



Does the principle of homologous groups allow a reduction of allergens in the skin prick test panel?

Katharina Eder¹ · Sabine Markmann¹ · Donata Gellrich¹ · Sophie-Louise Mann¹ · Catalina Högerle¹ · Moritz Gröger¹

Received: 4 June 2020 / Accepted: 30 July 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Purpose Regulatory and economic conditions drive the application of the concept of homologous groups on therapeutic and diagnostic formulations. In this context, a possible reduction of allergens in the skin prick test panel in the clinical routine should be investigated for the grass, birch and Dermatophagoides homologous group.

Methods Grass and cultivated rye skin prick test results of 1101 patients, birch/hazel/alder skin prick test results of 1131 patients and 1068 Dermatophagoides pteronyssinus and farinae skin prick tests were retrospectively evaluated with regard to sensitivity and false-negative rate detecting sensitization against cultivated rye with grass skin prick test, sensitization against hazel and/or alder with birch skin prick test and sensitization towards Dermatophagoides farinae with Dermatophagoides pteronyssinus skin prick test.

Results Skin prick test results in all groups highly correlated in Spearman Rank Order correlation. Sensitivity of grass skin prick test detecting sensitization against cultivated rye was highest with 98.9%, sensitivity of birch skin prick test detecting sensitization toward hazel and alder was 95.6 and 90.0%, respectively. Sensitivity of skin prick test with Dermatophagoides pteronyssinus detecting sensitization towards Dermatophagoides farinae was 93.6%. Only 26 patients with positive rye, hazel, alder or Dermatophagoides farinae skin prick test that were overlooked by grass, birch or Dermatophagoides pteronyssinus skin prick test reported allergic symptoms.

Conclusion In routine clinical practice, sensitization against cultivated rye can safely be detected by skin prick test with grass allergen. In addition, the sensitivity of birch skin prick test is high in terms of sensitization towards hazel, but lower for alder. Sensitivity of Dermatophagoides pteronyssinus skin prick test also is high to detect sensitization towards Dermatophagoides farinae. Further research will indicate if several skin prick test allergens will disappear and be completely replaced by a single skin prick test based on the principle of homologous groups.

Keywords Skin prick test · Homologous groups · Allergens · Birch · Grass · Dermatophagoides

Abbreviations

D. pter	Dermatophagoides pteronyssinus
SPT	Skin prick test
D. farinae	Dermatophagoides farinae
sIgE	Specific immunoglobulin E
NPT	Nasal provocation testing
FEIA	Fluorescence enzyme immunoassay
FNR	False-negative rate

FPR	False-positive rate
SLIT	Sublingual immunotherapy

Introduction

The prevalence of allergic disease has risen over the last years [1, 2], and sensitization to grass, rye, birch, hazel and alder are the most frequent in Germany and Europe next to Dermatophagoides species. Sensitization rates in the German population are 18.1% for timothy grass, 16.8% for rye, 17.4% for birch, 16.2% for hazel, 16.5% for alder and 15.9% for Dermatophagoides pteronyssinus (D. pter) [3]. These numbers reveal the importance of allergological diagnostics nowadays. Although serological methods have become more

✉ Katharina Eder
Katharina.Eder@med.uni-muenchen.de

¹ Department of Oto-Rhino-Laryngology, Head and Neck Surgery, Ludwig-Maximilians-University, Marchioninstr. 15, 81377 Munich, Germany

important, the skin prick test (SPT) still represents a basic tool to diagnose sensitization against various aeroallergens at the same time at low cost [4], and in combination with relevant clinical symptoms alone it is sufficient to initiate specific immunotherapy according to the current guideline in Germany [5]. The European and German standard of SPT panel includes grass mix, birch, hazel, alder, D. pter and *Dermatophagoides farinae* (*D. farinae*) next to other inhalant allergens [6, 7] and SPT with cultivated rye allergen has been widely used next to grass mix in the past [8] and is still used in clinical routine. However, cost recovery of a comprehensive diagnostic approach is a challenge for an allergologist. Furthermore, current European Union regulations have a tremendous impact on the availability of therapeutic and diagnostic allergen solutions, e.g. has decreased the number of available skin test allergens by more than a half [9]. These changes, amongst others, are based on the transformation of the so-called “principle of taxonomic families” to the “principle of homologous groups” [10]. In the past, allergens were grouped merely along with taxonomic relationship, whereas the classification of allergen sources in one homologous group on the basis of homology and cross-reactivity of allergens is found to be more specific and satisfactory [10]. Therefore, allergens of birch (*Betula verrucosa*), alder (*Alnus glutinosa*), and hazel (*Corylus avellana*) belong to the same homologous group, similarly as the allergens of grass species with timothy grass (*Phleum pratense*) and cultivated rye (*Secale cereale*), as well as D. pter and *D. farinae* are part of the same homologous group. For each homologous group, one representative allergen source is suggested, such as timothy grass for the “grass homologous group”. This classification is based on several studies indicating similar Ig-E binding properties and high cross-reactivity within homologous groups, as summarized by Lorenz et al. [10].

In this study, we questioned if it is acceptable in routine clinical practice to reduce the SPT panel to only birch for the diagnosis of sensitization towards birch, hazel and alder, to grass to detect sensitization towards grass species and cultivated rye, and to only D. pter as European house dust mite for both *Dermatophagoides* species.

