Correspondence to “Basophil activation test as predictor of severity and threshold of allergic reactions to egg”

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To the Editor,

We read the related papers by Radulovic *et al*[1] and Krawiec *et al*[2] with great interest. Double-blind controlled food challenges (DBPCFCs) remain the reference test to diagnose egg allergy. However, DBPCFCs are resource-intensive and can cause life-threatening reactions. We appreciate their pursuit for safe and reliable tests to reduce the number of DBPCFCs. According to these studies, the basophil activation test (BAT) is a robust tool that confers superior diagnostic and predictive performance compared with traditional egg allergy diagnostics. However, we would like to add some nuances to these conclusions. The intrinsic variability of the BAT can affect its performance when applied in clinical routine.

Methodological details about the execution and interpretation of BAT are of utmost importance for correct interpretation of the results and to follow the calculation of the test performance. For example, the minimal numbers of cells analysed should be provided to enable calculation of the coefficient of variation of the test. In addition, the authors are urged to provide information about standardization of the mean fluorescence intensity (MFI) for CD203c, and to indicate whether the percentages of $CD63^+$-cells are expressed as net percentages. Furthermore, a clear explanation of the way results in the figures are expressed is required to appreciate the results. An example of a detailed BAT protocol is described by Bridts et al. [3].

Another important issue relates to non-responders/non-releasers. The authors define a non-responder status on CD63, but not for CD203c. However, as shown in figure 1, upregulation of CD63 and CD203c can dissociate and upregulation of CD203c alone does not indicate histamine release[4, 5]. The authors excluded 10.8% of the cases because of a CD63 non-responder status of the basophils[2], or restricted inclusion to CD63-BAT responders[1]. This approach deviates from the 2015 EAACI position paper recommendation to report on non-responder patients and include them as "false negatives" when assessing test performance[6]. Non-responders should not stay hidden or unpublished, as this can profoundly embellish the performance of the test and imperil credibility of research and utility of the test[7]. It is important to realize that i) as shown in figure 1, there are two distinct forms of basophilic

non-responsiveness, ii) responder status might differ according to the read-out and that iii) the interpretation of a non-responsiveness depends on the clinical status of the individual participant. Non-responsiveness to the positive control (e.g., anti-IgE or anti-FcεRI) is not by definition associated with non-responsiveness to the allergen. In established patients, when there is an unresponsiveness to both the positive control and the allergen, it is impossible to correctly interpret the negative allergen stimulation. For such patients the test is lost as a diagnostic. For study purposes, such uninterpretable results should be considered as invalid and allocated to the group of false negatives for an adjusted calculation of test metrics. In contrast, when unresponsiveness is limited to the positive control stimulus, positive allergen stimulation can be considered as diagnostic, provided the tested allergen does not trigger nonspecific basophil degranulation in exposed control individuals. Positive allergen stimulation in uneventfully exposed control individuals, should be considered as clinically irrelevant, irrespective responder status to positive control. In exposed controls, when there is a complete unresponsiveness to both the positive control and the allergen, it is impossible to interpret the negative allergen stimulation. For study purposes, such results should be considered as inutile and encourage inclusion of other exposed controls responsive to positive control stimulation.

Taken together, studies reporting on the BAT as a diagnostic should provide methodological details that allow correct interpretation and appreciation of the results. In addition, non-responders should be defined correctly, reported, and, most importantly, included in calculation of the test performance metrics in order to avoid bias in the results.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

All authors participated in writing the paper and in proofreading and revising the final text.

