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Birch pollen-related foods can cause late eczematous reactions in patients with atopic dermatitis

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Abstract

Background: Previous studies have indicated that in patients with atopic dermatitis (AD) and birch pollen allergy related foods are able to cause a late eczematous response. However, the relevance of AD worsening by ingestion of birch pollen-related foods is still a matter of debate.

Objective: The purpose of this retrospective study was to determine how frequently birch pollen-related foods induce a deterioration of eczema. Additionally, the diagnostic value of specific IgE (sIgE) determination was evaluated.

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Methods: A total of 182 children and adults with AD and suspected birch pollen-related food allergy underwent 261 double-blind placebo-controlled food challenges (DBPCFC). Total and specific IgE levels were determined prior to DBPCFC.

Results: 65 patients developed allergic reactions (responders) upon DBPCFC with birch pollen-related foods (n=103 DBPCFC). Of these, 32 patients exhibited significant deterioration of AD defined as a median increase of 15.4 SCORAD index points (95% CI 12.4-16.3) from baseline making up 37% of all positive reactions. Responders showed significantly higher sIgE levels to birch pollen and apple as well as a higher prevalence of allergic rhinoconjunctivitis compared to non-responders (P<.05). However, patients with late eczematous response could not be differentiated from those with isolated immediate type reactions by specific IgE levels.

Conclusion: In a subpopulation of patients with AD and birch pollen sensitization, related foods should be considered as a trigger for an aggravation of eczema. Since sufficient markers for prediction of late eczematous reactions are still lacking, DBPCFC cannot be replaced in diagnosis of birch pollen-related foods in patients with AD.

Clinical Implications: In patients with AD and birch pollen-allergy, birch pollen-related foods should be considered as a provocation factor for an aggravation of disease signs and symptoms.

Key words: atopic dermatitis; birch pollen-related foods; food allergy; late eczematous reactions; food challenge; specific IgE; diagnostic value

Abbreviations:

AD:	atopic dermatitis
DBPCFC:	double-blind placebo-controlled food challenge
NPV:	negative predictive value
OAS:	oral allergy syndrome
PPV:	positive predictive value
RCA:	allergic rhinoconjunctivitis
SCORAD:	severity scoring of atopic dermatitis
sIgE:	specific IgE

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Introduction:

Foods can provoke a flare-up of eczema in a subgroup of sensitized patients with atopic dermatitis (AD).[1,2,3] In children, staple foods such as cow´s milk and hen´s egg belong to the most important causes of food allergies[2,4]. By contrast in adolescents and adults, food allergies commonly occur as a consequence of primary sensitizations to pollen allergens.[2] In central Europe, birch pollen-related food antigens are the most frequent elicitor of a pollen-related food allergy. The major birch pollen-allergen is Bet v 1, which belongs to the pathogenesis-related protein family 10.[5] Over the last decades, an abundance of birch pollen-related foods from different plant families such as *Rosaceae*, *Apiaceae* and *Fabaceae* have been described, and Bet v 1-homologue allergens in fruits, vegetables, legumes and nuts have been characterized.[5-14] Among these, apple, carrot, celery, and hazelnut most often induce allergic symptoms.[15-17] Typical symptoms of birch pollen-related food allergy are immediate type reactions such as oral allergy syndrome (OAS), urticaria, angioedema, rhinoconjunctivitis and asthma, but also anaphylactic reactions were reported.[12,13,16-19] In a previous study birch pollen-related foods were demonstrated to cause significant worsening of dermatitis in birch pollen-sensitized adult patients with AD even in the absence of immediate type reactions.[20] In lesional skin, Bet v 1–specific T-cell responses could be detected, which illustrates that in late eczematous reactions to birch pollen-related foods Bet v 1-specific T-lymphocytes are important key players.[20] Likewise, in a subsequent study including children with AD and birch pollen sensitization, Breuer et al. observed deterioration of dermatitis after ingestion of birch pollen-related foods in four out of twelve children.[21] Further data in this field indicate that even heat-treated birch pollen-related foods can cause a worsening of AD, whereas thermal processing abrogated their ability to elicit local immediate type reactions in forms of an OAS.[22]

Despite increasing evidence birch pollen-related foods are still a matter of debate as relevant provocation factor of AD.

In this retrospective study we focused on the relevance of birch pollen-related food allergy in a large cohort of children and adults with AD. We aimed to better define the frequency of birch pollen-related late-type reactions and possible predictors as well as the diagnostic validity of determination of sIgE.

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Methods:

Patients: A total of 182 patients met the following inclusion criteria and were enrolled in a retrospective data analysis: 1) Physician-diagnosed mild to severe AD measured by SORAD index. 2) Suspected diagnosis of birch pollen-related food allergy based on a history of seasonal respiratory or cutaneous symptoms during the birch pollen season. 3) All patients already had consecutively undergone oral food challenges with birch pollen-related foods as a routine diagnostic procedure in the Department of Dermatology and Allergy, Hannover Medical School, Germany according to the guidelines on food allergy[23-24]. Prior to oral food challenges, written informed consent had been obtained from all patients. As mentioned above, corresponding data was analyzed retrospectively.