Methods

Patient data

The database of the Department of Oto-Rhino-Laryngology, Head and Neck Surgery of the Ludwig-Maximilians-University in Munich was retrospectively scanned between January 2009 and December 2013 for patients with the following SPT data: grass mix, cultivated rye, birch, alder, hazel, D. pter, *D. farinae* besides positive and negative control. This

resulted in 1166 patients. The study population was divided into 4 overlapping groups: (1) grass analysis group (SPT data for grass mix and cultivated rye), (2) birch analysis group I (SPT data for birch and hazel), (3) birch analysis group II (SPT data for birch and alder), (4) *Dermatophagoides* analysis group (SPT for D. pter and *D. farinae*).

The local ethics committee and the local data protection commissioner approved the use of data from routine clinical practice. All patients provided written informed consent for the use of their parameters for scientific research and gave consent to publish these results.

Skin prick test (SPT)

The SPT solutions used were ALK-SQ-solutions (ALK-Abelló, Wedel, Germany) with native allergen extracts in case of birch/*Betula verrucosa*, hazel/*Corylus avellana*, alder/*Alnus glutinosa*, cultivated rye/*Secale cereale*, D. pter and *D. farinae*. Grass mix solution contained timothy grass/*Phleum pratense*, meadow oat grass/*Helictotrichon pratense*, cock's foot grass/*Dactylis glomerata*, meadow fescue/*Festuca pratensis*, perennial rye/*Lolium perenne* and smooth meadow grass/*Poa pratensis*. SPT was considered positive after 20 min with a wheal ≥ 3 mm in diameter (I = ≥ 3 to 4 mm, II = ≥ 4 to 5 mm, III = ≥ 5 to 6 mm, IV = ≥ 6 mm) in combination with Histamine dihydrochloride solution at 1 mg/ml as positive control and allergen-free saline solution as a negative control. The classification and procedure were in line with European standards and published guidelines [11, 12].

Fluorescence enzyme immunoassay

To exclude false-positive results in cases of a weakly positive SPT result (wheal 3–4 mm), the fluorescence enzyme immunoassay method (UniCAP-FEIA, Thermo Fisher Scientific, Freiburg, Germany) was used to confirm sensitization to a specific allergen by detecting specific Immunoglobulin E (sIgE) reactivity with a commercially available test kit (Thermo Fisher Scientific, Freiburg, Germany). The positive cutoff value was ≥ 0.35 kU/l, and the procedure was in accordance with the manufacturer's instructions. Patients with a SPT of I and sIgE against the according to allergen below the positive cutoff value were considered false positive in SPT and, therefore, were excluded from the group of sensitized patients. Patients with a SPT of I, but without available sIgE data were excluded from the study resulting in the following patient numbers per group:

1. grass analysis group (grass mix and cultivated rye) = 1101 patients
2. birch analysis group I (birch and hazel) = 1131 patients
3. birch analysis group II (birch and alder) = 1131 patients

4. Dermatophagoides analysis group (D. pter and D. farinae) = 1068 patients.

Statistical Analyses

Data analysis was performed by Excel (Microsoft, Redmond, WA, USA) and SigmaPlot (Jandel Corp., San Rafael, CA, USA). Correlation between SPT data within different groups was calculated by Spearman Rank Order Correlation. A *p* value of > 0.05 was considered positive.

Results

The study's patient collective was divided into 4 overlapping groups: (1) grass analysis group (grass mix and cultivated rye) with 1101 patients, (2) birch analysis group I (birch and hazel) with 1131 patients, (3) birch analysis group II (birch and alder) with the same 1131 patients, (4) Dermatophagoides analysis group (D. pter and D. farinae) with 1068 patients.

Table 1 summarizes the patient data, in terms of gender and age (Table 1).

SPT results within all 4 groups correlated highly in Spearman Rank Order Correlation analysis. The correlation coefficient for grass mix/cultivated rye group was $\rho = 0.924$ ($p < 0.001$), for birch/hazel group $\rho = 0.853$ ($p < 0.001$), for the birch/alder group $\rho = 0.842$ ($p < 0.001$), and for the D. pter/D. farinae group $\rho = 0.935$ ($p < 0.001$).

We found the following sensitization rates for grass of 54.9% ($n = 604$), for cultivated rye of 50.2% ($n = 553$), for birch 46.9% ($n = 530$), hazel 42.5% ($n = 481$) and alder 48.5% ($n = 548$), for D. pter of 40.8% ($n = 436$) and D. farinae of 42.2% ($n = 451$), respectively.

Table 2 summarizes the corresponding sensitization rates for the 4 evaluated groups (Table 2). Looking at corresponding sensitization rates within the grass/cultivated rye group we found a corresponding result for both allergens in 94.3% ($n = 1038$), 44.6% ($n = 491$) were neither sensitized against

Table 2 Sensitization rates in percent for each patient group divided into -/- (no sensitization against both allergens of the individual group), +/+ (sensitization against both allergens), ± (sensitization against the first allergen) and -/+ (sensitization against the second allergen of the individual group)

