

Diagnosis and Immunotherapy of Mould Allergy

III. Diagnosis of *Cladosporium* Allergy by Means of Symptom Score, Bronchial Provocation Test, Skin Prick Test, RAST, CRIE and Histamine Release

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Thirty-three adult asthmatic patients suspected of mould allergy were investigated by *in vivo* and *in vitro* tests in order to establish a specific diagnosis of asthma caused by the mould species *Cladosporium*. The patients were evaluated by daily symptom scores in the peak *Cladosporium* season, bronchial provocation test (BPT), skin prick test (SPT), RAST, histamine release from basophil granulocytes (HIST), and crossed radioimmuno-electrophoresis (CRIE), and the results were scored as negative (score 0), equivocal (score 1) or positive (score 2). Based on daily symptom scores and the result of BPT the patients were classified as being manifest allergic (asthma) to *Cladosporium* (positive allergy), inconclusive or negative. Positive allergy was defined as asthma symptoms oscillating with the spore concentration and a BPT score 2 (positive at allergen concentration < 10,000 BU). Negative allergy was defined as no asthma symptoms and a negative BPT (score 0) and inconclusive in the case of symptoms and BPT sum of score 1-3. According to the classification a final diagnosis (positive or negative) could be established in 85 % of the patients. "False positive" tests were found: for BPT in 27 %, SPT 18 %, RAST 0 %, HIST 18 %, and CRIE 0 %. The corresponding figures for "false negative" were: BPT 0 %, SPT 0 %, RAST 27 %, HIST 18 %, and CRIE 23 %. The relative risk of being allergic in spite of a negative test result was 0 % for BPT and SPT, and 25-30 % with RAST, HIST, and CRIE. In the case of positive test the risk was 90-100 %. Excluding BPT, SPT was found to be the optimal single test to predict/rule out clinical allergy. A stepwise combination of positive SPT and positive RAST was found exclusively in patients clinically evaluated as positive, and does not call for an additional BPT. Using a potent allergenic extract a negative SPT excluded clinically important allergy. The primary conclusion of the study, however, is that the final diagnosis of *Cladosporium* asthma could not be based on a positive BPT alone (due to "false positive"), but only on a combination of clinical symptoms during the spore season and a positive BPT.

Key words: asthma bronchiale; bronchial provocation test; *Cladosporium herbarum*; crossed radioimmuno-electrophoresis; diagnosis; histamine; release; RAST; skin prick test.

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The purpose of performing diagnostic tests in allergic patients is to obtain a precise diagnosis of the causative allergen (1, 20, 21, 24). Without a precise diagnosis neither allergen avoidance nor specific treatment are to be instituted.

For many years diagnostic procedures in allergy have implied a combination of history and skin test and in rare cases some sort of provocation test (1, 20, 21, 24). The introduction of *in vitro* tests within recent decades has widened the diagnostic spectrum, but the value

in relation to improving the diagnostic capability is still a subject of debate (3, 11, 13, 29, 31).

Diagnostic tests used in allergy all estimate specific IgE in different compartments. The skin prick test (SPT) is a result of IgE localized on skin mast cells (20, 24). Bronchial provocation test (BPT) is an expression of IgE bound to lung mast cells (1, 21, 24). Radio-allergosorbent test (RAST) estimates circulating IgE (13). Histamine release from basophil leukocytes (HIST) is a function of IgE fixed on circulating basophils (28) and finally, crossed radioimmunoelectrophoresis (CRIE) measures circulating IgE (7).

As pointed out by Aas et al. (2) the diagnosis of respiratory allergy to moulds might be difficult, as it is highly dependent on the potency and antigenic composition of the allergen extracts. The relative value of a test in relation to obtaining the "true" diagnosis depends on the sensitivity and the specificity being a function of the quality of the allergenic extract used. Recently, intense research on especially *Cladosporium* allergens has been carried out by Aukrust et al. (2, 7) resulting in the production of an extract with a high potency and optimum allergenic composition.

The present study presents a detailed investigation of the diagnostic capacity of allergological procedures and the relation between these tests in patients with clinical suspicion of mould allergy.

MATERIAL AND METHODS

Patients

From a population of 62 adult asthmatics screened for mould allergy (17) 33 patients were further evaluated. All patients had a history of aggravation of respiratory allergy in the peak mould season (June-September) for a minimum of 3 years. The clinical relevance of mould allergy was further evaluated by a detailed registration of subjective symptoms, consumption of anti-allergic drugs and peak-flow measurement mornings and evenings during the

peak *Cladosporium* season (14 weeks) in 1981 (32). Fourteen females and 19 males (median age 24 years (range 16-58)) were included in the study. Most patients were allergic to other allergens, but with the majority of symptoms elicited during the mould season. Previous immunotherapy with mould extracts within the last 2 years resulted in exclusion from the study. *In vivo* testing and blood sampling were performed simultaneously outside the mould season.