Medical history and scoring of AD: A detailed medical history of AD, respiratory and birch pollen-related food allergy was obtained. AD was diagnosed following the criteria of Hanifin and Rajka which include pruritus, a typical morphology and distribution.[25]

According to the position paper of the European Academy of Allergology and Clinical Immunology (EAACI) and the Global Allergy and Asthma European Network (GA2LEN) regarding eczematous reactions to food in patients with AD[26] the severity of AD was rated daily during DBPCFC (pre-challenge). In addition, AD was also scored one day after the DBPCFC had been finalized in order not to miss possible late-type reactions. This was done by a trained dermatologist using the ”Scoring of Atopic Dermatitis” (SCORAD) index[27] which assesses signs and subjective symptoms of AD.

Total and specific IgE: Total IgE and sIgE against birch pollen and birch pollen-related foods (apple, carrot, celery and hazelnut) were determined prior to the DBPCFC using the ImmunoCAP® fluorescence enzyme immunoassay (FEIA) according to the manufacturer’s instructions (Thermo Fisher Scientific Inc., Waltham, MA; Manufactured: Phadia AB, Uppsala, Sweden). Patients with a sIgE-titer of at least 0.35 kU/l were regarded as sensitized.

Double-blind placebo-controlled food challenges: Birch pollen-sensitized patients with suspected immediate type and/or late type allergy to at least one birch pollen-related food (apple, carrot, celery or hazelnut) underwent DBPCFC as a standard diagnostic procedure according to European guidelines[23-24] outside the birch pollen season after a 4-week elimination diet including all kinds of birch-pollen related food upon instruction of a dietitian.

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DBPCFC was performed during stable course of AD. If required, an ongoing topical therapy with mild corticosteroids (European class I or II) in addition to emollients was allowed in selected cases. The treatment regimen had to remain unchanged during the complete period of DBPCFC. Antihistamines were withdrawn 72 h prior to the DBPCFC.

Apple, carrot, celery and hazelnut were used for DBPCFC since these represent birch pollen related foods which are commonly consumed in Central Europe. In certain cases, only one to three birch pollen-related foods were administered. The reasons for this approach were: 1) exclusion of those foods known to induce clear immediate type reactions in a patient, 2) the patient refused to be tested with a certain kind of birch pollen-related food, or 3) consecutive DBPCFC with single food after positive DBPCFC with a meal consisting of a mixture of several birch pollen-related foods.

Verum and placebo were prepared identically regarding colour, consistency and taste as has been published previously.[20-21] Basically, DBPCFC with raw birch pollen-related food was performed with fresh ground apple, carrot, celery, and hazelnut. For oral food challenge with heat-treated foods, apple, carrot and celery were precooked for 5-15 minutes while hazelnut was heated in a microwave oven (700 W for 2 minutes). The amount of birch-pollen related foods was adapted to a representative daily intake as previously published[20]. Placebo meal consisted of carob, rice flakes, orange flavor and beta-carotene.

According to national and international guidelines[23-24,26,28-29] placebo and verum were administered each on two consecutive days in a random order. The first challenge day was performed titrated, and on the second day the full dose was given. The severity of AD was rated daily before starting DBPCFC using the SCORAD index.

DBPCFC were performed in a two-step procedure built up of a “spit and a swallow phase” as first described by Ballmer-Weber et al.[16] When starting the challenge with a new meal the patients first had to keep 15 g of the meal in their mouth and spit it out after 10 and 30 seconds, respectively. Subsequently, the swallow phase was performed in a titrated manner with increasing doses of verum/placebo every 30 minutes (15 g, 45 g, 90 g and 180 g). The challenge was stopped after the full dose had been given or immediately in case of symptoms of an allergic immediate type reaction. On the following day, the complete dose was administered all at once. On both days, patients were further observed up to 3 h after the last dose had been given. Allergic reactions that occurred within 6 h after ingesting the last dose were defined as immediate type reactions. An increase of ≥10 SCORAD index

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points within 6-48 h after administration of the last dose was considered as a significant deterioration of AD (late eczematous reaction) as defined in the position paper of EAACI and GA2LEN.[26] Combined reactions were defined as an immediate type reaction prior to a significant worsening of AD.

