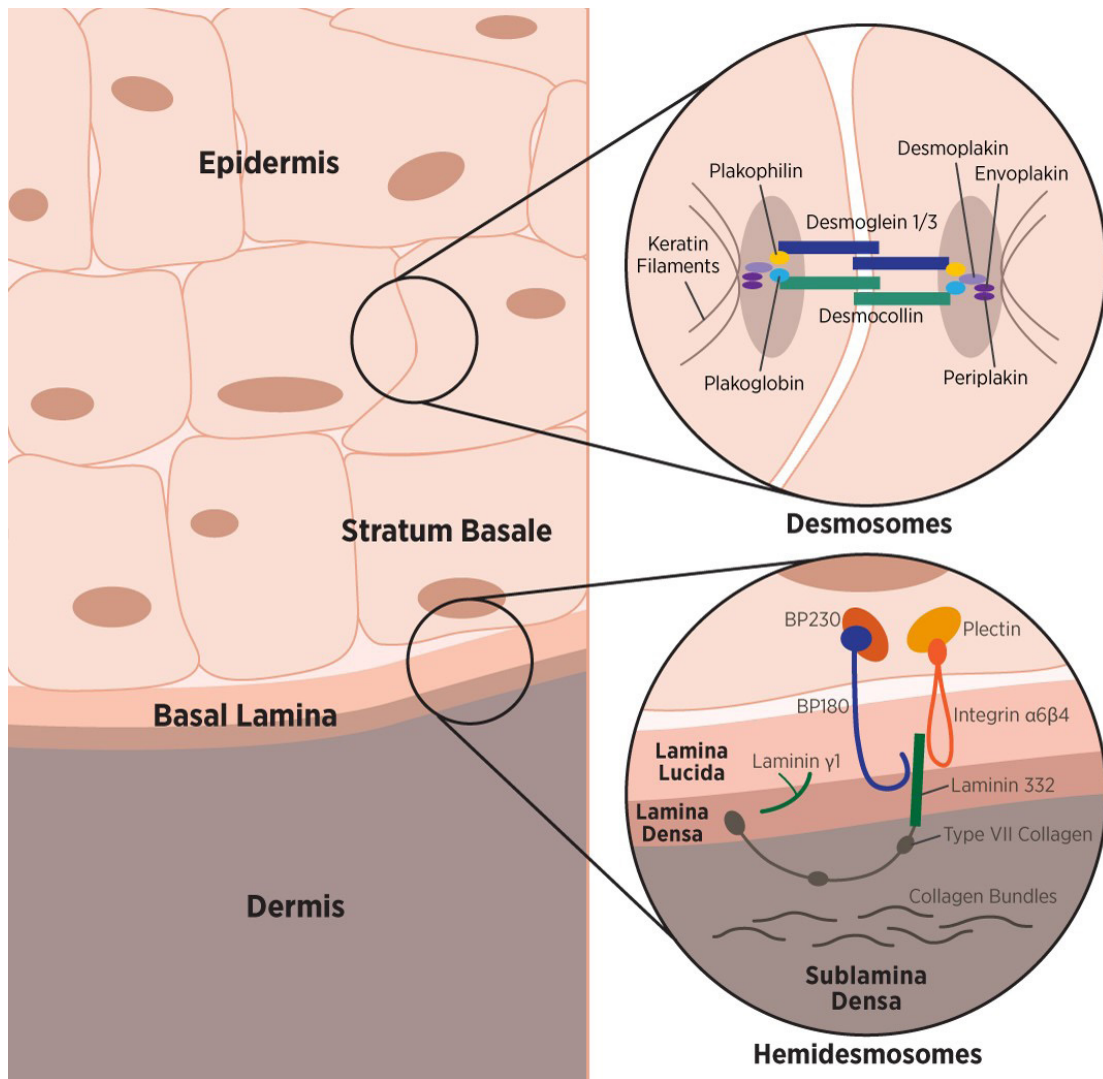


Figure 1. Graphical representation of human skin. Desmosomes connect adjacent keratinocytes and are critical to cellular adhesion (top circle within desmosomes: there is a vast network of cadherin proteins [desmogleins, desmocollins and desmoplakins]), linker proteins [eg plakoglobin, plakophilin] and keratin intermediate filaments). Hemidesmosomes facilitate the adhesion of basal keratinocytes to the underlying basal lamina. Key hemidesmosomal-associated proteins include the cytoplasmic protein BP230, the transmembrane protein BP180, laminin 332 and collagen type VII.

including cardiac muscle, gastrointestinal mucosa, and epithelia, all of which can be subject to significant mechanical stress during normal physiological and disease states [3].

Within desmosomes, there is a vast network of cadherin proteins (desmogleins, desmocollins and desmoplakins), linker proteins (eg plakoglobin, plakophilin) and keratin intermediate filaments, that connect as desmosome-intermediate filament complexes (DIFCs). Desmoplakin, which coordinates other cadherin proteins and keratin filaments, is the most prevalent protein within the desmosome [4].

