

Original article

Prediction of challenge test results by flour-specific IgE and skin prick test in symptomatic bakers

Background: Wheat and rye flours are among the most important allergens causing occupational asthma. Usually, the diagnosis of baker's asthma is based on inhalation challenge tests with flours.

Aims of the study: To evaluate the relevance of flour-specific serum immunoglobulin E (IgE) and skin prick test (SPT) in the diagnosis of baker's asthma and to define flour-specific IgE concentrations and wheal sizes that allow a prediction of the outcome of challenge testing.

Methods: Bronchial and nasal challenge tests with wheat (rye) flour were performed in 71 (95) symptomatic bakers. Determinations of flour-specific IgE as well as SPTs were performed in all subjects. Analyses included the calculation of sensitivity, specificity, positive (PPV) and negative predictive values (NPV) at different IgE concentrations and different wheal sizes, and receiver-operating characteristics (ROC) plots with the challenge result as gold standard.

Results: Thirty-seven bakers were positive in the challenge with wheat flour, while 63 were positive with rye flour. Depending on the flour-specific IgE concentrations (wheal size), PPV was 74–100% (74–100%) for wheat and 82–100% (91–100%) for rye flour, respectively. The minimal cut-off values with a PPV of 100% were 2.32 kU/l (5.0 mm) for wheat flour and 9.64 kU/l (4.5 mm) for rye flour. The shapes of the ROC plots were similar for wheat and rye flour.

Conclusion: High concentrations of flour-specific IgE and clear SPT results in symptomatic bakers are good predictors for a positive challenge test. Challenge tests with flours may be avoided in strongly sensitized bakers.

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Baker's asthma is one of the most frequent forms of occupational asthma. In 2005 49% of 571 confirmed cases of occupational asthma in Germany were caused by bakery-derived allergens (1). In general, for diagnosis of occupational asthma, especially within the scope of compensation claims, the specific challenge to suspected occupational agents is considered the gold standard (2, 3). Because there are relatively few specialized centers, and challenge tests are time-consuming, unpleasant for patients and may cause severe reactions, it was the aim of this study to investigate whether the concentration of flour-specific immunoglobulin E (IgE) or the results of skin prick test (SPT) can act as predictors for the outcome of the specific challenge tests.

While the general associations between the degree of bronchial responsiveness to allergens and the degree of

sensitization as assessed by SPT and/or *in vitro* tests are well known, it was shown only for some allergens, especially food allergens, that the results of specific IgE determination or SPT are useful to predict the result of the challenge test (4, 5). In this connection little is known about occupational allergens.

Materials and methods

Subjects

This multicentre study included 107 bakers (77% males) all suffering from work-related symptoms like rhinitis, conjunctivitis, cough, chest tightness, shortness of breath or wheezing. Eighty-three subjects (78%) complained of asthma and out of these 75 reported also rhinitis. Twenty-four bakers (22%) reported rhinitis, but no

symptoms of asthma. Ninety-nine bakers (93%) reported improvement during holidays or weekends. The mean age was 40 ± 13 years, 36% were current smokers, 15% ex-smokers. Fifty-six subjects (52%) were atopics according to SPT results. Thirty-eight bakers (36%) were still working as bakers at the time of the study. The mean time since leaving the job was 4 ± 3 years. All bakers were examined within the scope of claims for compensation due to occupational asthma in six different German and one Polish allergy centres. The examinations comprised flour-specific IgE determination, SPTs, and challenge tests (nasal, bronchial or workplace simulation) with flours. Seventy-one bakers (mean age: 41 years, 71% male) were challenged with wheat flour, 95 bakers (mean age: 40 years, 79% male) with rye flour. While SPTs and challenge tests were performed in the different centres, specific IgE measurements were done centrally in BGFA.

Specific IgE determination

Specific IgE antibodies to wheat and rye flour were measured by ImmunoCAP (Phadia, Uppsala, Sweden) according to the manufacturer's recommendations. In this assay, a value ≥ 0.35 kU/l is indicative of the presence of allergen-specific IgE.