Statistical analysis: As the data showed a non-normal distribution, statistical analysis was performed by nonparametric tests. The medians of metrically scaled variables (age, total and sIgE levels, SCORAD) were compared with the Mann-Whitney U-test. The history of respiratory atopic diseases of responders and non-responders was compared with the Chi-square test. The prevalence of birch pollen-related food allergies in the different age groups was compared with the Fisher´s exact test.
To evaluate the diagnostic validity of birch pollen-specific IgE-determination for the diagnosis of late eczematous reactions, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated according to the method of Thomas[30] by using the following equations:

$$\text{Sensitivity (\%)} = TP/(TP + FN) \times 100$$

$$\text{Specificity (\%)} = TN/(TN + FP) \times 100$$

$$\text{PPV (\%)} = (P \times \text{Sensitivity} \times 100)/[(P \times \text{Sensitivity}) + (100 - P) \times (100 - \text{Specificity})]$$

$$\text{NPV (\%)} = (100 - P) \times \text{Specificity} \times 100/[(100 - P) \times \text{Specificity} + P \times (100 - \text{Sensitivity})]$$

The prevalence (P) was defined as the number of responders with a deterioration of dermatitis divided by total patients who underwent a DBPCFC. True-positive (TP) results were defined as patients who exhibited an eczematous reaction upon DBPCFC and who had birch-specific IgE levels at a cut-off point of 17.5 kU/l. Patients who did not show a worsening of AD after DBPCFC but had birch pollen-specific IgE-determination of ≥17.5 kU/l were regarded as false-positive (FP) results. True-negative (TN) results were defined as patients without an eczematous reaction after DBPCFC and sIgE-determination <17.5 kU/l. Patients who showed an aggravation of AD after the DBPCFC but had a birch pollen-specific IgE levels <17.5 kU/l were regarded as false- negative (FN) results.

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Results:

Demographics and baseline characteristics

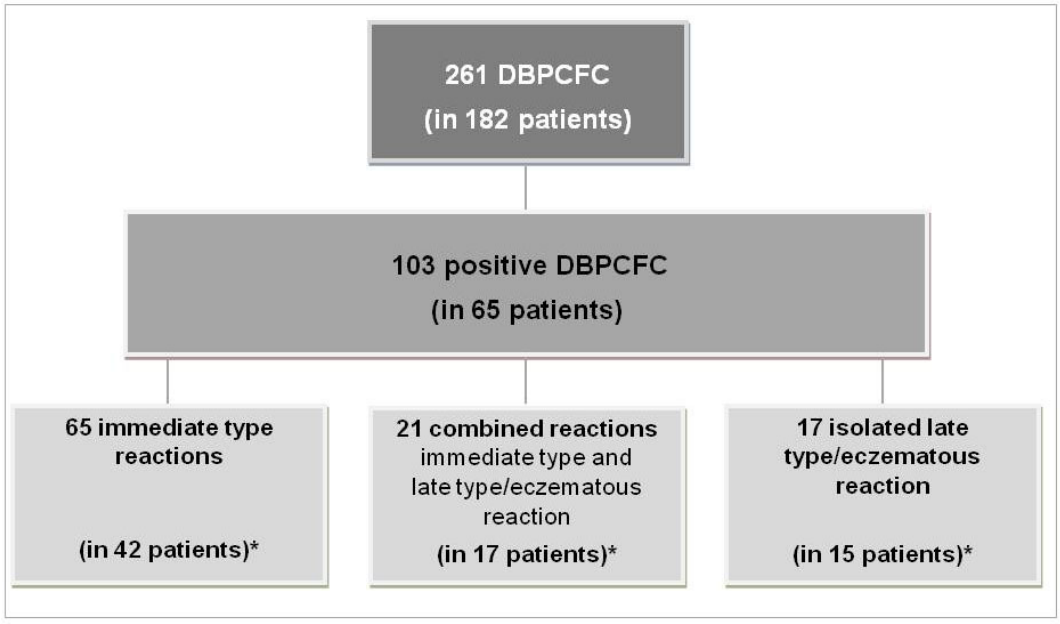
A total of 182 patients were included in this retrospective analysis (58% female, 42% male). The median age of the study population was 26 years (range 3-70 years; 82% ≥12 years and 18% <12 years). The median disease severity before oral food challenges assessed by SCORAD index was 28.0 points (range 8.3-65.0 points).

74% of the patients suffered from seasonal or perennial respiratory allergic symptoms (allergic rhinoconjunctivitis (RCA) or asthma bronchiale).

Clinical outcome of oral food challenges

In these 182 patients, consecutively 261 DBPCFC with at least one birch pollen-related food (apple, carrot, celery and hazelnut) were performed. 107 challenges were performed using all four kinds of birch pollen-related foods. 52 challenge tests were performed with two or three components, and 102 challenge tests were conducted with a single birch pollen-related food.

65 patients showed a clinical reaction (responders) with immediate and/or late type allergic symptoms upon ingestion of birch pollen-related foods (103 DBPCFC) (FIG 1).



*Some patients underwent several DBPCFC and showed different clinical reaction patterns. Hence, some responders are mentioned multiple times in FIG 2.