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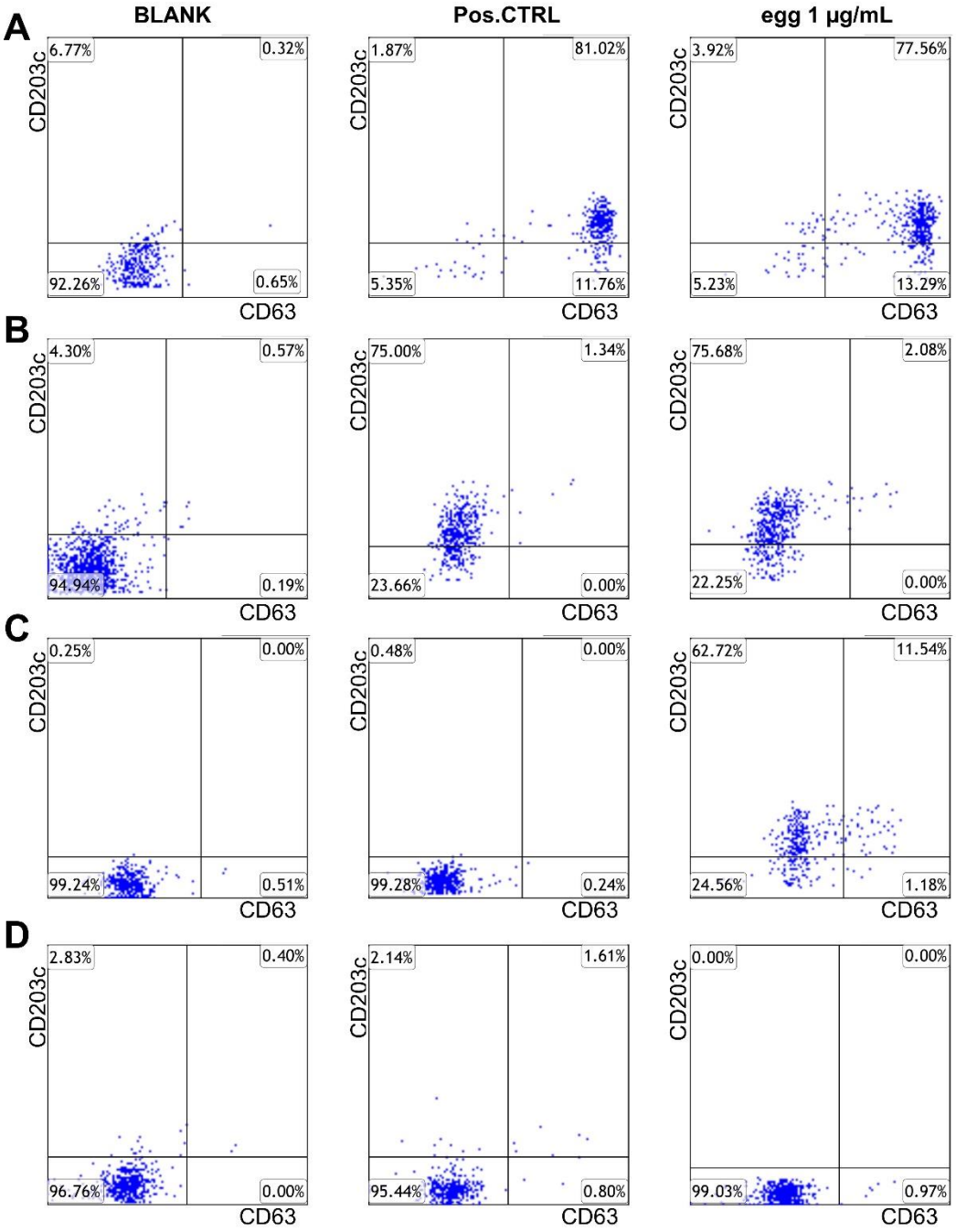

	Clinical characteristics	SPT egg	SIgE egg (kUA/L)
Panel A	Urticaria, angioedema, dyspnoea	3/10	0.24
Panel B	Urticaria, angioedema, dyspnoea	NA	2.55
Panel C	Urticaria, dyspnoea	NA	1.70
Panel D	Urticaria, angioedema	9/10	1.22
SPT, skin prick test (wheal and flare); NA, not available			

Figure 1: Basophilic responses to buffer (blank), positive control (anti-IgE) and egg (1µg/mL). Resting basophils barely express CD203c and CD63. [A]: traditional situation, i.e., basophils responding to both positive control and allergen with the upregulation of CD203c and CD63. [B]: a CD63 non-responder status is shown (unresponsiveness of CD63 to both positive control

and allergen although upregulation of CD203c). [C]: cells responsive to allergen but not to positive control. [D]: a complete non-responder status for CD63 and CD203c is shown (unresponsiveness to both positive control and allergen). Clinical and diagnostical characteristics of the patients shown in the different panels are displayed in the table.