



Research paper

Autoantibody detection in bullous pemphigoid: Clinical evaluation of the EUROPLUS™ Dermatology Mosaic

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ABSTRACT

Bullous pemphigoid (BP) is an autoimmune blistering skin disease. Autoantibodies to BP180 and BP230 can be detected by indirect immunofluorescence (IIF) on different substrates (oesophagus, salt-split-skin, BP180-antigen dots, BP230-transfected cells) and ELISA. Here, we compared test characteristics of these test systems.

We analysed sera from BP patients ($n=60$) in whom the clinical diagnosis had been confirmed histopathologically. The control cohort comprised sera from patients with other autoimmune-associated ($n=22$) or inflammatory ($n=35$) skin diseases. All samples were tested by IIF (EUROIMMUN™ Dermatology Mosaic) and ELISA (EUROIMMUN and MBL).

Anti-BP180 is best detected with BP180-antigen dots by IIF (sensitivity: 88%; specificity: 97%). As compared to IIF, the differences with both BP180 ELISA techniques are small though. Likelihood ratios (LRs) for positive and negative test results are >10 and between 0.1 and 0.2, respectively, for all test systems. Detection of anti-BP230 is highly variable (sensitivity range 38–60%; specificity range 83–98%). Only the IIF test reveals a LR for positive test results >10 . Since the LRs for a negative test are all ~ 0.5 , negative test results for anti-BP230 antibodies do not help to exclude BP.

In conclusion, the multi-parameter IIF test reveals a good diagnostic performance in BP. Since this test simultaneously allows for the detection of anti-Dsg1 and anti-Dsg3 antibodies, involved in pemphigus foliaceus and vulgaris, a single test-incubation may be sufficient to differentiate between the most frequent autoimmune blistering diseases.

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1. Introduction

Bullous pemphigoid (BP) is an acquired autoimmune blistering skin disease also affecting mucous membranes. BP is predominantly seen in the elderly, typically between the age of 60 and 80 years, and is characterized by the linear deposition of

IgG and C3 at the cutaneous basal membrane zone. In BP, autoantibodies are directed to two hemidesmosomal proteins, BP180 and BP230 (Schmidt and Zillikens, 2010; Sagi et al., 2011; Sticherling and Erfurt-Berge, 2012). BP180 is a type II transmembrane molecule. Autoantibodies typically react with the extracellular 16th non-collagenous (NC16A) domain of BP180 (Zillikens et al., 1997). BP230 is an intracellular component of the hemidesmosome. The globular C-terminal domain of BP230 is the main immunogenic target of the autoantibodies (Skaria et al., 2000).

Like in many other autoimmune diseases, in particular in systemic autoimmune diseases (Damoiseaux and Cohen Tervaert, 2006), screening for autoantibodies is performed by

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indirect immunofluorescence (IIF). If positive, and depending on the staining pattern, antigen-specific assays should be performed. In case of the blistering skin diseases two main patterns can be distinguished on an oesophagus substrate: an intercellular staining of the epidermis revealing a chickenwire appearance, or staining of the basal membrane delineating the border between dermis and epidermis. The first pattern is associated with pemphigus vulgaris and pemphigus foliaceus; the autoantibodies are directed to desmoglein (Dsg)1 and/or Dsg3 (Schmidt and Zillikens, 2010; Sagi et al., 2011; Sticherling and Erfurt-Berge, 2012). The basal membrane pattern is associated with BP. For detection of autoantibodies in BP, human skin, in which dermal–epidermal splitting was induced by incubation in a 1 M NaCl solution, has been identified as the optimal substrate (Kelly and Wojnarowska, 1988). Anti-BP180 and anti-BP230 antibodies typically bind to the roof, i.e., the epidermal site, of the salt-split skin substrate, but antigen-specificity needs to be confirmed.

As shown recently for antineutrophil cytoplasmic antibodies (ANCA), the biochip IIF methodology enables the use of multiple substrates, including antigen-specific substrates, in a single test (Damoiseaux et al., 2010). Likewise, the EUROPLUS™ Dermatology Mosaic combines monkey oesophagus sections, human salt-split skin sections, HEK293 cells transfected with Dsg1, Dsg3, and the C-terminal globular domain of BP230, respectively, and recombinant tetrameric NC16A spots (BP180). In the current study we evaluated the test characteristics of the EUROPLUS™ Dermatology Mosaic in relation to the commercially available ELISAs for anti-BP180 and anti-BP230 antibodies in a cohort of patients with BP (n = 60) and disease controls (n = 58).