Patient group	Sensitization in no. of patients (%)			
	-/-	+/+	+/-	-/+
Grass mix/cultivated rye	491 (44.6)	547 (49.7)	57 (5.2)	6 (0.5)
Birch/hazel	580 (51.3)	460 (40.7)	70 (6.2)	21 (1.8)
Birch/alder	546 (48.3)	493 (43.6)	37 (3.3)	55 (4.9)
D. pter/D. farinae	603 (56.5)	422 (39.5)	14 (1.3)	29 (2.7)

grass nor against cultivated rye, 49.7% ($n = 547$) were sensitized against both allergens. Only 5.7% ($n = 63$) of positive results did not correspond in this group: 6 patients (0.5%) were only sensitized against cultivated rye and did not show a positive result for grass and 57 patients (5.2%) showed a sensitization against grass but not cultivated rye. In the birch/hazel group, we found a corresponding result in 92.0% ($n = 1040$). 40.7% ($n = 460$) showed a sensitization towards birch and hazel and 51.3% ($n = 580$) did not show a sensitization against birch nor hazel. 8.0% ($n = 91$) did not show the same result, 21 patients (1.8%) were only sensitized against hazel without sensitization against birch and 70 patients (6.2%) had a positive SPT to birch but not for hazel. The birch/alder group showed a corresponding result in 91.8% ($n = 1039$), 43.6% ($n = 493$) were sensitized to birch and alder and 48.3% ($n = 546$) were neither sensitized against birch nor alder. 8.2% ($n = 92$) did not show a corresponding sensitization, 55 patients (4.9%) were only sensitized against alder and 37 patients (3.3%) were only sensitized against birch but not alder. Within the Dermatophagoides group 39.5% ($n = 422$) were tested positive for D. pter and D. farinae, 56.5% ($n = 603$) did not show any sensitization towards Dermatophagoides. 14 patients (1.3%) had a positive SPT towards D. pter but a negative test with D. farinae, whereas 29 patients (2.7%) were tested positive with D. farinae allergen but negative with D. pter (Table 2).

On this basis we performed a detection analysis questioning whether SPT with grass mix allergen could safely detect sensitization against cultivated rye, SPT with birch the sensitization against hazel or alder and SPT with D. pter the sensitization towards D. farinae, respectively. Figure 1 summarizes the results for the grass/cultivated rye group (Fig. 1).

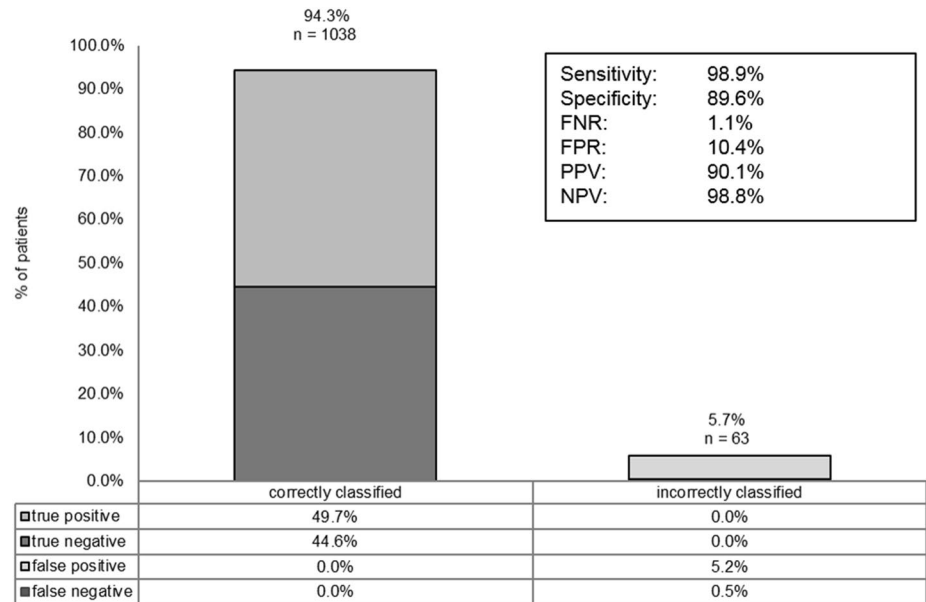
In this study, 98.9% of patients with a sensitization against grass mix and/or cultivated rye alone would have been detected with SPT with grass mix allergen alone (= sensitivity). Only 1.1% of patients showing a positive result when tested with cultivated rye allergen would have been overlooked with single testing with grass mix allergen

Table 1 Demographics and characteristics of patient groups

	Grass mix/cultivated rye	Birch/hazel Birch/alder	D. pter/D. farinae
No. of patients	1101 (100%)	1131 (100%)	1068 (100%)
Male	600 (55%)	613 (54%)	586 (55%)
Female	501 (45%)	518 (46%)	482 (45%)
Age			
Range	3–87	3–87	3–87
Median	35.5	35.8	35.5

Values are number of patients in total (percent of each evaluated group). Age in years is given as range and median

Fig. 1 Detection analysis of SPT with grass mix for sensitization towards grass mix and cultivated rye (*FNR* false negative rate, *FPR* false positive rate, *PPV* positive predictive value, *NPV* negative predictive value)



[false-negative rate (FNR)]. Specificity was 89.6%, the false positive rate (FPR) was 10.4% (Fig. 1).

Figure 2 shows the corresponding results for the birch/hazel group. The sensitivity for detecting a sensitization against birch and/or hazel with SPT with birch allergen alone was 95.6%, specificity 89.2%. The false-negative rate was calculated as 4.4% (Fig. 2).

Figure 3 summarizes the results for the birch/alder group. Sensitivity was 90.0%, specificity 93.7% and the false-negative rate was 10.0% (Fig. 3).

Figure 4 shows the results for the Dermatophagoides group. Sensitivity was 93.6%, specificity 97.7% and the false-negative rate was 6.4% (Fig. 4).