Hemidesmosomes resemble tiny stud-shaped structures and are similar in shape to desmosomes. However, there are several differences between these 2 structural components. Hemidesmosomes attach keratinocytes to the extracellular matrix and utilize integrins rather than desmogleins and desmocollins. Key hemidesmosomal-associated proteins include the cytoplasmic protein BP230, the transmembrane protein

BP180, laminin 332 and collagen type VII [5]. Another feature of hemidesmosomes is their role in signaling pathways, relevant for the migration of keratinocytes (Figure 1) [6].

Autoimmunity involves the presence of antibodies (produced by B lymphocytes) and T lymphocytes that have escaped mechanisms of self-tolerance, both centrally and peripherally, that are reactive to one's own self-antigens. When auto-reactive lymphocytes cause enough target tissue damage, autoimmune disease can occur.

There are over 80 human autoimmune diseases in existence, affecting over 20 million Americans [7]. Indicators of autoimmunity (ie antinuclear antibodies) suggest that the incidence of autoimmune disease has been increasing over the past few decades [8].

Collectively, autoimmune diseases present a tremendous, and likely under-estimated burden on healthcare costs: over \$100 billion annually in the United States [9]. These costs

reflect a multitude of factors which are impacted by delays in diagnosis, poor or infrequent monitoring of disease activity leading to less than optimal disease management.

Systemic autoimmune diseases affect multiple organs, whereas organ-specific diseases target a single organ such as the skin. Autoimmune diseases that affect the skin include vitiligo, scleroderma, lupus, psoriasis, vasculitis, and autoimmune bullous dermatoses (AIBDs). AIBDs are a collection of autoimmune skin specific disease characterized by the production of autoantibodies against structural components of the skin including desmosomes and hemidesmosomes [10]. Such an autoimmune reaction interferes with intercellular connections within the epidermis in addition to the crucial linkage between the epidermis and the dermis. AIBDs manifest as skin layer separation and blistering and are divided into 4 main groups according to their target antigens and localization of the blisters: pemphigus diseases, pemphigoid diseases, acquired epidermolysis bullosa (EBA), and dermatitis herpetiformis (DH) (Table 1) [10].

Clearly, there is need for a greater understanding of the epidemiology, pathophysiology, and natural history of AIBD. The continued evolution of methods for the reliable, accessible, and cost-effective detection of disease relevant autoantibodies is an ongoing endeavor to improve our ability to diagnose and monitor autoimmune activity, with impact in clinical management and decision-making accurately and rapidly.

Objectives

Here, we discuss the key autoantigens in AIBDs and highlight how serological testing can be used in conjunction with clinical symptoms for diagnostic purposes.

Pemphigus diseases

Pemphigus diseases are commonly characterized by the production of autoantibodies primarily against the desmosomal proteins desmoglein (Dsg)3 and Dsg1 which results in the loss of epidermal cell-cell adhesion and subsequent blister formation [11]. Some patients experience only mucous membrane erosions with minimal skin blistering, others exhibit lesions on both mucosal as well as non-mucosal surfaces, while others still may only show skin involvement without mucous membrane involvement [11]. The clinical phenotype of pemphigus has been linked to defined Dsg3 and Dsg1 antibody profiles [12]. Additionally, differences in the normal tissue distribution of Dsg1 and Dsg3 proteins (Dsg1 on the epidermal surface and Dsg3 in deep epidermal layers/mucous membranes) may explain the varying clinical manifestations of different pemphigus forms [13]. For example, in pemphigus foliaceus (PF), IgG antibodies are only directed against Dsg1 and blistering is confined to the skin surface. On the other hand, in pemphigus vulgaris (PV), autoantibodies against both Dsg1 and Dsg3 can be observed, and

Table 1. Target antigens in autoimmune bullous dermatoses

AIBD Subtype	Blister Location	Target Antigen ^a	Ig Type
Pemphigus			
PV (mucosal-dominant type)	Intraepidermal	Dsg3	IgG
PV (mucocutaneous type)	Intraepidermal	Dsg3, Dsg1, Dsc1, Dsc2, Dsc3	IgG
IgA Pemphigus	Intraepidermal	Dsg3, Dsg1, desmocollins	
PF	Intraepidermal	Dsg1	IgG
PNP	Intraepidermal	Envoplakin, Dsg3, Dsg1, periplakin, epiplakin, plectin, desmoplakins, Dsc(1-3), BP230, α 2-macroglobulin-like 1	IgG
Pemphigoid			
BP	Subepidermal	BP180, BP230	IgG
MMP	Subepidermal	BP180, BP230, laminin332, integrin α 6/ β 4, and collagen VII	IgG
EBA	Subepidermal	Type VII collagen	IgG
DH	Subepidermal	Epidermal/tissue transglutaminase, endomysium, deamidated gliadin	IgA/IgG
Pemphigoid gestationis	Subepidermal	BP180, BP230	IgG
Linear IgA bullous dermatosis	Subepidermal	Ectodomain fragment of BP180, BP230	IgA

^aMain target antigens are indicated in bold.