2. Materials and methods

2.1. Patient sera

Within a time-frame of twelve years (1997–2009) patients with bullous pemphigoid (n = 60) were retrospectively identified based on the presence of typical clinical manifestations (tense bullae, filled with clear fluid, localized in the skin and mucosal surfaces) (Vaillant et al., 1998) and histopathological features (subepidermal, nonacantholytic blisters), as well as positive immunofluorescence findings in the skin biopsies (linear deposition of immunoglobulin and/or C3 along the basement membrane) (Schmidt and Zillikens, 2010). In addition two age- and gender-matched control cohorts were included (Table 1). The first control cohort consisted of patients with other autoimmune-associated skin diseases (n = 22), like pemphigus vulgaris/foliaceus (n = 8), subacute cutaneous systemic lupus erythematosus (SLE) or chronic discoid lupus erythematosus (n = 5), vasculitis (n = 4), psoriasis (n = 2), IgA

dermatosis (n = 1), and dermatitis herpetiformis (n = 2). The second control cohort consisted of patients with inflammatory skin diseases (n = 35), like eczema (n = 11), prurigo (n = 6), erythema (n = 3), and several other miscellaneous skin manifestations (n = 15). From all patients the serum obtained at the time of diagnosis, i.e. before possible immunosuppressive therapy was instituted, was used in the current study. Since sera were obtained for diagnostic purposes and the rest-serum was used for analyses in an anonymous way, ethical approval and informed consent was not necessary according to the Dutch guidelines.

2.2. Detection of autoantibodies by EUROPLUS™ Dermatology Mosaic

Anti-skin autoantibody detection by indirect immunofluorescence (IIF) was performed on the EUROPLUS™ Dermatology Mosaic according to manufacturer's instructions (EUROIMMUN AG, Lübeck, Germany). This mosaic contains six biochips as substrate: monkey oesophagus sections, 1 mol/L NaCl split human skin sections, HEK293 cells transfected with Dsg1, Dsg3, and the C-terminal globular domain of BP230, respectively, and recombinant tetrameric NC16A spots. Serum samples were diluted 1/10 (cut-off) and 1/100. All slides were evaluated simultaneously by two observers using a discussion microscope; in case of a difference in opinion a third observer was decisive. All six biochips were evaluated independent from each other and each result was recorded separately for the study. The observers were blinded from the diagnosis of the patients.

2.3. Detection of anti-BP180 and anti-BP230 antibodies by ELISA

Anti-BP180 antibodies were determined by two distinct ELISA systems. The first ELISA employs a single recombinant NC16A domain (MBL, Nagoya, Japan), while in the second ELISA four copies of the NC16A domain were fused to enhance epitope exposure (EUROIMMUN) (Kobayashi et al., 2002; Sitaru et al., 2007). Both ELISAs were performed according to the manufacturer's instructions. The cut-off values were 9 U/mL and 20 RU/mL, respectively.

Anti-BP230 antibodies were determined by ELISA systems of the same companies. The ELISA plates from MBL (Nagoya, Japan) were coated with N-terminal and C-terminal human recombinant proteins (Tampona et al., 2009). A fragment of the C-terminal globular domain of BP230 was cloned, expressed in *E. coli*, and coated in the microwells of the ELISA from EUROIMMUN (Lübeck, Germany). Both ELISAs were performed according to the manufacturer's instructions. The cut-off values were 9 U/mL and 20 RU/mL, respectively.

3. Results

3.1. Detection of anti-BP autoantibodies by EUROPLUS™ Dermatology Mosaic

Results obtained by IIF on the dermatology mosaic are summarized in Table 2. Using monkey oesophagus as substrate, 57/60 BP sera (95.0%) revealed immunofluorescence of the basal membrane in the 1/10 and/or 1/100 serum dilution. One sample was only positive in the 1/10 dilution, while another sample was negative in the 1/10 dilution, but strongly positive

Table 1
Patient characteristics.

