

Original paper

The usefulness of the basophil activation test in monitoring specific immunotherapy with house dust mite allergens

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Abstract

Introduction: In clinical practice, reliable tools for monitoring specific immunotherapy (SIT) are of utmost importance.

Aim: To assess the usefulness of the basophil activation test (BAT) in monitoring SIT in paediatric patients with allergy to house dust mites (HDM).

Material and methods: Thirty-one children qualified for SIT with HDM, of whom 21 completed the SIT during the observation period. The BAT was carried out prior to commencing the SIT (time point BAT1) and upon finishing the initial pack of allergy vaccine (cumulative dose of allergen 12487.5 PNU; BAT2), as well as after the second vaccine pack (cumulative dose of allergen 23750.0 PNU; BAT3). Peripheral blood of the patients was stimulated with allergen solutions in five concentrations from 0.00225 ng/ml to 22.5 ng/ml. Basophil activation was measured by CD63 expression in flow cytometry.

Results: For the allergen concentration of 0.225 ng/ml, a statistically significant decrease in median basophil activation was observed, from 51.29% at BAT1 to 8.48% at BAT2 ($p = 0.004$) and 4.21% at BAT3 ($p < 0.001$). For the allergen concentration of 0.0225 ng/ml, a statistically significant decrease was seen between BAT1 (1.72%) and BAT3 (0.21%, $p = 0.01$). Median CD-sens index decreased significantly from 1099.02 at BAT1 to 179.31 at BAT2 ($p < 0.002$) and 168.04 at BAT3 ($p < 0.001$).

Conclusions: There is a significant decrease in BAT results in the course of specific immunotherapy with HDM allergens in children, with the optimum allergen concentration for monitoring basophil response at 0.225 ng/ml. The CD-sens index seems to be a better monitoring parameter than the plain percentage of CD63-expressing basophils.

Key words: basophil activation test, CD-sens, monitoring of specific immunotherapy.

Introduction

Allergic symptoms may be alleviated with medications; however, only specific immunotherapy (SIT) constitutes a causal treatment of atopic diseases. The SIT may interrupt the “allergic march” and cause clinical improvement to persist years after cessation of the treatment [1]. Unfortunately, providing a univocal answer to the question of the optimum duration of immunotherapy in a given patient proves difficult in clinical conditions. The clinical effectiveness of SIT seems to be related to the administered dose of allergen, rather than merely the duration of the therapy; therefore, assessing the therapeutic effects after administering defined cumula-

tive doses of allergen seems reasonable [2]. In practice, the time of administration of the same cumulative dose may vary substantially, which is due to individual tolerance and compliance, as well as complicating health conditions (e.g. infections). The role of basophils in the allergic reaction and in development of immunological tolerance during SIT has been studied intensively [3]. It is assumed that basophil stimulation *in vitro* replicates an *in vivo* mechanism that leads to the development of allergic symptoms. The SIT has been demonstrated to modulate basophil and mastocyte activation thresholds and decreases IgE-mediated histamine release [4, 5]. Studies into the course of immunotherapy demonstrated that SIT initially decreases basophil activation by the al-

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lergen with a subsequent decrease in basophil count and production of interleukin (IL)-4 and IL-13 [6]. The decrease in basophil activation as well as depletion of mediator reserves due to repetitive allergen stimulation is considered as the key mechanism at early stages of SIT [7]. Hence, a decrease in basophil activation in response to the allergen *in vitro* seems a promising marker of clinical tolerance developing during the course of SIT [8].

Despite basophil activation test (BAT) being considered as an aid in diagnosis of airborne allergy, drug allergy, Hymenoptera venom allergy, and in autoimmune urticaria, as well as in qualification for SIT, the protocols used and results obtained still vary from centre to centre [9]. Basophil activation markers with proven sensitivity and specificity are CD63 and CD203c. CD63, i.e. lysosomal-associated membrane glycoprotein-3, LAMP-3 in a resting basophil is located in the membrane of intracellular secretory granules. Upon FcεRI activation, it is expressed on the basophil surface [10]. CD203c is a transmembranous, glycosylated molecule, which is constitutively present on basophil surfaces, and upon being activated by allergen its expression increases [11]. Both markers are characterised by similar sensitivity and specificity. CD63 is used more widely due to the direct connection between its membrane expression and basophil degranulation [12, 13].

