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In reply

We thank Dr Khumalo for the thoughtful words about our recent article on CCCA. The comments are especially meaningful because of Dr Khumalo's long experience with and deep understanding of CCCA, and we appreciate the feedback. We wish herein to address several of the issues raised.

First, we propose an explanation for the discrepancy in the prevalence of CCCA found by Kyei et al¹ (17%) compared with that found by Olsen et al² (5.6%) and Khumalo et al^{3,4} (2.7% in 604 women). Kyei et al¹ enrolled their subjects through advertisement of a hair study, whereas Olsen et al² screened people from an annual health and beauty symposium for African American women who were unaware of the study prior to their attendance at that symposium and 2 church meetings for African American women who did not know about the hair screenings prior to their attendance at those meetings. In spite of this, Olsen et al² touted a 95% participation rate from the church meetings. The assumption here is that no advertisement was made prior to the hair screenings; thus, selection bias was not an issue.

There are several reasons why we chose to advertise for our hair screenings. First, it allowed participants to prepare their hair prior to the screenings. Many African Americans use hair weaves, braids, and wigs on their hair, especially for outings, and we wanted a more accurate assessment of scalp hair loss. Second, it is difficult to have high participation when people do not know what you are going to do prior to the study. Moreover, few studies are truly without bias. I would argue that even Olsen et al² experienced some self-selection within the various groups screened, namely the beauty symposium and the church groups, because not everyone within those groups chose to participate in the study. We chose to accept some selection bias to get a more accurate assessment of hair loss.

The second issue is regarding geographic locations of participants. It is true that Olsen et al² recruited participants from 2 geographic regions of the United States, the Midwest (Ohio) and the Southeast (Maryland and North Carolina), while Kyei et al¹ recruited only from the Midwest. However, Olsen et al² did not include the Cleveland subjects in their calculation of the prevalence; thus, their prevalence data

can only present the Southeastern section of the United States. A truly geographically diverse study would include states from all regions of the United States.

One key point that Kyei et al¹ make is that there is a lot to discover on the topic of CCCA, and they have revealed that the causes of CCCA not lie in only hair grooming but also in the general health of the individual (ie, diabetes, infections). Further studies are needed to generate more data and subsequent solutions to this disfiguring disorder.

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Sensitivity of Immunofluorescence Studies vs Enzyme-Linked Immunosorbent Assay for Diagnosis of Bullous Pemphigoid

We read with interest the Practice Gap comments of Dr Chan published in the March issue of the *Archives*.¹ We agree that there is a lack of consensus on diagnostic criteria for bullous pemphigoid (BP) and a need to provide better information in this area. However, the author's recommendation to routinely perform the commercially available enzyme-linked immunosorbent assay (ELISA) for BP180 instead of using indirect immunofluorescence (IIF) microscopy studies on a split-skin sample for the diagnosis of BP cannot be fully supported.

The first question to address is whether characterization of circulating autoantibodies is required to establish the diagnosis of BP. This is not the case, since clinical features are sufficient to make the diagnosis of BP in patients with positive direct immunofluorescence findings.² Previous studies have shown that when 3 of 4 clinical criteria are present, the diagnosis of BP can be made with a sensitivity of 90%, a specificity of 83%, and a high positive predictive value.² Hence, the routine performance of the ELISA for BP180 represents an academic diagnostic exercise. Interestingly, a minor adaptation of the direct immunofluorescence microscopy technique with analysis of the fluorescence pattern ("n" and "u" serrated patterns) would also allow the clinician to rapidly and easily distinguish BP from other conditions with almost no extra costs or blood sampling.³

Second, there are a minority of studies⁴⁻⁶ (Table) that have shown ELISA for BP180 to be a more sensitive test

Table. A Survey of BP Diagnostic Sensitivity and Specificity of ELISA for BP180

Source	BP Serum Samples, No.	Control Serum Samples, No.	IIF, % ^a	Recombinant Protein ^b	Se, % ^c	Sp, %
Zillikens et al, ⁵ 1997 ^d	50	107	84	P-NC16A (AA 490-562)	94	100
Nakatani et al, ⁶ 1998	110	50	89	P-NC16A (AA 507-548)	96	100
Hata et al, ⁷ 2000	83	40	100	P-NC16A (AA 490-566)	70	93
Kobayashi et al, ⁸ 2002	64	538	100	P-NC16A (AA 490-566)	84	99
Hofmann et al, ⁹ 2002	116	100	100	E-NC16A+ Col 15 (AA 490-811)	80	99
Sakuma-Oyama et al, ¹⁰ 2004	102	94	100	BP180-NC16A ^e	89	98
Mariotti et al, ¹¹ 2004	78	107	100	P-NC16A (AA 490-562)	82	100
Tsuji-Abe et al, ¹² 2005	14	447	100	BP180-NC16A ^e	79	99
Yoshida et al, ¹³ 2006	239	430	100	BP180-NC16A ^e	70	99
Sitaru et al, ¹⁴ 2007	118	494	94	Tetramers of P-NC16A	90	98
Di Zenzo et al, ¹⁵ 2008	49	80	96	BP180-NC16A ^e	90	NK
Feng et al, ¹⁶ 2008	42	24	100	BP180-NC16A ^e	98	96
Tampoia et al, ¹⁷ 2009	20	82	100	BP180-NC16A ^e	90	98
Charneux et al, ⁴ 2011	138	0	62 (70/113)	BP180-NC16A ^e	86	NK
Roussel et al, ¹⁸ 2011	190	78	81	BP180-NC16A ^e	79	90

Abbreviations: AA, amino acid; BP, bullous pemphigoid; IIF, indirect immunofluorescence; NK, not known; Se, sensitivity; Sp, specificity.