	Bullous pemphigoid n = 60	Control cohort 1 ^a n = 22	Control cohort 2 ^a n = 35
Age ^b			
Median	80.0	73.5	76.0
IQR	76–85	69–78	74–82
F/M ratio	15/45	7/15	8/28

^a Control cohort 1 consisted of patients with other autoimmune-mediated skin diseases, while control cohort 2 consisted of patients with other skin diseases.

^b Age was defined at the time of diagnosis.

in the 1/100 dilution. There was complete concordance (100%) within the BP sera with respect to staining of the epidermal site of the salt-split skin substrate, although 7 sera were only positive in the 1/10 dilution. Immunofluorescence of the basal membrane was observed in 9/57 control sera (15.8%). Only one of these samples was positive in both the 1/10 and 1/100 dilutions. Epidermal staining in the salt-split skin was observed in 13/57 control samples (22.8%); only 2 sera were positive in both the 1/10 and 1/100 dilutions. Concordance between both substrates was 82.5%; 6 samples (10.5%) were positive on both substrates and 41 samples (71.9%) were negative on both substrates.

Reactivity towards BP180 and BP230 was 53/60 (88.3%) and 23/60 (38.3%), respectively, in the BP cohort. All anti-BP180 antibody positive samples were also positive by IIF on the skin tissues; similarly, all anti-BP230 antibody positive samples were also positive for anti-BP180 antibodies. In the control cohort one sample (patient with psoriasis) was positive for both anti-BP180 and anti-BP230 antibodies; this sample also revealed staining of the basal membrane, but was negative by IIF in the salt-split skin. A second control sample (patient with urticaria) was only positive for anti-BP180 antibodies; this sample was also positive by IIF on both skin tissues.

3.2. Detection of anti-BP180 antibodies

Anti-BP180 antibodies were detected in 52 (86.7%) and 48 (80.0%) of the BP sera by the EUROIMMUN and MBL ELISAs, respectively (Table 3). This was accompanied by a positive reaction in 4 (7.0%) and 1 (1.8%) of the control sera, respectively. The specificity of the EUROIMMUN ELISA could be increased from 93.0% to 94.7%, without affecting the sensitivity, by increasing the cut-off from 20 to 25 RU/mL as based on receiver operating characteristics (ROC) curve analysis.

Although sensitivity and specificity are most often used as test characteristics in clinical evaluations of assays for auto-antibodies, in clinical practice likelihood ratios (LRs) are more valuable (van Paassen et al., 2003; Bossuyt, 2009). All three antigen-specific tests for anti-BP180 antibodies have LRs for a positive test result > 10, which makes results clinically significant with respect to a change in pre-test to post-test probability; LRs

for a negative test result are between 0.1 and 0.2, indicating modest clinical significance.

Concordance between assays was the best for the IIF–MBL ELISA combination (93.2%); concordance for the other two combinations (IIF–EUROIMMUN ELISA and EUROIMMUN ELISA–MBL ELISA) was 88.9%. Interestingly, none of the control samples were positive in more than one test for anti-BP180 antibodies.

3.3. Detection of anti-BP230 antibodies

The sensitivity of anti-BP230 antibodies for BP was 60.0% and 58.3% in the EUROIMMUN and MBL ELISAs, respectively (Table 3). The respective specificities were 82.5% and 93.0%. Again, the cut-off of the EUROIMMUN ELISA could be slightly optimised by ROC-curve analysis: an increase from 20 to 25 RU/mL resulted in a somewhat lower sensitivity of 56.7% and a slightly increased specificity of 87.7%.

Only detection of anti-BP230 antibodies by indirect immunofluorescence on a substrate of HEK293 cells, transfected with the C-terminal globular domain of BP230, revealed a LR for a positive test result > 10; the LRs for a negative test result were all close to 0.5, which hampers the usefulness of negative test results.

Concordance of the EUROIMMUN ELISA with IIF and the MBL ELISA was 79.5% and 80.3%, respectively. This relatively low concordance was predominantly due to the lower specificity of the EUROIMMUN ELISA. The concordance between the MBL ELISA and IIF was 87.2%. Two control samples were positive in two distinct assays for anti-BP230 antibodies. One of these samples (patient with psoriasis) was positive on the oesophagus substrate, but not on the salt-split skin; the other sample (patient with eczema) was completely negative by IIF.

