

OCCUPATIONAL ALLERGY TO WHEAT FLOUR. NASAL RESPONSE TO SPECIFIC INHALATIVE CHALLENGE IN ASTHMA AND RHINITIS VS. ISOLATED RHINITIS: A COMPARATIVE STUDY

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Abstract

Objectives: The purpose of the study was to compare cytological and biochemical changes in nasal lavage fluid induced by wheat flour inhalatory challenge in bakers with allergic rhinitis and with asthma accompanied by rhinitis. **Materials and Methods:** A single-blind, placebo controlled study was conducted in 64 bakers with allergic rhinitis ($n = 17$), bronchial asthma and rhinitis ($n = 24$) and without occupational allergy ($n = 23$). Nasal washings were examined before, 30 min, 4 and 24 h after the specific provocation, whereas non-specific bronchial hyperreactivity (PC20) before and after 24 h. **Results:** A significant decrease in PC20 after the challenge test was observed only in patients with asthma and rhinitis. Eosinophil count and percentage, basophil count and the permeability index induced by specific provocation were significantly increased in both rhinitis patients and asthmatics. Moreover, the increase especially in total count and proportion of eosinophils as well as in the permeability index was more pronounced in subjects suffering from asthma and rhinitis than in those with rhinitis alone, although the changes were not statistically significant. **Conclusions:** The results indicate the applicability of the “nasal pool” technique as a simple diagnostic procedure in flour-induced airway allergy. However, the evaluation of nasal lavage fluid, although a very sensitive and specific method of diagnosing respiratory allergic disease, cannot be used to distinguish patients with upper and lower airway allergy.

Key words:

Bakers' asthma, Nasal lavage, Non-specific bronchial hyperreactivity, Specific inhalation challenge test, Wheat flour

INTRODUCTION

Baker's respiratory allergy is reported to be the most common form of occupational allergy in many countries. The prevalence of work-related rhinitis ranges from 18 to 29%, whereas of occupational asthma from 4.9 to 7% [1–4].

However, specific sensitisation to occupational allergens is not found in about one third of patients reporting work-related respiratory symptoms [1]. For instance, in the longi-

tudinal study performed by Cullinan et al. [5], only 2 of the 45 bakers with work-related rhinoconjunctivitis with onset during the first 4 years of bakery work were sensitized to flour. Thus, nonallergic mechanisms are also likely to be involved in respiratory tract among bakers [6]. On the other hand, 32% of bakers sensitized to occupational allergens, do not report any respiratory symptoms [1]. In many countries, including Poland, recognition of occupational disease

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implicates in financial compensation to a patient. Hence an accurate diagnosis of occupational respiratory allergy with objective methods is needed. Inflammatory process, present in allergic airway disease, can be monitored with the use of nasal lavage fluid (NLF) examination. Allergen provocation of either the nose or the bronchi results in generalized airway inflammation. Moreover, the eosinophilic inflammation of the nasal mucosa exists even in asthma patients without any symptoms [7]. In patients with moderate-severe asthma, the process is more pronounced in the bronchi than in the nasal mucosa, whereas in patients with mild asthma inflammation it appears to be similar at both sites [8]. As the evaluation of NLF is frequently used in the diagnosis of occupational respiratory allergy, the question arises if the inflammatory process is different in patients suffering from occupational allergy involving whole respiratory tract or only upper airways.

The aim of the study was to compare cellular and biochemical changes in NLF induced by specific inhalative challenge with wheat flour in subjects with allergic rhinitis and with bronchial asthma accompanied by rhinitis.

MATERIALS AND METHODS

Subjects

Sixty-four bakers reporting work-related respiratory symptoms participated in this study: 17 of them suffered from occupational allergic rhinitis (group A), 24 from both occupational asthma and rhinitis (group B), and in 23 subjects with atopic asthma occupational allergy was not found (group C). The baseline characteristics are summarized in Table 1.

Study protocol

The study was designed as a biphasic, crossover, single blind trial. At phase I, the subjects were challenged with placebo and wheat flour. The diagnosis was based on the results of the nasal symptom score, skin prick tests (SPT), spirometry and histamine challenge test.