^aPercentage of positive findings by IIF microscopy.

^bRecombinant proteins used as substrate. Protein name beginning with P indicates prokaryotic; E, eukaryotic expressed protein.

^cNoteworthy, in most studies, sensitivity is largely overestimated because selected serum samples from patients with BP and positive indirect IF findings were used.

^dOnly 3 (boldface) of 15 studies reported the ELISA sensitivity to be higher than IIF sensitivity.

^eA commercially available ELISA kit (MBL Co).

than IIF for BP. The recently reported low sensitivity of IIF (62%) was owing to the use of monkey esophagus, which is not the substrate of reference for the search of BP autoantibodies.⁴ As a matter of fact, because selected serum samples were used, most studies have overestimated the sensitivity of commercially available ELISAs. In the only prospective study available to our knowledge, our research group found that findings in ELISA for BP180 and IIF studies using split-skin samples were comparable.¹⁵

In another retrospective study of 190 patients, positive findings in ELISA for BP180 and IIF using split-skin samples were found in 79% and 81% of cases, respectively.¹⁸ Even if ELISA for BP180 has advantages (eg, multiple sample testing, ease of performance, standardization), the commercially available kits cost \$46.00 to \$65.00 per test. Furthermore, ELISA for BP180 does not provide clues for specific conditions, such as p200 pemphigoid or epidermolysis bullosa acquisita. Of note, a recent prospective study¹⁹ has provided evidence that ELISA for BP180 should be performed at the time of cessation of therapy as a prognostic test (rather than a diagnostic test). In fact, patients with increased BP180 values found on ELISA have an increased risk for relapse.

Finally, since the combination test of ELISA for BP180 and the newly available ELISA for BP230 only slightly increases the diagnostic sensitivity (plus 5% to 10%) achieved with ELISA for BP180 alone, ELISA for BP230 should be performed only in the subset of patients with negative ELISA findings for BP180.^{4,18}

In conclusion, (1) the diagnosis of BP can be made with high sensitivity and specificity based on certain clinical features when direct immunofluorescence study findings are positive; (2) the ELISA for BP180 (NC16A, MBL Co) does not have a higher sensitivity than salt-split skin IIF studies for the diagnosis of BP; and (3) its usefulness

in the management of patients during the decrease of corticosteroid doses remains to be demonstrated.

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In reply

Di Zenzo and colleagues properly raise their concern about the relative importance of ELISA and IIF for the initial diagnosis of BP. However, they seem to misread the essence of my article in the March 2011 issue of the Archives.¹

First, what was my recommendation? As stated in the last paragraph, my recommendation was to urge the experts in the field to develop a consensus regarding the usefulness of ELISA to properly guide the practice of dermatologists.¹ I further made this point abundantly clear in the text: "If the expert community does not consider the current evidence about the use of ELISA to be sufficient for inclusion in the diagnostic criteria, then recommendations should be made as to the direction of future research regarding ELISA."¹(p292)

Regarding the diagnostic criteria, Di Zenzo and colleague's paper argued about this point in a self-conflicting manner. In the first paragraph, they state "We agree that there is a lack of consensus on diagnostic criteria for bullous pemphigoid (BP) and a need to provide better information in this area," yet in the second paragraph, they affirm that "clinical features are sufficient to make the diagnosis of BP in patients with positive direct immunofluorescence findings." The second statement is obviously incorrect, since 3 other autoimmune subepidermal blistering skin diseases that can clinically resemble BP with a positive direct immunofluorescence pattern identical to that of BP.²⁻⁴ The inability of a positive direct immunofluorescence finding (of skin basement membrane deposit of IgG) to distinguish one form of subepidermal blistering disease from another is pre-

cisely the reason why IIF on salt-split skin and methods to identify the target antigens of the autoantibodies (such as Western blot and ELISA) were used in the first place.²⁻⁵

With respect to relative sensitivity of ELISA and IIF in detecting BP antigens and anti-basement membrane autoantibodies, respectively, there are obviously different results reported in the literature, and I have urged the development of consensus.¹ But before we sort this out in an objective scientific manner, the conclusion that IIF studies are not less sensitive than ELISA for BP180 is hard to make.

Regarding method specificity, ELISA definitely has the advantage because IIF on salt-split skin substrate can only distinguish the autoantibodies targeting upper lamina lucida antigens (eg, BP180, a6b4 integrins) from those targeting lower lamina lucida or sublamina densa antigens (eg, laminin-5, p105, P200, type VII collagen),²⁻⁵ while ELISA data provide a definite target antigen detected by patients' autoantibodies. If ELISA can provide more specific information regarding a patient's diagnosis, it is puzzling that Di Zenzo and colleagues argued to return to the less specific method.

With regard to the inability of BP180 ELISA to detect other anti-basement membrane autoantibodies (eg, anti-P200, anti-type VII collagen), the argument of Di Zenzo and colleague is not relevant because my article was not about using ELISA as a screening tool for all possible subepidermal autoimmune blistering diseases, but rather specifically for BP, as stated explicitly in its title.

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VIGNETTES

Erysipeloid (*Erysipelothrix rhusiopathiae* Infection) Acquired From a Dead Kakapo

Erysipeloid is a zoonotic infection caused by the gram-positive rod *Erysipelothrix rhusiopathiae*. Human cases are generally acquired from domesticated animals, swine and poultry in particular (in