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CHAPTER 2
Significantly higher prevalence of circulating bullous pemphigoid-specific IgG autoantibodies in elderly patients with a nonbullous skin disorder

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Dear editor, we read with interest the recent article of Van Beek *et al*. on "*Serum autoantibodies against the dermal–epidermal junction in patients with chronic pruritic disorders, elderly individuals and blood donors prospectively recruited*" and the recent review article of Schmidt *et al.* on "*BP180- and BP230-specific IgG autoantibodies in pruritic disorders of the elderly: a preclinical stage of bullous pemphigoid?*" about the association between pruritus in the elderly and the presence of bullous pemphigoid (BP)-specific IgG autoantibodies.[1,2]

Van Beek *et al.* studied autoantibody reactivity against the epidermal basement membrane zone (EBMZ) by indirect immunofluorescence microscopy (IIF), enzyme-linked immunosorbent assay (ELISA) and immunoblot. Positive reactivity in any test was found in 31.2% of the sera of elderly individuals (≥ 70 years; n=93), 16.7% of the sera of patients with chronic pruritic disorders (n=78) and in 26% of the sera of healthy blood donors of all ages (n=50), respectively. In our opinion these are remarkably high percentages, probably due to false-positive rates of immuno-assays, as mentioned in the discussion of their article. Van Beek *et al.* concluded that neither advanced age nor chronic pruritus have been verified as risk factors for autoantibodies against the EBMZ.[1]

Recently Schmidt *et al.* reviewed clinical and experimental studies about the possible association between senile pruritus and BP IgG autoantibodies, and question whether this could be a preclinical stage of BP.[2] Prior studies by Rieckhoff-Cantoni *et al.* (1992), Hofmann *et al*. (2003) and Feliciani *et al.* (2009) on the presence of circulating BP autoantibodies in elderly patients with pruritic disorders, but without blistering, reported IgG reactivity against BP180 or BP230 in 10 of 43 patients (23%), 3 of 25 patients (12%) and 5 of 15 patients (33%), respectively.[3-5] The question remains whether circulating autoantibodies against BP antigens in the elderly and patients with pruritic disorders indicate the presence of a BP subtype, may identify patients with an increased risk of developing BP, or may have no clinical relevance at all.

As an extension to these studies we present our results of a retrospective database study, which included 374 patients who consulted our department for a skin disorder, without blistering. Data was collected from patients in our dermatology database in whom direct immunofluorescence (DIF) and serological testing were performed at the University Medical Center Groningen (UMCG). Patients were excluded if DIF was positive or if they clinically presented with blisters or erosions on skin or mucous membranes, to exclude those with an

evident diagnosis of autoimmune blistering disease. Patient characteristics are shown in table 1. The following serological test results were studied: IIF on monkey esophagus, IIF on salt-split skin (SSS), immunoblot testing on BP180 and BP230 antibodies, and BP180 NC16A- and BP230-specific enzyme-linked immunosorbent assay (ELISA, MBL, Nagoya, Japan, cut-off <9 U/ml) (Fig. 1).

In our study, we found at least one positive serological test in 13.6% (n=51) of the dermatology patients with a non-bullous skin disorder. The median age of these patients (71.1 years, n=51) was significantly higher (p=0.009, Mann-Whitney U-test) than the median age of patients with no positive test results (64.0 years, n=323). Moreover, logistic regression showed that age above 75 years had a significant predictive value for positive reactivity for at least one serological test (p=0.025; odds ratio 3.8) compared to patients aged < 45 years. The higher prevalence of BP IgG autoantibodies in patients aged above 75 years was confirmed with X^2-test (p=0.012). In contrast, no relation was found between the presence of pruritus and BP IgG autoantibodies. Furthermore, no relation was found between the combined presence of pruritus and older age (>65 years) and BP IgG autoantibodies.

Table 1. Patient characteristics with sex, pruritus and age vs. serological test results

	Total	No reactivity	At least one positive test result	P-value[a]
Total population	374	323 (86.4)	51 (13.6)	-
Sex				
Female	218 (58.3)	190 (87.2)	28 (12.8)	0.60
Male	156 (41.7)	133 (85.3)	23 (14.7)	
Pruritus				
No	67 (17.9)	59 (88)	8 (12)	0.66
Yes	307 (82.1)	264 (86.0)	43 (14.0)	
Age (years)				
Mean (range)	62.2 (5.5-96.0)			
≤ 75 years	268 (71.7)	239 (89.2)	29 (10.8)	0.012*
> 75 years	106 (28.3)	84 (79.2)	22 (20.8)	

*Values are n (%) unless stated otherwise. [a] P-value by X^2-test. *Significant (p < 0.05).*

Van Beek *et al*. stated that positive ELISA results could not be confirmed with ELISA tests of other manufacturers, nevertheless, these values were considered positive. In our study ELISA results were only considered positive if the result remained positive after replication of the tests. This does not exclude nonspecific binding of antibodies, but minimizes false-positive results due to technical errors. Therefore another 15 former positive ELISA results were negative after replicated testing. In 13 of 374 patients (3.5%) multiple positive reactivity was seen in tests of different methodology, of which seven patients had positive reactivity in two different tests, three patients in three tests, two patients in four tests and one patient with reactivity in five different tests.

