

Comparison of immunologic tests in the diagnosis of occupational asthma and rhinitis

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In this study, three immunologic tests, skin prick test, RAST, and basophil histamine-release test (BHRT), were compared by provocation in the diagnosis of occupational asthma and rhinitis. Twenty-three positive bronchial or nasal challenges were performed on 16 patients (six farmers, six bakery workers, and four food industry workers) and asthma or rhinitis was diagnosed as caused by cereal flour or grain, cow epithelium, storage mites, garlic, or soy dust. A control group consisted of 12 patients, of whom four (two bakery workers, one food industry worker, and one farmer) were challenge-negative, and the rest suffered from pollen allergy and seasonal rhinitis and were not challenged. The sensitivity and specificity of the prick test, RAST, BHRT, and a panel of them were as follows: 74 and 89%, 57 and 86%, 78 and 93%, and 91 and 71%, respectively. The overall concordance among these three type I allergy tests or between immunologic tests and challenge was relatively good.

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Organic dusts are common causes of occupational respiratory diseases. Asthma and rhinitis from cereal flour among bakers (12, 15, 16) and from storage mites among farmers (2, 7, 9, 22, 26) are well-known examples of occupational sensitization. Allergic rhinitis or asthma typically represents a type I hypersensitivity reaction. Ideally, the diagnosis of occupational asthma or rhinitis should be based on positive response to challenge. In addition, skin and laboratory tests are performed to confirm type I sensitivity to suspected allergens. Conventional tests such as the prick test and measurement of specific IgE antibodies have been utilized in the diagnosis of occupational asthma or rhinitis attributable to cereal flour and storage mites (1, 3, 23, 25). More novel laboratory tests such as determination of allergen-induced basophil histamine release have also been applied (3, 23).

For the present study, patients working in a dusty environment and belonging to a risk group with regard to the development of occupational respiratory diseases were chosen. The patients were farmers, bakery workers, or other food industry workers. In these subjects, allergic asthma or rhinitis was verified by nasal or bronchial challenge, and the usefulness

of prick test, RAST, and basophil histamine-release test (BHRT) as diagnostic aids was evaluated.

Material and methods

Study populations

Sixteen patients with challenge-proven occupational rhinitis or asthma and 12 patients with seasonal rhinitis or other respiratory symptoms were included in the present study (Table 1). The occupations of the patients with work-related asthma or rhinitis were farmer (six subjects), bakery employee (six), and other food industry employee (four). The control group of patients with respiratory symptoms included eight patients with seasonal rhinitis and without suspicion of work-related allergy and four patients with suspected occupational exacerbation of their symptoms. These latter subjects (two bakery workers, one food industry worker with seasonal rhinitis, and one farmer) were challenged with work-related antigens with negative outcome. The eight seasonal rhinitis patients had no occupational contact with flour, animal epithelia, or storage mites and no symptoms suggestive of sensitization to these al-

Table 1. Background information on study populations

Study group	Number of subjects/ women	Mean age and range (years)	Respiratory and skin diseases Disease	Number
Patients with occupational asthma and/or rhinitis	16/8	38.4, 22–60	Asthma	3
			Rhinitis	9
			Asthma, rhinitis	4
			Atopic eczema	1
			Hand eczema	7
Patients with respiratory symptoms	12/9	36.5, 24–57	Asthma	1
			Rhinitis	9
			Cough	2
			Chronic bronchitis	1
			Urticaria	1
			Atopic eczema	4
			Hand eczema	2

lergens. No bronchial or nasal provocations were therefore applied to these patients.

Diagnosis of asthma

Bronchial hyperresponsiveness to methacholine was examined by a dosimeter method with controlled tidal breathing, as previously described (14).

The cornerstones for diagnosis of bronchial asthma were a typical history with chronic or repeated symptoms, and a documented variation in the FEV₁ or PEFR of more than 15% after medication, or a repeatedly $\geq 20\%$ spontaneous daily variation in PEFR monitoring for 2 weeks (13). In addition, an immediate decrease of $\geq 15\%$ or a delayed decrease of $\geq 20\%$ in air flow after provocation with a work-related allergen was a criterion for diagnosing occupational bronchial asthma.

