



## Basophil activation test for inhalant allergens in pediatric patients with allergic rhinitis



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### ABSTRACT

**Objective:** Flow cytometric quantification of *in vitro* basophil activation can be quite performant and reliable tool to measure IgE-dependent allergen-specific responses in allergic patients. Current study aimed to evaluate the clinical relevance of basophil activation test (BAT) for the diagnosis of pediatric grass pollen and house dust mite (HDM) allergies.

**Methods:** Forty-seven patients suffering from allergic rhinitis with HDM and grass pollen co-sensitization with clinical history of allergic rhinitis and/or asthma and 15 non-allergic healthy subjects were enrolled. BAT was determined by flow cytometry upon double staining with anti-IgE/anti-CD63 mAb.

**Results:** Regarding HDM with cut-off point greater than 12.5% for CD63<sup>+</sup> basophils sensitivity and specificity of the BAT were 90% and 73%, with positive predictive value (PPV) and negative predictive value (NPV) as 0.70 and 0.91, respectively. The analysis of concordance of being either allergic or healthy in comparison to BAT results for HDM revealed a substantial concordance ( $\kappa$  index = 0.61,  $p < 0.001$ ). Grass pollen with cut-off point greater than 11%, BAT attained a sensitivity, specificity, PPV, and NPV of 96%, 93%, 0.98, and 0.88, respectively. The analysis of concordance of being either allergic or healthy in comparison to BAT results for grass pollen revealed an almost perfect concordance ( $\kappa$  index = 0.87,  $p < 0.001$ ).

**Conclusion:** Our findings concluded that BAT is reliable technique in the diagnosis of sensitization to grass pollen and HDM. The sensitivity of BAT in pollen allergic children was found to be remarkably higher in our cohort compared to other studies.

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### 1. Introduction

Allergy diagnosis generally relies upon the patient's clinical history and appropriate confirmatory tests such as skin prick tests (SPTs), specific immunoglobulin E (IgE) quantification and challenge tests. However, identification of the causative allergen(s) and potentially cross-reactive structures is not always straightforward [1]. The specificities of SPT and specific IgE are nearly 90% in patients with IgE mediated allergic disorder [2], and the alternative

tests can be useful when these tests are negative in allergic patients. Functional *in vitro* assays detecting release of mediators have been also used for the allergy diagnosis [3,4]. In general, these assays cannot be used routinely in the clinical practice due to cost and time consuming procedures [5]. Since none of the laboratory tests have reached adequate diagnostic accuracy, requirement of alternative tests are needed to study allergic patients.

Basophils are one of the effector cells involved in immediate type allergic reactions. These cells bear IgE bound to their membrane Fc $\epsilon$  type I receptors. On challenge with a specific allergen, basophils release mediators and change the phenotype due to the expression of several basophil surface molecules [6]. Following the discovery of CD63 as an activation marker of basophils in 1991 [7], the basophil activation test (BAT) was developed for Type-I-allergy diagnosis by Sainte-Laudy et al. [8]. CD63 (gp53) is a member of the

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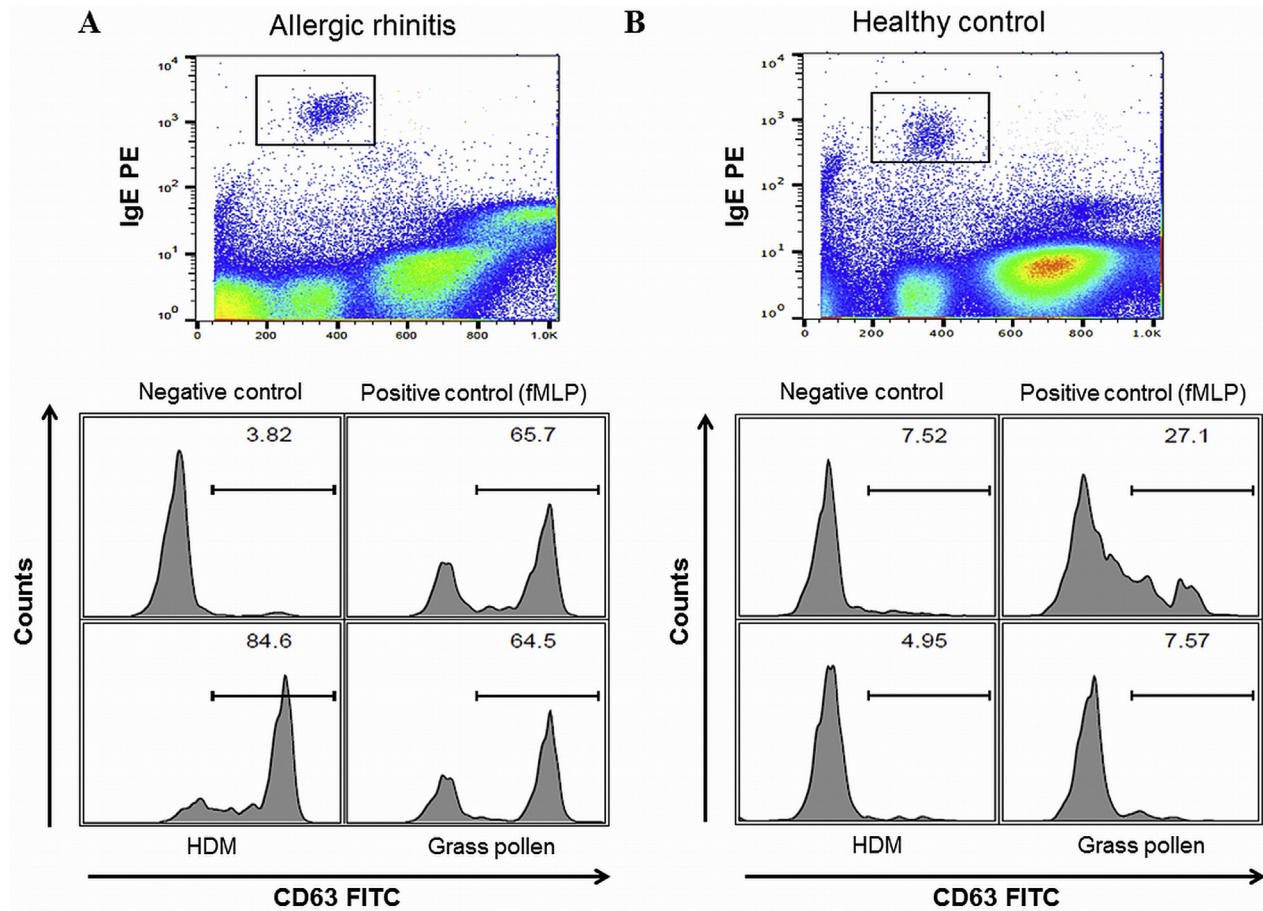