Two outcome measures of basophil activations are used: basophil reactivity, i.e. the number of basophils activated by allergen, and basophil sensitivity, i.e. allergen concentration causing half of the basophils to degranulate. Basophil sensitivity provides additional information about the sensitivity to allergen – how much allergen basophils can tolerate before they react. On the curve of reactivity vs. allergen concentration, the eliciting con-

centration at which 50% of basophils respond (EC50) is determined. EC50 can be expressed as ‘CD-sens’ by inversion and multiplication by 100. Basophil reactivity may be expressed as ‘CD-max’ (see below) [9].

Aim

The aim of the present study was to assess the usefulness of a BAT in monitoring SIT in paediatric patients with allergy to house dust mites (HDM).

Material and methods

Study protocol

During the first stage of the study conducted in the years 2010–2012, the results of which were published elsewhere [14], out of the group of 52 children with respiratory allergy aged 7–18 years, 31 had been qualified for SIT with HDM allergens based on the clinical symptoms, skin prick tests (SPT) results, and sIgE concentration. The first basophil activation test (BAT1) was carried out in those patients before commencing SIT. Control BATs were performed after administering to the subjects defined cumulative doses of the allergen – respectively 12487.5 PNU (the total allergen amount in the initial pack of allergy vaccines; measuring point (BAT2)) and 23750.0 PNU (cumulative dose from the first and second packs of the vaccine (BAT3)). Figure 1 presents the study design.

Changes in two parameters of basophil activation upon stimulation were analysed during the SIT. The first was calculated by subtracting the baseline basophil activation recorded in the negative control from the maximum activation after stimulation with the allergen at defined concentrations. The second parameter – the CD-sens used to describe subjects’ sensitivity to the allergen – was calculated by reversing the allergen concentration required to cause CD63 expression on 50% of all basophils in the sample and multiplying the product by 100 [15]. The study project was approved by the Committee of Bioethics of the Jagiellonian University; opinion no.: KBET/119/B/2011 of 1 July 2011. Written informed consent was obtained for each participant from a parent.

Basophil activation test

Patients’ blood (each 400 µl) was collected in test tubes containing ethylenediaminetetraacetic acid (EDTA) before commencing SIT (BAT1), after completing the initial pack of allergy vaccine (BAT2), as well as after completing the second pack (BAT3). The test was performed within 2 h after the blood collection. The assessment of basophil activation by measuring CD63 antigen expression was carried out by means of the Flow2 CAST test (Bühlmann Laboratories AG, Schönenbuch, Switzerland). Anti-FcεRI monoclonal antibodies and fMLP oligopeptides were used as a positive control, while an unstimulated

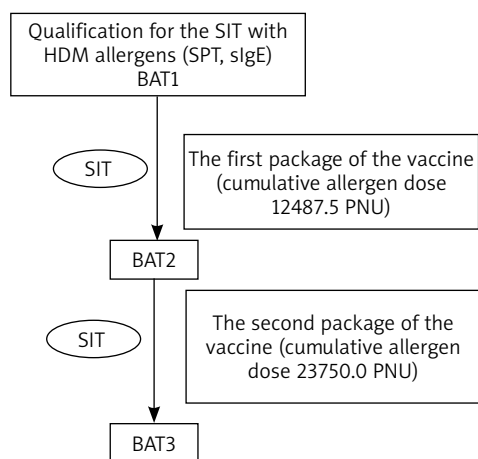


Figure 1. Design of the study. The BAT, SPT, and sIgE were carried out prior to commencing the SIT (BAT1) and after completing the initial pack (BAT2; cumulative allergen dose 12487.5 PNU), and after completing two packages (BAT3; cumulative allergen dose 23750 PNU)

sample served as a negative control. Cells were stimulated with an allergen of *Dermatophagoides pteronyssinus* (Dp) at the following concentrations: 22.5 ng/ml, 2.25 ng/ml, 0.225 ng/ml, 0.0225 ng/ml, and 0.00225 ng/ml. A mixture of FITC-conjugated anti-CD63 monoclonal antibodies, and PE-conjugated anti-CCR3 antibodies was added to the cellular suspension and incubated for 15 min at 37°C, after which 2 ml of erythrocyte lysing solution were added, samples were centrifuged, resuspended in 0.3 ml of washing buffer, and analysed within 1 h on a flow cytometer (FACSCalibur, Becton, Dickinson and Company, New Jersey, USA) with basophils gated as CCR3⁺/SSC^{low} cells.