Before bronchial challenge the patients were kept under observation in the pulmonary ward for at least 2 days. They had to be asymptomatic without any medication for asthma. The PEFR was measured every 2 h during daytime, and the diurnal variation was not allowed to exceed 10%. Before provocation the FEV₁ had to be $\geq 70\%$ of the predictive value, and the PEFR was not allowed to differ more than 15% from the previous day's value. Challenges were performed with the following foodstuffs and allergen extracts: cereal flour, soy flour, powdered garlic, storage mites (Laboratorium Diephuis, supplied through Allergologisk Laboratorium, Copenhagen, Denmark), and cow epithelium (ALK). Placebo challenges were performed with lactose or corresponding diluents for allergen extracts. If the challenge with placebo was clearly positive, further provocation with allergen was withdrawn.

The patient was exposed to lactose or powdered foodstuffs in a 5.5 m³ cabin, where compressed air at a flow rate of 10 l/min was directed into an open

bowl containing these materials. The provocation schedule included a 5-min initial exposure, a 20-min pause, and a second exposure of 20 min if the first provocative step was negative (6). The symptoms and FEV₁ and PEFR values were recorded, and the response was considered to be positive if there was a decrease of at least 15% in these values. After provocation the patient was followed in the pulmonary ward, and PEFR values were recorded hourly for at least 8 h. A decrease of $\geq 20\%$ in the PEFR in the late phase was considered to be positive.

Bronchial inhalation challenges with storage mite and cow epithelium allergens were performed by a dosimeter method with controlled tidal breathing (14). For nebulization, an automated, inhalation-synchronized dosimeter jet nebulizer was used. The breath-actuated, variable timing circuit regulates air through a solenoid valve to the nebulizer with a flow rate of 7.5 l/min to give aerosol particles with a mass median aerodynamic diameter of 1.6 μ m. The dosimeter was adjusted to nebulize for 0.4 s from the beginning of each inhalation. With this nebulization period, the mean output was 7.1 μ l/breath. Patients controlled their tidal breathing with the flow indicator and digital readout, so that the inspiratory flow rate reached but did not exceed 0.5 l/s. The concentrations of allergen extracts were 1, 10, 100, and 1000 Noon (N) units/ml for storage mites and 0.001, 0.01, 0.1, and 1% for cow epithelium. Increasing allergen doses were administered every 10 min in 60 inhalations until the highest dose was achieved or an early asthmatic reaction occurred. The patients were followed in the pulmonary ward as described above.

Patient no. 16, a dairy farmer, was not challenged in hospital since the diagnosis of cow-epithelium-induced asthma was evident on the basis of a 2-week follow-up and laboratory tests. Variations over 20% in PEFR were recorded during working days, whereas no variation could be detected on days off work. In addition, the RAST to cow epithelium exceeded 300 kU/l.

Diagnosis of allergic rhinitis

The diagnosis of allergic rhinitis was based on a positive outcome in either nasal or bronchial provocation. Nasal provocations were so timed that the rhinitis symptoms of the patients should be as slight as possible. In addition, the patients were not on oral or local antiallergic medication. Nasal provocations were started with lactose powder or diluent, depending on the subsequent allergen. If the patient developed symptoms on placebo challenge, provocation with allergen was withdrawn. The challenges were performed with pure cereal flour, cow epithelium (1/100 w/v, ALK), and storage mite allergens (1000 NU/ml, Diephuis). Powdered allergens were

applied to the mucosa of the anterior nasal cavity with two cotton-tip pins moistened with saline. With aqueous allergens, 0.2 ml of the solution was sprayed into the anterior nasal cavity with a syringe. Sneezing, rhinorrhea, itching, and mucosal edema at rhinoscopy were evaluated. The challenge was considered to be positive if at least two of these symptoms or signs clearly appeared.