AIBD = autoimmune bullous dermatoses; PV = pemphigus vulgaris; PF = pemphigus foliaceus; Dsc = desmocollins; PNP = paraneoplastic pemphigus; BP = bullous pemphigoid; MMP = mucous membrane pemphigoid; EBA = epidermolysis bullosa acquisita; DH = dermatitis herpetiformis; Dsg = desmoglein.

the degree of blistering and mucous membrane involvement varies based on the prevalence of either anti-Dsg1 and anti-Dsg3 [12]. This framework, correlating the clinical presentation of pemphigus to antibody profile, is known as the Dsg compensation hypothesis (DCH), featured prominently in dermatology textbooks and previous research studies [14].

As elegant as the hypothesis may be, however, recent studies have identified exceptions to this hypothesis [15,16].

PV accounts for 80% of all pemphigus cases and mainly affects middle-aged and elderly populations [17]. While Dsg3 (89% - 90% of patients) and Dsg1 (50% - 60% of patients) are the major autoantigens in PV, additional structural and metabolic autoantigens have been identified including desmocolins (Dsc) 1 and 3, muscarinic and nicotinic acetylcholine receptors, mitochondrial antigens, thyroid peroxidase, hSPCA1, plakophilin 3, plakoglobin, and E-cadherin [18]. Studies have shown that autoantibodies against these additional targets may complement the effects of anti-Dsg autoantibodies and explain individual variations in pemphigus disease severity [18].

In paraneoplastic pemphigus (PNP), autoantibodies are directed against desmosomes including Dsg1 and Dsg3, α 2-macroglobulin-like 1, and the plakins envoplakin, desmoplakin I and II, plectin, periplakin and the hemidesmosome BP230. The presence of desmoplakin autoantibodies is also common to PV, PF, and BP. However, autoantibodies for envoplakin and periplakin on immunoblot, as well as autoantibodies for desmoplakin (on indirect immunofluorescence and rat bladder epithelium), appear to be sensitive and specific for PNP diagnosis [19]. This has led to the development of an enzyme-linked immunosorbent assay (ELISA) that detects envoplakin in consideration for a diagnostic tool for PNP.

PNP is associated in a majority of cases with non-Hodgkin lymphoma, chronic lymphocytic leukemia and Castleman disease [20]. A common clinical feature is stomatitis which presents with painful erosions and ulcerations of the oropharynx. Anti-envoplakin antibodies are highly specific for PNP and are used for the differentiation of PNP from other AIBDs [21].

In IgA pemphigus, a rare form of pemphigus with unclear etiology, serum IgA autoantibodies are associated with reactivity against the desmosomal cadherins Dsc1, Dsc2, Dsc3, Dsg1, and Dsg3 [17]. These circulating IgA antibodies lead to formation of pruritic and painful eruptions that present as vesicles and pustules on the skin [22]. As IgA pemphigus is so rare, there is currently no reported sex, age, or race distribution of this disease. However, IgA pemphigus has been observed in all age demographics [23].

Pemphigoid diseases

Pemphigoid diseases are characterized by subepidermal blister formation in the skin and mucous membranes [24]. Pemphigoid

diseases occur when the immune system produces autoantibodies against proteins involved in the linkage between the epidermis and dermis. As a result of this autoimmune reaction, the epidermal layer separates from the dermis. Several different types of pemphigoid diseases exist including bullous pemphigoid (BP), pemphigoid gestationis, mucous membrane pemphigoid, linear IgA dermatosis and p200 pemphigoid [10].

The hemidesmosomal proteins, BP180 and BP230, which tether the 2 skin layers together, are the common autoantibody targets in BP. BP is the most common AIBD in the general population, with an annual incidence ranging between 2.3 to 23 cases per million. BP disproportionately affects elderly people, with an incidence of 190-312 cases per million among those 80 years and older [14]. This disease manifests with bulging skin blisters and minimal mucous membrane involvement [25]. Unlike pemphigus, BP shows a negative Nikolsky sign (ie no splitting of skin upon applying pressure) [26].

