

Original article

Comparison of wheat and rye flour skin prick test solutions for diagnosis of baker's asthma

Background: Skin prick tests (SPTs) play an important role in the diagnosis of baker's asthma and in the investigation of sensitization frequencies in field studies. It was the aim of our study to compare different SPT solutions for wheat and rye flour sensitization and to assess the validity of test results.

Methods: Skin prick tests with wheat and rye flour were performed in parallel with extracts from different companies and compared with the results of bronchial challenge tests with both flours (69 rye flour and 51 wheat flour challenge tests). Additionally, specific immunoglobulin E (sIgE) to wheat and rye flour were tested. SPT solutions were analysed for protein content and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Results: Skin prick test solutions for diagnosis of wheat and rye flour sensitization from three companies differed in protein concentrations and composition with the consequence of widely differing SPT results. Sensitivity of SPTs in comparison with allergen-specific bronchial challenge as a gold standard was between 40 and 67%, specificity was between 86 and 100%, the positive predictive value (PPV) ranged from 81 to 100% and the negative predictive value (NPV) from 44 to 70%. These numbers were only marginally affected by using a combination of challenge test result and sIgE value as a more specific gold standard.

Conclusion: Improvement and standardization of SPT extracts for wheat and rye flour is highly recommended.

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Skin prick tests (SPTs) play an important role in the diagnosis of baker's asthma and epidemiological field studies on frequencies of sensitization to wheat or rye flour. Sensitization frequencies to wheat flour by SPT in epidemiological studies of bakers varied from 5 to 15% (1). This may be caused at least partially by different test extracts containing a varying amount of the relevant single allergens (2). Additionally, serological assessment of sensitization to wheat and rye flour was more sensitive (3). Thus, it was the aim of our study to compare different commercial SPT extracts and to assess the validity of the test results.

Materials and methods

Patients

A total of 115 bakers complaining of workplace-related respiratory symptoms were admitted for a medical expert opinion. Part of the examination were SPTs and specific immunoglobulin E (sIgE) measurements with bakery allergens and, depending on anamnestic data, specific inhalative challenge tests with wheat flour (in 51 of the patients) or rye flour (in 69 of the patients).

Skin prick test

Skin prick tests were performed with wheat and rye flour extracts from ALK (Copenhagen, Denmark) for all patients, Bencard (Neuss, Germany) for about 80% of the patients and Allergopharma (Reinbek, Germany) for 40 of them. SPT responses with at least one-half of that of the histamine control (10 mg/ml) were considered as positive.

Specific IgE determination

Human IgE antibodies to wheat and rye flour were measured for 90% of patients by IgE-Enzyme Allergo Sorbent Test (EAST) (Phadezym RAST kit; Pharmacia, Uppsala, Sweden) and 10% of patients by the Pharmacia CAP-system according to the manufacturer's recommendations. EAST/CAP values >0.35 kU/l were considered as positive.

Inhalative challenge tests

Body plethysmography was performed at rest, after application of a placebo (sucrose powder), and six times after allergen application (15, 30, 60, 120, 240 and 360 min afterwards). Challenges were performed by a work-site simulation (room size, 10 m³) with native

flour type 1150 (rye flour) and type 550 (wheat flour) obtained from one bakery. Measurement of wheat and rye flour particle size distribution using a Sympatec particle size analyser (Sympatec GmbH, Clausthal-Zellerfeld, Germany) with Helos measuring device detected 75% below 102 μm , 19% below 10 μm and 12% below 5 μm diameter in rye flour while in wheat flour 66% of particles were below 102 μm , 10% below 10 μm and 7% below 5.2 μm diameter. These flours did not contain fungal α -amylase or xylanase according to our retrospective measurements with two-sited immunoassays (4). The patients handled approximately 100 g sucrose powder for 30 min and afterwards the same flour quantity for up to 30 min. Measurements of dust concentrations in the provocation chamber showed mean air concentrations between about 60 and 120 mg/m^3 . Measurements with a cascade impactor during wheat flour provocation tests found 22–33% of the total dust exposure to derive from particles below 32.4 μm , 12–17% below 15.8 μm , 4–9% below 8.13 μm and 1–2.5% below 4.06 μm . A positive test reaction was defined as the occurrence of shortness of breath together with a rise of specific airway resistance $\geq 100\%$ and to at least 2.0 kPa s.

Validation of tests

For the evaluation of SPTs (or sIgE-values) the result of the specific bronchial challenge was taken as the gold standard. Right positives (*rp*) were bakers with positive challenge and SPT (sIgE); right negatives (*rn*) were bakers with negative challenge and negative SPT (sIgE); false positives (*fp*) were bakers with negative challenge and positive SPT (sIgE); false negatives (*fn*) were bakers with positive challenge and negative SPT (sIgE). Sensitivity [$rp/(rp + fn)$], specificity [$rn/(rn + fp)$], positive [$rp/(rp + fp)$] and negative [$rn/(rn + fn)$] predictive values and test efficiency [$(rp + rn)/(rp + fp + rn + fn)$] were calculated in percentage.