In patients of group A, occupational allergic rhinitis was recognized, when work-related nasal symptoms were accompanied by sensitization to wheat flour (positive SPT or

Table 1. Characteristics of subjects with allergic rhinitis (group A), asthma and rhinitis (group B) and in patients without occupational respiratory allergy (group C) participating in the study

| | Group A | Group B | Group C |
|------------------------------------|-------------|-------------|------------|
| N | 17 | 24 | 23 |
| Age (years) | 37.8 ± 10.6 | 34.2 ± 12.7 | 43.9 ± 9.5 |
| Gender, M: F | 13:4 | 8:16 | 11:12 |
| Smoking: | | | |
| active smokers | 3 (17.6%) | 5 (20.8%) | 3 (13%) |
| ex-smokers | 5 (29.4%) | 6 (25%) | 5 (21.7%) |
| passive smokers | 9 (52.9%) | 13 (54.2%) | 15 (65.2%) |
| Family history of atopy | 4 (23.5%) | 4 (16.7%) | 4 (17.4%) |
| Latency period of symptoms (years) | 14.3 ± 9.4 | 9.5 ± 7.8 | 13.6 ± 7.7 |
| Duration of symptoms (years) | 5 ± 4.6 | 6.4 ± 7.4 | 9.4 ± 7.7 |

M – males.
F – females.

CAP-RAST) and positive nasal response to provocation test, i.e. total score of more than 3 points.

Group B comprised patients reporting work-related nasal and chest symptoms, in whom a specific challenge test induced significant bronchial response (at least a 20% decrease in FEV₁) – early or dual asthmatic reaction, or a threefold increase in non-specific bronchial hyperreactivity.

Group C consisted of patients with atopic asthma, sensitized to house dust mites, without changes in spirometry and non-specific bronchial hyperreactivity after specific challenge. To exclude patients with false negative results of the challenge test, additional testing according to Vandenas et al. [9] was performed. In brief, the subjects who did not show significant ($\geq 20\%$) fall in forced expiratory volume in one second (FEV₁) during the first day underwent a repeated challenge test for two hours on the next day. In patients with changes in FEV₁ ranging between 10 and 20% after that challenge, the exposure was prolonged up to three hours.

At least 6 weeks later, a subsequent specific inhalative challenge, placebo-controlled with the evaluation of NLF, was performed. The study participants did not receive any systemic or local medication, except inhaled short-acting β_2 -agonists 14 days prior to the study. The Regional Bioethical Committee approved the study protocol. All

the participants gave their informed consent prior to the study.

Skin prick tests

Skin prick tests (SPT) were performed on the volar part of the forearm with a standard battery of common allergens and bakery series, including tree and grass pollens, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, moulds, feathers, oatmeal, wheat, corn, barley, and rye flour (Allergopharma, Germany), house dust and bakery dust (Stallergen, France). The negative control was allergen diluent and the positive one – 1 mg/ml histamine dihydrochloride solution. The largest wheal diameter was assessed after 15 min. Positive reaction was defined as a wheal diameter of at least 3 mm with no reaction to the diluent and a positive reaction to histamine.

Total and specific IgE

Total serum IgE was evaluated using the Uni-CAP system (Uppsala, Pharmacia Diagnostics, Sweden). The results were expressed quantitatively in kilo units per liter and considered positive at values higher than 0.35 kU/l. Specific antibodies against flour and α -amylase were measured by allergen CAPS (Phadezym fx20, k87, Pharmacia, Diagnostics, Uppsala, Sweden).

Inhalation challenge tests

Provocation tests with wheat flour were performed in a worksite simulation setting (room space 6 m²) with the patient's own samples. The patient was sifting approximately 100 g of wheat flour for 30 min. Potato flour was used as placebo. The total dust concentration during the challenge was estimated at 33.6 ± 12.3 mg/m³.

Symptom score

The number of sneezes and the degree of mucosal oedema, rhinorrhea and itching were evaluated. Total symptom score (SS) ranged from 0 to 8 and represented the sum of the scores for sneezing (0 sneezes – 0 points, 1–4 sneezes – 1 point, > 4 sneezes – 2 points), rhinorrhea (none – 0 points, mild – 1 point, abundant – 2 points), mucosal oedema (none – 0 points, mild – 1 point, nasal block – 2 points)

and itching (none – 0 points, itching of the nose or throat – 1 point, itching of the nose and throat – 2 points) [10].