Autoantibodies against BP180 represent the most significant biomarker in BP due to their high prevalence [27]. Additional screening for anti-BP230 antibodies is important as they occur in 40% of patients who are seronegative for anti-BP180 antibodies. The parallel detection of both anti-BP230 antibodies and anti-BP180 antibodies increases the sensitivity of BP detection significantly, to a combined 97.1% [28]. Pemphigoid gestationis is the manifestation of BP in pregnant women and is characterized by autoantibodies predominately against epitopes in the immunodominant NC16A domain of BP180 (BP180-NC16A) [29]. In children, linear IgA dermatosis occurs from the autoantibody recognition of the ectodomain fragment of BP180 [30]. In addition to BP180, laminin 332 is a major target in mucous membrane pemphigoid (MMP) [31]. Additionally, patients with mucous membrane pemphigoid may show antibodies against BP230, integrin α 6/ β 4, and collagen VII [31,32]. The identification of anti-laminin 332 is important for determining a patient's prognosis as anti-laminin 332 positive patients seem to be at an increased risk of malignancies [33].

Epidermolysis bullosa acquisita

EBA is a severe blistering dermatosis characterized by autoantibodies against type VII collagen [34]. EBA manifests as subepidermal blisters and erosions in response to the minor irritation of skin and affects both the skin and mucous membranes. The level of the cleavage in the basal membrane contributes to the various phenotypes of EBA, including the most common inflammatory and mechanobullous (noninflammatory) variants [35].

Dermatitis herpetiformis

DH is an itchy dermatosis affecting 10% of celiac patients. It manifests as blisters in the subepidermis of areas such as the

elbows, knees, and buttocks. There is also minimal blistering of the mucous membranes. DH is one of many manifestations of gluten-sensitivity and is characterized by IgA autoantibodies against endomysium tissue/epidermal transglutaminase (anti-tTG/-eTG) and/or deamidated gliadin (IgA/IgG) [36]. In contrast to the increase in diagnosis of celiac disease, DH incidence appears to be decreasing (Table 1) [37].

Diagnostic approach

The diagnosis of AIBDs requires the detection of both circulating and tissue-bound antibodies, and histopathology, in conjunction with clinical symptoms [38]. The pathway to AIBD diagnosis can be broken down into 4 pillars. First, the clinical manifestations of the disease must be assessed. Second, histopathology can be performed to provide information on the location of skin involvement (sub- or intraepidermal separation). Third, the detection of tissue bound autoantibodies by direct immunofluorescence (DIF) is done. DIF is the current diagnostic gold standard for AIBDs but gives limited information on the target antigens. DIF has a sensitivity of 82% - 91% and a specificity of 98% [17]. The fourth pillar is the identification of autoantibodies by serological testing such as indirect immunofluorescence (IIF) microscopy, monospecific ELISA or immunoblot techniques.¹⁷

Serological testing for the detection of circulating antibodies in AIBDs has the advantage of being minimally invasive and may be suitable for diagnostic purposes in conjunction with the clinical manifestations, and for aiding therapy decisions and disease prognosis [17]. Conventional serological detection of AIBD-specific antibodies involves an initial IIF screen using tissue substrates, followed by an antigen-specific assay such as ELISA.

In 2016, the International Bullous Diseases Consensus Group met to standardize the diagnosis and management of pemphigus [39]. The diagnosis of pemphigus was agreed to be based on the clinical presentation and histopathology consistent with pemphigus and either a positive DIF microscopy or serologic detection of autoantibodies against epithelial cell surface antigens [39]. The determination of serum autoantibodies was recommended for therapeutic decision making as serum levels of IgG against Dsg1 and Dsg3 correlate with the clinical activity of pemphigus.

AIBD autoantibody screening using tissue IIF

Due to their high sensitivity, tissue substrates are ideal for screening for AIBDs autoantibodies (esophagus, salt-split skin, bladder mucosa) [40]. The esophagus substrate yields characteristic honeycomb-like immunofluorescence patterns which can be differentiated when screening for antibodies in suspected cases of PV or PF. IIF using esophagus as a substrate has proven to be useful for the detection of

autoantibodies against Dsg1 and Dsg3, with a sensitivity of 81% - 100% and a specificity of 89% - 100% [17,41]. For the differentiation of autoantibodies in subepidermal AIBDs, tissue sections of salt-split skin are used [42]. Salt-split skin substrate has a sensitivity of 73% - 96% and a specificity of 97% for such subepidermal antibodies. Additionally, as antibodies have varying antigenic binding properties on salt-split skin, this allows for the differentiation between the subepidermal AIBDs BP, pemphigoid gestationis, linear IgA dermatosis and other subepidermal AIBDs such as EBA, and anti-laminin-332-type MMP. Where BP180 and BP230 are located on the epidermal side of salt-split skin, collagen type VII and laminin 332 remain on the dermal side.

Urinary bladder is an ideal substrate for distinguishing between PNP and other pemphigus diseases as plakins like envoplakin are highly expressed in the bladder while Dsg1 and Dgs3 are not [43]. Urinary bladder is therefore a highly specific substrate for PNP (99% - 100%) and having a sensitivity of 74% [17]. Finally, liver tissue is useful for the detection of IgA autoantibodies against endomysium in DH [44].