Although detection of circulating BP IgG antibodies has been reported previously in patients with various (chronic) pruritic dermatoses and in elderly individuals, the mechanism and relevance are not yet fully understood. Both Hofmann *et al.* and Schmidt *et al.* discussed the mechanism of repeated cell injury to the EBMZ due to itching in pruritic dermatoses, which may lead to exposure of hidden epitopes. In combination with a loss of self-tolerance related to the ageing process, these patients could be at risk of developing anti-EBMZ antibodies and, eventually, BP.[2,4,6] Conversely, anti-BP230 IgG autoantibodies may trigger pruritus, leading to the development of pruritic skin lesions and possibly anti-BP180 IgG antibodies, by the process of epitope spreading.[2,6]

Our findings did not show a relation between pruritus and circulating BP IgG autoantibodies in our non-bullous dermatology population. A possible association might have been obscured due to the selection bias, since our study was based on a population that consulted a dermatologist for a dermatosis that may be pruritic due to various causes.

The non-bullous clinical variant of BP and the minimal criteria to diagnose BP are a topic of recent discussion.[7-9] Positive epidermal binding of IIF on SSS may play an important diagnostic role, with a reported high specificity and positive predictive value in typical BP patients.[10] The finding in our study of a significantly higher prevalence of BP-specific IgG autoantibodies in elderly dermatology patients with non-bullous skin disorders raises the question whether a diagnosis of pemphigoid in elderly with pruritus and a negative DIF is often missed. However, the results of testing sera of elderly patients with pruritic disorders should be interpreted with caution, and additional studies are needed in the general

population (as control) and in a high-risk population for developing BP (elderly persons in nursing homes).

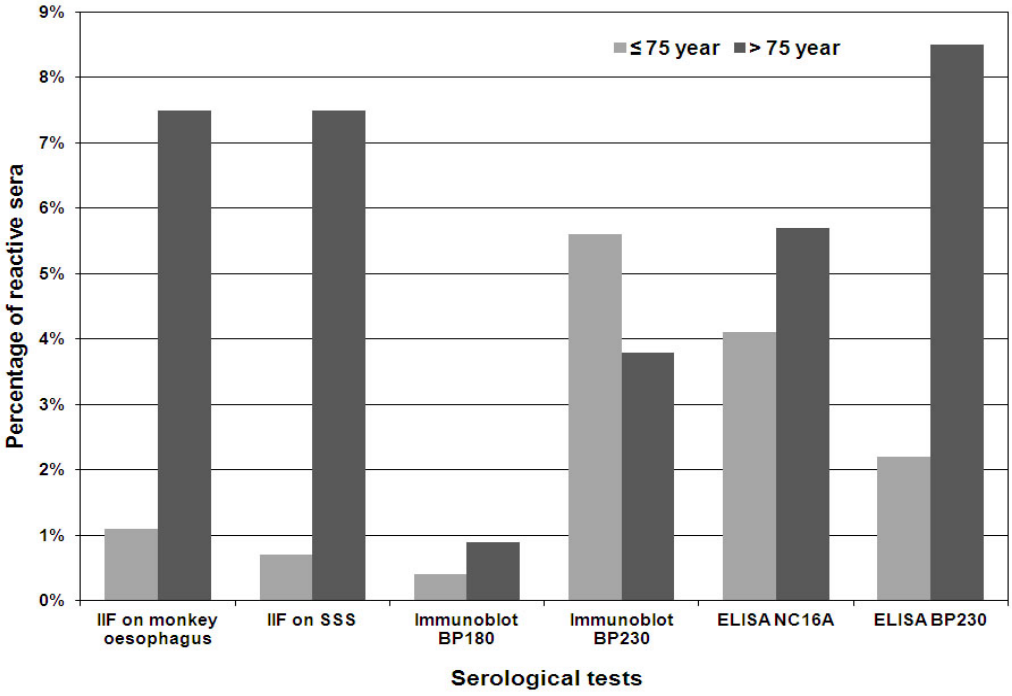

Figure 1. Bullous pemphigoid-specific IgG autoantibody reactivity in our study population of dermatology patients with nonbullous skin disorders, divided into age >75 years and ≤75 years. Sera were tested with six different serological tests, including indirect immunofluorescence (IIF) microscopy on monkey oesophagus (n = 308), IIF on human salt-split skin (SSS, n = 359), immunoblot (n = 306) and BP180 and BP230 enzyme-linked immunosorbent assay (ELISA, n = 239 and 227, respectively).

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