Pulmonary function

Resting spirometry (Vicatet 2A, Mijnhardt, the Netherlands) was performed in all subjects reporting chest symptoms. Bronchial response was measured by serial monitoring of FEV₁ and peak expiratory flow rate (PEFR) before and 5 min, 30 min, 1, 2, 4, 6 and 24h after the provocation. All the subjects were instructed beforehand how to use the peak flow meter for hourly PEF measurements. Histamine challenge was performed according to Cockcroft et al. [11]. Bronchial response was measured by FEV₁ monitoring. The non-specific bronchial hyperreactivity was evaluated on the day before specific challenge test and 24 h after the test.

Nasal lavage and challenge procedure

All the procedures were performed as in the “nasal pool” method [12]. Before the provocation, each nostril was washed 10 times with 5 ml of saline solution using the “nasal pool” device, 10 ml syringe closely fitting the nostril. Nasal washings were collected immediately before the provocation and 30 min, 4 and 24 h afterwards. The washing procedure has been described in detail elsewhere [13].

Statistical analysis

The data obtained from NLF examination were analyzed with repeated measures ANOVA. The changes in non-specific bronchial hyperreactivity were analyzed with two-tailed paired Student's test (Statistica 4.5 for Windows). For all the tests a level of significance was established at a value of $\alpha = 0.05$.

RESULTS

A significant decrease in PC20 after the challenge test was observed only in group B ($p < 0.001$). In neither group A nor C significant changes in bronchial hyperreactivity were observed. Individual changes in histamine challenge tests are presented in Figs. 1–3. Also only in group B, changes in FEV₁ induced by specific challenge could be observed.

In 8 bakers, isolated early reaction was recorded, while the other 16 subjects revealed dual asthmatic reaction. In group C, immediate decrease in FEV₁ was noted, however, it did not exceed 15% and did not last longer than an hour.

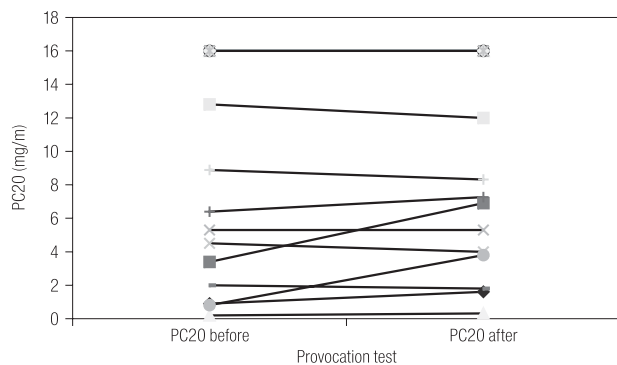


Fig. 1. Results of the histamine challenge test before and 24 h after specific challenge test with wheat flour in subjects with occupational allergic rhinitis (group A – individual results are presented).

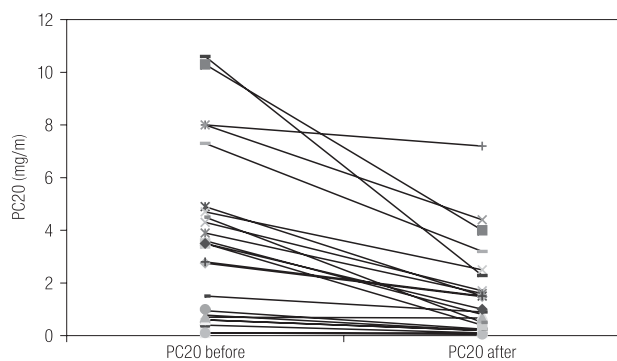


Fig. 2. Results of the histamine challenge test before and 24 h after specific challenge test with wheat flour in subjects with occupational asthma and rhinitis (group B – individual results are presented).

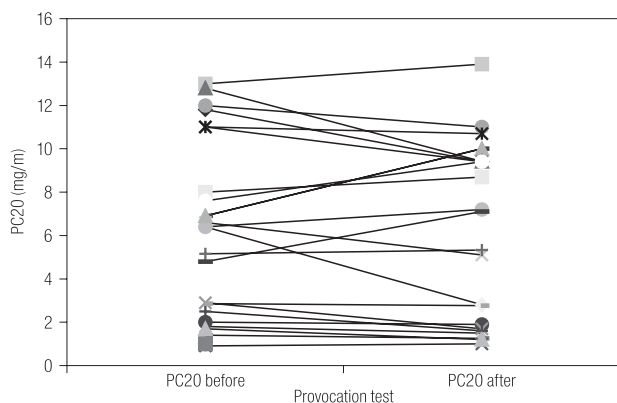


Fig. 3. Results of the histamine challenge test before and 24 h after specific challenge test with wheat flour in subjects without occupational respiratory allergy (group C – individual results are presented).