The International Bullous Diseases Consensus Group recommends using IIF microscopy on monkey esophagus or human skin to detect autoantibodies against surface proteins of epidermal keratinocytes [39]. In cases of atypical presentation or the suspicion of another AIBD, the use of IIF microscopy on rat bladder and immunoblot/immunoprecipitation is discussed. They also describe the use of recombinantly expressed Dsg1, Dsg3, or envoplakin substrates (EUROIMMUN) when Dsg- or envoplakin-specific ELISA cannot be used [39].

Antigen-specific detection of AIBDs

The detection of antigen-specific autoantibodies in AIBDs can be achieved using monospecific IIF and ELISAs [17]. Monospecific IIF can be accomplished using transfected cells as a substrate in which the target antigen has been recombinantly expressed. Additionally, designer antigens have been created to enhance diagnostic sensitivity and specificity of IIF. Such purified recombinant designer antigens are utilized as monospecific IIF substrates. BIOCHIPS, which are coated with an IIF substrate and arranged onto microscope slides, allow for autoantibody screening and confirmatory discrimination in a single incubation. In this way, various types of AIBDs can be screened for in one test. IIF BIOCHIP mosaics contain combinations of different substrates (esophagus, salt-split skin, bladder mucosa, transfected cells, purified designer antigens). A study which compared the performance of the "Dermatology Mosaic 7" with a multi-step serum analysis using single antibody tests, found a 94% diagnostic agreement between both methods. Therefore, multiparametric BIOCHIP mosaics offer a cost and time effective IIF method.

Autoantibodies can be mono-specifically identified using ELISAs which utilize purified recombinant proteins [17]. Commercial assays which utilize the recombinant ectodomains of Dsg1 and Dsg3 have a high sensitivity and specificity for the detection of pemphigus foliaceus and pemphigus vulgaris (96% - 100%, 96% - 100% and 85% - 100%, 96 - 100%, respectively) [17]. In addition to their use as monospecific substrates in IIF, designer antigens have been developed for ELISAs (for the detection of antibodies against BP180 and BP230, deamidated gliadin peptides) to improve immunoreactivity. ELISA techniques provide quantitative measurement which is useful for the application of therapy monitoring. Profile ELISAs containing a combination of antigens enables the simultaneous detection of multiple AIBDs subtypes in patients with suspected AIBDs.

Experts recommend determining anti-Dsg1 and/or anti-Dsg3 IgG antibodies by ELISA for the detection of PF and mucosal/mucocutaneous PV (Mannose-Binding Lectin, EUROIMMUN) [39]. Serum concentrations of antibodies against Dsg1 and Dsg3 are associated with pemphigus disease activity in and high levels of anti-Dsg1 by ELISA has a positive predictive value for skin relapses. Therefore, the determination of serum autoantibodies against skin structural proteins by ELISA has a prognostic value for guiding pemphigus treatment.

Conclusions

A definitive diagnosis of AIBD is based on a combination of clinical signs and symptoms and the analysis of autoantibodies using IIF and ELISA. IIF, using various tissue substrates, is a useful application for antibody screening while transfected cells and purified antigen substrates are suitable for antigen-specific IIF. ELISA allows for the quantitative measurement of antibody levels to support the detection of different AIBD subtypes. Serological antibody testing is important for distinguishing between the various AIBD subtypes due to differences in their prognosis and treatment.

Immunologic testing has also a key role in providing an accurate diagnosis as blistering skin diseases are easily misdiagnosed. Oral blisters are often misdiagnosed as an infection such as candidiasis or herpes. Without a proper diagnosis, a patient is at risk of being mistreated, potentially with a chronic overexposure to steroids which may reduce some symptoms without fully addressing the underlying problem.

Additionally, serological testing allows for the monitoring of AIBD disease. Serum levels of anti-BP180 antibodies correlate with disease activity of BP while anti-BP230 levels correlate with the disease duration [45]. Moreover, levels of anti-Dsg1 and Dsg3 are associated with severity of pemphigus diseases and response to therapy while anti-envoplakin titers correlate with the degree of PNP symptoms as well as

differential diagnostic clarification [46]. The detection of anti-collagen type VII antibodies aids in the detection of EBA and allows for the differentiation of EBA from other AIBDs [47,48]. In addition to disease monitoring, correlations between lowered levels of AIBDs specific autoantibodies in response to therapy point to the use of serological testing for therapy monitoring purposes [49,50].

Continued efforts to develop and deploy increasingly accurate and multi-parameter methods for the detection of the comprehensive set of AIBD-associated autoantibodies can be expected to enhance diagnostic efforts and further our understanding of disease mechanisms, progression and response to therapy.