Skin prick test

Skin prick tests were performed with wheat and rye flour extracts from Bencard (Munich, Germany) as well as a panel of common inhalant allergens, including grass pollen, birch pollen, house dust mite (*Dermatophagoides pteronyssinus*), and cat dander (Allergopharma, Reinbek, Germany). Atopy was defined as a wheal diameter ≥ 3 mm to at least one of these common aeroallergens. Histamine (10 mg/ml) and saline were used as positive and negative controls, respectively. In each centre, identical extracts were pricked according to a standardized procedure twice in a predetermined order on the untreated skin on both volar forearms in opposite direction. After 15 min, test solutions were wiped off with alcohol, and contours of wheals were transferred with transparent tape to a blank sheet of paper. Assessment of SPT results was carried out at BGFA. The mean value of the largest diameter and the diameter at the midpoint, at a right angle, was recorded in mm. A subject was considered positive if at least one of the two SPT wheals was above the chosen cut-off value. The larger wheal of the double estimation was considered for analysis.

Challenge tests

Overall, 126 challenge tests with flour were performed in 107 symptomatic bakers (Table 1). Whereas SPT and specific IgE measurements were standardized and comparable between centres,

standardization of challenge tests was not targeted, and therefore some heterogeneities of test procedure were unavoidable.

Nasal challenge tests using commercial aqueous flour solutions were performed in two cases (wheat) and eight cases (rye), respectively. Nasal resistance was measured by anterior rhinomanometry. A nasal challenge test was considered positive if nasal symptoms were followed by a decrease of nasal flow by at least 40% from baseline. Bronchial challenge tests with nebulization of commercial aqueous flour solutions in increasing concentrations were performed in 15 cases (wheat) and 24 cases (rye) while a workplace simulation with flour dust or inhalation of flour via spinhaler was performed in 54 cases (wheat) and in 63 cases (rye), respectively. In all cases a control challenge using sucrose powder, pea flour or potato flour was performed without a bronchial reaction. Allergen-induced airway responsiveness was measured in all except one centre by body plethysmography. A positive test result was assumed if specific airway resistance (sRt) doubled and increased simultaneously to ≥ 2.0 kPa s, or if the fall in FEV₁ was $\geq 20\%$.

Forty bakers were challenged with a mixture of wheat and rye flour dusts. The results of these workplace simulations were judged positive or negative for both flours because in 19 bakers who had been separately challenged with both flours, in no case a discordant result was detectable. Overall decisions whether the challenge test was positive or negative were provided by the respective examiner.

Statistical methods

Calculations were performed using SAS/STAT software, version 9.1 (Cary, NC, USA). Two-by-two tables were used to calculate sensitivity [rp/(rp + fn)], specificity [rn/(rn + fp)], positive (PPV) [rp/(rp + fp)] and negative [rn/(rn + fn)] predictive values (NPV) of specific IgE concentrations or SPT results using challenge test results as gold standard. Right positives (rp) were bakers with positive challenge and positive specific IgE/positive SPT; right negatives (rn) were bakers with negative challenge and negative specific IgE/negative SPT; false positives (fp) were bakers with negative challenge and positive specific IgE/positive SPT; false negatives (fn) were bakers with positive challenge and negative specific IgE/negative SPT. Concentrations of flour-specific IgE and wheal sizes in bakers with a positive or a negative challenge were compared with the Wilcoxon rank sum test. Receiver operating characteristic (ROC) plots are one possible graphical representation for describing and comparing diagnostic tests (6). For creating ROC curves, sensitivity was plotted vs 1-specificity over all specific IgE concentrations and wheal sizes (here both wheals of each subject were considered). The area under the curve (AUC), which is a global measure for the test's accuracy, ranges from 0 to 1 and can be interpreted as the likelihood that a randomly selected subject will be assessed correctly.

Table 1. Performance characteristics and results of the 126 challenge tests with flours performed in 107 symptomatic bakers

	n	Wheat* (n = 31)		Rye* (n = 55)		Wheat and rye (n = 40)	
		Positive	Negative	Positive	Negative	Positive	Negative
Nasal challenge	10	2	0	7	1	0	0
Bronchial challenge	39	7	8	18	6	0	0
Workplace simulation	77	8 (na: 0, b: 3, nab: 5)		18 (na: 2, b: 3, nab: 13)		20 (na: 5, b: 2, nab: 13)	
Total	126	17	14	43	12	20	20

na: isolated nasal, b: isolated bronchial, nab: nasal and bronchial reaction.

*19 bakers have been separately challenged with both flours and in no case a discordant result was detectable.