Symptom score, cellular and biochemical findings in nasal lavage fluid induced by flour and placebo in subjects with asthma and rhinitis, isolated rhinitis and in apprentices without occupational allergy participating in the study are presented in Table 2. Symptom score was the criterion used for recognition of allergic rhinitis, so that parameter was not analyzed. Placebo provocation did not induce significant changes in cellular or biochemical composition of NLF in any of the groups.

Cellular findings

The provocation with flour resulted in the increased total count of leukocytes in nasal washings from all the groups at all time points. The differences between the groups were statistically significant 24 h after the provocation: total leukocyte count in group B after specific challenge was significantly higher than in group C ($p < 0.01$). Significant differences induced by flour in the total leukocyte count were observed in group A between 0, 4 and 24 h ($p < 0.001$), in group B between 0 and 24 h ($p < 0.001$), and in group C between 0 and 4 h ($p < 0.05$).

The neutrophil proportion changed significantly after flour challenge in group A, after 30 min, 4 and 24 h ($p < 0.001$) it was higher than before the challenge.

An analysis of the total eosinophil count revealed significant differences between subsequent time points after the challenge – after 30 min, 4 and 24 h the number of eosinophils was significantly higher than before the provocation ($p < 0.001$). However, the interaction was not significant, so the simple effects were not tested.

An analysis of the proportion of eosinophils revealed significant differences between the groups 30 min, 4 and 24 h after flour challenge – after the provocation, the percentage in groups A and B was higher than in group C ($p < 0.001$). Within-groups comparisons revealed significant differences in groups A and B after specific provocation ($p < 0.001$). The eosinophil percentage in both groups was higher 30 min, 4 and 24 h after than before the challenge.

An analysis of the total basophil count revealed significant differences between placebo and flour provocation results in groups A and B, 4 and 24 h after provocation ($p < 0.05$). At these time points the basophil count was significantly

Table 2. Symptom score, cellular and biochemical findings in nasal lavage fluid induced by flour and placebo in subjects with allergic rhinitis (group A), asthma and rhinitis (group B) and in patients without occupational respiratory allergy (group C) participating in the study

| Parameter | Group A | | | | | | Group B | | | | | | Group C | | | | | | | | |
|---|---------|--------|------|-------------|------|--------|---------|------|------|-------------|------|------|---------|--------|------|-------------|------|--------|------|------|--|
| | Placebo | | | Wheat flour | | | Placebo | | | Wheat flour | | | Placebo | | | Wheat flour | | | | | |
| | 0 | 30 min | 4 h | 24 h | 0 | 30 min | 4 h | 24 h | 0 | 30 min | 4 h | 24 h | 0 | 30 min | 4 h | 24 h | 0 | 30 min | 4 h | 24 h | |
| Score | 0.4 | 1.5 | 0.9 | 0.1 | 0.7 | 7.3 | 5.3 | 1.9 | 0.5 | 2.2 | 1.6 | 0.2 | 0.4 | 7.6 | 5.9 | 2.7 | 0 | 1.1 | 0.1 | 0 | |
| Total cell count ($\bullet 10^3/\text{mm}^3$) | | | | | | | | | | | | | | | | | | | | | |
| Leucocytes | 5.0 | 8.1 | 7.3 | 7.1 | 5.1 | 9.5 | 25.2 | 70.8 | 7.5 | 18.4 | 15.2 | 22.3 | 7.3 | 22.4 | 60.6 | 95.2 | 6.4 | 28.8 | 35.1 | 29.1 | |
| Eosinophils | 0.1 | 0.2 | 0.4 | 0.6 | 0.2 | 1.4 | 3.3 | 7.9 | 0.4 | 0.3 | 0.5 | 0.1 | 1.0 | 3.5 | 6.4 | 21.9 | 0.2 | 1.4 | 3.9 | 3.7 | |
| Basophils | 0 | 0.1 | 0.1 | 0 | 0 | 0.9 | 1.4 | 2.3 | 0 | 0 | 0.1 | 0 | 0 | 2.5 | 2.3 | 3.7 | 0 | 0.6 | 0.7 | 0.6 | |
| Cell percentage (%) | | | | | | | | | | | | | | | | | | | | | |
| Neutrophils | 37.0 | 51.2 | 54.2 | 54.0 | 38.8 | 59.8 | 55.7 | 67.6 | 48.5 | 66.3 | 69.4 | 74.1 | 50.8 | 72.4 | 84.4 | 75.8 | 50.1 | 62.4 | 74.7 | 65.9 | |
| Eosinophils | 2.4 | 2.9 | 3.2 | 2.6 | 2.5 | 9.1 | 14.8 | 17.1 | 4.2 | 3.8 | 4.3 | 3.5 | 4.1 | 15.3 | 16.7 | 19.2 | 1.5 | 1.3 | 1.6 | 1.2 | |
| Basophils | 0.1 | 0 | 0.1 | 0 | 0.4 | 1.6 | 1.9 | 1.9 | 0 | 0 | 0.1 | 0 | 0.1 | 0.1 | 1.5 | 2.6 | 0.5 | 0.3 | 0.4 | 0.4 | |
| Permeability Index (%) | 5.8 | 7.1 | 6.6 | 7.2 | 17.8 | 23 | 29.8 | 26.7 | 6.9 | 7.4 | 6.6 | 7.94 | 18.5 | 29 | 37.7 | 32.3 | 18.7 | 17.4 | 17.3 | 18.4 | |