Fig. 2. Percent of stimulation index (SI) after adding fMLP, HDM and grass pollen in patients (P, n = 47) and healthy controls (HC, n = 15), (\*p < 0.05, \*\*\*p < 0.001).

**Table 1**  
Demographic and laboratory features of the patients with allergic rhinitis (AR).

|                           | Mean ± SD | Median (Min–Max) |
|---------------------------|-----------|------------------|
| Age (yr)                  | 11 ± 3    | 11 (5–18)        |
| Age at diagnosis (yr)     | 7 ± 3     | 7 (2–17)         |
| Total IgE (IU/ml)         | 523 ± 645 | 308 (24–3338)    |
| Eosinophils (/ul)         | 452 ± 284 | 400 (10–1400)    |
| Specific IgE Hp-1 (IU/ml) | 28 ± 30   | 18 (0.1–100)     |
| Specific IgE GP3 (IU/ml)  | 27 ± 36   | 8 (0.1–100)      |
| SPT DP (mm)               | 6.7 ± 2.6 | 6.2 (3–15)       |
| SPT DF (mm)               | 6.6 ± 2.2 | 6 (3.5–14)       |
| SPT Grass mix (mm)        | 6.9 ± 2.5 | 6.5 (3.5–13)     |

42 patients (89%) were reactive to HDM and 45 patients (96%) were reactive to grass pollen. The percent of stimulation index (SI) results of BAT with/without fMLP, HDM and grass pollen stimulations for patients and healthy controls were represented in Fig. 1. A low level of basophil activation to the positive control (fMLP) was seen in healthy controls as compared with the allergic rhinitis patients ( $p < 0.05$ ). Basophils from healthy controls did not respond to HDM and grass pollen (Fig. 1). The mean percentage of activated basophils in allergic children to HDM was  $4.2 \pm 3\%$  for negative control,  $42 \pm 24\%$  for positive control. The mean percentage of activated basophils in allergic children to grass pollen was  $4.2 \pm 3\%$  for negative control, and  $41 \pm 25\%$  for positive control. Accordingly, in the group of healthy controls, the mean percentage of cells with CD63 expression was  $6 \pm 4\%$  for negative control and  $22 \pm 15\%$  for positive control of the test kit. The expression of CD63 on basophils

was resulted as  $70 \pm 21\%$  for HDM and  $64.5 \pm 23\%$  for grass pollen after incubation with specific allergens at 1:100 dilutions according to the manufacturer's instruction. However, in the control group CD63 expression on basophils was  $2.3 \pm 1.6\%$  and  $3.7 \pm 2.3\%$  for HDM and grass pollen, respectively. Representative increase in expressions of CD63 on basophils following fMLP, HDM and grass pollen stimulations and the gating strategy for the flow cytometry were given in Fig. 2.

Positive technical cut-off in BAT to HDM and grass pollen allergens were recommended as increase more than 15% in CD63 expression of basophils in the manufacturer's instructions of the kit and obtained from ROC curve for inhalant allergens [18]. According to our results, positive technical cut-off in BAT to HDM was greater than 12.5% and to grass pollen was greater than 11% according to ROC curve of our data.