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FIG 1: Clinical outcome of 261 DBPCFC performed with birch pollen-related foods (apple, carrot, celery and hazelnut) in a total of 182 patients with AD. In total, 65 patients showed an allergic reaction upon birch pollen-related food ingestion in 103 DBPCFC. Of these, 42 patients showed an allergic immediate type reaction, and 32 patients displayed an isolated and/or a combined late type reaction with a deterioration of AD within 6-48 h after DBPCFC.

Together, 32 patients developed a late type reaction with a significant increase in SCORAD index from pre-challenge to post-challenge (median increase 15.4 points, 95% CI 12.4-16.3), whereas no significant increase of SCORAD index has been observed upon placebo challenges.

Of 103 positive oral food challenges, 63% were associated with an immediate type reaction, and 37% of positive food challenges were accompanied with a late eczematous reaction (FIG 2).

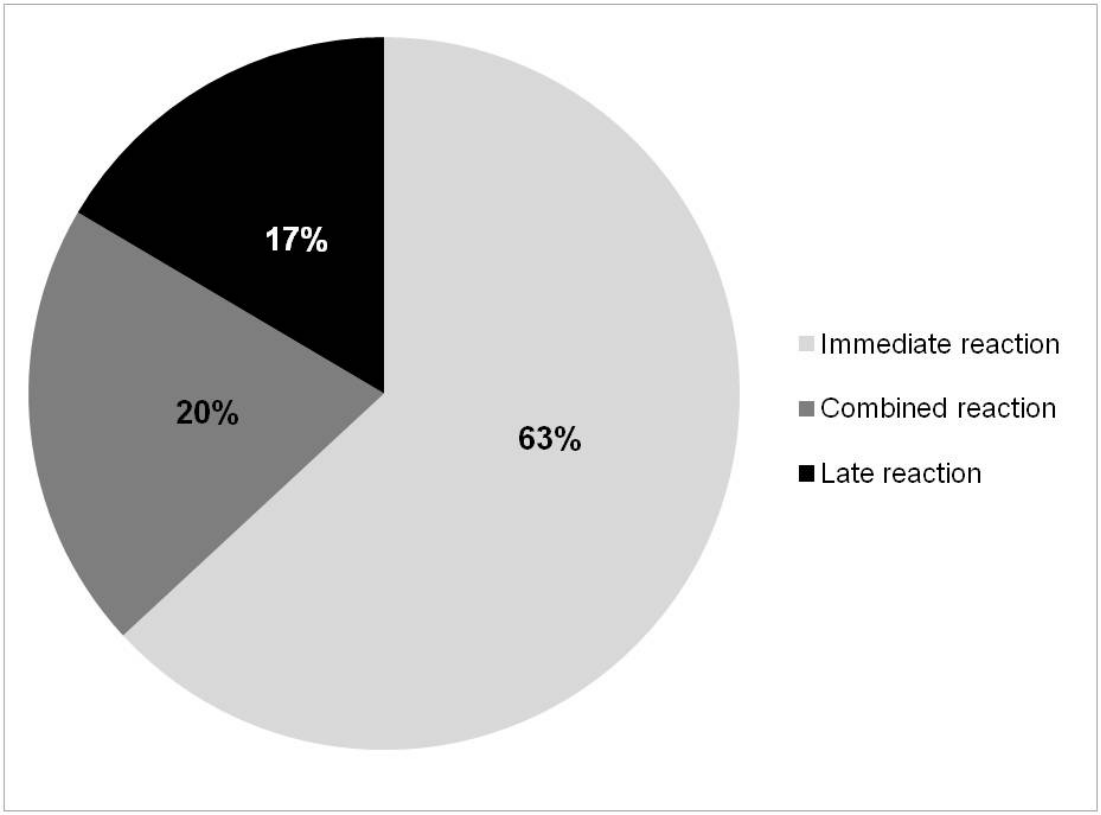


FIG 2: Pattern of clinical reactions (n=103 DBPCFC): Immediate reactions including delayed symptoms are defined as reactions occurring within 0-6 h after DBPCFC. Combined reactions are defined as immediate reactions developed within 0-6 h followed by an eczematous reaction within 6-48 h after DBPCFC, and isolated late cutaneous reactions are defined as a deterioration of AD within 6-48 h after DBPCFC without prior immediate type reaction.

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Symptoms of immediate type reactions in this study were OAS (58%), pruritus (12%), flush (9%), urticaria and/or angioedema (5%), gastrointestinal symptoms (5%) and asthma/dyspnea (3%). In one patient an anaphylactic reaction with flush and hypotension occurred after ingestion of a verum meal consisting of all four birch pollen-related foods apple, carrot, celery and hazelnut. In this case, no late eczematous reaction was observed.