Prick tests

The subjects were prick-tested with the standard series (ALK), mold series (ALK), storage mites (*Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, Diephuis), cereal flour, and, in some cases, soy (ALK) or garlic (fresh food material). In the prick tests with flour, our own noncommercial preparations were used, and some patients were also tested with commercial flour extracts (ALK). To prepare prick test reagents from wheat, rye, barley, and oats, grains of these cereals were ground, and the flour was suspended in saline, about 200 mg/0.3 ml. Prick tests were read at 15 min. The reactions were graded as follows: + corresponding to a reaction of one-fourth the histamine standard, ++ reaction half the size, +++ equal to, and ++++ twice as large as the wheal of the histamine standard (10 mg/ml). Reactions $\geq$ ++ were regarded as positive.

Laboratory tests

Specific and total IgE antibodies were measured with commercial radioimmunoassays (CAP RAST RIA for specific IgE, IgE RIA for total IgE, Kabi Pharmacia, Uppsala, Sweden). Allergen-specific IgE values $\geq$ 0.7 kU/l were regarded as positive.

The allergens for the BHRT were prepared as follows. Prick test reagents of soy (ALK) and storage mites (Diephuis), and an aqueous extract of cow epithelium (1:100 w/v, ALK) were dialyzed against phosphate-buffered saline (PBS) to remove the preservatives. An amount of 2 g finely ground garlic in 10 ml PBS was extracted overnight at 4°C, after which the suspension was centrifuged and the supernatant filter-sterilized. To obtain cereal flour antigens, wheat, rye, barley, and oats flour was extracted with sodium acetate buffer, pH 3.8, followed by extraction with sodium phosphate buffer, pH 7.0, as previously described (1). The extracts were concentrated by ultrafiltration with a cutoff of 1000 Da and freeze-dried. In the final cereal flour antigen preparations, the acetate- and phosphate-soluble fractions were combined.

The BHRT was performed as previously described (20). In brief, washed whole blood cell suspensions containing basophils were incubated with

serial dilutions of allergens or medium only (tubes for spontaneous histamine release) at 37°C for 30 min. For measurement of total blood histamine, the samples were heated at 100°C for 10 min. The histamine content of the supernatants was measured by radioenzymatic assay (8). The final results were expressed as the percentage of released histamine of total blood histamine. Values were regarded as positive when the difference between spontaneous and allergen-induced histamine release was at least 8%.

Statistics

Correlations between different test results were calculated by the Spearman rank correlation test. Sensitivity and specificity of skin and laboratory tests were calculated as follows:

- Sensitivity = proportion of positive tests in patients with occupational asthma or rhinitis.
- Specificity = proportion of negative tests in patients without occupational asthma or rhinitis.

When sensitivity and specificity values for a panel of three immunologic tests were calculated, the panel was regarded as positive if at least one test was positive, and as negative if all three tests were negative.

Results

Twenty-three bronchial or nasal provocations induced a positive response in 16 patients. Five out of seven patients with occupational asthma exhibited nonspecific bronchial hyperreactivity in the methacholine test. Upon challenge, the patients with occupational asthma developed immediate reactions with Δ PEFR values ranging from -19 to -36% and with Δ FEV₁ values from -26 to -71%. None of them developed late-phase reactions.

Background data on the patients are shown in Table 1. Table 2 shows the main results of the present work. Three farmers were allergic to flour or grain dust, three to cow epithelium, and two to storage mites. Patients working in the food industry were sensitized to cereal flour (nine cases), and, in addition, there were one case of soy-induced asthma and one case of garlic-induced asthma and rhinitis. In the group of patients with occupational diseases, prick test, RAST, and BHRT were positive in 17/23, 13/23, and 18/23 positive challenges, respectively. Patients 1, 3, 7, 9, and 15 were prick-tested in parallel with commercial and our own flour allergen preparations. The former yielded weaker reactions in most cases. Patients with nonoccupational respiratory symptoms exhibited 3/28 false-positive values in the prick test (no. 26), 4/28 in RAST (nos. 21, 26), and 2/27 in BHRT (nos. 24, 25).