Looking at the subgroup of patients showing a sensitization in SPT against cultivated rye, hazel, alder or *D. farinae* that would have been overlooked by SPT with grass mix, birch allergen or *D. pter* we found the following reported clinical symptoms in each group: out of 6 patients with sensitization against cultivated rye in SPT but with negative SPT result for grass mix 3 patients reported rhinoconjunctival symptoms during rye season. In the birch/hazel group, 21 patients with positive SPT results for hazel would have been missed with SPT with birch allergen. 4 of these patients reported symptoms during the hazel season. In the birch/alder group, 10 out of 55 missed patients reported symptoms during alder season. 9 out 29 *D. pter* negative patients with

Fig. 2 Detection analysis of SPT with birch for sensitization towards birch and hazel (*FNR* false-negative rate, *FPR* false positive rate, *PPV* positive predictive value, *NPV* negative predictive value)

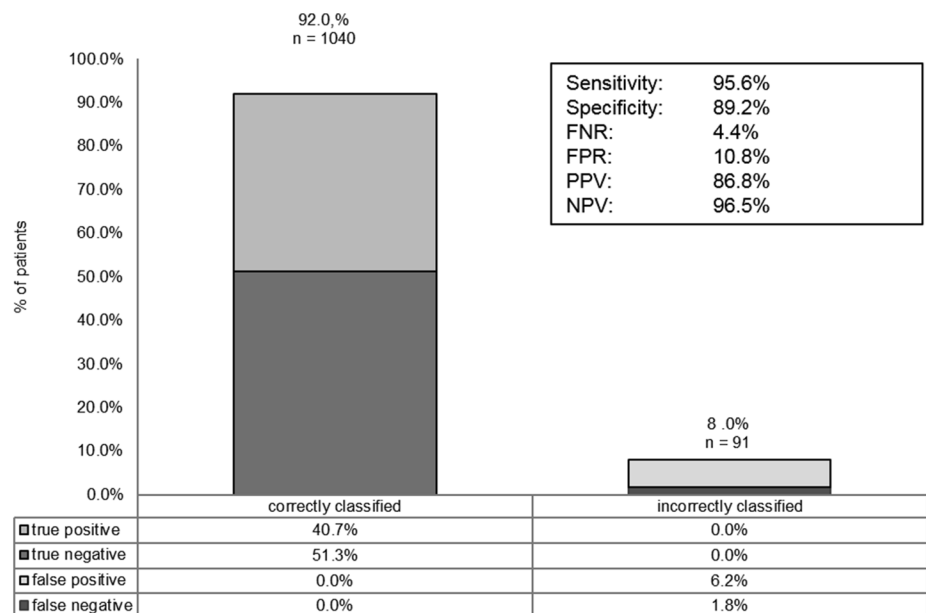


Fig. 3 Detection analysis of SPT with birch for sensitization towards birch and alder (*FNR* false-negative rate, *FPR* false-positive rate, *PPV* positive predictive value, *NPV* negative predictive value)

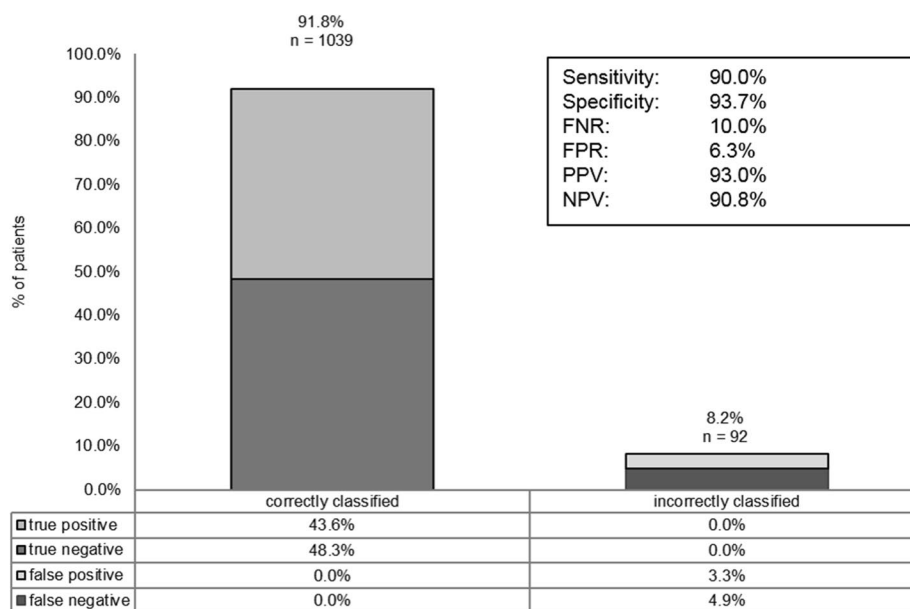
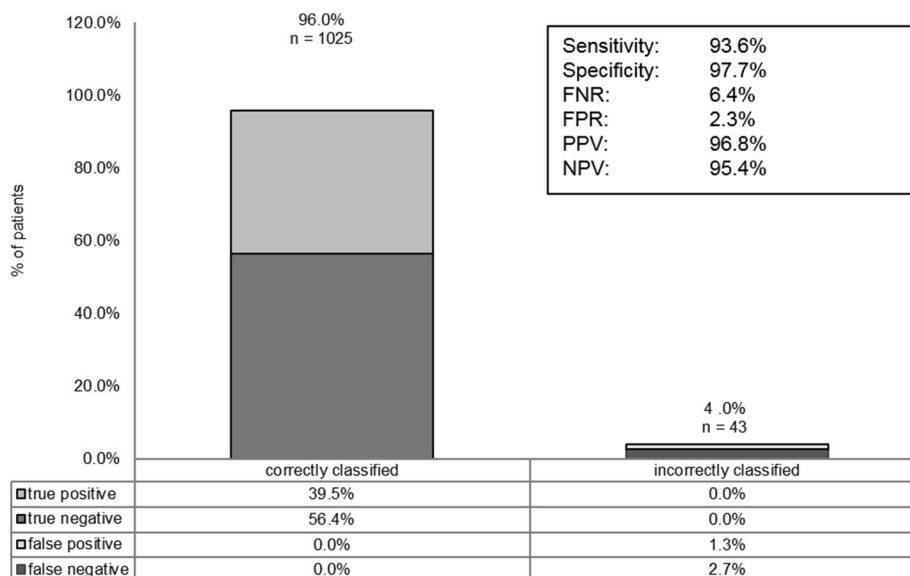