Clinical reaction patterns related to different birch pollen-related foods

49% of all positive DBPCFC with a verum meal containing two to four kinds of birch pollen-related foods were associated with a deterioration of AD whereas DBPCFC with only one kind of food led to a broad variety of immediate and/or late-type symptoms (TABLE 1).

With regard to the DBPCFC with single birch pollen-related foods, raw and cooked celery were most frequently a trigger for an exacerbation of dermatitis (39% of positive food challenges with celery), while raw apple as well as raw and cooked carrot predominantly caused immediate type reactions (100% and 91% of positive DBPCFC with these foods, respectively). DBPCFC with raw and heat-treated hazelnut caused a deterioration of AD in 20% of positive oral food challenges with this birch pollen-related food (TABLE 1).

TABLE 1: Clinical reaction pattern of oral food challenges with the different kinds of raw and heat-treated birch pollen-related foods

	N	**Positive reactions**	**Immediate reactions**	**Combined reactions**	**Late reactions**
DBPCFC with four kinds of birch pollen-related foods (raw and heat-treated)	**107**	40 (37%)	20 (50%)	11 (28%)	9 (22%)
DBPCFC with two to three kinds of birch pollen-related foods (raw and heat-treated)	**52**	17 (33%)	9 (53%)	5 (29%)	3 (18%)
	n	**Positive reactions**	**Immediate reactions**	**Combined reactions**	**Late reactions**
Apple (raw and heat-treated)	**14**	7 (50%)	7 (100%)	0 (0%)	0 (0%)
Carrot (raw and heat-treated)	**29**	11 (38%)	10 (91%)	0 (0%)	1 (9%)
Celery (raw and heat-treated)	**30**	18 (60%)	11 (61%)	5 (28%)	2 (11%)
Hazelnut (raw and heat-treated)	**29**	10 (35%)	8 (80%)	0 (0%)	2 (20%)

n, number of oral food challenges. DBPCFC, double-blind placebo-controlled food challenge.

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Oral food challenges with heat-treated birch pollen-related foods

24 of 261 oral food challenges were performed with heat-treated birch pollen-related foods. In total, eight (33%) of these were positive with immediate type symptoms in six cases. A combined reaction and an isolated late eczematous reaction occurred in another two DBPCFC.

In more detail, celery was particularly associated with a high rate of allergic symptoms despite heat-treatment. Six out of seven DBPCFC with heat-treated celery were positive: In four cases an immediate type reaction was induced while in one patient significant deterioration of AD and in another patient a combined reaction was observed after ingestion of cooked celery. Heat-treated apple caused an immediate type allergic reaction in one out of three tested patients while cooked carrot elicited an immediate type reaction and in one out of eight performed DBPCFC. Contrarily, no allergic reaction appeared upon DBPCFC with heated hazelnut (n=5 DBPCFC).

Characterization of responders and non-responders

Patients who developed an allergic reaction upon DBPCFC were compared to non-responders with regard to age, history of respiratory allergic diseases and total and sIgE levels.

Age and severity of AD

Responders were significantly older than non-responders (P=.018; median age of responders and non-responders: 29 (range 3-66 years) and 23 (range 3-70 years) years, respectively (FIG 3).

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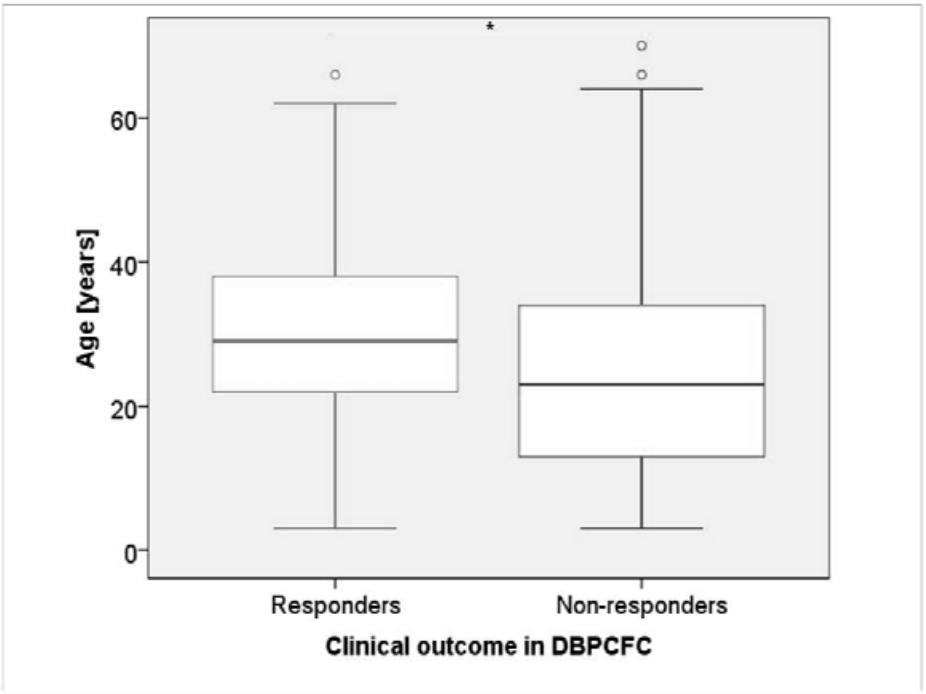