Statistical parameters were calculated on the basis

Table 2. Provocation and laboratory test results of patients with occupational and nonoccupational respiratory diseases

Subjects'	Occupation	Total IgE (kU/l)	Provocation result	Allergen	Prick	RAST (kU/l)	BHRT (%)
Patients with occupational asthma and/or rhinitis							
1.	Bakery worker	23	R	Rye	++ (-)	<0.4	6.1
2.	Bakery worker	30	R	Rye	++	<0.4	16.6
			R	Wheat	+	0.8	19.4
3.	Bakery worker	21	R	Wheat	++ (++)	0.7	45.0
4.	Bakery worker	446	R	Rye	+++	18.2	84.8
			R	Wheat	+++	59.0	77.4
5.	Bakery worker	40	R	Barley	++	<0.4	5.4
6.	Bakery worker	38	A	Rye	-	<0.4	35.6
7.	Food industry worker	214	A, R	Garlic	++++	11.4	44.0
			R	Wheat	++ (+)	12.7	60.3
8.	Food industry worker	40	A	Soy	+	6.6	61.6
9.	Food industry worker	762	A, R	Rye	+++ (++)	150.0	74.7
			R	Wheat	+++ (++)	133.0	82.5
10.	Food industry worker	91	R	Wheat	+	3.4	25.3
			R	Rye	+++	0.4	12.1
11.	Farmer	299	A, R	<i>L. destructor</i>	++	0.8	58.9
12.	Farmer	323	R	Barley	++	0.7	49.8
			R	Cow epithelium	-	<0.4	0.9
13.	Farmer	226	R	<i>A. siro</i>	++	0.4	43.0
			R	Cow epithelium	+	<0.4	2.7
14.	Farmer	91	A, R	Barley	++	<0.4	40.5
15.	Farmer	23	R	Barley	++ (+)	<0.4	7.6
16.	Farmer	813	A	Cow epithelium	+++	334.0	26.4
Patients with respiratory symptoms							
17.	Bakery worker	23	Negative	Wheat	-	0.4	6.1
18.	Bakery worker	89	Negative	Wheat	-	<0.4	0.5
			Negative	Rye	-	<0.4	0.3
19.	Food industry worker	404	Negative	<i>L. destructor</i>	-	<0.4	1.0
20.	Farmer	6	Negative	Cow epithelium	-	<0.4	n.d.
21.	Salesman	9240	n.d.	Wheat	-	0.8	10.8
			n.d.	Rye	-	0.7	9.0
			n.d.	Barley	+	0.8	3.2
22.	Engineer	455	n.d.	Wheat	-	<0.4	9.2
			n.d.	Rye	-	<0.4	6.4
23.	Physician	16	n.d.	Wheat	-	<0.4	1.6
			n.d.	Rye	-	<0.4	1.3
			n.d.	Barley	-	<0.4	3.2
24.	Housewife	430	n.d.	Wheat	-	0.5	15.7
25.	Technician	881	n.d.	Wheat	+	<0.4	17.9
26.	Nurse	1305	n.d.	Wheat	++	0.8	6.9
			n.d.	Rye	++	<0.4	8.4
			n.d.	Barley	++	0.5	7.5
27.	Secretary	323	n.d.	Soy	-	<0.4	1.8
			n.d.	Garlic	-	<0.4	2.6
			n.d.	<i>L. destructor</i>	-	<0.4	0.1
			n.d.	<i>A. siro</i>	+	<0.4	0.1
			n.d.	Cow epithelium	-	<0.4	1.7
28.	Medical student	1525	n.d.	Soy	-	<0.4	0.5
			n.d.	Garlic	-	<0.4	2.6
			n.d.	<i>L. destructor</i>	-	<0.4	2.5
			n.d.	<i>A. siro</i>	-	<0.4	1.2
			n.d.	Cow epithelium	+	<0.4	3.0

¹ A: asthma; R: rhinitis; n.d.: not done.

² In prick test and RAST, values $\geq ++$ and ≥ 0.7 were considered to be positive, respectively. Positive BHRT values are italicized. In prick test, values obtained with commercial flour allergens are shown in parentheses.