Results

Challenge tests

A positive challenge test to any flour was found in 72 (67%) of 107 symptomatic bakers. Thirty-seven (52%) of 71 bakers challenged with wheat flour showed a positive test while this was the case in 63 (66%) of 95 bakers challenged with rye flour. After workplace simulations, 31 (67%) of 46 bakers with positive challenge tests showed both bronchial and nasal reactions (Table 1). There was no difference in specific IgE concentrations and wheal sizes between these patients and those with an isolated nasal or bronchial reaction (data not shown).

Specific IgE

Wheat flour-specific IgE concentrations in bakers challenged with wheat flour ranged from < 0.35 to 27.10 kU/l (median 0.56 kU/l). In those bakers chal-

lenged with rye flour, rye flour-specific IgE concentrations ranged from < 0.35 to > 100 kU/l (median 1.31 kU/l). The AUC of the ROC plots was 0.83 for both wheat and rye flour-specific IgE (Fig. 1).

Sensitivity, specificity, PPV and NPV were first calculated for a specific IgE cut-off value of 0.35 kU/l (Table 2a). At this (minimal) cut-off value for wheat and rye flour-specific IgE, specificity was 68% and 62%, PPV 74% and 82% and NPV was 82% and 71%, respectively, whereas sensitivity was 87% for both flours. Specific IgE concentrations were significantly higher in bakers with positive challenge tests (wheat flour: median 2.32 kU/l, range < 0.35 to 27.1 kU/l; rye flour: median 3.3 kU/l, range < 0.35 to > 100 kU/l), than in those with negative challenge tests (wheat flour: median < 0.35 kU/l, range < 0.35 to 2.11 kU/l, $P < 0.0001$; rye flour: median < 0.35 kU/l, range < 0.35 to 7.86 kU/l, $P < 0.0001$) (Fig. 2).

According to ROC analysis, the optimal cut-off level that maximizes the sum of sensitivity and specificity was

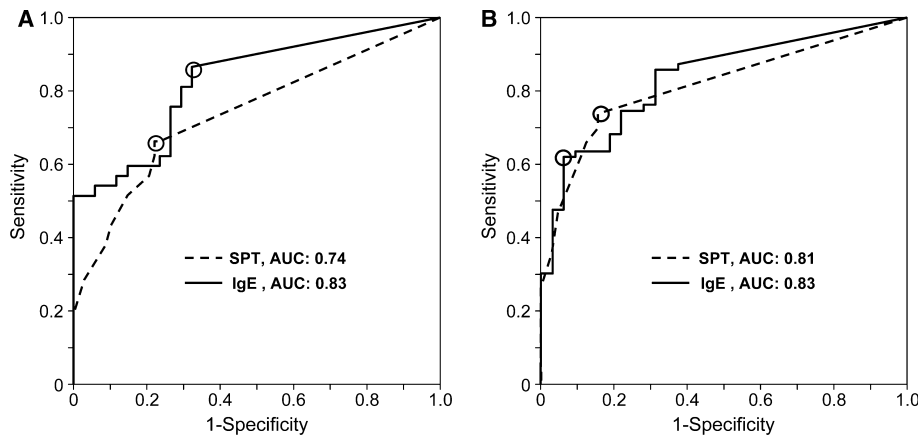


Figure 1. ROC (receiver-operating characteristic) plots: specific IgE and SPT for wheat flour (A) and rye flour (B) with the specific challenge test as gold-standard. The area under the curve (AUC) is a global measure for the test's accuracy that ranges between 0 and 1. The optimal cut-off values are encircled.

Table 2. Sensitivity, specificity, positive (PPV) and negative predictive value (NPV) of wheat and rye flour-specific IgE based on the gold-standard challenge test. Evaluation was performed using different cut-off values for specific IgE: (a) minimal cut-off, (b) optimal* cut-off, (c) cut-off at which 100% PPV was obtained

	Cut-off value	Positive specific IgE		Negative specific IgE		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive challenge (n)	Negative challenge (n)	Positive challenge (n)	Negative challenge (n)				
(a)	Wheat (n = 71)	32	11	5	23	87	68	74	82
	≥0.35 kU/l								
	Rye (n = 95)	55	12	8	20	87	62	82	71
	≥0.35 kU/l								
(b)	Wheat (n = 71)	32	11	5	23	87	68	74	82
	≥0.37 kU/l*								
	Rye (n = 95)	39	2	24	30	61	94	95	56
	≥2.46 kU/l*								
(c)	Wheat (n = 71)	19	0	18	34	51	100	100	65
	≥2.32 kU/l								
	Rye (n = 95)	19	0	44	32	30	100	100	42
	≥9.64 kU/l								

*The definition of optimal cut-off value is derived from the ROC plot (Fig. 1).