Statistical analysis

As the distribution of raw data was non-Gaussian, medians were used in the description of results and Wilcoxon's test was applied in testing the statistical significance of differences observed. The analysis was performed using the GraphPad Prism 6 package.

Results

A total of 31 patients were qualified for the SIT; their results are presented in Table 1. Of these patients, 21 subjects actually underwent the SIT, of whom 14 (67%) patients reported for the BAT2 testing 4 weeks after administration of the last dose from the initial pack of the vaccine, and 21 (100%) patients reported for the BAT3 carried out 4 weeks after administration of the last dose from the second pack of the vaccine. An overview of the results is shown in Figure 2. Significant changes were observed for BAT results expressed as the percentage of activated basophils at the allergen concentrations of 0.225 ng/ml and 0.0225 ng/ml, as well as for CD-sens (Table 2). A comparison of indices that decreased significantly in the course of SIT is shown in Figure 3.

Discussion

In our study, the BAT turned out to be a good objective parameter for the monitoring of SIT with an HDM allergen in children. We applied a protocol measuring basophil activation in whole blood, used a CD63 as a basophil activation marker [14, 16–19], and measured SIT effects after

having administered an earlier planned identical cumulative dose of the allergen. In SIT monitoring, we used five 10-fold allergen dilutions identified in the preliminary tests [20]. The basophil activation thus calculated decreased significantly in the second measurement for the concentration of 0.225 ng/ml, and in the third for concentrations of 0.225 ng/ml and 0.0225 ng/ml respectively.

So far there have been few studies using a BAT to assess SIT effectiveness in allergy to HDM. In the study by Ciepiela *et al.* basophil activation after stimulation with Dp or *Dermatophagoides farinae* (Df) or grass pollen allergens in 0.2 IR/ml concentration, decreased in the majority of children after a year of sublingual immunotherapy (SLIT) [21]. Sánchez and Cardona observed that basophil activation after Dp stimulation in 1 ng/ml concentration is lower than in the control group after a year of the SIT with Dp/Df allergens in children with atopic dermatitis [22].

The BAT is more often applied in monitoring of immunotherapy for seasonal aeroallergens and Hymenoptera venom. Gokmen *et al.* for the olive pollen allergen concentration of 1 ng/ml obtained a decrease in basophil activation after 7 preseasonal injections [23]. In the study Kepil Özdemir *et al.* basophil activation in response to stimulation with grass allergens in 4.5×10^{-4} and 4.5×10^{-5} µg/ml concentrations decreased after the completed preseasonal immunotherapy and remained at a low level during the next grass pollination season [24]. Zitnik *et al.* demonstrated a decrease in CD63 expression output values in response to a submaximal concentration of 0.1 µg/ml after 6 months of desensitisation to bee venom [25]. In a study by Ebo *et al.*, after 6 months of venom immunotherapy (VIT), a decrease in basophil activation, also limited to a submaximal concentration of 0.01 mg/ml, was recorded, which, according to the authors, suggests that 6 months of immunotherapy induces a decrease in a basophil response, but not total suppression of these cells [26].

Not all researchers record decreases in basophil activation during immunotherapy. Van Overtvelt *et al.* did not observe significant changes in basophil activation despite the sIgG induction in patients receiving SLIT with grass pollens for 4 months [27]. Erdmann *et al.* did not observe a correlation between a change in basophil activation during VIT and a response to a control sting provocation test. Unfortunately, basophil activation was performed with two allergen concentrations, 100 ng and 1 µg, and due to

Table 1. Basophil activation test results in all 31 patients who were qualified for SIT

| Basophil activation* | Allergen concentration | | | | |
|----------------------|------------------------|------------|-------------|--------------|---------------|
| | 22.5 ng/ml | 2.25 ng/ml | 0.225 ng/ml | 0.0225 ng/ml | 0.00225 ng/ml |
| Median | 64.01% | 67.49% | 46.42% | 4.87% | -0.19% |
| Minimum | 5.20% | 1.80% | -2.67% | -5.48% | -6.77% |
| Maximum | 92.09% | 89.10% | 87.46% | 52.32% | 8.00% |

*Basophil activation expressed as percent of basophils expressing CD63 on their surface in response to allergen, after subtracting the baseline activity (negative control).

