group 1 and 5 allergens in a 13 grass extract and to standardise the extraction method.

Methods: The grass pollens were extracted in isolation and pooled also in combination and analysed using a variety of techniques including enzyme-linked immunoabsorbent assay (ELISA), liquid chromatography-mass spectrometry (LC-MS) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Results: Gold-staining and IgE immunoblotting revealed a high degree of homology of protein bands between the 13 species and the presence of a densely stained doublet at 25 to 35 kD along with protein bands at approximately 12.5, 17 and 50 kD. The doublet from each grass species demonstrated a high level of group 1 and 5 interspecies homology. However, there were a number of bands unique to specific grasses consistent with evolutionary change and indicative that a grass mix immunotherapeutic could be considered broad spectrum.

Conclusions: SDS-PAGE and IgE immunoblotting showed all 13 grasses share a high degree of homology particularly in terms of group 1 and 5 allergens. IgE and IgG ELISA potencies were shown to be independent of extraction method.

10 Seasonal Versus Symptom-based Evaluation of a Depigmented Grass–Birch Allergoid

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Background: The safety and efficacy of specific immunotherapy (SIT) with depigmented and polymerized allergen extracts of pollen is well documented in several clinical trials. The results of such clinical studies are highly dependent on the quantity and quality of pollen exposure and their measurements. To identify a pollen-independent efficacy assessment we compared the combined symptom and medication score (SMS) measured during the pollen season with the SMS defined by a minimum symptom score of the placebo group in a subcutaneous immunotherapy with a depigmented Grass-Birch allergoid over 2 years.

Methods: 269 (ITT) patients with confirmed rhinitis and/or rhinoconjunctivitis were treated during 2009 and 2010 in Germany, Romania, Poland, Lithuania, and Bulgaria. Patients were randomised to the treatment groups on a 2:1 basis (175 verum: 94 placebo). The main parameter in this study was the combined symptom and medication score during the birch and grass pollen season 20101. In addition the SMS of the actively treated patients was analysed following the placebo treated patients eliciting a considerable symptom burden >2.

Results: The clinical results following the seasonal approach are given in the other abstract of our group.1. Taking all days with mean symptom score >2 in the placebo group as calculation basis, the combined SMS values were considerably lower for actively treated patients than for placebo treated patients. For both seasons, the differences between the treatment groups were highly statistically significant (median: 2009: 5.06 vs 7.97, 2010: 4.26 vs 6.43; ITT set) with P-values of 0.0038 and 0.003.

Conclusions: The results show that the efficacy assessment of specific immunotherapy might be better discriminated in relation to the actual symptoms of the placebo group rather than following the days of pollen exposure.

REFERENCE

1. Abstract # 3093: Biedermann T, Pfarr O, Sager A. "Comparison of efficacy and safety of a depigmented polymerized allergen extract of grass and birch with placebo in patients with type-I allergic rhinoconjunctivitis."

11 Efficacy and Tolerability of HDM Injectable Immunotherapy With Monomeric Allergoid

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Background: Subcutaneous immunotherapy (SCIT) is an effective treatment of respiratory allergy and carbamylated monomeric allergoids (monoids), by virtue of their reduced IgE-binding activity, resulted clinically safe by sublingual administration. Purpose of this study was to investigate the efficacy and tolerability of immunotherapy with house dust mites (HDM) monoid administered by injective route in patients with allergic rhinoconjunctivitis (AR).

Methods: A preparation of 0.70 mL of 10 BU/mL containing modified extract with 50% Dermatophagoides pteronyssinus and 50% Dermatophagoides farinae (amount of major allergen: 4 µg of group 1 per milliliter) was delivered monthly for 12 months, following a 5-week build-up induction phase (0.10–0.20–0.30–0.50–0.70 mL), to 58 patients (60% males, mean age 25.1 ± 12.7) suffering from AR due to mites for at least 2 years, whereas 60 patients with similar baseline characteristics were observed as controls. All patients were allowed to assume traditional drug therapy for their condition. At the end of the study changes from baseline in symptoms scores, in number of days with drug assumption, in severity of AR (according to ARIA classification) were compared between the 2 groups; moreover an overall assessment of clinical efficacy and tolerability was based on patients’ and physicians’ judgements (unsatisfactory, mild, good, optimal).

Results: In respect to baseline both groups showed, after 1 year, an improvement in symptoms score (P < 0.001) with a significant difference in favour of SCIT group (P < 0.05). Days of drug intake were significantly lower in patients receiving SCIT (P < 0.05). The number of patients with severe AR decreased in the first group while no variation was observed in controls. The subjective clinical overall assessment was optimal in 31 cases and good in 24 according to physicians’ and patients’ judgements; similarly 38 patients judged tolerability as optimal and 18 as good, whereas according to physicians it was optimal in 37 patients and good in 19; in only 1 patient the treatment was considered unsatisfactory.

Conclusions: In this prospective controlled study, SCIT with HDM carbamylated allergoid was associated with a significant clinical benefit observed through objective and subjective outcomes; the traditional safety of monomeric allergoids was confirmed by the subjective judgements of tolerability.

ALLERGEN STRUCTURE 1

12 Comprehensive Detection of Allergens in Grass Pollen Extracts by Mass Spectrometry

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Background: More than 40% of type I-allergic individuals suffer from hypersensitivity to grass pollen. Patients are treated traditionally with specific
immunotherapy using pollen extracts derived from one or several different Poaceae species. While for several species the most important allergens (group 1 and group 5) have been identified, other allergens have either not been identified or sequence data are still missing. We have used mass spectrometry (MS) together with genetic and immunological methods to identify allergens in various grass pollen extracts.

**Methods:** Pollen extracts of 6 different grass species (Phleum pratense, Holcus lanatus, Lolium perenne, Dactylus glomerata, Festuca pratensis, Poa pratensis) and a mixture thereof were analyzed. For identification of allergens by MS, extracts were subjected to enzymatic digestion. Resulting peptides were separated by liquid chromatography and analyzed by tandem mass spectrometry. Protein identification was performed by searching both the NCBIPlant release and an individually designed database. The presence of individual allergens was confirmed with allergen-specific monoclonal antibodies. Unknown sequences were determined following cDNA synthesis from pollen RNA and allergen sequence amplification by PCR.

**Results:** Fes p 1 and Fes p 5 were identified by the PCR approach. MS analysis of pollen extracts from the 6 individual species resulted in detection of all known allergens including the newly identified Fes p 1 and Fes p 5. Based on the homology of allergens from different grass species, previously unknown sequences of representatives of groups 2, 3, 4, 7, 11, 12 and 13 were detected by MS in investigated extracts with high sequence coverage. Group 6 allergens could not be identified in some of the analyzed extracts. These findings are supported by immunological analyses and thus demonstrate the specificity of the applied method. Members of all allergen groups were identified in an extract mix prepared from pollen of all 6 grass species studied.

**Conclusions:** The most important grass allergens (group 1 and group 5) were detected in all extracts. In addition all other known allergens of the assayed species and homologues thereof could also be identified, thus demonstrating the quality of the tested extracts.