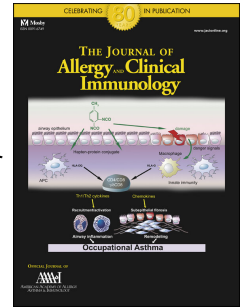


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Letter to the Editor

Basophil activation test determination of CD63 combined with CD203c is not superior to CD203c alone in identifying ABPA in cystic fibrosis

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Reply to letter from Gillian M. Lavelle et al. and letter from Dr. Chirumbolo

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26 **Capsule summary**

27 CD203c level at the surface of allergen-activated basophils is the best marker to
28 identify allergic bronchopulmonary aspergillosis (ABPA) and discriminate from
29 *Aspergillus fumigatus* colonization in patients with cystic fibrosis.

30

31 **Key words**32 - *Aspergillus Fumigatus*

33 - Biomarker

34 - CD203c

35 - CD63

36 - Cystic fibrosis

37 - Immunoglobulin E

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42 To the Editor (673 words),

43

44 We would like to comment on the letter from Professor Chirumbolo ¹ regarding
45 the work of our collaborators Mirkovic *et al.*. ² Mirkovic *et al.* demonstrated that
46 *Aspergillus fumigatus* (Af)-sensitized individuals display an increased
47 upregulation of the surface-expressed basophil marker CD203c in response to ex
48 vivo challenge with Af extract, a response readily measurable by flow cytometry
49 in the basophil activation test (BAT). However, Mirkovic *et al.* did not report on
50 evaluation of basophil CD63 expression, which is also upregulated by allergen
51 challenge, as shown in our prior published work. ³

52 It is known that CD203c and CD63 have independent kinetics upon basophil
53 activation. CD63 appears to be contained in the same cytosolic granules as
54 histamine; CD63 surface upregulation and basophil histamine release thus have
55 similar kinetics. ⁴ CD203c is an ectonucleotidase (E-NPP3) that is rapidly
56 induced at the surface of activated basophils to hydrolyze ATP. ⁵ It has recently
57 been shown that E-NPP3 is responsible for the suppression of ATP-dependent
58 chronic allergic inflammation. ⁶ CD203c and CD63 responses in the BAT assay
59 have shown to be informative in identifying patients with insect venom allergy,
60 food allergy, latex allergy and major immediate-type drug allergies. However, in
61 patients with cystic fibrosis (CF), Professor Chirumbolo raised the important
62 question as to whether measuring CD63 upregulation at the surface of the
63 basophil in association with CD203c levels would be helpful in identifying ABPA
64 and/or discriminating patients with ABPA from those colonized with *Aspergillus*

65 *fumigatus* (without ABPA). To bring insight into this question, we reviewed our
66 data on both markers.

67 We conducted a 2-year prospective longitudinal study comparing BAT
68 biomarkers by flow cytometry before and after activation with *Af* allergen extract
69 and recombinant *Asp f1* in CF-ABPA patients (n=20) and in two comparison
70 groups: CF patients colonized with *Af* but without ABPA (CF-AC; n=13) and CF
71 patients without either *Af* colonization or ABPA (CF; n=12). Patients were tested
72 every six months and when ill with a pulmonary exacerbation. In this study, a
73 number of basophil markers, including CD203c and CD63, were evaluated,
74 although only results for CD203c were reported in our recent publication.⁷ We
75 here summarize our results evaluating the receiver operating characteristics
76 (ROC) of BAT CD203c responses alone or combined with CD63 (Table).

77 In brief, the area under the ROC curve to identify CF-ABPA patients as
78 compared to CF patients without ABPA or *Af* colonization, based on CD63 levels
79 combined with CD203c, was only marginally superior to the ROC curve
80 generated with CD203c alone at visit 2 (0.94 compared to 0.86) and the two
81 ROC curves were identical at visit 1 (0.98). The ROC curve which resulted from
82 CD203c combined with CD63 was similarly only slightly better than the ROC
83 curve using CD203c only to discriminate CF-ABPA from CF patients with *Af*
84 colonization (CF-AC) in visit 1 (0.90 compared to 0.87) and both ROC curves
85 were almost identical at visit 2 (0.96 compared to 0.95). Finally, the ROC curve
86 which resulted from CD203c combined with CD63 was again only slightly better
87 compared to the ROC curve using CD203c alone in discriminating CF-AC from
88 CF patients at visit 1 (0.64 compared to 0.60) and the two ROC curves were

identical at visit 2 (0.73). The same results were obtained following 30 minutes of *ex vivo* stimulation with *Aspergillus Fumigatus* extract (data not shown).

Thus, from this study, allergen-induced BAT CD203c surface upregulation appears to be a sufficient single biomarker for diagnostic purposes, and does not require additional measurement of CD63 to identify ABPA and/or *Aspergillus fumigatus* colonization in patients with cystic fibrosis. Our data suggest that the differences between a single CD203c BAT and a combined CD203c-CD63 BAT are neither highly significant nor stable over time, and therefore the added effort and expense needed to run a combination assay (along with the statistical handling of that data) is not justified. Further studies enrolling large numbers of patients will need to be conducted in order to definitively determine the clinical value of the CD203c and CD63 BAT assays. This is particularly the case of APBA occurring with asthma, which remains to be investigated as aggressively as ABPA occurring in people with CF has been to date.

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Table I. Predictive values of individual and combined blood basophil CD203c and CD63 levels following 10 min *ex vivo* stimulation with *Af* extract for subject group for visit 1 (A) and visit 2 (B).

A

Groups to identify	Proposed	P value	Area under the
Visit 1	predictor(s)	(log likelihood)	ROC curve
CF-ABPA vs CF	CD203c(10 min)	$<10^{-3}$	.98
	CD63 (10 min)	.22	.57
	CD203c x CD63 (10 min)	$<10^{-3}$	.98
CF-ABPA vs CF-AC	CD203c (10 min)	.003	.87
	CD63 (10 min)	.16	.61
	CD203c x CD63 (10 min)	$<10^{-3}$	.90

CF-AC vs CF	CD203c (10 min)	.12	.60
	CD63 (10 min)	.91	.52
	CD203c x CD63 (10 min)	.08	.64

B

Groups to identify (Visit 2)	Proposed predictor(s)	P value (log likelihood)	Area under the ROC curve
CF-ABPA vs CF	CD203c (10 min)	$<10^{-3}$	.86
	CD63 (10 min)	$<10^{-3}$	.85
	CD203c x CD63 (10 min)	$<10^{-3}$	.94
CF-ABPA vs CF-AC	CD203c (10 min)	$<10^{-3}$	.96
	CD63 (10 min)	.002	.82

	CD203c x CD63	<10⁻³	.95
	(10 min)		
CF-AC vs CF	CD203c (10 min)	.03	.73
	CD63 (10 min)	.48	.51
	CD203c x CD63	.07	.73
	(10 min)		

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