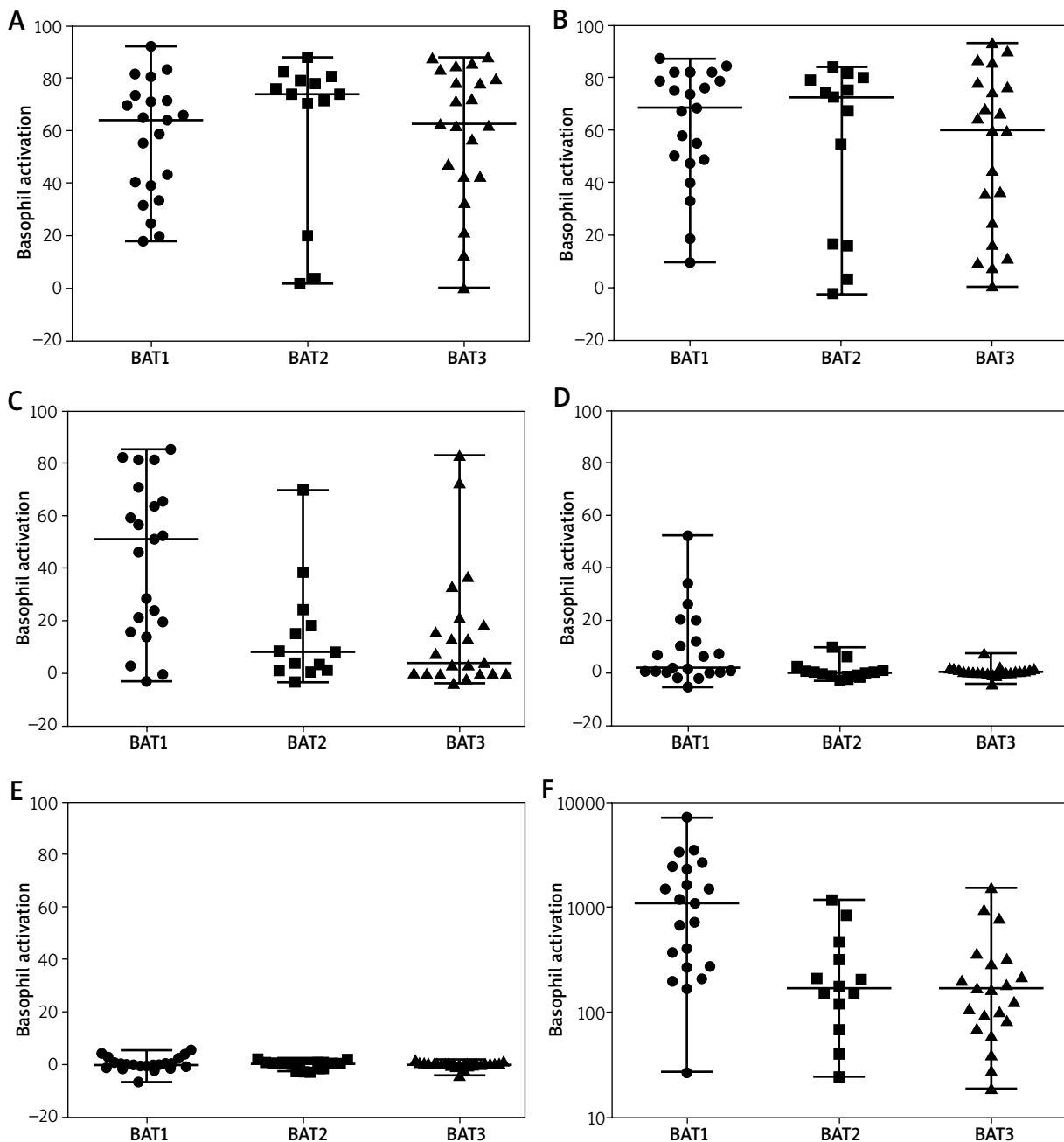


Figure 2. Overview of changes in basophil activation for each concentration (A – 22.5 ng/ml, B – 2.25 ng/ml, C – 0.225 ng/ml, D – 0.0225 ng/ml, E – 0.00225 ng/ml) and changes in CD-sens (F) during the SIT. Horizontal lines indicate medians and ranges