References

1. Yousef H, Alhadj M, Sharma S. Anatomy, Skin (Integument), Epidermis. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.
2. Schmidt A, Koch PJ. Desmosomes: just cell adhesion or is there more? *Cell Adh Migr.* 2007;1(1):28-32. DOI: 10.4161/cam.1.1.4204. Epub 2007 Jan 26. PMID: 19262094. PMCID: PMC2633677.
3. Delva E, Tucker DK, Kowalczyk AP. The desmosome. *Cold Spring Harb Perspect Biol.* 2009;1(2):a002543. DOI: 10.1101/cshperspect.a002543. PMID: 20066089. PMCID: PMC2742091.
4. Garrod D, Chidgey M. Desmosome structure, composition and function. *Biochim Biophys Acta.* 2008;1778(3):572-87. DOI: 10.1016/j.bbamem.2007.07.014. PMID: 17854763.
5. Borradori L, Sonnenberg A. Structure and Function of Hemidesmosomes: More Than Simple Adhesion Complexes. *J Invest Dermatol.* 1999;112(4):411-418. DOI: 10.1046/j.1523-1747.1999.00546.x. PMID: 10201522.
6. Harrison OJ, Brasch J, Lasso G, et al. Structural basis of adhesive binding by desmocollins and desmogleins. *Proc Natl Acad Sci U S A.* 2016;113(26):7160-7165. DOI: 10.1073/pnas.1606272113. PMID: 27298358. PMCID: PMC4932976.
7. Rose NR. Autoimmune Diseases. In: Quah SR, ed. International Encyclopedia of Public Health (Second Edition). Oxford: Academic Press; 2017:192-195.
8. Dinse G, Parks C, Weinberg C, et al. Increasing Prevalence of Antinuclear Antibodies in the United States. *Arthritis Rheumatol.* 2020;72(6):1026-1035. DOI: 10.1002/art.41214. PMID: 32266792. PMCID: PMC7255943.
9. Julian MK. Autoimmune disease: Cost-effective care. *Nurs Manage.* 2014;45(11):24-29; quiz 30. DOI: 10.1097/01.NU-MA.0000455740.32485.9c. PMID: 25304753.
10. Egami S, Yamagami J, Amagai M. Autoimmune bullous skin diseases, pemphigus and pemphigoid. *J Allergy Clin Immunol.* 2020;145(4):1031-1047. DOI: 10.1016/j.jaci.2020.02.013. PMID: 32272980.
11. Kasperkiewicz M, Ellebrecht CT, Takahashi H, et al. Pemphigus. *Nat Rev Dis Primers.* 2017;3:17026. DOI: 10.1038/nrdp.2017.26. PMID: 28492232. PMCID: PMC5901732.
12. Amagai M, Tsunoda K, Zillikens D, Nagai T, Nishikawa T. The clinical phenotype of pemphigus is defined by the anti-desmoglein autoantibody profile. *J Am Acad Dermatol.* 1999;40(2 Pt 1):167-170. DOI: 10.1016/s0190-9622(99)70183-0. PMID: 10025740.

