

Monitoring of Allergen-Specific Immunotherapy using Basophil Activation Test: A Case Study

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Introduction

We used commercially available flow cytometry detection BasoFlowEx® Kit (EXBIO Praha) based on combination CD63FITC/CD203cPE to monitor changes in basophil specificity and reactivity of the patient undergoing immunotherapy against Timothy grass (*Phleum pratense*) pollen. Stimulated blood samples were assayed by detection kit followed by analysis using flow cytometry. Basophil specificity and reactivity changes were calculated from dose-response curves as an area under curve (AUC) parameter for the comparison purpose. A correlation between the changes of basophil specificity and reactivity and changing levels of Timothy grass specific IgEs during immunotherapy was also checked.

Objective

The use of BAT for a monitoring of allergen-specific immunotherapy was described elsewhere^{1, 2}. To validate compatibility of EXBIO Praha products (ED7043 BasoFlowEx® Kit, ED7443 Timothy grass (g6), ED7458 rPhl p 1 (g205), ED7459 rPhl p 5 (g215) and ED7460 rPhl p 7 (g210) in BAT a monitoring of immunotherapy efficacy has been chosen as a proper experimental design.

Experimental set-up

- Single patient hypersensitive to Timothy grass (*Phleum pratense*) with grass pollen-induced rhinitis and allergic conjunctivitis was chosen for immunotherapy monitoring.

- Sublingual immunotherapy is performed with an oral lyophilisate tablets of Grazax (ALK-Abello A/S, Denmark).

- Treatment is set for 3 year with start on 01/2017 and ongoing.

- Basophil activation test (BAT) is performed once per month during treatment by *in-vitro* stimulation of basophilic granulocytes in freshly taken heparinized blood sample with Timothy grass pollen extract and recombinant molecules rPhl p 1, rPhl p 5 and rPhl p 7.

- To obtain statistically significant data BAT was performed for each of ten points of concentration range of stimulating allergen in triplicates and average value was calculated (CD63+ cells). For that reason BAT was accommodated to be performed in 96-well test plate. Minimum of 160 basophilic granulocytes (CD203c+ cells) of each individual BAT was used to evaluate cells response at each concentration point.

- Basophil expression of CD63 and CD203c was evaluated with the FACS BD Canto SORP flow cytometer with HTS and data were analyzed with FACSDiva software (BD Biosciences, USA). Dose-response curves and AUCs were calculated with SigmaPlot software, version 13 (Systat Software, USA).

- Specific IgEs against Timothy grass pollen and rPhl p 1 were measured every three month from blood plasma using ImmunoCAP LAB assay to correlate the data obtained from BAT measurements with the sIgE level changes and patient's symptoms in response to treatment.

- Written informed consent was obtained from the patient.