Allergen extracts

A lyophilised, partially purified and standardized preparation of *Cladosporium herbarum* (Pharmalgen®, Pharmacia, Uppsala, Sweden) reconstituted to a concentration of 100,000 BU/ml and diluted in HSA buffer (0.03 % human serum albumin, 0.9 % sodium chloride and 0.4 % phenol) was used for SPT, BPT and HIST. To ensure maximum potency, extracts were reconstituted and diluted at the day of testing and then discarded.

An intermediate product of the raw extract used for producing the Pharmalgen quality mentioned above was used for RAST and CRIE. The only discrepancy from the Pharmalgen estimated by CIE (crossed immunoelectrophoresis) being a few more precipitates in the intermediate product. The IgE-binding (CRIE) to these precipitates was negligible.

Bronchial provocation test (BPT)

Bronchial provocation was carried out at the same time as SPT and blood sampling. Treatment with antihistamines, inhaled corticosteroids, sodium cromoglycate, beta-2-agonists and xanthine derivatives was discontinued for a minimum of 72, 72, 24, 18 and 18 h, respectively prior to BPT. Systemic steroid < 10 mg prednisolone used by two patients was not supposed to interfere with the immediate response (24). Using the tidal volume the patients inhaled 1 ml of aerosolized material (Pari nebulizer, Pariwerk, W. Germany) for 5 min. The flow was 5 l/min and the output 0.221 ml/min. Forced expiratory volume in 1 sec

(FEV₁) was measured using a Vitalograph spirometer (Buckingham, England). The procedure of BPT was to obtain a baseline FEV₁ before and after inhalation of HSA buffer. After an interval of 10 min 1 ml of *Cladosporium* 1 BU/ml was inhaled and FEV₁ registered. After another 10 min FEV₁ was repeated, and if not reduced > 20 % from the baseline value, the provocation was continued with 10-fold more concentrated extract until either a positive reaction (> 20 % decrease in FEV₁) or up to a concentration of 10,000 BU/ml. When interrupting BPT before nebulization of 1 ml the residual volume was recorded and the BPT result corrected accordingly. The bronchial obstruction induced by the provocation was treated by inhalation of beta-adrenergic medication. FEV₁ obtained after inhalation of allergen was plotted as percent of the baseline value obtained by HSA diluent as a function of the allergen concentration, and the allergen concentration eliciting a decrease in FEV₁ of 20 % was calculated (the bronchial sensitivity = PC₂₀). Late asthmatic response was only observed in one patient. The reproducibility of repeated BPT was within 1/2 log step of allergen dilution. Challenge with doses up to 100,000 BU/ml did not result in unspecific reactions in non-asthmatic normal persons.

Skin prick test (SPT)

SPT was performed as previously described (18). In short, quadruplicate tests with at least three 10-fold dilutions of Pharnalgen (10–100,000 BU/ml) were performed on the patients' forearms. The wheal area was calculated by planimetry and plotted in a log-log-system as a function of the allergen concentration. Linear regression analysis was performed and tests with a coefficient of correlation < 0.9 were discharged as non-valid. The skin sensitivity (SSH) was calculated as the allergen concentration eliciting a wheal equal to the one produced by histamine HCl 1 mg/ml. Anti-histamines and steroid ointments were withdrawn at least 72 h prior to skin testing. Steroid doses < 10 mg prednisolone per day were allowed (20).

Patient serum

Venous blood was drawn immediately prior to SPT and BPT. Serum was separated and stored in small aliquots at -20°C until analysed. Samples were only frozen and thawed once.

Radioallergosorbent test (RAST)

RAST was performed according to Ceska et al. (12). In brief, the intermediate product of the *Cladosporium* extract was coupled to CNBr-activated paper discs. The discs were incubated with patient serum undiluted and diluted 5- and 25-fold overnight at room temperature in duplicate. After washing the discs were incubated overnight at room temperature with ¹²⁵I-labelled affinity-purified rabbit anti-human IgE (Phadebas® RAST Isotope). The assay was calibrated by use of a human reference and the results were expressed in RAST classes according to the PRU value (Phadebas RAST units) in undiluted serum. Dilution of patient serum was performed in order to read the binding of ¹²⁵I-anti-IgE at the steepest part of the dose response curve. All serum samples were run in one set up, and the reproducibility of double determination was within 10 % of the mean.