higher after flour challenge than after placebo administration. Within-groups analysis revealed significant differences only in group A after specific provocation. Basophil count was significantly higher 24 h after than before the challenge.

Similarly, an analysis of the proportion of basophils revealed significant differences only between the groups 24 h after flour challenge ($p < 0.05$) – the percentage was higher in group A than after placebo challenge and than in group C. Within-groups comparisons revealed significant differences only in group A after the provocation ($p < 0.05$). The basophil percentage in that group was higher 24 h after than before the challenge.

Permeability index

Significant differences between the groups were observed 24 h after flour challenge ($p < 0.05$) – in groups A and B the permeability index was significantly higher than after placebo administration and than in group C after flour challenge. Moreover, in groups A and B significant differences could be observed between permeability indices at successive time points ($p < 0.05$) – after 4 and 24 h, the values were higher than before the challenge.

Moreover, an increase, especially in the total count and proportion of eosinophils as well as in permeability index, was more pronounced in subjects suffering from asthma and rhinitis than in those only with rhinitis, although the changes were not statistically significant. The baseline parameters were also lower in rhinitic patients, albeit not significantly.

DISCUSSION

Specific inhalation challenge is the gold standard for the diagnosis of bakers' asthma [14]. The test is usually performed by reproducing the patients' work exposure in a challenge chamber. The tests are often performed in a non-standardized way, without the measurement of airborne dust levels. So there is a risk of irritating effect of the flour, especially in subjects with bronchial hyperreactivity. De Zotti et al. [14] showed that the risk of such effect could be ruled out among patients not sensitized to

flour and without non-specific bronchial hyperreactivity. However, there are some patients suffering from atopic bronchial asthma due to common allergens without sensitization to occupational allergens in whom specific sensitization may induce respiratory symptoms. In those subjects an objective method of the evaluation of the test may be needed. Therefore, our control group comprised patients with atopic asthma, excluded occupational asthma. It seems unlikely that some of the bakers, showing no changes in FEV₁ or in PC20 during the two test days would have developed an asthmatic reaction after further challenge exposures [9].

As asthma and rhinitis commonly coexist, the concept of "one airway, one disease" has emerged and the terms "allergic rhinobronchitis" [15] or "united airway disease" (UAD) have been proposed [16]. The most important link between rhinitis and asthma is the presence of inflammation of the nasal and bronchial mucosa [17]. Gaga et al. [18] showed eosinophil infiltration in the nasal mucosa of asthmatic patients irrespective of any signs of rhinitis. On the other hand, bronchial hyperresponsiveness can be present in patients with allergic rhinitis without clinical evidence of asthma [19]. Nasal symptoms occur in 28 to 78% of patients with asthma in comparison with 5 to 20% of the general population [20]. It has also been suggested that defining allergic rhinitis as a risk factor for developing asthma is not completely correct, because rhinitis represents an early stage of UAD and the subsequent onset of asthma may be its evolution [21].