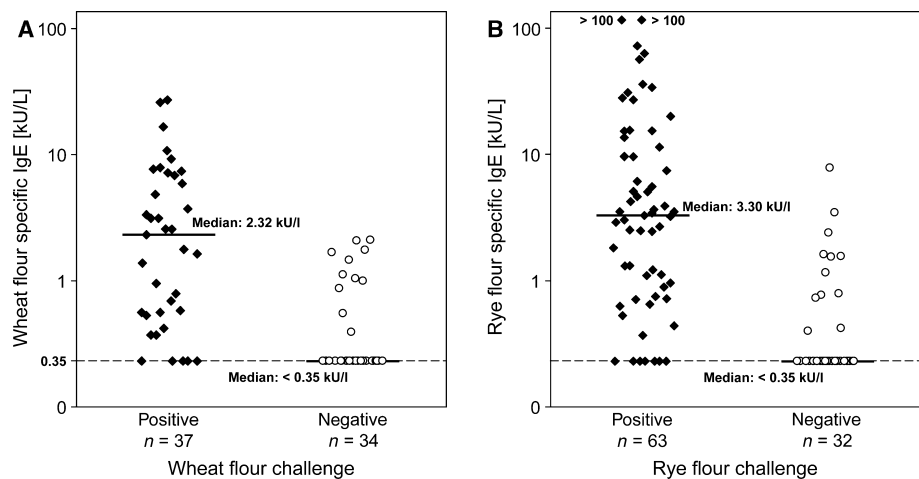


Figure 2. Comparison of concentrations of flour-specific IgE antibodies between bakers with a positive challenge test (■) and those with a negative challenge test (○) to wheat flour (A) and rye flour (B), respectively.

0.37 kU/L for wheat flour and 2.46 kU/L for rye flour (Table 2b). The minimal cut-off value, at which a PPV of 100% could be obtained, was 2.32 kU/l for wheat flour and 9.64 kU/l for rye flour (Table 2c). This means that all bakers with wheat flour-specific IgE ≥ 2.32 kU/l ($n = 19$) and all bakers with rye flour-specific IgE ≥ 9.64 kU/l ($n = 20$) had a positive challenge test.

Skin prick test

In bakers challenged with wheat flour, wheal sizes due to wheat flour ranged from 0 to 12.5 mm (median 1.0 mm) while in the group of bakers challenged with rye flour, wheal sizes between 0 and 11.0 mm (median 3.0 mm) were obtained using rye flour SPT-solution. The AUC of the ROC plots for SPT was 0.74 for wheat flour and 0.81 for rye flour (Fig. 1).

Sensitivity, specificity, PPV and NPV were calculated for a cut-off value of ≥ 2 mm (Table 3a). Because small wheal sizes maximize the sum of sensitivity and specificity according to ROC analysis, this was the minimal and the optimal cut-off value at the same time. Using wheat (rye) flour SPT-solution, sensitivity was 68% (78%), specificity

74% (84%), PPV 74% (91%), and NPV was 68% (66%). Wheal sizes were significantly greater in bakers with positive challenge tests (wheat flour: median 3.0 mm, range 0.0–12.5 mm; rye flour: median 3.5 mm, range 0.0–11.0 mm), than in those with negative challenge tests (wheat flour: median 0.0 mm, range 0.0–4.5 mm, $P = 0.0003$; rye flour: median 0.0 mm, range 0.0–4.0 mm, $P < 0.0001$) (Fig. 3).

The minimal cut-off value for a PPV of 100% was 5.0 mm for wheat flour and 4.5 mm for rye flour (Table 3b). This means that all bakers with a wheal size ≥ 5 mm to wheat flour ($n = 8$) and all bakers with a wheal size ≥ 4.5 mm to rye flour ($n = 24$) had a positive challenge test.