Histamine release (HIST)

HIST from basophils was carried out as previously described (16, 28). In short, Ficoll-Hypaque isolated mononuclear cells were incubated for 40 min at 37°C with 10-fold dilutions of *Cladosporium* extract. The release of histamine was expressed as the percentage of the total histamine contents of the sample. Correction for spontaneous histamine release amounting from 0–5 % was made. The cell sensitivity was expressed as the allergen concentration eliciting a 20 % histamine release.

Crossed radioimmunoelectrophoresis (CRIE)

CRIE was performed with 5 µl dialysed *Cladosporium* extract (60 mg/ml) applied in the well and run in the 1st dimension for 25 min (10 v/cm) using a 1 % A-agarose gel (Pharmacia Fine Chemicals) in TRIS-tricine buffer, pH 7.8. The 2nd dimension electrophoresis was run at 2 v/cm overnight at

room temperature against a 10% rabbit anti-serum raised against the extract used in the 1st dimension. CRIE was carried out as described by Aukrust (7) by incubating the plates with patient serum overnight at room temperature. After washing a further incubation with ^{125} I-labelled anti-IgE (Phadebas RAST isotope) followed by autoradiography for 14 days and 1 month. The degree of radiostaining is an expression of the specific IgE-binding and the CRIE sensitivity. Controls included incubation with serum from a pool of non-allergies in order to study the degree of non-specific binding of ^{125} I-anti-IgE to the precipitates.

Score system

The results obtained with the various tests were scored in the following way: score 0 = negative test, score 1 represented an equivocal positive result of the test, and score 2 an unequivocal positive test. Relation between scores and the results of the different tests are seen in Table 1.

Clinical evaluation

Based on the daily symptom scores and use of anti-allergic drugs during the peak *Cladosporium* season the patients were evaluated as having clinically manifest symptoms (score 2) or no symptoms (score 0). Patients presenting symptoms during

Table 1

The results of BPT, SPT, RAST, HIST and CRIE are scored as negative (score 0), equivocal (score 1), or positive (score 2) according to the degree of sensitivity

Score	Negative 0	Equivocal 1	Positive 2
BPT	negative	$\geq 10,000$ BU	$< 10,000$ BU
SPT	negative	$\geq 100,000$ BU	$< 100,000$ BU
RAST	class 0	class 1	class 2-4
HIST	$< 5\%$	$< 20\%$ or ≥ 100 BU	$> 20\%$ and < 100 BU
CRIE	no radiostaining	doubtful radiostaining	certain radiostaining

Definition: allergy (asthma) to *Cladosporium*

Positive allergy: clinical score 2 plus BPT score 2.

Negative allergy: clinical score 0 plus BPT score 0.

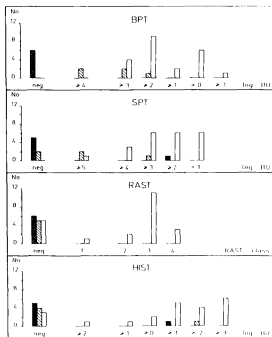


Fig. 1. The result of the diagnostic tests (degree of sensitivity) related to the clinical evaluation

■ negative allergy by clinical evaluation; ▨ inconclusive allergy; □ positive allergy.

The results of BPT, SPT and HIST are given in log BU and RAST in classes. For further details on the estimation of sensitivity, see text.

the mould season, but without oscillation of symptoms according to variations in the *Cladosporium* spore counts were considered clinically inconclusive (score 1).

To ensure certainty of a definite positive allergy (asthma) to *Cladosporium* only patients with an unequivocal positive clinical evaluation (score 2) and positive BPT (score 2) were accepted. Patients without symptoms in the mould season (clinical score 0) and negative BPT (score 0) were considered as not having asthma caused by *Cladosporium*. An equivocal (inconclusive) allergy to *Cladosporium* appeared when the sum of clinical evaluation and BPT were scored 1-3.

Statistics

Comparison of the results of diagnostic tests and the clinical diagnosis was performed using a Chi-square test. Correlation between the

degree of sensitivity of the various tests was calculated by Spearman's Rho.

RESULTS

Positive clinical allergy (asthma) to *Cladosporium* was found in 22 patients (67%), a negative in six (19%). An unequivocal diagnosis could not be established in five patients (15%).

The relation between the clinical classification in positive, inconclusive and negative allergy to *Cladosporium* and the result of the individual diagnostic tests is evident from Fig. 1. Using BPT a positive test was found in three patients clinically classified as inconclusive (3/11 = 27% "false positive"). The SPT results showed positive tests in two patients classified as negative and inconclusive respectively (2/11 = 18% "false positive"), and an equivocal test was found in a patient classified as positive (1/22 = 5% "false negative"). RAST was positive in all clinically

positive patients (0/11 = 0% "false positive"), but six patients had negative (5) or equivocal (1) tests in spite of a clinical classification as positive (6/22 = 27% "false negative"). Two patients defined as having negative or inconclusive allergy to *Cladosporium* produced a positive HST (2/11 = 18% "false positive") and a negative (3) or equivocal (1) HST appeared in four patients classified as clinically positive (4/22 = 18% "false negative"). No "false positive" GRIE was found, but 5/22 = 23% had a "false negative" test (data not shown).