FIG 3: Age comparison of responders and non-responders
*P=.018, Mann-Whitney U-test

A significantly higher proportion of responders could be observed in adolescents ≥12 years and adults in comparison to children <12 years of age (DBPCFC response rate 39% vs. 14%, Fisher´s exact test, P=.01). However, no significant difference in age could be observed between patients with an isolated immediate type reaction and those with a deterioration of AD. In more detail, there was a trend towards a higher proportion of late eczematous reactions to birch pollen-related foods in adolescent and adults compared to children <12 years of age without reaching the level of significance (Fisher´s exact test, P=.613). Likewise, the patients who experienced an aggravation of AD upon DBPCFC did not differ significantly from those with an isolated immediate type reaction in terms of severity of AD before oral food challenge (Mann-Whitney U-test, P=.309).

Patient`s history of respiratory atopic diseases

Regarding the patient´s history, responders suffered significantly more often from RCA than non-responders (FIG 4). No significant difference in the prevalence of RCA could be observed between responders with a deterioration of AD and those with an isolated immediate type reaction (68% vs. 84%, Fisher´s exact test, P=.213).

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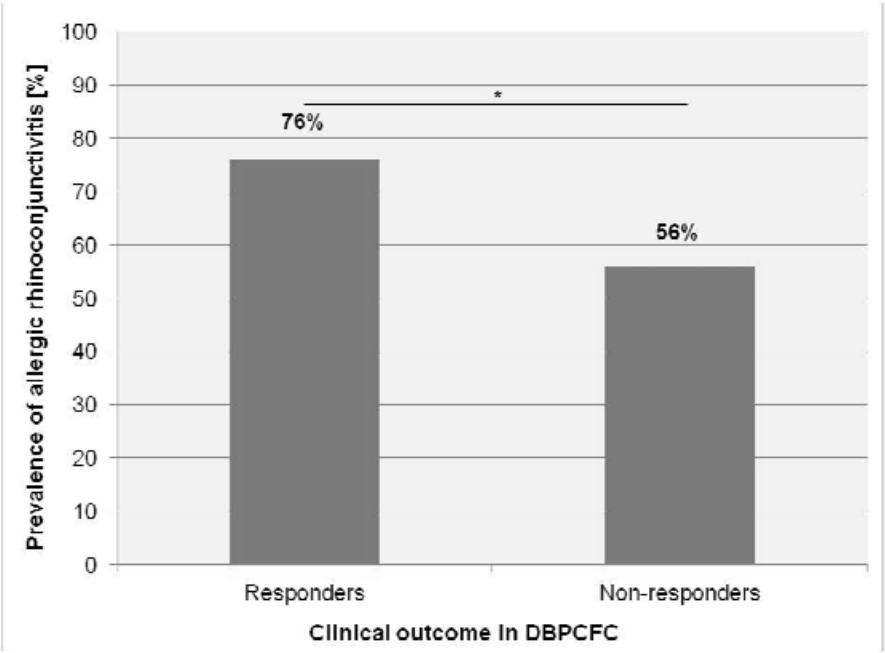


FIG 4: Patient's history of RCA in responders and non-responders
*P=.030; Chi-square test

With regard to both age groups, subjects ≥12 years of age showed a significantly higher prevalence of RCA and asthma bronchiale in comparison to subjects <12 years of age (RCA 76% vs. 14%, Chi-square test P<.001; asthma 54% vs. 14%, Chi-square test P=.001). Responders and non-responders did not significantly differ in terms of history of asthma bronchiale (data not shown).

Specific IgE levels to birch pollen and birch pollen-related foods

sIgE levels against birch pollen and apple turned out to be significantly higher in responders compared to non-responders, whereas no significant differences between both groups were found in terms of total IgE levels as well as sIgE to carrot, celery and hazelnut (TABLE 2).