For an additional evaluation, a combination of bronchial challenge test and sIgE was used as gold standard: positives were patients with a positive challenge and sIgE to the respective flour; negatives were patients with a negative challenge without sIgE.

Protein detection and sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Protein in prick test solutions was measured by ESL protein assay (Roche Molecular Biochemicals, Mannheim, Germany).

Briefly, 10 μl of each wheat flour and rye flour solution mixed with 5 μl 3 $\times$ concentrated Laemmli sample buffer were separated per lane electrophoretically in a pre-cast NuPAGE[®] Bis-Tris gel (Invitrogen, Groningen, The Netherlands). Proteins were silver stained according to the method of Blum et al. (5).

Results

Sensitivity and specificity of wheat and rye flour SPTs

The results of SPT extracts for wheat and rye flour from two companies (ALK and Bencard) were compared with the corresponding inhalative challenge test results as a gold standard (Table 1a–d). While specificity was in all cases above 85%, sensitivity of SPTs was 50 or 40% for rye flour solutions and 67 or 45% for wheat flour solution.

Sensitivity was higher for sIgE results (Table 2). If sIgE and inhalative challenge test results were combined to

Table 1. Results of skin prick test (a: ALK wheat flour; b: Bencard wheat flour; c: ALK rye flour; d: Bencard rye flour) compared with provocation test with native flour

	Challenge wheat flour		Σ
	Positive	Negative	
(a) ALK*			
Prick +	13	3	16
Prick –	16	19	35
Σ	29	22	51
(b) Bencard†			
Prick +	16	2	18
Prick –	8	19	27
Σ	24	21	45
	Challenge rye flour		Σ
	Positive	Negative	
(c) ALK‡			
Prick +	19	1	20
Prick –	29	20	49
Σ	48	21	69
(d) Bencard§			
Prick +	19	0	19
Prick –	19	15	34
Σ	38	15	53

* Sensitivity: 45%; specificity: 86%; positive predictive value: 81%; negative predictive value: 54%; test efficiency: 63%.

† Sensitivity: 67%; specificity: 90%; positive predictive value: 89%; negative predictive value: 70%; test efficiency: 78%.

‡ Sensitivity: 40%; specificity: 95%; positive predictive value: 95%; negative predictive value: 41%; test efficiency: 57%.

§ Sensitivity: 50%; specificity: 100%; positive predictive value: 100%; negative predictive value: 44%; test efficiency: 64%.

Table 2. Specific IgE compared with provocation test with native flour

Pharmacia	Challenge wheat flour*			Challenge rye flour†		
	Positive	Negative	Σ	Positive	Negative	Σ
Specific IgE +	24	9	33	34	4	38
Specific IgE –	5	13	18	13	17	30
Σ	29	22	51	47	21	68

* Sensitivity: 83%; specificity: 59%; positive predictive value: 73%; negative predictive value: 72%; test efficiency: 73%.

† Sensitivity: 72%; specificity: 81%; positive predictive value: 89%; negative predictive value: 57%; test efficiency: 75%.

evaluate SPT results, specificity of Bencard SPTs reached 92% (wheat flour) and 100% (rye flour) with a sensitivity of 75% (wheat flour) and 53% (rye flour). The SPTs with ALK solutions showed a specificity of 100% (wheat flour) and 94% (rye flour) while sensitivity was 50% (wheat flour) and 47% (rye flour).

Comparison of SPT results with solutions from different companies

Parallel SPTs were performed in 40 patients using SPT solutions of three companies. In total, 17 patients reacted

with any of the wheat SPT extracts. Only six of them reacted on all wheat flour extracts and only three of 13 patients with any positive rye flour result reacted on all rye flour extracts (Fig. 1). Comparing rye and wheat flour SPTs within companies, all rye flour positives with ALK and Allergopharma solutions reacted also to the wheat flour extract of the respective company and 10 of 12 rye flour positives with the Bencard extract reacted also to the wheat flour extract from Bencard.

Protein content and characterization

Two batches of each SPT solution were analysed concerning protein content (Table 3) and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). While batch to batch variations were small, the protein patterns (Fig. 2) and protein content of SPT extracts from different companies showed great differences, protein concentrations ranging from 0.06 to 0.49 mg/ml.