4. Discussion

The clinical evaluation of the EUROPLUS™ Dermatology Mosaic revealed that the test characteristics for the classical substrates, i.e. oesophagus and salt-split skin, fulfil the requirements for a screenings test: high sensitivity with reasonable specificity. Only one of the three sera that were negative on both tissue substrates revealed positive results in the EUROIMMUNE ELISAs, but not in the other antigen-specific assays. In addition, the detection of anti-BP180 and anti-BP230 antibodies by IIF revealed better test characteristics than the respective antigen-specific ELISAs. Although not the focus of the current study, the EUROPLUS™ Dermatology Mosaic also allows for the detection of anti-Dsg1 and Dsg3 antibodies, involved in pemphigus foliaceus and vulgaris. Thus far, no studies are available on the diagnostic performance of the EUROPLUS™ Dermatology Mosaic for these two diseases. Importantly, none of the BP sera reacted with a typical chicken-wire epidermal staining pattern or with the HEK293 cells transfected with Dsg1 or Dsg3, while in the control cohort this reactivity was confined to the sera from patients with a diagnosis of pemphigus foliaceus or vulgaris (data not shown). These data indicate that at least the specificity of these substrates is good.

The sensitivity for anti-BP180 antibodies varied in our study between 80 and 90%, depending on the assay used. This is in line with other reports (Sitaru et al., 2007; Feliciani et al., 2009; Tampoia et al., 2009; Charneux et al., 2011). Also the observed sensitivities of the anti-BP230 antibody ELISAs

Table 2
Autoantibody reactivity on the EUROPLUS™ Dermatology Mosaic.

	Test dilution	Basal membrane (oesophagus)	Epidermal staining (SSS)	BP180 NC16A dots	BP230 globC HEK293
BP ^a (n = 60)	1/10	57 (95%)	57 (95%)	53 (88%)	23 (38%) ^b
	and/or 1/100				
	1/10 and 1/100	55 (92%)	50 (83%)	47 (78%)	21 (35%)
DC (n = 57)	1/10	9 (16%)	13 (23%)	2 (3%)	1 (2%)
	and/or 1/100				
	1/10 and 1/100	1 (2%)	2 (3%)	0 (0%)	0 (0%)

^a Abbreviations: BP, bullous pemphigoid; DC, disease controls; SSS, salt-split skin.

^b Two samples revealed inconclusive results in the 1/10 dilution.

Table 3

Test characteristics of anti-BP180 and anti-BP230 antibody assays.

	BP180			BP230		
	EUROIMMUN IIF ^a	EUROIMMUN ELISA	MBL ELISA	EUROIMMUN IIF	EUROIMMUN ELISA	MBL ELISA
Sensitivity	88.3	86.7	80.0	38.3	60.0	58.3
Specificity	96.5	93.0	98.2	98.3	82.5	93.0
LR (pos)	25.2	12.4	44.4	22.5	3.4	8.3
LR (neg)	0.12	0.14	0.20	0.63	0.48	0.45

^a Abbreviations: ELISA, enzyme-linked immuno-sorbent assay; IIF, indirect immunofluorescence; LR, likelihood ratio.

(~60%) were according to previous publications (Kromminga et al., 2004; Feliciani et al., 2009; Tampoia et al., 2009; Charneux et al., 2011). It should be stressed, though, that the EUROIMMUN ELISA for anti-BP180 antibodies reveals high levels of antibodies in the majority of BP patients (53% > 100 RU/mL; 72% > 50 RU/mL), while disease controls only had low levels of these antibodies (<40 RU/mL). The sensitivity of the anti-BP230 antibody detection by the EUROPLUS™ Dermatology Mosaic was low (38%). Combined with the high specificity, however, this results in a LR for a positive test result of 22.5, which implies that a positive result is of clinical significance. Except the MBL ELISA for anti-BP180 antibodies, the other ELISAs have a relatively low specificity (<95%) in our study, but not in previous studies (Kromminga et al., 2004; Sitaru et al., 2007; Feliciani et al., 2009; Tampoia et al., 2009). The strength of our study, however, is the inclusion of disease controls that are matched for age and gender. Because of the high median age of patients with BP this matching is important since the prevalence of autoantibodies increases upon ageing (Tan et al., 1997). However, there may be two alternative explanations for these false-positive results. First, as is the case in several other autoimmune diseases (Arbuckle et al., 2003; Rantapää-Dahlqvist et al., 2003), the presence of autoantibodies may precede the clinical manifestations of BP. Indeed, IgG autoantibodies against BP180 and BP230 are more frequent in elderly patients with pruritic dermatoses, which may be considered a prodromal phase of BP (Feliciani et al., 2009). In our study false-positive results in any of the antigen-specific tests were obtained in 14/57 controls (25%). The frequency was threefold higher in the controls with other autoimmune skin diseases versus the inflammatory controls (data not shown). Second, there is an ongoing debate on the interpretation of positive results in the absence of clinical manifestations of BP (Fine, 2010; Wieland et al., 2010).