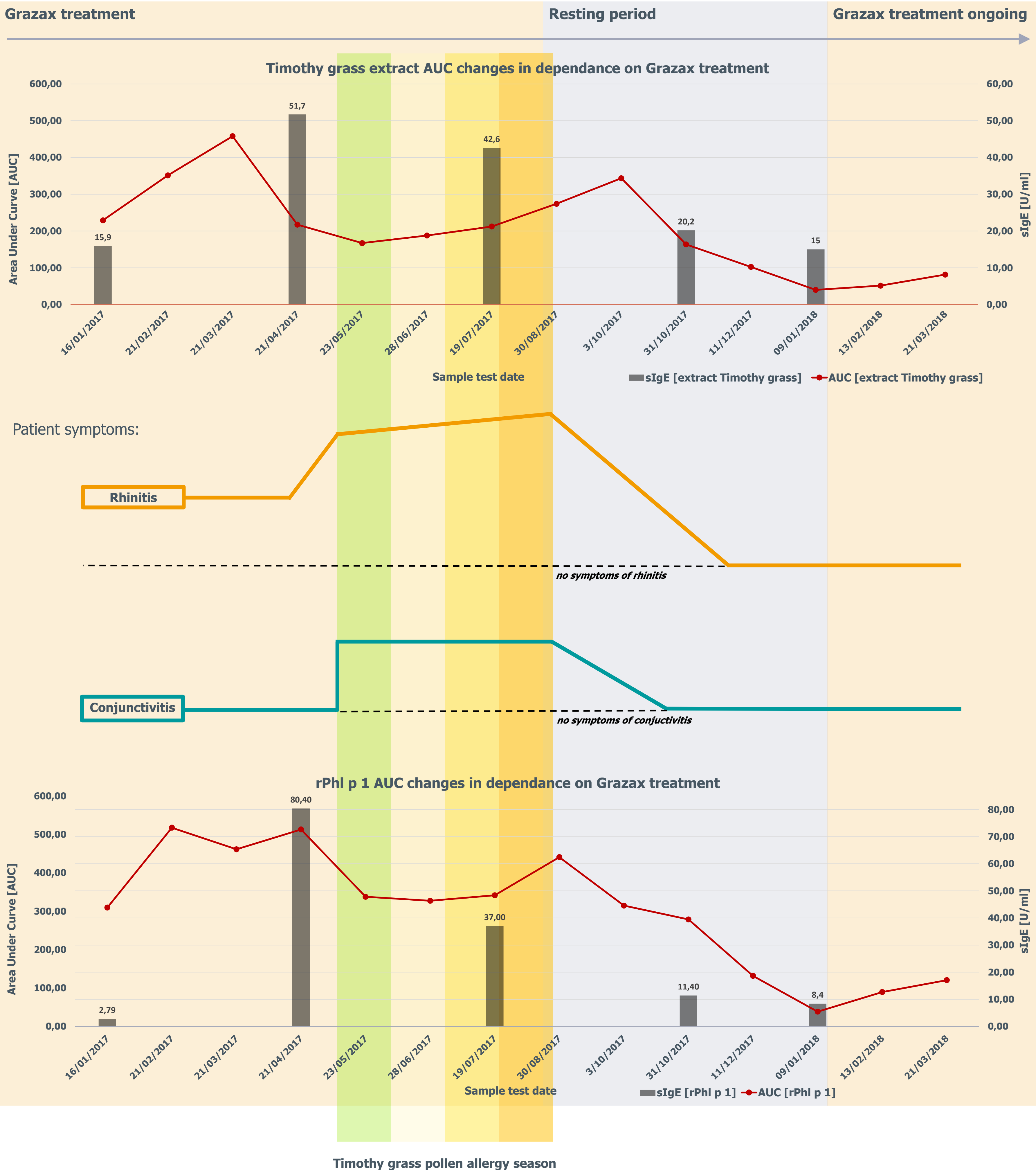
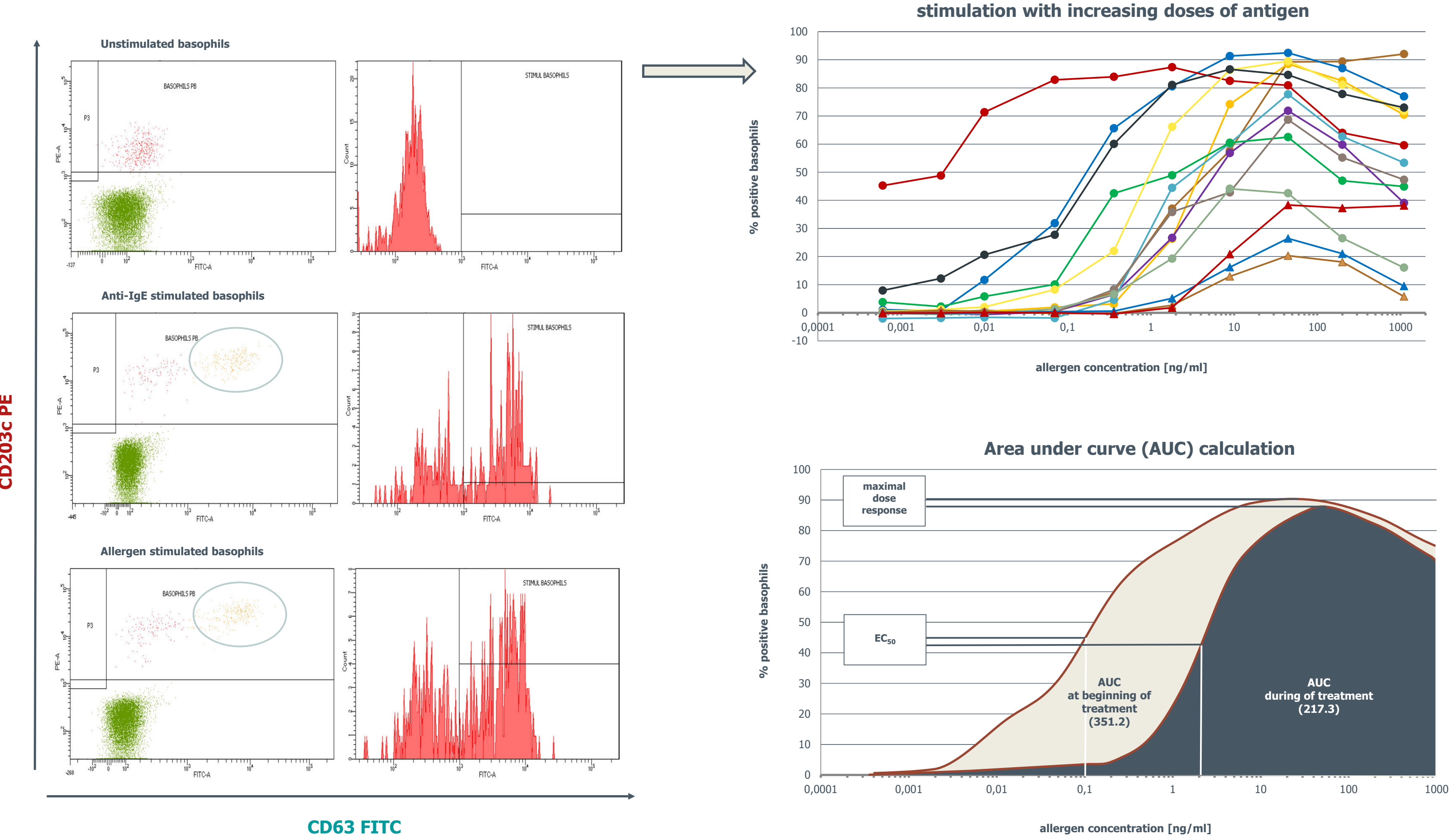
Results