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TABLE 2: Comparison of the *in vitro*-diagnostic of responders and non-responders (medians)

IgE [kU/l]	Responders	Non-responders	*P* value[1]
Total IgE (n_R=54; n_N=96)	858.00 (95% CI 507.50-1499.50)	1008.50 (95% CI 431.51-1623.00)	.565
sIgE birch (n_R=57; n_N=97)	**58.0 (95% CI 38.70-88.80)**	**34.20 (95% CI 26.00-47.70)**	**.047**
Ratio sIgE birch/ total IgE (n_R=54; n_N=96)	0.046 (95% CI 0.023-0.082)	0.024 (95% CI 0.017-0.034)	.100
sIgE apple (n_R=54; n_N=72)	**5.00 (95% CI 3.65-7.75)**	**2.67 (95% CI 1.24-3.48)**	**.014**
sIgE carrot (n_R=54; n_N=70)	1.87 (95% CI 1.15-3.79)	1.19 (95% CI 0.66-2.00)	.099
sIgE celery (n_R=53; n_N=72)	1.61 (95% CI 0.93-2.54)	1.00 (95% CI 0.45-1.61)	.070
sIgE hazelnut (n_R=55; n_N=75)	2.70 (95% CI 1.37-5.68)	1.68 (95% CI 1.06-2.87)	.087

sIgE, specific IgE
n_R, number of responders
n_N, number of non-responders
[1]Mann-Whitney U-test

Responders with an eczematous late type reaction (n=32) and those with an isolated immediate type reaction (n=33) did not significantly differ in serum total IgE and sIgE levels (63.20 kU/l (95% CI 33.30->100.00) vs. 53.35 kU/l (95% CI 38.35->100.00), Mann-Whitney U-test *P*=.928). Likewise, no differences could be observed in serum birch pollen-specific antibodies between children and adolescent/adult patients (data not shown).

Diagnostic value of birch pollen-specific IgE

In this retrospective study, IgE-sensitization to birch pollen at a cut-off point of 17.5 kU/l showed a high sensitivity (81%) and NPV (88%) for the diagnosis of deterioration of dermatitis, while the specificity (31%) and PPV (21%) here were low.

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Discussion:

This work confirms previous observations[20,21,22] that birch pollen-related foods can cause immediate type reactions in patients with AD and, moreover, may serve as a relevant provocation factor for an aggravation of AD. In the present study, 37% of 103 positive DBPCFC with birch pollen-related foods resulted in a worsening of AD. With respect to all tested patients, 18% reacted with a late eczematous reaction combined with or without a prior immediate type reaction upon DBPCFC. This rate is lower than the frequency of eczematous reactions observed in former studies in adult patients (46%)[20] and in children with AD (33%).[21] Such variable incidence of late type reactions might be due to different study inclusion criteria: Reekers et al. only included adult patients who were highly sensitized against birch pollen (sIgE ≥17.5 kU/l), and who did not have experienced an immediate type reaction after ingestion of birch pollen-related foods before.[20] Breuer et al. only included children who were not supposed to suffer from an immediate type reaction after ingestion of the tested foods.[21] In the present study, the defined threshold for sIgE to birch pollen was lower than in the trial of Reekers et al.[20]. Moreover, 63% of responders retrospectively analyzed had exhibited an immediate type reaction after the DBPCFC and, for safety reasons, had finally not undergone a repetitive provocation over two consecutive days. Subsequently, it could be assumed that in these patients the amount of allergen uptake was probably too low to elicit an eczematous reaction.

In accordance with former studies[31-35] OAS symptoms in the present study appeared as main immediate type symptom of birch pollen-related food allergy. Only one DBPCFC performed with four kinds of birch pollen-related foods (apple, carrot, celery and hazelnut) led to an anaphylactic reaction. It may be speculated that in this patient a late type reaction was suppressed through antiallergic systemic treatment including corticosteroids. Overall, epinephrine administration was not required in any patient of this study. The dominance of mild allergic symptoms in terms of an OAS and the small number of systemic immediate type reactions results from the instability of Bet v 1-homologue allergens against gastrointestinal digestion.[36] Pepsin completely abrogates the IgE-binding capacity of recombinant Mal d 1, Api g 1 and Cor a 1.04 within one second. Equally, trypsin destroys the IgE-binding of rMal d 1 and rApi g 1 after incubation for 15 minutes, whereas the major hazelnut allergen rCor a 1.04 shows a higher stability towards trypsinolysis. Moreover, it could previously be demonstrated that digested Mal d 1 and Cor a 1.04 still activate Bet v 1–specific T cells, whereas digested Api g 1 does not.[36] With regard to sequence similarity, this is lower between the major celery allergen Api g 1 and Bet v 1 compared to the major hazelnut allergen Cor a 1.04 (61% and 79%,

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respectively).[35] However, we demonstrated *in vivo* that celery served as the most frequent elicitor of late eczematous reactions, whereas apple exclusively induced mild oropharyngeal symptoms. Likewise, carrot and hazelnut induced predominantly immediate type reactions (91% and 80%, respectively). Hence, our data confirm previous results from Bohle et al. who found that the degree of sequence similarity between Bet v 1–related food allergens and the major birch pollen allergen cannot be used as a marker to predict their potential to induce T cell–mediated symptoms.[22]

Despite the fact that in all birch pollen-sensitized patients included in the present study birch pollen-related food allergy was suspected, the overall response rate in DBPCFC was only 36%. However, those foods known to induce clear immediate type reactions were excluded from oral food challenges. This approach probably contributed to the relatively low proportion of responders in this study.

