

CORRESPONDENCE

Reply to “On the reliability of the CD123-endowed basophil activation test (BAT) and its application in food allergy”

To the Editor,

In response to the comments of Chirumbolo et al.¹ concerning our recent paper,² we note that this group has published comments with similar concerns, in reply to various papers over the past several years.^{3–10} Their general concern is that basophil gating strategies employed for the basophil activation test (BAT) vary between different publications, with potential for non-maximal basophil purity to affect the interpretation of the BAT and thus its overall accuracy.

We contend that the concerns raised by Chirumbolo et al. regarding our paper are limited. Firstly, there appears to be a misunderstanding by Chirumbolo et al. who write at the end of their comments that we demonstrated that BAT could “...diagnose almost 67% of sesame allergic patients, a range very close to the one obtained with HPSE-SPT (≥ 13 mm), ie. 72%.” In fact, we show in table 1 of our paper that at the optimal critical value for CD63 as BAT activation marker (10.9%), a sensitivity of 86%, and a specificity of 85% were obtained. CD203c at its critical value (5.2%) yielded similar results (84% sensitivity, 80% specificity). At the critical value for HPSE-SPT (10 mm weal), performance was slightly worse (79% sensitivity, 75% specificity). However, while the overall performance of BAT exceeded that of HPSE-SPT, the latter test performed better at extreme test results. The 13 mm value for the HPSE-SPT referred to by Chirumbolo et al. was not its optimal critical value, but rather the value yielding 100% positive predictive value. Similarly, as we highlight in figure 4 of our paper, an HPSE-SPT weal size of ≤ 3 mm completely ruled out sesame allergy. In contrast, neither CD63 nor CD203c-based BAT was capable of completely ruling in or ruling out sesame allergy. These findings form the bases of our proposed diagnostic approach, which utilizes the strengths of each test.

Chirumbolo et al. raise the concern that our gating of the CD123⁺ cells may not have been sufficiently separated from the remainder of the side-scatter^{low} cell population, and they speculate that addition of IL3 to the stimulation media caused true basophils to dissociate from the gated population. After reexamining the flow cytometry files obtained for 7 patients whose blood was incubated in the presence or absence of IL3, we did not observe any notable difference in the geometric mean fluorescent intensity for FITC (the fluorochrome attached to anti CD123) between the gated populations in these 2 conditions. Furthermore, we disagree with the premise that the false-negative and false-positive results we encountered in BAT were due to basophil gating. In all BAT false-

negative patients, for whom both CD63 and CD203c test values were below their respective optimum cut-offs, CD63 was less than 1.5% and CD203c was less than 2.5% ($n = 4$). Likewise, for all BAT false-positive patients, for whom either CD63 or CD203c was above its cut-off, CD63 exceeded 32.5% and CD203c exceeded 10.5% ($n = 4$). With the exception of 3 BAT non-responders who were excluded from the BAT section of our algorithm, a positive control (anti-IgE or anti-Fc ϵ R1) response was observed in the BAT assay for all patients, using the same basophil gating, indicating that basophils were in fact captured. The test values for the BAT-misclassified patients were sufficiently distant from the cut-offs such that any possible modifications in basophil gating would not likely have affected the results.

Finally, we do not believe that BAT optimization will obviate the need for SPT. Skin prick testing is a well-recognized allergy test, yielding rapid results without the need for specialized equipment, or drawing of blood. Our paper shows that the reliability of SPT results may depend on the source of extract, and that HPSE-SPT in particular performs very well as a first-line test for sesame allergy. BAT can function to complement equivocal HPSE-SPT results. We proposed a similar approach with SPT a first diagnostic step followed by BAT, in our recent publication on treenut allergy diagnosis.¹¹ The variability among publications regarding basophil identification strategies and BAT protocols remains as a challenge. Yet, efforts to standardize these elements of BAT will strengthen its reliability and facilitate its broader implementation to the clinic.

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