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Isolated NC16a-ELISA testing is of little value to identify bullous pemphigoid in elderly patients with chronic pruritus

We read with interest the recent article of Jedlickova *et al.* on "Anti-basement membrane zone antibodies in elderly patients with pruritic disorders and diabetes mellitus" [1]. By indirect immunofluorescence (IF) microscopy using monkey oesophagus they were able to detect circulating antibodies against basement membrane zone (BMZ) proteins in up to 19% of 31 serum samples obtained from elderly patients with pruritic skin disorders, but without diabetes. Half of the latter also showed reactivity with recombinant forms of BP180 (collagen XVII) and/or BP230 by ELISA. In extension to this study, we here report our preliminary results of a follow-up study on the usefulness of the NC16a-ELISA in identifying elderly patients with pruritus who are potentially destined to develop bullous pemphigoid (BP). BP is the most common autoimmune subepidermal bullous dermatosis affecting predominantly the elderly, in which pruritus may be one of the leading initial symptoms of the disease [2]. Its diagnosis is usually based on combined clinical features, histological and immunopathological findings. The major antigenic tar-

get in BP is the NC16A-domain of BP180, recombinant forms of which have been used to establish ELISAs for rapid sensitive and specific diagnosis of BP as well as for monitoring disease activity in affected patients [3]. In this study, 15 patients aged ≥ 65 years (80.3 ± 10.7 years) with pruritus of at least 6-week-duration, and 34 age-matched controls without pruritus were assessed by NC16A-ELISA and indirect IF microscopy at the first visit and after 6 months. Most patients had dry skin and excoriations. Individuals with eczematous, urticarial or prurigo-like skin lesions, or with underlying infectious, metabolic or allergic disorders were excluded from the study. For ELISA, a prokaryotic GST-tagged NC16A-protein encompassing amino acids 490-567 of BP180 was used as described [4]. The cut-off value was determined as mean + 3 SD of 50 healthy individuals (OD 0.30). At first presentation, 4/15 (27%) patients with chronic pruritus displayed low-titer antibodies against the NC16A-domain (OD < 0.57). After 6 months, two of these patients still had persisting low-titer antibodies (OD 0.40), despite the disappearance of pruritus at that time (*table 1*). In the control group, 2/34 sera (6%) reacted slightly positively with the NC16a-domain (OD 0.31 and 0.44). None of the patients and control subjects showed positive indirect IF microscopy findings using human salt-split-skin as substrate and none developed BP during the observational period. Although the duration of pruritus varied from 6 weeks to 10 years, most patients were in clinical remission after 6 months of follow up, probably as a result of improved skin care with hydrating creams. Some patients had also been treated intermittently with non-sedating antihistaminics. Our study was motivated by previous findings demonstrating reactivity to the NC16a-domain of BP180 by ELISA in 3/25 (12%) sera from elderly patients with itch [5]. We wondered whether these patients suffered from an initial form of BP and therefore initiated this follow-up study. Considering the relatively low incidence of BP, even in the elderly [6], the number of prospectively followed patients in this study is insufficient to draw any conclusion about the predictive value of positive NC16A-

Table 1. Characteristics of patients

Patient	Age	Duration of itch (months)	Severity of itch I	NC16a-ELISA I	Severity of itch II	NC16a-ELISA II
1	93	13	++	neg	0	neg
2	81	1.5	+	0.57	0	0,4
3	89	5	++	0.53	0	0,4
4	64	120	+	neg	0	neg
5	81	12	++	neg	+	neg
6	66	36	++	0.39	+	neg
7	72	24	++	neg	0	neg
8	84	3	++	neg	+	neg
9	63	1.5	++	neg	0	neg
10	91	2	++	0.31	0	neg
11	78	6	++	neg	+	neg
12	85	1.5	++	neg	0	neg
13	75	18	++	neg	+	neg
14	84	n.d.	++	neg	0	neg
15	99	n.d.	++	neg	0	neg

I, at first presentation; II, after 6 months; 0, absence of pruritus; +, moderate pruritus; ++, intense pruritus, n.d., not documented

Table 2. Autoreactivity against BP antigens in patients with pruritic diseases of unknown origin

Patients	Method	Reactive sera	Reference
Patients with chronic pruritus or prurigo (n = 43)	IIF on human skin WB using KE	1/10 (10%) 10/43 (23%) to BP180 or BP230	Rieckhoff-Cantoni <i>et al.</i> , 1992
Elderly patients with pruritus (n = 25)	IIF on SSS NC16a-ELISA	0/25 (0%) 3/25 (12%)	Hofmann <i>et al.</i> , 2003
Elderly patients with pruritus (n = 31)	IIF on MO BP180, BP230 ELISA	6/31 (19%) 3/31 (10%)	Jedlickova <i>et al.</i> , 2008
Elderly patients with pruritus (n = 15)	BP180, BP230 ELISA	5/15 (33%)	Feliciani <i>et al.</i> , in press
Elderly patients with pruritus (n = 15)	IIF on SSS NC16a-ELISA	0/15 (0%) 4/15 (27%)	This study

IIF, indirect immunofluorescence; SSS, salt-split-skin; MO, monkey oesophagus; KE, keratinocyte extract

ELISA results to identify elderly subjects who will develop BP. Although the NC16a-ELISA has been claimed to have a specificity of >95% in the diagnosis of BP, the data reported here and previous data (summarized in *table 2*) clearly indicate that slightly positive NC16a-ELISA values or low-titer anti-BMZ-antibodies in general are frequently found in elderly patients with itch. The varying percentage of sera with circulating antibodies against basement membrane components (*table 2*) may depend on several factors including differences in patient selection and test systems. ELISA systems used in these studies are not directly comparable, since recombinant proteins were differentially expressed and represented various domains of BP180 or BP230.

In summary, isolated ELISA testing for BP antibodies is too expensive for little return in clinical practice [1, 5, 7, 8]. Therefore, diagnosis of BP should always be based on a combination of clinical features and positive direct IF microscopy studies. ■

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Is it really possible to differentiate insect bite-like reaction and nodular variant of eosinophilic cellulitis in a healthy person?

Exaggerated insect bite reaction and Wells' syndrome were considered distinct entities. Recently it has been suggested that there is considerable overlap [1]. We present a patient with an exaggerated insect bite reaction which seems to be an overlapping condition with nodular variant of eosinophilic cellulitis and we suggest that this condition should be named an "insect bite-like reaction".

A 52-year-old woman was referred to our clinic with a 7-day history of a tender lesion with swelling, erythema and ulceration on her abdomen. The patient complained of reduced appetite, fatigue and myalgia, together with the onset of this lesion. She denied a history of recent travel, contact with animals, trauma or insect bites. Systemic sulbactam-ampicilline was prescribed by her general practitioner had been unhelpful. Dermatological examination revealed an indurated nodule with central ulceration on her right abdominal region. There was no obvious surrounding erythema and the affected area was cool and tender to touch (*figure 1A*). Microbial swabs from the lesions were negative and tissue culture revealed cutaneous micro flora organisms. Laboratory studies were within normal limits and there was no peripheral eosinophilia. A skin biopsy revealed epidermal spongiosis, intraepidermal multilocate vesicles and dermal inflammatory infiltrate. The dermal infiltrate was angiocentric and interstitial and consisted of lymphocytes, plasmacytes and numerous eosinophil leukocytes. Eosinophil leukocytes infiltrated between dermal collagen fibres and invaded deep dermal and subcutaneous tissue. There were also eosinophils in