this fact the authors only assessed the changes in basophil reactivity, but not basophil sensitivity, which may have resulted in obtaining false results [26, 28].

The latest publications emphasise that based on CD-max, clinicians may draw erroneous conclusions pertaining to continuation/cessation of a SIT or anti-IgE treatment [29]. In assessing changes in basophil activation during immunotherapy, the CD-sens should be analysed

[30–32]. In our study changes in CD-sens were significant in all the subjects already at the first measurement during the SIT, while in the following determination we observed a further decrease in CD-sens. In the paediatric population, due to the lack of cooperation, it is frequently difficult to perform a specific provocation, constituting a gold standard in assessment of an allergen’s impact on the target organ, and, for this reason, application of a CD-

Table 2. Basophil activation test parameters that changed significantly during specific immunotherapy

| Variable | | Measuring point | | |
|-------------------------------------|--------|-----------------|---------------|---------------|
| | | BAT1 | BAT2 | BAT3 |
| Basophil activation at 0.225 ng/ml | Median | 51.29% | 8.48%* | 4.21%** |
| | Range | -2.67–85.48% | -2.98–70.13% | -3.4–83.27% |
| Basophil activation at 0.0225 ng/ml | Median | 1.72% | 0% | 0.21%* |
| | Range | -5.48–52.32% | -2.99–9.55% | -4.2–7.29% |
| CD-sens | Median | 1099.02 | 179.31* | 168.04** |
| | Range | 27.26–7256.89 | 24.52–1181.89 | 18.90–1544.88 |

* $p < 0.05$, ** $p < 0.001$.

sens reflecting the sensitivity to an allergen is of peculiar importance in this group. Our results correlated with a clinical improvement experienced by patients. Similar results were obtained by other authors in the case of SIT monitoring with birch and timothy as well as with Hymenoptera venom. In the study by Nopp *et al.* a decrease in CD-sens in the early stage of immunotherapy was accompanied by an increase in IgG4 antibody concentration and lack of changes in basophil reactivity [33]. Schmid *et al.* observed a decrease in basophil sensitivity already after 3 weeks, while Lalek *et al.* observed a decrease after two months of SIT, which correlated with a reduction in clinical symptoms [34, 35].

Conclusions

There is a significant decrease in BAT results in the course of specific immunotherapy with HDM allergens in children, with the optimum allergen concentration for monitoring the basophil response at 0.225 ng/ml. The CD-sens index seems a better monitoring parameter than the plain percentage of CD63-expressing basophils.

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Conflict of interest

The authors declare no conflict of interest.

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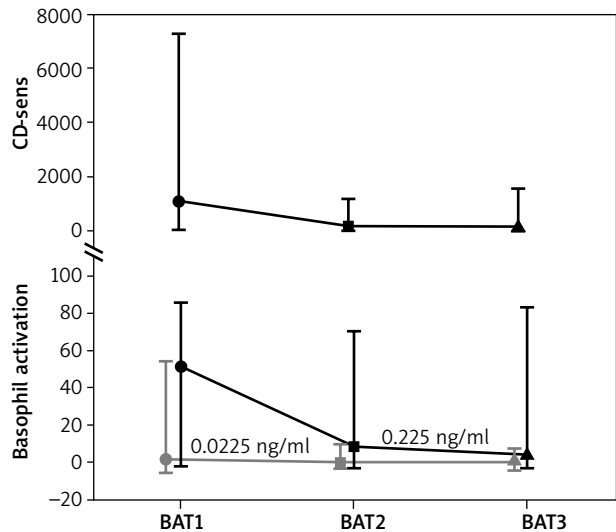


Figure 3. Significant changes in percentages of activated basophils and CD-sens in the course of SIT

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