Fig. 4 Detection analysis of SPT with *D. pter* for sensitization towards *D. pter* and *D. farinae* (*FNR* false-negative rate, *FPR* false-positive rate, *PPV* positive predictive value, *NPV* negative predictive value)



positive SPT with *D. farinae* allergen reported symptoms during the whole year.

Discussion

The aim of the study was to investigate redundancies in SPT results in a large patient sample for the diagnosis of sensitization to birch, alder, hazel, grass, cultivated rye and *Dermatophagoides* species. This study is of great importance since regulatory and economic aspects have an immense impact on an allergologist and the availability of diagnostic skin test allergens [9, 10]. In this context, it is of high relevance whether a limited SPT panel with the only birch,

grass and *D. pter* allergen (instead of birch, alder, hazel, grass, cultivated rye, *D. pter* and *D. farinae*) has an acceptable sensitivity.

Sensitization rates diagnosed by SPT in our patient sample were relevantly higher than sensitization rates reported by Haftenberger et al. [3]. We found a sensitization rate against grasses of 54.9%, cultivated rye of 50.2%, birch of 46.9%, hazel of 42.5%, alder of 48.5% *D. pter* of 40.8% and *D. farinae* of 42.2%. We assume the difference is caused by a selection bias. Patients who are visiting our allergy department clearly do not represent the general German population, but rather consult an allergologist because of certain rhinological symptoms; further, they are pre-selected by positive screening tests. More than 90% of our study patients

had a positive Immunocap®-SX1 (Thermo Fisher Scientific, Freiburg, Germany), a screening which was performed before detailed allergy testing in most cases. Therefore, the ratio of patients with a sensitization against one or more allergens is likely to be much higher than in the general population as it is in other study groups with a specific patient collective. Exemplary, sensitization rates from the Global Allergy and Asthma European Network for Germany are named as follows: grasses 37.9%, birch 37.6%, hazel 35.9%, alder 34.8%, *D. pter* 23.5% and *D. farinae* 21.1% [13].

There is a trend towards a “clean” use of allergen solutions for specific immunotherapy. Already in 1984, it was published that pollen allergens from rye and other common grasses have a close immunochemical relationship so that skin test reactivity against common grasses alone could serve as a rationale to diagnose and treat hay fever supposedly elicited by rye alone [14]. Lately, Couroux et al. published a randomized, double-blind trial on 214 patients to determine the optimal dose for clinical efficacy of the SQ tree sublingual immunotherapy (SLIT)-tablet and investigate the symptom reduction towards different species from the birch homologous group [15]. They found that the SQ tree SLIT-tablet reduced average allergic rhinoconjunctivitis symptoms triggered by birch or oak pollen, also a member of the birch homologous group. Also, clinical and immunological findings suggested that the tablet may be used to treat allergies to all species within the birch homologous group and not birch alone, but concluded that further studies will have to verify this evidence and its transferability to other homologous groups [15]. Therefore, the principle of homologous groups is already followed in the therapeutic strategy of allergic disease.

Our results support that the principle of homologous groups can also be applied to diagnosing sensitization against allergens within the birch, grass and *Dermatophagoides* homologous groups. In our collective, correlation of SPT results between grasses/cultivated rye, birch/hazel, birch/alder and *D. pter*/*D. farinae* were high and in line with the literature [10, 16, 17]. Furthermore, especially the sensitivity of detecting sensitization towards cultivated rye with grass mix SPT was 98.9%. Sensitivity of birch SPT for detection of sensitization towards hazel was slightly lower with 95.6%, and lowest for alder with 90.0% respectively. Sensitivity of *D. pter* SPT for detecting sensitization towards *D. farinae* was 93.6%. Only 1.1% of cultivated rye SPT positive patients would not have been detected with grass SPT. FNR for birch SPT detecting sensitization against hazel was 4.4% and as high as 10% for alder. 6.4% of patients with positive SPT with *D. farinae* allergen would not have detected with *D. pter* SPT. Do these numbers allow a reduction in SPT panel allergens? Already Bousquet et al. found in a large Pan-European Multi-center study that a limited number of allergens allowed the

identification of the majority of sensitized patients [18]. However, the authors named as limitation of the study that the number of subjects tested per individual center was restricted, ranging from 116 to 393, and concluded that still the suggested whole test battery of allergens is needed to appropriately assess patients.

The difference in FNR of SPT with birch allergen detecting sensitization against hazel or alder in our study cannot be explained by homology or cross-reactivity. Already in 1991, Valenta et al. investigated high homology at the nucleic acid level of the major allergens of birch, hazel and alder by cross-hybridization [19]. Since then, several studies showed high cross-reactivity and homology between birch, hazel and alder major allergen components, well summarized by Biedermann et al. [20]. Rather, the spectrum of different allergen components in the test solution could affect the sensitivity of SPT with birch allergen detecting sensitization particularly against alder. In addition, we only compared sensitization rates of SPT solutions of one manufacturer.