Skin prick test/specific IgE

Out of the eight bakers who showed wheal sizes ≥ 5.0 mm to wheat flour, seven had also high wheat flour-specific IgE concentrations (median 6.38 kU/l, range 3.14–26.0 kU/l). In the case of rye flour, all bakers showing wheal sizes ≥ 4.5 mm ($n = 24$) were also positive in IgE-determination with rye flour (median 10.61 kU/l, range

Table 3. Sensitivity, specificity, positive (PPV) and negative predictive value (NPV) of SPT using wheat and rye flour based on the gold-standard challenge test. Evaluation was performed using different cut-off values for SPT: (a) minimal/optimal* cut-off, (b) cut-off at which 100% PPV was obtained

	Cut-off value	Positive SPT		Negative SPT		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive challenge (n)	Negative challenge (n)	Positive challenge (n)	Negative challenge (n)				
(a)	Wheat (n = 71)	25	9	12	25	68	74	74	68
	≥ 2.0 mm*								
	Rye (n = 95)	49	5	14	27	78	84	91	66
	≥ 2.0 mm*								
(b)	Wheat (n = 71)	8	0	29	34	22	100	100	54
	≥ 5.0 mm								
	Rye (n = 95)	24	0	39	32	38	100	100	45
	≥ 4.5 mm								

*The definition of optimal cut-off value is derived from the ROC plot (Fig. 1).

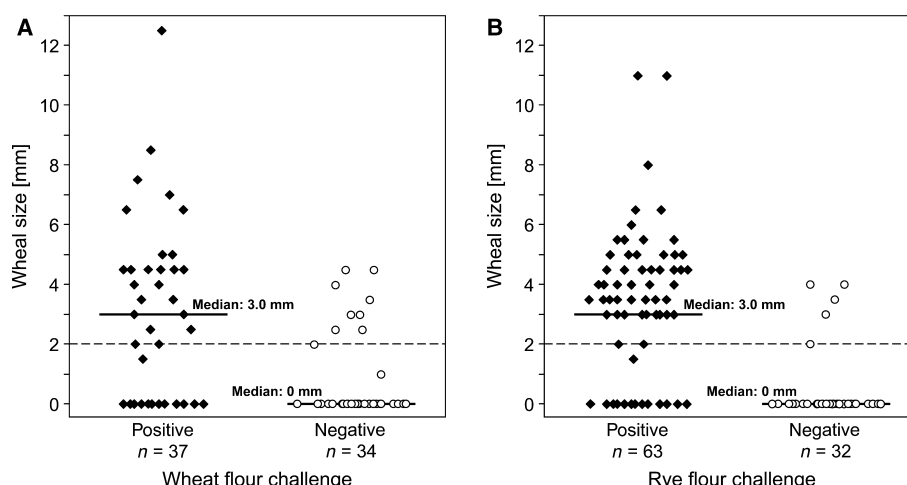


Figure 3. Comparison of results of SPTs with flours between bakers with a positive challenge test (■) and those with a negative challenge test (○) to wheat flour (A) and rye flour (B), respectively.

0.75 to >110 kU/l). Using the minimal cut-off values (≥ 0.35 kU/l and ≥ 2 mm, respectively) in the case of wheat flour, the PPV of specific IgE determination and of SPT was identical (74%) while in the case of rye flour SPT reached a higher PPV than specific IgE (91% vs 82%).

Using a more stringent positive criterion (both SPT and specific IgE determination had to be positive), in the case of rye flour, specificity and PPV remained the same while sensitivity and NPV were marginally lower than using results of SPT alone. In the case of wheat flour, there was a slight increase in specificity (74% vs 79%) and PPV (74% vs 77%) if results of SPT and specific IgE were combined in comparison with SPT alone.

Discussion

The results of this study demonstrate that both, flour-specific IgE and SPT with flours, can be used effectively for the prediction of the outcome of specific challenge tests with flours in symptomatic bakers. It was shown that all bakers with flour-specific IgE or wheal sizes in SPT with flours above a defined cut-off had a positive challenge test.

Even if the predictive values were not clearly higher with a combination of specific IgE and SPT as positive criterion than with the single criteria alone, we recommend both diagnostic tests for reasons of quality control. Our findings indicate that SPT provide little, although additional diagnostic information compared with specific IgE. While specific IgE determination is the more sensitive test, SPT reached at least in the case of rye flour a higher PPV using the minimal cut-off values.