³ In basophil histamine-release test, results obtained at optimal antigen concentrations are shown (flour antigens: 1–100 $\mu\text{g/ml}$; cow epithelium: 1/10–1/1000; storage mites: 1/10–1/100; soy: 1/10–1/100, and garlic: 1/1000–110 000 of stock solution).

of the data in Table 2. The prick test was positively correlated with RAST ($P < 0.001$) and BHRT

($P < 0.001$). In addition, there was a significant correlation between the RAST and BHRT values

($P < 0.001$). The sensitivity and specificity of prick test, RAST, and BHRT were 74 and 89%, 57 and 86%, and 78 and 93%, respectively. The panel of three immunologic tests detected 21/23 positive challenges and gave 8/28 false-positive results; thus, the sensitivity and specificity of the panel were 91 and 71%, respectively.

Discussion

Most patients presented here were sensitized to cereal flour or grain dusts. All of them could eat cereal products with impunity. The panel of three immunologic tests used revealed 91% of patients with respiratory allergy and yielded false-positive results in 29% of cases.

In Finland, all new cases of occupational disease are reported to the National Register. Animal epithelia, especially cow dander and cereal flour and grain have been leading causes of occupational asthma and rhinitis, accounting for about two-thirds of new cases. During recent years the proportion of occupational diseases caused by storage mites has clearly been increasing in Finland, evidently because of improved diagnosis. In a Finnish investigation, cow epithelium and storage mites were equally common causes of allergic rhinitis among dairy farmers (22), whereas, according to Danish findings, storage mite allergy was more frequent in this occupational group (9). Epidemiologic investigations from several countries have established that sensitization to storage mites is common, especially among farmers (2, 7, 9, 22, 26) and mill (18) and bakery (19) workers. Soy is a rare cause of bronchial asthma (4), but allergy to ingested soy products is common. Sensitization to garlic occurs more often via the oral route or skin contact than by inhalation; therefore, garlic-induced asthma is infrequently diagnosed.

Three type I allergy tests, i.e. the prick test, RAST, and BHRT, were compared in the present study. The specificity and sensitivity of the prick test and BHRT were similar and slightly better than those obtained with RAST. When the applicability of prick test, RAST, and BHRT was compared in previous studies, the diagnostic value of these tests in baker's asthma or storage mite allergy has varied from moderate to good (1, 2, 23, 25). Some investigators may have preferred one laboratory test to another, but no test has so far been superior. The BHRT is more laborious and expensive than the prick test and RAST, and this has limited its use. Under certain circumstances, e.g., when discordant or uncertain results have been obtained with other allergy tests, BHRT may be valuable. In addition, blood samples from patients on antihistamines can be examined with BHRT, and this test can be performed in many cases when commercial RAST or commercial aller-

gens for the prick test are not available. We are not aware of previous studies in which BHRT has been used to verify allergy to storage mites, soy, or garlic. The test seems to work quite well with these allergens.

The quality of allergen preparations is important in laboratory and *in vivo* tests. We have compared our own and commercial prick test reagents for wheat, rye, barley, and oats in patients with allergy to ingested cereals (unpublished) and in some patients with respiratory allergy (present study). In these comparisons the commercial flour preparations (ALK) yielded 33% false-negative results, but there were no false-positives. The percentages of false-negative and false-positive cases with our own flour allergens were 9 and 15%, respectively. We prefer our own reagents to the commercial ones, but dilution or some degree of purification might help to diminish the proportion of false-positive results.

Although the prick test, RAST, and BHRT represent parallel methods to measure type I allergy, they often give discordant results. There are several reasons for discrepancies among different allergy test results. Firstly, many organic allergens are complex mixtures of antigenic molecules. The quality and composition of allergen extracts may vary in different test assays, and thus the responses measured may be dissimilar. In our material the skin prick test and BHRT were more often positive than RAST. RAST measures overproduction of specific IgE in circulation after entrapment of Fc ϵ -receptor-positive cells, and may therefore be less sensitive than the prick test and BHRT. Escape from detection in RAST can be explained either by low levels of free IgE in circulation or by the formation of IgE antibodies against antigenic determinants which are present in low concentrations in the allergen preparation used (24). It has also been found that high levels of allergen-specific IgG antibodies (27) or anti-IgE antibodies (10) may interfere with the RAST assay and lead to diminished or negative values. In a few subjects, BHRT remains negative even if RAST is positive. These subjects possess IgE antibodies which are able to sensitize the basophils, but the latter are functionally defective (21).

