**Simplified AIT for allergy to several tree pollens - Arguments from the immune outcome analyses following treatment with SQ tree SLIT-tablet**

**Online supplement**

**Authors:** Peter Adler Würtzen1, Pernille Milvang Grønager1, Gitte Lund1, Shashank Gupta1, Peter Sejer Andersen1, Tilo Biedermann2, and Henrik Ipsen1

**Affiliations:**

1: ALK, Hoersholm, Denmark, 2: Department of Dermatology and Allergology, Technical University of Munich, Munich, Germany and Clinical Unit Allergology, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH, Neuherberg, Germany

**Author e-mails:** [PAWDK@alk.net](mailto:PAWDK@alk.net), [PMGDK@alk.net](mailto:PAWDK@alk.net), [GILDK@alk.net](mailto:PAWDK@alk.net), [SGUDK@alk.net](mailto:PAWDK@alk.net), [PADDK@alk.net](mailto:PAWDK@alk.net), tilo.biedermann@tum.de, [HHIDK@alk.net](mailto:HIDK@alk.net)

**\*Corresponding author:** Peter Adler Wurtzen (PAWDK@alk.net)

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**Performance of the Cor a IgG4 ImmunoCAP assay**

The average level of serum IgG4 induced by tree tablet treatment differs somewhat when measured by the ImmunoCAP using birch, alder, and hazel allergen extracts even though this difference is less pronounced for serum IgE concentrations. The performance of the IgG4 assay for hazel extract may be evaluated to some extend by comparing serum IgG4 concentrations to hazel extract with concentrations to hazel major allergen Cor a 1 because both of these assays are available for the ImmunoCAP assay. In addition, the IgG4 responses towards major allergens Bet v 1 and Cor a 1 and towards the corresponding tree pollen extracts Bet v and Cor a were compared to further understand the differences observed.

**Methods**

50 actively treated patients from the phase III trial (ref) were selected at random for evaluation of the method used for IgG4 measurements.

Serum samples collected at baseline and after 1, 4, 7, and 9 month treatment from this patient subset were analysed for Bet v 1 and Cor a 1 serum IgG4 antibodies by ImmunoCAP (Phadia 250, Thermo Fischer Scientific, Uppsala, Sweden).

The data were compared to previously obtained data on serum IgG4 antibodies towards the allergen extracts Bet v and Cor a also measured by ImmunoCAP.

The change from baseline during treatment was assessed by fitting to a linear mixed model similar to the statistical method for IgE and IgG4 towards the allergen extracts described in the main manuscript.

**Results and discussion**

As seen from figure E1, Bet v 1 IgG4 serum concentrations are lower that Bet v IgG4 (right-shift in the scatter-plot), which would be expected considering that Bet v 1 is an individual protein among the multiple allergen/protein components present in an allergen extract. In contrast serum IgG4 concentrations to Cor a 1 are higher than to the corresponding hazel extract Cor a (left-shift in the scatter-plot). This is surprizing and suggests that a part of the Cor a 1-specific IgG4 is not detected in the Cor a IgG4 assay and that the latter seem to underestimate the sum of hazel component specific IgG4 in the serum samples. Furthermore, if only a part of the total hazel-specific IgG4 is detected by the Cor a IgG4 assay it may be the reason why lower average serum IgG4 concentration towards hazel are measured during SQ tree SLIT-tablet treatment than the concentrations measured for birch.



Scatter-plots of Bet v or Cor a serum IgG4 (x-axis) and serum IgG4 specific to either Bet v 1 or Cor a 1 (y-axis). Lower level of quantification (LLQ) is indicated by dashed lines (0.15 mgA/L) for both axes. Solid lines indicate line of identity (x=y).

This was further investigated by comparing the Least Squares Means modelled kinetics of IgG4 towards Bet v 1 and Cor a 1 with the kinetics for birch extracts (Bet v) where data were available for all 50 patients (figure E2). The data clearly shows that the average change from baseline in Bet v, Bet v 1 and Cor a 1-specific IgG4 are almost identical throughout the treatment duration with a maximum fold change above three after 7 and 9 month treatment. This further supports the notion that the only 2-fold increases seen towards hazel extract (figure 2, main manuscript) would have been higher if all the Cor a 1-specific IgG4 was detected by the Cor a assay.



Data are represented as Least Squares Means (LSM) fold change from baseline of allergen extract specific IgG4 (mean +/- 95% conf). Bet v=Betula verrucosa/birch (circles), Bet v 1=birch group 1 major allergen (diamonds) Cor a 1=hazel group 1 major allergen (squares).

T-cell production of cytokines in response to Bet v

Online fig E3: Predominant generation of Th2 T-cell lines 

T-cell cytokine production was evaluated by fluoroSPOT analysis 10 T-cell lines with sufficient cell numbers. Cells were isolated at day 14 after initial stimulation with Bet v and the number of cells producing IL-5 or IFN-g (per mio cells) on day 3 after restimulation with Bet v are depictured with backgrounds subtracted. 9/10 T-cell lines had cells produced IL-5, 7/10 mainly contained IL-5 producing cells, 1/10 equal numbers of IL-5 and IFN-g producing cells, and two cell lines were dominated by/exclusively contained IFN-g producing cells. Cytokine producing cells per mio cells varied from around 100-6000 for IFN-g and around 200-8000 for IL-5.

**Additional online figs – data from individual patients**

Online fig E4: Change from baseline in serum IgE

A, Alder



B, Birch



C, Hazel



Changes in IgE antibody responses were investigated as described in the material and method section of the main manuscript and data for the individual subjects in the active (blue) and the placebo group (red) are depictured for transparency regarding modelling of the data (fig 1). Dark blue/red dots for birch-specific IgE measurements are indicating the subset used in the current investigation matching the subset analysed for alder- and hazel-specific IgE.

Online fig E5: Change from baseline in serum IgG4

A, Alder



B, Birch



C, Hazel



Changes in IgG4 antibody responses were investigated as described in the material and method section of the main manuscript and data for the individual subjects in the active (blue) and the placebo group (red) are depictured for transparency regarding modelling of the data (fig 2). Dark blue/red dots for birch-specific IgE measurements are indicating the subset used in the current investigation matching the subset analysed for alder- and hazel-specific IgG4.