As already indicated by the fact that NPV in no case reached a value higher than 82%, IgE concentrations and wheal sizes below the minimal cut-off values did not exclude a positive reaction in the challenge. Using the

lowest cut-off values (0.35 kU/l; 2 mm), five bakers without specific IgE and 13 bakers with negative SPT to wheat, as well as eight bakers without specific IgE and 14 bakers with negative SPT to rye flour showed a positive challenge test. This could be either due to insufficient sensitivity of determination procedure (specific IgE test or SPT) or to irritative effects (7).

There are a number of reports about predictive values of specific IgE concentrations for the outcome of oral food challenges, predominantly performed in children. For egg, milk, peanut, and fish allergy, the diagnostic levels of specific IgE which were shown to predict clinical reactivity in a group of 196 children and adolescents with greater than 95% certainty were 6, 32, 15 and 20 kU/l, respectively (8). In another study group, neither with specific IgE (9) nor with SPT (4) a predictive probability of 95% for wheat and soy was reached.

Little is known about the relationship between specific IgE or SPT and the outcome of challenge tests with inhalation allergens. For cat allergy it could be shown in 49 asthmatic subjects that a probability of a positive bronchial challenge of $\geq 93\%$ could be reached at a cut-off value of ≥ 17 kU/l. Using SPT results, a maximum PPV of 71% at an 8 mm cut-off could be reached (10). In a further study, cat-exposed persons with a history of asthma and a positive methacholine test were investigated. Positive challenge tests occurred in all 27 participants with cat-specific IgE, compared with 12 of 44 subjects with negative IgE result. This analysis revealed also that 38 of 41 subjects with positive SPT to cat, but only 10 of 39 with negative SPT had a positive challenge result (11).

The relationship between specific IgE concentration or SPT and the outcome of the specific challenge test has been rarely investigated with occupational allergens. Out of 27 dairy farmers with suspected occupational asthma due to bovine allergens, 11 farmers showed a positive result in the specific challenge test. The minimal cut-off value for

bovine-specific IgE, at which a PPV of 100% could be obtained, was 5 kU/l. All farmers with higher IgE concentrations ($n = 9$) had positive challenge tests. However, PPV of SPT with bovine dander solution calculated for a cut-off value of ≥ 3 mm was only 46% (12).

A small number of studies found correlations between the degree of sensitization as assessed by SPT and the degree of the bronchial reactions during challenge tests with occupational allergens. The authors of a study including 24 bakers with positive SPT to different bakery-derived allergens and bronchial hyperresponsiveness described a formula showing the allergen concentration causing a 20% fall in FEV₁ during inhalative challenge test (allergen PC₂₀) as a function of skin sensitivity to allergen and methacholine PC₂₀. A highly significant correlation ($r = 0.92$, $P < 0.001$) was found between the measured allergen PC₂₀ and the predicted allergen PC₂₀ calculated with this formula. A similar formula using specific IgE instead of skin sensitivity could not be established (13). Shirai et al. demonstrated also a correlation ($r = 0.796$, $P < 0.05$) among allergen PC₂₀, methacholine PC₂₀ and skin reactivity to epigallocatechine gallate, the causative agent of green tea-induced asthma (14).

In a study with 57 workers with occupational asthma due to platinum salts, an univariate correlation between skin reactivity and bronchial responsiveness due to platinum salts ($r = 0.6$, $P < 0.0001$) could be observed.

However, responsiveness to platinum was not correlated with the degree of bronchial hyperresponsiveness (15).

Because of its multicentre design we could rely in our study on a rather large group of subjects and therefore we accepted heterogeneity in challenge test procedures. However, a PPV of 100% was reached at relatively low cut-off values: 2.32 kU/l for wheat and 9.64 kU/l for rye within specific IgE determination and ≥ 5.0 mm (wheat) and ≥ 4.5 mm (rye) within SPT, respectively. The difference between the specific IgE cut-off values for so similar allergens like wheat and rye flour could be explained by one single subject with higher concentration of rye flour-specific IgE, but a negative challenge. However, analysis of cut-off values has to be carried out for each allergen separately.

In conclusion, a high concentration of flour-specific IgE in the sera of bakers suffering from work-related symptoms is a good indicator for a positive inhalation challenge test with flours. Also clearly positive SPT results with flour extracts in symptomatic bakers indicate a great likelihood that a subject will show a positive reaction in specific challenge test. Thus, in such cases specific challenges may be dispensable. On the other hand, positive challenges with flours may occur also at low-grade sensitization, and in rare instances also without sensitization. Challenge tests with flours should be considered preferably in these subjects.

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