Although the current study is based on two serum dilutions for evaluating the EUROPLUS™ Dermatology Mosaic, it is sufficient to use the 1/10 dilution in clinical practice for routine screening. This dilution reveals a higher sensitivity, in particular with respect to the epidermal staining of the salt-split-skin, but at the cost of specificity on both tissues. The lack of specificity on the tissues, however, is compensated for by the presence of BP180-specific test results. While the clinical performance of the EUROPLUS™ Dermatology Mosaic is good for diagnostic purposes it is important to realize that only qualitative results are obtained, which in case of titration can be considered at best semi-quantitative. Quantification of results by ELISA therefore remains required, in particular follow-up of patients with BP since anti-BP180 antibody levels have been shown to correlate with disease activity (Schmidt et al., 2000; Tampoia et al., 2009). Quantification by

the EUROIMMUN ELISA is probably more reliable since the assay employs a standard curve based on three standards varying from 2 to 200 RU/mL. Quantification by the MBL ELISA, on the other hand, is based on a negative calibrator (0 U/mL) in combination with a single positive calibrator (100 U/mL). This may explain the difference in inter-assay variation, as supplied by the manufacturers, being <5% and <15% for the EUROIMMUN and MBL ELISAs, respectively.

5. Conclusion

Altogether it can be concluded that the EUROPLUS™ Dermatology Mosaic shares the advantage of obtaining results with several distinct substrates in a single incubation, with good assay characteristics. When considering LR as the clinically most useful test parameter, the EUROPLUS™ Dermatology Mosaic performs better for diagnostic purposes as compared to antigen-specific ELISAs.

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References

- Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., Harley, J.B., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* 349, 1526.
- Bossuyt, X., 2009. Clinical characteristics of a laboratory test. A practical approach in the autoimmune laboratory. *Autoimmun. Rev.* 8, 543.
- Charneux, J., Lorin, J., Vitry, F., Antonicelli, F., Reguiat, Z., Barbe, C., Tabray, T., Grange, F., Bernard, P., 2011. Usefulness of BP230 and BP180-NC16a enzyme-linked immunosorbent assays in the initial diagnosis of bullous pemphigoid. *Arch. Dermatol.* 147, 286.
- Damoiseaux, J., Cohen Tervaert, J.W., 2006. From ANA to ENA: how to proceed? *Autoimmun. Rev.* 5, 10.
- Damoiseaux, J., Steller, U., Buschütz, M., Vaessen, M., Rosemann, A., van Paassen, P., Stöcker, W., Fechner, K., Cohen Tervaert, J.W., 2010. EUROPLUS™ ANCA BIOCHIP mosaic: PR3 and MPO antigen microdots improve the laboratory diagnostics of ANCA-associated vasculitis. *J. Immunol. Methods* 348, 67.
- Feliciani, C., Caldarola, G., Kneisel, A., Podstawa, E., Pfütze, M., Pfützner, W., Hertl, M., 2009. IgG autoantibody reactivity against bullous pemphigoid (BP) 180 and BP230 in elderly patients with pruritic dermatoses. *Br. J. Dermatol.* 161, 306.
- Fine, J.D., 2010. Prevalence of autoantibodies to bullous pemphigoid antigens within the normal population. *Arch. Dermatol.* 146, 74.
- Kelly, S.E., Wojnarowska, F., 1988. The use of chemically split tissue in the detection of circulating anti-basement membrane zone antibodies in bullous pemphigoid and cicatricial pemphigoid. *Br. J. Dermatol.* 118, 31.
- Kobayashi, M., Amagai, M., Kuroda-Kinoshita, K., Hashimoto, T., Shirakata, Y., Hashimoto, K., Nishikawa, T., 2002. BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and monitoring tool for bullous pemphigoid. *J. Dermatol. Sci.* 30, 224.