Cut-off value for BAT test with HDM was considered as greater than 12.5% for a positive result with an area under the curve 0.94. Using this cut-off point, we obtained a sensitivity of 90% and a specificity of 73%. PPV for this value was 0.70 and NPV as 0.91. The analysis of concordance of BAT results for HDM allergic patients and healthy controls revealed a substantial concordance ( $\kappa$  index = 0.61,  $P < 0.001$ ) (Table 2).

Cut-off value for BAT test with grass pollen was considered as greater than 11% for a positive result with an area under the curve 0.94. Using this cut-off point, BAT sensitivity was 96%, with a specificity of 93%. PPV for this group was 0.98 and NPV as 0.88. The analysis of concordance of BAT results for allergic patients and healthy controls revealed an almost perfect concordance ( $\kappa$

**Table 2**  
Sensitivity, specificity, PPV and NPV for HDM and grass pollen.

| BAT (SI)           | Sensitivity (%) | Specificity (%) | PPV  | NPV  | P (Chi Sq) | Kappa agreement |
|--------------------|-----------------|-----------------|------|------|------------|-----------------|
| HDM (12.5%)        | 89              | 73              | 0.70 | 0.91 | <0.001     | 0.61            |
| Grass pollen (11%) | 96              | 93              | 0.98 | 0.88 | <0.001     | 0.87            |

index = 0.87,  $P < 0.001$ ) (Table 2).

#### 4. Discussion

Our study demonstrated that the analysis of allergen-induced CD63 up-regulation by flow cytometry is a reliable tool for diagnosis of HDM and grass pollen sensitization in pediatric patients, with high specificity and sensitivity. As far as we know, the highest sensitivity value compared to others using CD63 in pollen allergic patients was obtained in current study with a cut-off value of 11%.

Basophil activation with specific allergens has proven to be useful diagnostic test for mite, pollen, food, latex, venom, and drug allergies [1,4]. Several studies have evaluated the application of flow cytometry in the diagnosis of inhalant allergens, such as HDM and grass pollen [5,18–25]. Overall, flow cytometric quantification of CD63 and/or CD203c on basophils exposed to natural and/or recombinant pollen allergens demonstrated a highly reliable approach for pollen allergy [22–25]. However, the CD203c protocol is not always straightforward due to low spontaneous expression of CD203c at resting, less prominent upregulation of CD203c by activated basophils, and false-negative results [1]. For these reasons, CD203c marker was also used to define purity of basophils in our validation studies as previously reported [26].

In the current study, sensitivity and specificity of the flow cytometric detection of basophil CD63 surface expression for grass pollen were 96% and 93%, respectively. Michova et al. using CD63 as an activation marker reported a sensitivity of 85% and a specificity of 100% to determine grass pollen allergic patients [23]. The highest sensitivity value compared to other studies in the literature using CD63 in pollen allergic patients was obtained in this study; moreover the specificity value (93%) was relatively high. Whereas, Ozdemir et al. using CD203c as an activation marker demonstrated a sensitivity and specificity of 95% to find out pollen allergic patients [24] which was very similar to our results. Additionally, corresponding PPV and NPV to determine grass pollen sensitization in children with allergic rhinitis were resulted as 0.98 and 0.88, respectively.

Two studies investigating the flow cytometric analysis of *in vitro*-activated basophils in the diagnosis of HDM allergy resulted that the overall sensitivity and specificity equaled or exceeded 90% [18,21]. Additionally, Cozon et al. also determined sensitivity and specificity of basophil CD63 surface expression with IL-3 pre-incubation induced by HDM and concluded as 78% and 81%, respectively for the diagnosis of atopic patients [19]. In current study, sensitivity and specificity for HDM were 90% and 73% to determine the sensitization in children with allergic rhinitis. Our results driven from evaluation of CD63 as an activation marker of basophils were in accordance with Paris-Köhler et al. [21] and Sanz et al. [18]. However, our specificity result for HDM was slightly lower than these studies above. The corresponding PPV and NPV for HDM, in current study, were found to be 0.70 and 0.91, respectively.

A relatively large proportion of the healthy controls showed significantly low level of basophil activation to the positive control (fMLP) as compared with the allergic rhinitis patients. This observation is in line with previous data shown in the literature [11,27,28]. This situation may be related to less sensitization in basophils of healthy controls in response to a stimulation with a

cross-linking antibody to IgE and fMLP [27].

All patients with allergic rhinitis symptoms in our cohort had SPT and specific IgE positivity for both grass pollen and HDM. Two of the grass pollen allergic and five of the HDM allergic patients did not respond to activation upon the relevant allergens. Positive SPT and detectable IgE concentrations to grass pollen and HDM allergens, but a negative outcome for CD63 expression may be explained by disturbed intracellular signaling commonly observed in around 5% of the general population (non-responders) [29].

In conclusion, our results were consistent with the description of BAT as highly reliable and easily applicable method to detect *in vitro* sensitization to grass pollen and HDM as a complementary tool to specific IgE and SPT. The obtained specificity, sensitivity, PPV and NPV values for these allergens were also found to be comparable with the levels in the literature.

#### Conflict of interest

The authors declare no conflict of interest directly relevant to this paper.

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