Occupational diseases represent an interesting model to study the relationship between rhinitis and asthma. High molecular weight allergens usually induce both nasal and bronchial symptoms. Therefore, in most cases, when occupational respiratory allergy is suspected, diagnostic procedures should include the evaluation of NLF. Objective methods are necessary in case of patients claiming for compensation, because the clinical history may be far from conclusive, hence appropriate provocation testing should be carried out [22]. The examination of nasal lavage fluid can be an alternative to broncho-alveolar lavage in asthmatic patients with concomitant rhinitis [22]. The nasal challenge method, along with the estimation of cell

count and the biochemical examination of the lavage fluid or secretion, renders it feasible to distinguish between the allergic and irritant effects of various substances on the respiratory system [23–25].

The allergic inflammation involves migration of inflammatory cells to the mucosa, which begins to become apparent approximately 30 min after specific challenge, continues to increase during the following 24 h and then slowly subsides [21].

In the present study, we observed a typical allergic reaction of a prolonged increase in the total cell and eosinophil counts, a less pronounced but very specific increase in metachromatic cell count and an increase in albumin/protein ratio for up to 24 h after allergen challenge in all the patients with occupational asthma and rhinitis as well as with isolated rhinitis. An increase in the count of leucocytes and total protein level in the nasal lavage fluid has been observed also in subjects without occupational allergy. It was, however, brief and did not affect the relative count of eosinophils and basophils or the relative concentration of albumin and may be attributed to the irritant-type reaction. It should be furthermore stressed that none of the subjects from the group without occupational allergy displayed prolonged increase in the proportion of eosinophils or in the permeability index. That observation confirms very high specificity of the specific challenge test evaluated by changes in NLF. Even though basophils and neutrophils also increased after challenge, eosinophils showed the most significant and persistent rise as well as the most significant correlations with clinical findings.

To our knowledge, none of the studies carried out to date compared the changes in NLF in subjects with occupational asthma and rhinitis with those found in isolated rhinitis. Certainly, it would be of interest to include also a group of subjects with asthma alone for further comparisons, but this was not possible because of the lack of subjects with isolated baker's asthma. The most common feature of flour-induced allergic and non-allergic respiratory disease is mucosal inflammation beginning in the nose [6].

Cellular and biochemical changes induced by allergen challenge found in subjects suffering from asthma and rhinitis were more pronounced than those found in patients

with rhinitis alone. It might be concluded that inflammation process was more intensive in longer lasting disease, including the whole respiratory system. Also Boulay and Boulet [26] demonstrated that non-asthmatic subjects with allergic rhinitis showed reduced airway inflammation following a repeated inhalation of very low doses of allergen, even when the exposure to allergen continued. They conclude that some rhinitic patients have protective or dampening mechanisms against the allergen-induced airway inflammatory process, possibly an immunological tolerance to the inhaled allergen. However, the lack of statistically significant differences between these two groups in our study provides evidence that the inflammatory process is not so much enhanced in asthmatics as compared with rhinitic patients. Bearing this mind, the evaluation of NLF, although a very sensitive and specific method of diagnosing respiratory allergic disease, cannot be used to distinguish the patients with upper and lower airway allergy. Our data confirm that an increase in non-specific bronchial hyperresponsiveness is the most reliable marker of subsequent bronchial response to occupational agent. Only in the group of subjects with both occupational asthma and rhinitis, we observed a significant decrease in PC20 in eight patients with allergic rhinitis, although they had shown bronchial hyperactivity before specific challenge test, it was not changed after the test. The same data also showed that occupational asthma could not be recognized in subjects with bronchial hyperreactivity, sensitized to bakery allergens without the specific challenge test.

CONCLUSIONS

The results indicate the applicability of the “nasal pool” technique as a simple diagnostic procedure in flour-induced airway allergy. However, the test does not allow to distinguish subjects with asthma and rhinitis from patients with isolated rhinitis. Therefore, the evaluation of spirometry and non-specific bronchial hyperreactivity is also necessary when diagnosing bakers’ respiratory allergy.

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