Discussion

Parallel tests of 40 patients with SPT extracts of wheat and rye flour from three different companies revealed that a positive SPT result strongly depends on the test extract. Whereas with the SPT solution from Bencard 37.5% of the bakers were sensitized to wheat flour and 30% to rye flour, with the solutions from ALK only 17.5% (wheat

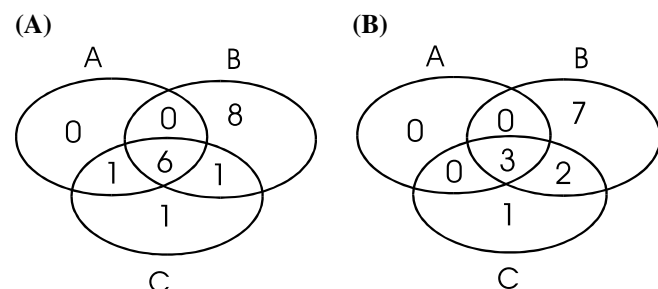


Figure 1. Hasse-diagrams of skin prick test (SPT) results with wheat flour solutions (A) und rye flour solutions (B). Presented are positive SPT results in a group of 40 patients with solutions from A: ALK, B: Bencard, and C: Allergopharma.

Table 3. Protein concentrations (mg/ml) of skin prick test solutions (batches from different years)

	Year 2000	Year 2002
Bencard rye flour	0.39	0.49
Allergopharma rye flour	0.17	0.19
ALK rye flour	0.04	0.06
Bencard wheat flour	0.38	0.35
Allergopharma wheat flour	0.09	0.06
ALK wheat flour	0.13	0.16

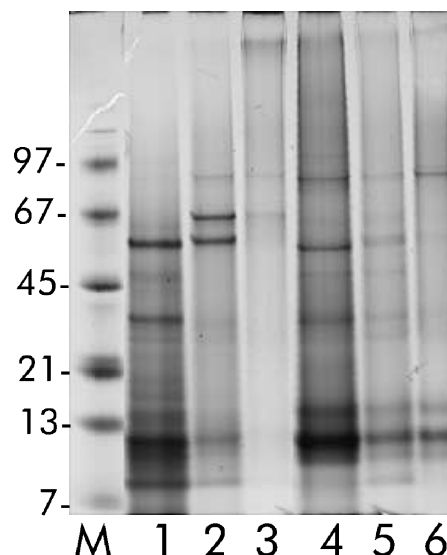


Figure 2. Equal volumes of SPT solutions (batches from 2002; lane 1, Bencard rye flour; lane 2, Allergopharma rye flour; lane 3, ALK rye flour; lane 4, Bencard wheat flour; lane 5, Allergopharma wheat flour; lane 6, ALK wheat flour) were separated in SDS-PAGE and silver stained.

flour) and 7.5% (rye flour) were sensitized, and according to the Allergopharma results 22.5% reacted to wheat flour and 15% to rye flour. Analysis of the SPT solutions showed striking differences in protein content and the pattern of protein bands. As a rule, solutions with a higher protein content and more bands in SDS-PAGE also showed a higher number of positive tests. Similarities between wheat and rye flour extracts from one producer concerning protein amount, band pattern and test results are greater than between the same cereal species of different producers.

Evaluation of SPTs for diagnosis of baker's asthma needs a gold standard. For confirmation of occupational asthma the specific bronchial provocation tests are presently considered as the gold standard although false positive or false negative challenges can occur (6). However, the duration and quantification of flour exposure during challenges varies considerably. Cloutier et al. (7) used a closed-circuit exposure system with concentrations below or close to 10 mg/m^3 and an exposure duration of up to 180 min, whereas Choudat et al. (8) exposed 3 mg/m^3 flour up to 104 min with a similar equipment and computer-controlled aerosolizer. Often exposure times were shorter while levels were higher and showed a great variation. De Zotti et al. (9) measured dust levels of $26\text{--}46 \text{ mg/m}^3$ as 25–75 percentiles of 100 challenge tests with a negative outcome after 30-min exposure, whereas Talini et al. (10) challenged a cumulative duration of 30 min and dust concentrations of $0.9\text{--}35 \text{ mg/m}^3$ and Merget et al. (11) exposed 160 mg up to more than 1000 mg inhaled wheat flour administered by Spinhaler. Although these exposure doses were certainly

very high, no nonspecific reactions were reported. The concentrations measured during our challenge tests (60–120 mg/m³) may be considered very high, therefore the possibility of false positive challenge reactions cannot be ruled out.

Thus, by using the inhalative challenge as gold standard, the low sensitivity of SPTs we found (wheat flour ALK 45%, wheat flour Bencard 67%, rye flour ALK 40%, rye flour Bencard 50%) could be questioned. However, by using a combination of sIgE and challenge test result as an improved gold standard, sensitivity remains poor (only 3–8% better than with challenge test alone).

Low sensitivity of commercial wheat and rye flour SPT solutions were also found by Tasman et al. (12) for the diagnosis of baker's rhinitis. In comparison with the result of a nasal provocation test, they reported sensitivities of 53 and 52% with wheat flour SPTs (ALK and Allergopharma) and 38 and 59% with rye flour SPTs (ALK and Allergopharma).

These data suggest that at present commercial wheat and rye flour SPT solutions differ in protein content and band patterns and fail to detect about 30–60% of patients with a positive allergen challenge. Improvement and standardization of SPTs for wheat and rye flour is highly recommended.

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