To our knowledge, there are no studies regarding prevalence and relevance of isolated allergy towards cultivated rye, hazel or alder without allergy against other grasses or birch, the representative allergen of the referring homologous group. Although the FNR detecting sensitization towards alder with birch SPT is rather high with 10%, considering reported clinical symptoms in these patients as a potential sign for clinically relevant sensitization, only a few allergic patients would have been overlooked in this study reducing the SPT panel to grass mix and birch. 3 out of 6 patients (50%), who would have been overlooked with grass SPT, reported rhinoconjunctival symptoms during rye season; as well 4 out of 21 patients (19%) during hazel season and 10 out of 55 patients (18%) during alder season would have been overlooked with birch SPT, respectively. This finding would support the questionable relevance of isolated sensitization against cultivated rye, hazel and alder since the clinical relevance of sensitization towards hazel and alder for Germany are around 90% as presented by Burbach et al. [13].

For *Dermatophagoides* genus Lorenz et al. summarized that the high number of 15–20 homologous allergen components of each species *D. pter* and *D. farinae* justifies grouping these two and contradictory results from cross-reactivity studies are not expected [10]. Also, the impact of SPT with *D. farinae* in addition to *D. pter* was described as lower as expected by Bousquet et al. [18]. On the other hand, this study aimed at defining the minimal number of SPT allergen to identify a patient as sensitized in general and did not compare *D. pter* and *D. farinae* individually. In our study, one could argue that *D. farinae* would be the allergen to test with, since sensitization is higher compared to *D. pter* (42.2% compared to 40.8%), although *D. farinae* is the “American house dust mite”. The FNR of SPT with

D. farinae to detect sensitization towards *D. pter* would be only 3.2% compared to 6.4% the other way around.

However, as outlined above, a FNR of 5–10% is not related to the general German population, but rather referenced to a pre-selected patient collective with possible higher sensitization rates towards aeroallergens. Since a negative SPT, in general, can reliably exclude IgE mediated allergy [21–23], we conclude, that SPT with grass allergen can serve as the only diagnostic tool also identifying an allergy towards rye and SPT with birch allergen could also serve as the first dependable diagnostic approach identifying an allergy against other pollen from the referring homologous group, such as hazel. Evaluating the impact of SPT without alder and *D. farinae*, on the other hand, might need a prospective study approach, also with regard to the clinical relevance. In addition, the European Academy of Allergy and Clinical Immunology (EAACI) and national European Allergy Societies just published a position paper, claiming the need for the availability of a wide range of high-quality diagnostic allergens for in-vivo diagnosis of IgE-mediated allergies not only covering predominant but also less frequent allergen sources [24].

The specificity and false positive rate were calculated for all evaluated groups for methodological purpose but will not be further discussed because of limited clinical relevance., as patients with positive SPT with grasses, birch and/or *D. pter* would receive anti-allergic therapy in case of allergic complaints irrespective of a negative SPT with cultivated rye, hazel, alder and/or *D. farinae*.

The retrospective design of the study clearly is a limitation. In terms of evaluating the clinical relevance of falsely negative tested patients, provocation testing would have been the preferable criterium. However, because of the retrospective design, data were limited, reported rhinitic symptoms during a certain period of the year or all year long were the only measures to estimate clinical relevance. Also, we did not compare sensitization rates by different SPT solution manufacturers. It has been investigated by immunochemical analysis and confirmed by in vivo testing, that mite extracts from different manufacturers show a heterogenous amount of components, affecting the clinical diagnosis of sensitization against mite allergen [25]. Therefore, our results by one manufacturer cannot be generalized with regard to all solutions available on the market.

In summary, our study suggests, that in the diagnostic cascade of rhinological symptoms with regard to sensitization to grass and cultivated rye, the SPT panel can be limited to grass alone. Regarding SPT with birch, alder, hazel, *D. pter* and *D. farinae* allergen, we conclude, that sensitization towards hazel can safely be detected via birch SPT. Concerning a possible exclusion of alder and either *D. pter* or *D. farinae* from SPT panel further research would be desirable.

Acknowledgements We thank the medical technician Elisabeth Pfrogner and Gabriele Bähr of the Department of Oto-Rhino-Laryngology, Head and Neck Surgery of the Ludwig-Maximilians-University in Munich.

Author contributions All authors contributed to the study conception and design. KE participated in the study design, analysed the data, performed the statistical analysis, interpreted the data, and wrote the manuscript. MG participated in the study design, was a major contributor in writing the manuscript, provided critical revisions, holds overall responsibility for the project. DG collected the data, gave critical advice interpreting the data and critically revised the manuscript. SM, CH and PZ S-LM collected the data, provided critical advice in analysing the data and revised the manuscript. All authors read and approved the final manuscript.

Funding This study did not receive specific funding.

Availability of data and material Data can be received on request.