- Kromminga, A., Sitaru, C., Hagel, C., Herzog, S., Zillikens, D., 2004. Development of an ELISA for the detection of autoantibodies to BP230. *Clin. Immunol.* 111, 146.
- Rantapää-Dahlqvist, S., de Jong, B.A., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., Sundin, U., van Venrooij, W.J., 2003. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* 48, 2741.
- Sagi, L., Baum, S., Agmon-Levin, N., Sherer, Y., Porat Katz, B.S., Barzilai, O., Ram, M., Bizzaro, N., SanMarco, M., Trau, H., Shoenfeld, Y., 2011. Autoimmune bullous diseases. The spectrum of infectious agent antibodies and review of the literature. *Autoimmun. Rev.* 10, 527.
- Schmidt, E., Zillikens, D., 2010. Modern diagnosis of autoimmune blistering skin diseases. *Autoimmun. Rev.* 10, 84.
- Schmidt, E., Obe, K., Bocker, E.B., Zillikens, D., 2000. Serum levels of autoantibodies to BP180 correlate with disease activity in patients with bullous pemphigoid. *Arch. Dermatol.* 136, 174.
- Sitaru, C., Dähnrich, C., Probst, C., Komorowski, L., Blocker, I., Schmidt, E., Schlumberger, W., Rose, C., Stöcker, W., Zillikens, D., 2007. Enzyme-linked immunosorbent assay using multimers of the 16th non-collagenous domain of the BP180 antigen for sensitive and specific detection of pemphigoid autoantibodies. *Exp. Dermatol.* 16, 770.
- Skaria, M., Jaunin, F., Hunziker, T., Riou, S., Schumann, H., Bruckner-Tuderman, L., Hertl, M., Bernard, P., Saurat, J.H., Favre, B., Borradori, L., 2000. IgG autoantibodies from bullous pemphigoid patients recognize multiple antigenic reactive sites located predominantly within the B and C subdomains of the COOH-terminus of BP230. *J. Invest. Dermatol.* 114, 998.
- Sticherling, M., Erfurt-Berge, C., 2012. Autoimmune blistering diseases of the skin. *Autoimmun. Rev.* 11, 226.
- Tampoia, M., Lattanzi, V., Zucano, A., Villalta, D., Filotico, R., Fontana, A., Vena, G.A., Di Serio, F., 2009. Evaluation of a new ELISA assay for detection of BP230 autoantibodies in bullous pemphigoid. *Ann. N. Y. Acad. Sci.* 1173, 15.
- Tan, E.M., Feltkamp, T.E.W., Smolen, J.S., Butcher, B., Dawkins, R., Fritzler, M.J., Gordon, T., Hardin, J.A., Kalden, J.R., Lahita, R.G., Maini, R.N., McDougal, J.S., Rothfield, N.F., Smeenk, R.J., Takasaki, Y., Wiik, A., Wilson, M.R., Koziol, J.A., 1997. Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum.* 40, 1601.
- Vaillant, L., Bernard, P., Joly, P., Prost, P., Labeille, B., Bedane, C., Arbeille, B., Thomine, E., Bertrand, P., Lok, C., Roujeau, J.C., French Bullous Study Group, 1998. Evaluation of clinical criteria for diagnosis of bullous pemphigoid. *Arch. Dermatol.* 134, 1075.
- van Paassen, P., Damoiseaux, J., Cohen Tervaert, J.W., 2003. Laboratory assessment in musculoskeletal disorders. *Best Pract. Res. Clin. Rheumatol.* 17, 475.
- Wieland, C.N., Confere, N.I., Gibson, L.E., Weaver, A.L., Krause, P.K., Murray, J.A., 2010. Anti-bullous pemphigoid 180 and 230 antibodies in a sample of unaffected subjects. *Arch. Dermatol.* 146, 21.
- Zillikens, D., Rose, P.A., Balding, S.D., Liu, Z., Olague-Marchan, M., Diaz, L.A., Giudice, G.J., 1997. Tight clustering of extracellular BP180 epitopes recognized by bullous pemphigoid autoantibodies. *J. Invest. Dermatol.* 109, 573.