From our data we can also conclude that raw as well as cooked celery is able to cause a deterioration of the skin lesions. Consistently, it has already been demonstrated that thermal processing at 80°C for 15 minutes and at 100°C for 5 minutes abolishes IgE-binding and mediator release capacity of Bet v 1-homolgue allergens Mal d 1, Dau c 1 and Api g 1 through changing their protein structure, whereas cooking does not influence their ability to stimulate Bet v 1-specific T-cells and cause a deterioration of dermatitis.[22] Thus, T-cell cross-reactivity between Bet v 1 and related food allergens occurs independently of IgE cross-reactivity *in vitro* and *in vivo*.

Data from Bohle et al. further indicate that heating irreversibly destroys the conformational structure of Mal d 1 and Dau c 1, while Api g 1 shows a partial restoration of the protein structure.[22] In our study, apple, carrot, and celery were cooked at 100°C for 5-15 minutes prior to the oral food challenge. This might explain previously published data[38,39] and our finding on cooked celery that still exhibited a distinct allergenic potential with induction of immediate type reactions in 5 out of 7 patients. Surprisingly, one DBPCFC with heat-treated apple was accompanied with a mild immediate type reaction of an OAS. It might be speculated that the apple used for the test meal was not heated sufficiently to abolish the IgE-binding capacity of Mal d 1. Moreover, subjective psychological factors cannot be excluded in this case since symptoms of an OAS cannot be verified by objective measurements. However, with respect to the mild severity of subjective symptoms in this birch-pollen allergic patient sensitization to heat-resistant apple allergen Mal d 3 from the allergen family of lipid transfer proteins (LTP) is rather unlikely. In fact, LTP sensitization is predominantly found in non-birch pollen sensitized patients from Mediterranean areas exhibiting more severe clinical reactions.[40] Nevertheless, in future studies in this field assessment of specific IgE-sensitization to rBet v 1 and the

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corresponding homologue molecular food allergens from the PR-10 family (i.e. rMal d 1, rDau c 1, rApi g 1 and rCor a 1) should be performed to improve the diagnostic accuracy. It might be supposed that molecular allergy diagnosis would also allow to identify and to characterize distinct sensitization patterns in birch pollen-sensitized subjects facilitating the diagnosis of birch pollen-related food allergy.

In contrast to previous studies[20,21], we found that the responders were significantly higher IgE-sensitized against birch pollen and apple compared to the non-responders while total IgE- and sIgE levels against carrot, celery, and hazelnut did not differ between both groups. The latter two results are in line with the data from Guhsl et al. who showed that sIgE testing with food extracts from celery and hazelnut is not suitable to discriminate between food-allergic and tolerant patients sensitized against Bet v 1.[35] As expected, responders in the present study were significantly older than non-responders and showed a significantly higher prevalence of RCA. This outcome reflects the natural course of food allergy and respiratory allergy.[41] However, age, birch pollen sIgE levels, and the presence of a RCA could not be used to differentiate between responders with isolated immediate type reactions and those with late eczematous reactions. In accordance to previous research[20,21], no differences could be observed between responders and non-responders in terms of the disease severity, total IgE, or food specific IgE against carrot, celery, or hazelnut.

The main limitation of the study that should be considered in the interpretation of our results is its retrospective and monocentric design. Therefore, our results cannot be transferred to other patient populations, especially not to those outside birch-endemic regions. Further prospective multicenter studies are needed to reproduce these results in larger cohorts from different geographical areas.

In conclusion, our data confirm that in a subpopulation of patients with AD and IgE-sensitization to birch pollen corresponding foods should be considered as potential trigger for an aggravation of dermatitis. Even if the responders with immediate and/or late type reaction tended to have higher sIgE levels against birch pollen compared to the non-responders, the diagnostic value of the sIgE determination as a single parameter is insufficient for the diagnosis of a late type reaction. Thus, DBPCFC cannot be replaced in patients with AD and suspected late eczematous reaction after ingestion of birch pollen-related foods.

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Conflict of Interests:

Anja Wassmann-Otto has no conflicts of interests to declare.

Annice Heratizadeh has no conflicts of interests to declare.

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Thomas Werfel has no conflicts of interests to declare.

Authors contribution:

Anja Wassmann-Otto conceived and designed the retrospective analyses, analyzed, interpreted and discussed the data and wrote the manuscript.

Annice Heratizadeh conceived and designed the retrospective analyses discussed the data and revised the article critically.

Katja Wichmann discussed the data and revised the article critically.

Thomas Werfel conceived and designed the retrospective analyses, discussed the data and revised the article critically.

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