Code availability Not applicable

Compliance with ethical standards

Conflict of interest Moritz Gröger has received speaker honoraria from ALK-Abelló, Allergy Therapeutics, HAL, Leti, Thermo Fisher Scientific, and Stallergenes, received financial support for attending symposia from ALK-Abelló, Allergopharma, Allergy Therapeutics, HAL, Lofarma, Thermo Fisher Scientific, and Shire, received research grants from ALK-Abelló, GSK, Sanofi, and Thermo Fisher Scientific, and is a member of the advisory board of ALK-Abelló. Donata Gellrich has received speaker honoraria from ALK-Abelló and Bencard Allergy and financial support for attending symposia from HAL Allergy, Phadia diagnostics and Shire. All other authors declare that they have no conflict of interest.

Ethics approval The local ethics committee and the local data protection commissioner approved the use of data from routine clinical practice.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication All patients gave written informed consent to publish the results of data analysis.

References

1. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, Williams H, Group IPTS (2006) Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. *Lancet* 368(9537):733–743. [https://doi.org/10.1016/S0140-6736\(06\)69283-0](https://doi.org/10.1016/S0140-6736(06)69283-0)
2. Bjorksten B, Clayton T, Ellwood P, Stewart A, Strachan D, Group IPIS (2008) Worldwide time trends for symptoms of rhinitis and conjunctivitis: Phase III of the International Study of Asthma and Allergies in Childhood. *Pediatr Allergy Immunol* 19(2):110–124. <https://doi.org/10.1111/j.1399-3038.2007.00601.x>
3. Haftenberger M, Laussmann D, Ellert U, Kalcklosch M, Langen U, Schlaud M, Schmitz R, Thamm M (2013) Prevalence of