13. Amagai M, Koch PJ, Nishikawa T, Stanley JR. Pemphigus vulgaris antigen (desmoglein 3) is localized in the lower epidermis, the site of blister formation in patients. *J Invest Dermatol*. 1996;106(2):351-355. DOI: 10.1111/1523-1747.ep12343081. PMID: 8601740.
14. Mahoney MG, Wang Z, Rothenberger K, Koch PJ, Amagai M, Stanley JR. Explanations for the clinical and microscopic localization of lesions in pemphigus foliaceus and vulgaris. *J Clin Invest*. 1999;103(4):461-468. DOI: 10.1172/JCI5252. PMID: 10021453. PMCID: PMC408100.
15. Sinha AA, Sajda T. The Evolving Story of Autoantibodies in Pemphigus Vulgaris: Development of the “Super Compensation Hypothesis”. *Front Med (Lausanne)*. 2018;5:218. DOI: 10.3389/fmed.2018.00218. PMID: 30155465. PMCID: PMC6102394.
16. Ahmed AR, Carrozzo M, Caux F, et al. Monopathogenic vs multipathogenic explanations of pemphigus pathophysiology. *Exp Dermatol*. 2016;25(11):839-846. DOI: 10.1111/exd.13106. PMID: 27305362.
17. Saschenbrecker S, Karl I, Komorowski L, et al. Serological Diagnosis of Autoimmune Bullous Skin Diseases. *Front Immunol*. 2019;10:1974. DOI: 10.3389/fimmu.2019.01974. PMID: 31552014. PMCID: PMC6736620.
18. Amber KT, Valdebran M, Grando SA. Non-Desmoglein Antibodies in Patients With Pemphigus Vulgaris. *Front Immunol*. 2018;9:1190. DOI: 10.3389/fimmu.2018.01190. PMID: 29915578. PMCID: PMC5994403.
19. Powell JG, Grover RK, Plunkett RW, Seiffert-Sinha K, Sinha AA. Evaluation of a Newly Available ELISA for Envoplakin Autoantibodies for the Diagnosis of Paraneoplastic Pemphigus. *J Drugs Dermatol*. 2015;14(10):1103-1106. PMID: 26461820.
20. Anhalt GJ. Paraneoplastic pemphigus. *J Investig Dermatol Symp Proc*. 2004;9(1):29-33. DOI: 10.1111/j.1087-0024.2004.00832.x. PMID: 14870982.
21. Poot AM, Diercks GF, Kramer D, et al. Laboratory diagnosis of paraneoplastic pemphigus. *Br J Dermatol*. 2013;169(5):1016-1024. DOI: 10.1111/bjd.12479. PMID: 23796242.
22. Hashimoto T, Teye K, Hashimoto K, et al. Clinical and Immunological Study of 30 Cases With Both IgG and IgA Anti-Keratinocyte Cell Surface Autoantibodies Toward the Definition of Intercellular IgG/IgA Dermatitis. *Front Immunol*. 2018;9:994. DOI: 10.3389/fimmu.2018.00994. PMID: 29867971. PMCID: PMC5950707.
23. Kridin K. Pemphigus group: overview, epidemiology, mortality, and comorbidities. *Immunol Res*. 2018;66(2):255-270. DOI: 10.1007/s12026-018-8986-7. PMID: 29479654.
24. Miyamoto D, Santi CG, Aoki V, Maruta CW. Bullous pemphigoid. *An Bras Dermatol*. 2019;94(2):133-146. DOI: 10.1590/abd1806-4841.20199007. PMID: 31090818. PMCID: PMC6486083.
25. Kridin K, Ludwig RJ. The Growing Incidence of Bullous Pemphigoid: Overview and Potential Explanations. *Front Med (Lausanne)*. 2018;5:220. DOI: 10.3389/fmed.2018.00220. PMID: 30177969. PMCID: PMC6109638.
26. Kershenovich R, Hodak E, Mimouni D. Diagnosis and classification of pemphigus and bullous pemphigoid. *Autoimmun Rev*. 2014;13(4-5):477-481. DOI: 10.1016/j.autrev.2014.01.011. PMID: 24424192.
27. Ishiura N, Fujimoto M, Watanabe R, et al. Serum levels of IgE anti-BP180 and anti-BP230 autoantibodies in patients with bullous pemphigoid. *J Dermatol Sci*. 2008;49(2):153-161. DOI: 10.1016/j.jdermsci.2007.08.008. PMID: 17920818.
28. Yoshida M, Hamada T, Amagai M, et al. Enzyme-linked immunosorbent assay using bacterial recombinant proteins of human BP230 as a diagnostic tool for bullous pemphigoid. *J Dermatol Sci*. 2006;41(1):21-30. DOI: 10.1016/j.jdermsci.2005.11.002. PMID: 16364599.
29. Shimanovich I, Skrobek C, Rose C, et al. Pemphigoid gestationis with predominant involvement of oral mucous membranes and IgA autoantibodies targeting the C-terminus of BP180. *J Am Acad Dermatol*. 2002;47(5):780-784. DOI: 10.1067/mjd.2002.113677. PMID: 12399776.
30. Schumann H, Baetge J, Tasanen K, et al. The shed ectodomain of collagen XVII/BP180 is targeted by autoantibodies in different blistering skin diseases. *Am J Pathol*. 2000;156(2):685-695. DOI: 10.1016/S0002-9440(10)64772-4. PMID: 10666397. PMCID: PMC1850053.
31. Chiorean R, Danescu S, Virtic O, et al. Molecular diagnosis of anti-laminin 332 (epiligrin) mucous membrane pemphigoid. *Orphanet J Rare Dis*. 2018;13(1):111. DOI: 10.1186/s13023-018-0855-x. PMID: 29980216. PMCID: PMC6035451.
32. Kamaguchi M, Iwata H. The Diagnosis and Blistering Mechanisms of Mucous Membrane Pemphigoid. *Front Immunol*. 2019;10:34. DOI: 10.3389/fimmu.2019.00034. PMID: 30740099. PMCID: PMC6357922.
33. Egan CA, Lazarova Z, Darling TN, Yee C, Coté T, Yancey KB. Anti-epiligrin cicatricial pemphigoid and relative risk for cancer. *Lancet*. 2001;357(9271):1850-1851. DOI: 10.1016/S0140-6736(00)04971-0. PMID: 11410196.
34. Mehren CR, Gniadecki R. Epidermolysis bullosa acquisita: current diagnosis and therapy. *Dermatol Reports*. 2011;3(3):e38. DOI: 10.4081/dr.2011.e38. PMID: 25386290. PMCID: PMC4211502.
35. Kridin K, Kneiber D, Kowalski EH, Valdebran M, Amber KT. Epidermolysis bullosa acquisita: A comprehensive review. *Autoimmunity Reviews*. 2019;18(8):786-795. DOI: 10.1016/j.autrev.2019.06.007. PMID: 31181325.
36. Antiga E, Caproni M. The diagnosis and treatment of dermatitis herpetiformis. *Clin Cosmet Investig Dermatol*. 2015;8:257-265. DOI: 10.2147/CCID.S69127. PMID: 25999753. PMCID: PMC4435051.
37. Salmi TT. Dermatitis herpetiformis. *Clin Exp Dermatol*. 2019;44(7):728-731. DOI: 10.1111/ced.13992. PMID: 31093998.
38. Witte M, Zillikens D, Schmidt E. Diagnosis of Autoimmune Blistering Diseases. *Front Med (Lausanne)*. 2018;5:296. DOI: 10.3389/fmed.2018.00296. PMID: 30450358. PMCID: PMC6224342.
39. Murrell DF, Peña S, Joly P, et al. Diagnosis and management of pemphigus: Recommendations of an international panel of experts. *J Am Acad Dermatol*. 2020;82(3):575-585.e1. DOI: 10.1016/j.jaad.2018.02.021. PMID: 29438767. PMCID: PMC7313440.
40. Shetty VM, Subramaniam K, Rao R. Utility of immunofluorescence in dermatology. *Indian Dermatol Online J*. 2017;8(1):1-8. DOI: 10.4103/2229-5178.198774. PMID: 28217464. PMCID: PMC5297263.
41. Feibelman C, Stolzner G, Provost TT. Pemphigus Vulgaris: Superior Sensitivity of Monkey Esophagus in the Determination of Pemphigus Antibody. *Arch Dermatol*. 1981;117(9):561-562. DOI: 10.1001/archderm.117.9.561. PMID: 7027966.
42. Ghohestani RF, Nicolas JF, Rousselle P, Claudy AL. Diagnostic value of indirect immunofluorescence on sodium chloride-split