A positive challenge response in nasal or bronchial provocation was regarded as the standard for occupational respiratory disease. Like all tests, challenges may sometimes yield false-positive or false-negative results. In the present material, sensitization to an allergen could be verified by one or more immunologic tests in 21/23 cases of challenge-proven asthma or rhinitis. It is possible that the challenge reactions of the two rhinitis patients with negative skin and blood tests were not allergic. However, localized allergic responses are not exceptional, and these patients are more likely to represent such cases.

There is an obvious need for challenge schedules in which mast cell products released *in situ* can be measured to confirm the involvement of type I allergy mechanisms. Mast-cell-derived mediators such as histamine and tryptase are already being measured in nasal lavage fluids obtained in provocation situations (5, 11, 17).

Patients for the present study were collected from those attending departments of pulmonary diseases, otorhinolaryngology, and dermatology, and they represent a heterogeneous sample. Patients with challenge-proven, work-related rhinitis or asthma had been exposed to a variety of organic dusts, whereas most control subjects were without occupational exposure. When unexposed subjects are studied, the specificity of immunologic tests will be greater. It may also be possible that the reliability of different tests depends on the allergen involved. Furthermore, the selection of cutoff points for immunologic tests directly affects sensitivity and specificity values. A representative workforce sample, including both symptomatic and asymptomatic subjects, would be necessary to assess better the value of different tests.

On the basis of the present preliminary study, the overall concordance of skin and blood tests with challenge seems to be relatively good in allergic asthma and rhinitis. These tests cannot, however, replace the challenge but serve as additional aids. Even parallel immunologic tests may give discordant information; therefore, the availability of more than one test is an advantage.