- sensitisation to aeroallergens and food allergens: results of the German Health Interview and Examination Survey for Adults (DEGSI). Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 56(5–6):687–697. <https://doi.org/10.1007/s00103-012-1658-1>
4. Cardona V, Demoly P, Dreborg S, Kalpaklioglu AF, Klimek L, Muraro A, Pfaar O, Popov TA, Hoffmann HJ (2018) Current practice of allergy diagnosis and the potential impact of regulation in Europe. Allergy 73(2):323–327. <https://doi.org/10.1111/all.13306>
 5. Pfaar O, Bachert C, Bufe A, Buhl R, Ebner C, Eng P, Friedrichs F, Fuchs T, Hamelmann E, Hartwig-Bade D, Hering T, Huttegger I, Jung K, Klimek L, Kopp MV, Merk H, Rabe U, Saloga J, Schmid-Grendelmeier P, Schuster A, Schwerk N, Sitter H, Umpfenbach U, Wedi B, Wohl S, Worm M, Kleine-Tebbe J, Kaul S, Schwalfenberg A (2014) Guideline on allergen-specific immunotherapy in IgE-mediated allergic diseases: S2k Guideline of the German Society for Allergology and Clinical Immunology (DGAKI), the Society for Pediatric Allergy and Environmental Medicine (GPA), the Medical Association of German Allergologists (AeDA), the Austrian Society for Allergy and Immunology (OGAI), the Swiss Society for Allergy and Immunology (SGAI), the German Society of Dermatology (DDG), the German Society of Oto-Rhino-Laryngology, Head and Neck Surgery (DGHN-KHC), the German Society of Pediatrics and Adolescent Medicine (DGKJ), the Society for Pediatric Pneumology (GPP), the German Respiratory Society (DGP), the German Association of ENT Surgeons (BV-HNO), the Professional Federation of Paediatricians and Youth Doctors (BVKJ), the Federal Association of Pulmonologists (BDP) and the German Dermatologists Association (BVDD). Allergo J Int 23(8):282–319. <https://doi.org/10.1007/s40629-014-0032-2>
 6. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, Durham S, Fokkens W, Gjomarkaj M, Haahtela T, Bom AT, Wohl S, Maibach H, Lockey R (2013) The skin prick test—European standards. Clin Transl Allergy 3(1):3. <https://doi.org/10.1186/2045-7022-3-3>
 7. Rueff F, Bergmann KC, Brockow K, Fuchs T, Gröbl A, Jung K, Klimek L, Mücken H, Pfaar O, Przybilla B, Sitter H, Wehrmann W (2010) Hauttests zur Diagnostik von allergischen Sofortreaktionen. Allergo J 19:402–415
 8. Heinzerling L, Frew AJ, Bindslev-Jensen C, Bonini S, Bousquet J, Bresciani M, Carlsen KH, van Cauwenberge P, Darsow U, Fokkens WJ, Haahtela T, van Hoecke H, Jessberger B, Kowalski ML, Kopp T, Lahoz CN, Lodrup Carlsen KC, Papadopoulos NG, Ring J, Schmid-Grendelmeier P, Vignola AM, Wohl S, Zuberbier T (2005) Standard skin prick testing and sensitization to inhalant allergens across Europe—a survey from the GALEN network. Allergy 60(10):1287–1300. <https://doi.org/10.1111/j.1398-9995.2005.00895.x>
 9. Klimek L, Hoffmann HJ, Kugler A, Muraro A, Hellings PW (2016) Impact of changed legislation on skin tests: the present and future. Curr Opin Allergy Clin Immunol 16(5):465–468. <https://doi.org/10.1097/ACI.0000000000000312>
 10. Lorenz AR, Luttkopf D, May S, Scheurer S, Vieths S (2009) The principle of homologous groups in regulatory affairs of allergen products—a proposal. Int Arch Allergy Immunol 148(1):1–17. <https://doi.org/10.1159/000151243>
 11. Bernstein IL, Storms WW (1995) Practice parameters for allergy diagnostic testing. Joint Task Force on Practice Parameters for the Diagnosis and Treatment of Asthma. The American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 75(6 Pt 2):543–625
 12. EAACI (1989) Skin tests used in type 1 allergy testing. Position paper prepared by the subcommittee on skin tests of the European Academy of Allergology and Clinical Immunology. Allergy 44(s10):11–59
 13. Burbach GJ, Heinzerling LM, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, Bousquet J, Bousquet-Rouanet L, Bousquet PJ, Bresciani M, Bruno A, Canonica GW, Darsow U, Demoly P, Durham S, Fokkens WJ, Giavi S, Gjomarkaj M, Gramiccioni C, Haahtela T, Kowalski ML, Magyar P, Murakozi G, Orosz M, Papadopoulos NG, Rohnelt C, Stingl G, Todo-Bom A, von Mutius E, Wiesner A, Wohl S, Zuberbier T (2009) GA(2)LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe. Allergy 64(10):1507–1515. <https://doi.org/10.1111/j.1398-9995.2009.02089.x>
 14. Lowenstein H, Wihl JA, Bache Billesbolle K, Bowadt H (1984) Rationale for specific immunotherapy of grass pollen allergy with extracts of rye pollen. Skin test reactivity and immunochemical relationship between pollen allergens from rye and other common grasses. Allergy 39(6):421–432. <https://doi.org/10.1111/j.1398-9995.1984.tb01964.x>
 15. Couroux P, Ipsen H, Stage BS, Damkjaer JT, Steffensen MA, Salapatek AM, Lund K, Wurtzen PA (2019) A birch sublingual allergy immunotherapy tablet reduces rhinoconjunctivitis symptoms when exposed to birch and oak and induces IgG4 to allergens from all trees in the birch homologous group. Allergy 74(2):361–369. <https://doi.org/10.1111/all.13606>
 16. Eriksson NE, Wihl JA, Arrendal H, Strandhede SO (1987) Tree pollen allergy. III. Cross reactions based on results from skin prick tests and the RAST in hay fever patients. A multi-centre study. Allergy 42(3):205–214
 17. Sade K, Roitman D, Kivity S (2010) Sensitization to Dermatophagoides, *Blomia tropicalis*, and other mites in atopic patients. J Asthma 47(8):849–852. <https://doi.org/10.3109/0277093.2010.506683>
 18. Bousquet PJ, Burbach G, Heinzerling LM, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, Bousquet-Rouanet L, Demoly P, Bresciani M, Bruno A, Gjomarkaj M, Canonica GW, Darsow U, Durham S, Fokkens WJ, Giavi S, Gramiccioni C, Papadopoulos NG, Haahtela T, Kowalski ML, Magyar P, Murakozi G, Orosz M, Rohnelt C, Stingl G, Todo-Bom A, von Mutius E, Wiesner A, Wohl S, Bousquet J, Zuberbier T (2009) GA2LEN skin test study III: minimum battery of test inhalant allergens needed in epidemiological studies in patients. Allergy 64(11):1656–1662. <https://doi.org/10.1111/j.1398-9995.2009.02169.x>
 19. Valenta R, Breiteneder H, Pettenburger K, Breitenbach M, Rumpold H, Kraft D, Scheiner O (1991) Homology of the major birch-pollen allergen, Bet v I, with the major pollen allergens of alder, hazel, and hornbeam at the nucleic acid level as determined by cross-hybridization. J Allergy Clin Immunol 87(3):677–682. [https://doi.org/10.1016/0091-6749\(91\)90388-5](https://doi.org/10.1016/0091-6749(91)90388-5)
 20. Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E (2019) Birch pollen allergy in Europe. Allergy 74(7):1237–1248. <https://doi.org/10.1111/all.13758>
 21. Ferguson AC, Murray AB (1986) Predictive value of skin prick tests and radioallergen sorbent tests for clinical allergy to dogs and cats. CMAJ 134(12):1365–1368
 22. Rueff F, Bergmann KC, Brockow K, Fuchs T, Gröbl A, Jung K, Klimek L, Mücken H, Pfaar O, Przybilla B, Sitter H, Wehrmann W, German Society for A, Clinical I (2011) Skin tests for diagnostics of allergic immediate-type reactions. Guideline of the German Society for Allergology and Clinical Immunology. Pneumologie 65(8):484–495. <https://doi.org/10.1055/s-0030-1256476>
 23. Schafer T, Hoelscher B, Adam H, Ring J, Wichmann HE, Heinrich J (2003) Hay fever and predictive value of prick test and specific IgE antibodies: a prospective study in children. Pediatr Allergy Immunol 14(2):120–129. <https://doi.org/10.1034/j.1399-3038.2003.00024.x>

24. Klimek L, Hoffmann HJ, Kalpaklioglu AF, Demoly P, Agache L, Popov T, Muraro A, Schmid-Grendelmeier P, Bonini S, Bonertz A, Mahler V, Vieths S, Pfaar O, Zuberbier T, Jutel M, Schmidt-Weber C, Hellings P, Dreborg S, Bonini M, Brough HA, Bousquet J, Hoffmann-Sommergruber K, Palomares O, Ollert M, Shamji MH, Cardona V (2020) In-vivo diagnostic test allergens in Europe: a call to action and proposal for recovery plan—an EAACI position paper. *Allergy*. <https://doi.org/10.1111/all.14329>
25. Brunetto B, Tinghino R, Braschi MC, Antonicelli L, Pini C, Iacovacci P (2010) Characterization and comparison of commercially

available mite extracts for in vivo diagnosis. *Allergy* 65(2):184–190. <https://doi.org/10.1111/j.1398-9995.2009.02150.x>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.