Changes in basophil specificity and reactivity, which were detected using BAT method with EXBIO products, correlate with patient symptoms described during allergen-specific immunotherapy as well as with changes of allergen specific IgEs levels (measured with the ImmunoCAP Lab assay).

Conclusion

A wide variability of basophil sensitivity and reactivity to same stimulating antigen (allergen) during immunotherapy of single patient was observed when BAT analysis was used to monitor changes of basophil activation. Therefore it occurs to us that most complex tool to evaluate changes in IgE-mediated basophil activation in a reaction to specific immunotherapy is to calculate and compare area-under-curve (AUC) parameter values. The Basophil Activation Test thus can be very convenient method to evaluate efficacy of the disease treatment during allergen-specific immunotherapy.

Results



The patient's clinical symptoms of IgE-mediated Timothy grass hypersensitivity correspond to changes in basophil specificity and reactivity on stimulation with pollen extract and rPhl p 1 observed during ongoing allergen-specific immunotherapy. The data for rPhl p 5 and rPhl p 7 are not published since patient's basophils stimulation signal to these two allergens is negative.

References

¹Patil SU, Shreffler WG. Immunology in the Clinic Review Series; focus on allergies: basophils as biomarkers for assessing immune modulation. Clin Exp Immunol 2012; 167:59-66.
²Santos AF, et al. Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. J Allergy Clin Immunol 2015; 135:179-186.