- skin in differential diagnosis of subepidermal autoimmune bullous dermatoses. *Arch Dermatol.* 1997;133(9):1102-1107. PMID: 9301587.
43. Liu AY, Valenzuela R, Helm TN, Camisa C, Melton AL, Bergfeld WF. Indirect immunofluorescence on rat bladder transitional epithelium: a test with high specificity for paraneoplastic pemphigus. *J Am Acad Dermatol.* 1993;28(5 Pt 1):696-699. DOI: 10.1016/0190-9622(93)70095-b. PMID: 7684408.
 44. Wolf J, Jahnke A, Fechner K, et al. Primate liver tissue as an alternative substrate for endomysium antibody immunofluorescence testing in diagnostics of paediatric coeliac disease. *Clin Chim Acta.* 2016;460:72-77. DOI: 10.1016/j.cca.2016.06.023. PMID: 27346479.
 45. Chanprapaph K, Ounsakul V, Pruettivorawongse D, Thadanipon K. Anti-BP180 and anti-BP230 enzyme-linked immunosorbent assays for diagnosis and disease activity tracking of bullous pemphigoid: A prospective cohort study. *Asian Pac J Allergy Immunol.* 2019; 39(4):272-278. DOI: 10.12932/AP-231118-0446. PMID: 31175713.
 46. Delavarian Z, Layegh P, Pakfetrat A, Zarghi N, Khorashadizadeh M, Ghazi A. Evaluation of desmoglein 1 and 3 autoantibodies in pemphigus vulgaris: correlation with disease severity. *J Clin Exp Dent.* 2020;12(5):e440-e445. DOI: 10.4317/jced.56289. PMID: 32509225. PMCID: PMC7263783.
 47. Koga H, Teye K, Yamashita K, Ishii N, Tsuruta D, Nakama T. Detection of anti-type VII collagen IgE antibodies in epidermolysis bullosa acquisita. *Br J Dermatol.* 2019;180(5):1107-1113. DOI: 10.1111/bjd.17310. PMID: 30311191.
 48. Kim JH, Kim YH, Kim S, et al. Serum levels of anti-type VII collagen antibodies detected by enzyme-linked immunosorbent assay in patients with epidermolysis bullosa acquisita are correlated with the severity of skin lesions. *J Eur Acad Dermatol Venereol.* 2013;27(2):e224-e230. DOI: 10.1111/j.1468-3083.2012.04617.x. PMID: 22731917.
 49. Mutasim DF. Therapy of autoimmune bullous diseases. *Ther Clin Risk Manag.* 2007;3(1):29-40. DOI: 10.2147/tcrm.2007.3.1.29. PMID: 18360613. PMCID: PMC1936286.
 50. Alaeen H, Toosi R, Mahmoudi H, et al. Short-term clinical and serological follow-up with conventional and conformational anti-desmoglein antibodies in treatment-naïve and previously treated patients with pemphigus vulgaris after receiving rituximab. *Int J Womens Dermatol.* 2019;5(5):372-377. DOI: 10.1016/j.ijwd.2019.05.008. PMID: 31909160. PMCID: PMC6938821.