References

1. BJÖRKSTÉN F, BACKMAN A, JÄRVINEN KAJ, et al. Immunoglobulin E specific to wheat and rye flour proteins. *Clin Allergy* 1977; 7: 473–83.
2. BLAINEY AD, TOPPING MD, OLLIER S, DAVIES RJ. Respiratory symptoms in arable farmworkers: role of storage mites. *Thorax* 1988; 43: 697–702.
3. BLANDS J, DIAMANT B, KALLÓS P, KALLÓS-DEFFNER L, LÖWENSTEIN H. Flour allergy in bakers. I. Identification of allergenic fractions in flour and comparison of diagnostic methods. *Int Arch Allergy Appl Immunol* 1976; 52: 392–406.
4. BUSH RK, SCHROECKENSTEIN D, MEIER-DAVIS S, BALMES J, REMPEL D. Soybean flour asthma: detection of allergens by immunoblotting. *J Allergy Clin Immunol* 1988; 82: 251–5.
5. CASTELLS M, SCHWARTZ LB. Tryptase levels in nasal-lavage fluid as an indicator of the immediate allergic response. *J Allergy Clin Immunol* 1988; 82: 348–55.
6. CLOUTIER Y, LAGIER F, LEMIEUX R, et al. New methodology for specific inhalation challenges with occupational agents in powder form. *Eur Respir J* 1989; 2: 769–77.
7. CUTHBERT OD, JEFFREY IG, MCNEILL HB, WOOD J, TOPPING MD. Barn allergy among Scottish farmers. *Clin Allergy* 1984; 14: 197–206.
8. HARVIMA RJ, KAJANDER EO, HARVIMA IT, FRÄKI JE. Purification and partial characterization of rat kidney histamine-N-methyltransferase. *Biochim Biophys Acta* 1985; 841: 42–9.
9. IVERSEN M, PEDERSEN B. The prevalence of allergy in Danish farmers. *Allergy* 1990; 45: 347–53.
10. JENSEN-JAROLIM E, VOGEL M, DE WECK AL, STADLER BM. Anti-IgE autoantibodies mistaken for specific IgG. *J Allergy Clin Immunol* 1992; 89: 31–43.
11. JULIUSSEN S, HOLMBERG K, BAUMGARTEN CR, OLSSON M, ENANDER I, PIPKORN U. Tryptase in nasal lavage fluid after local allergen challenge. *Allergy* 1991; 46: 459–65.
12. JÄRVINEN KAJ, PIRILÄ V, BJÖRKSTÉN F, KESKINEN H, LEHTINEN M, STUBB S. Unsuitability of bakery work for a person with atopy: a study of 234 bakery workers. *Ann Allergy* 1979; 42: 192–5.
13. MENEELY GR, RENZETTI AD, STEELE JD, WYATT JP, HARRIS HW. Chronic bronchitis, asthma and pulmonary emphysema: definition and classification of chronic bronchitis, asthma and pulmonary emphysema. *Am Rev Respir Dis* 1962; 85: 762–8.
14. NIEMINEN MM, LAHDENSUO A, KELLOMÄKI L, KARVONEN J, MUITTARI A. Methacholine bronchial challenge using a dosimeter with controlled tidal breathing. *Thorax* 1988; 43: 896–900.
15. POPA V, GEORGE SA, GAVANESCU O. Occupational and nonoccupational respiratory allergy in bakers. *Acta Allergologica* 1970; 25: 159–77.
16. PRICHARD MG, RYAN G, MUSK AW. Wheat flour sensitisation and airways disease in urban bakers. *Br J Ind Med* 1984; 41: 450–4.
17. PROUD D, BAILEY GS, NACLERIO RM, et al. Tryptase and histamine as markers to evaluate mast cell activation during the responses to nasal challenge with allergen, cold, dry air, and hyperosmolar solutions. *J Allergy Clin Immunol* 1992; 89: 1098–110.
18. REVSBECH P, ANDERSEN G. Storage mite allergy among grain elevator workers. *Allergy* 1987; 42: 423–9.
19. REVSBECH P, DUEHOLM M. Storage mite allergy among bakers. *Allergy* 1990; 45: 204–8.
20. RÄSÄNEN L, LEHTO M, REUNALA T. Diagnostic value of skin and laboratory tests in cow's milk allergy/intolerance. *Clin Exp Allergy* 1992; 22: 385–90.
21. STAHL SKOV P, NORN S, WEEKE B, NOLTE H. Impaired basophil histamine release from allergic patients. *Agents Actions* 1987; 20: 303–6.
22. TERHO EO, HUSMAN K, VOHLONEN I, RAUTALAHTI M, TUKIAINEN H. Allergy to storage mites or cow dander as a cause of rhinitis among Finnish dairy farmers. *Allergy* 1985; 40: 23–6.
23. THIEL H, ZIMMERMANN I, RASCHE B, ULMER WT. Allergen-specific histamine release from whole blood in flour-sensitive bakers. *Respiration* 1982; 43: 208–20.
24. VAN DER ZEE JS, DE GROOT H, VAN SWIETEN P, JANSEN HM, AALBERSE RC. Discrepancies between the skin test and IgE antibody assays: study of histamine release, complement activation *in vitro*, and occurrence of allergen-specific IgG. *J Allergy Clin Immunol* 1988; 82: 270–81.
25. VAN HAGE-HAMSTEN M, IHRE E, ZETTERSTRÖM O, JOHANSSON SGO. Bronchial provocation studies in farmers with positive RAST to the storage mite *Lepidoglyphus destructor*. *Allergy* 1988; 43: 545–51.
26. VAN HAGE-HAMSTEN M, JOHANSSON SGO, HÖGLUND S, TULL P, WIRÉN A, ZETTERSTRÖM O. Storage mite allergy is common in a farming population. *Clin Allergy* 1985; 15: 555–64.
27. ZIMMERMANN EM, YUNGINGER JW, GLEICH GJ. Interference in ragweed pollen and honeybee venom radioallergosorbent tests. *J Allergy Clin Immunol* 1980; 66: 386–93.

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