The relation between the clinical diagnosis and the score obtained in the individual tests is evident from Table 2. The Chi-square and the *P*-values are also stated.

Based on Table 2 the relative risk of being clinically allergic (definition) was calculated for test results negative, equivocal and positive (Table 3). Clinical allergy was excluded in the case of negative test results of BPT and SPT,

Table 2

Agreement between the result (score 0, 1, or 2) of individual diagnostic tests in relation to the clinical diagnosis stated as positive, inconclusive or negative

		Score		
		0	1	2
Clinical diagnosis	pos	-	-	22
	incl	-	2	3
	neg	6	-	-

$$\chi^2 = 44.6, P < 0.0001$$

		Score		
		0	1	2
Clinical diagnosis	pos	-	1	21
	incl	2	2	1
	neg	5	-	1

$$\chi^2 = 29.0, P < 0.0001$$

		Score		
		0	1	2
Clinical diagnosis	pos	5	1	16
	incl	5	-	-
	neg	6	-	-

$$\chi^2 = 17.5, P = 0.002$$

		Score		
		0	1	2
Clinical diagnosis	pos	3	1	18
	incl	4	-	1
	neg	5	-	1

$$\chi^2 = 14.8, P = 0.005$$

		Score		
		0	1	2
Clinical diagnosis	pos	4	1	17
	incl	5	-	-
	neg	6	-	-

$$\chi^2 = 19.8, P = 0.001$$

whereas 25–30 % of the patients with negative RAST, HIST and CRIE were found to be allergic to *Cladosporium* by clinical evaluation. The risk of being allergic with regard to an equivocal test result was found in 0 %, 33 % and 100 % using BPT (definition), SPT and RAST, HIST and CRIE respectively (based on a few observations). Positive tests indicated clinical allergy in 90–100 %.

The reliability of the individual tests, i.e. the percentage of final diagnosis (clear-cut positive or negative) established by an unequivocal result of the test in question, is shown in Table 4. Figures between 67 and 85 % were found with BPT being the optimum and RAST the least useful test.

The sum of scores obtained by combination of tests in relation to the clinical diagnosis is given in Table 5. The Chi-square values obtained by combination of SPT + RAST, SPT + HIST, and SPT + RAST + CRIE + HIST are also evident. From Table 5 it is also seen that a complete concordance (positive or negative) between all four diagnostic procedures was found only in 21/33 (64 %) of the patients. Even if minor discrepancies are accepted, the diagnostic capacity by combination of four tests does not

exceed figures found using single tests only (Table 4).

The common practice in allergological diagnostic work of stepwise combination of tests in order to build up a final diagnosis was also investigated. The results of combining SPT (1st screening test) with RAST or HIST in patients with a final clinical diagnosis are given in Table 6. The outcome of SPT, RAST and HIST is stated as positive, equivocal or negative according to the definitions. As is evident (Table 3) a positive SPT test indicated clinical allergy in 91 % and a negative test in 0 %. Combination of a positive SPT and positive RAST was only found in allergic patients. Twenty-four percent of the patients with clinical allergy, however, were missed due to negative or equivocal RAST. The figures of a combined positive SPT and HIST result in clinical allergy in 90 % thus "catching" 86 % of all allergics. The combination of SPT + RAST + HIST results in a diagnostic reliability between SPT + RAST and SPT + HIST.

The relationship between the exact degree of allergic sensitivity as estimated by the different tests was calculated by performing paired correlation (Spearman's Rho). The highest correlation (Rho = 0.85) was found between HIST vs SPT ($P < 0.001$). In decreasing order the correlation (Rho) was RAST vs SPT 0.68, RAST vs HIST 0.66 and SPT vs BPT 0.59 ($P < 0.001$). The correlation between HIST vs BPT was calculated as 0.52 and RAST vs BPT as low as 0.48 ($P < 0.01$) (Fig. 2).

DISCUSSION

The problems involved in establishing a specific diagnosis in patients suspected of mould allergy

Table 3

The relative risk of being allergic to *Cladosporium* calculated for diagnostic test result negative, equivocal, and positive

Predictive value of diagnostic tests		
Risk of being allergic with negative test result:		
BPT	0/6	0 % (definition)
SPT	0/7	0 %
RAST	5/16	31 %
HIST	3/12	25 %
CRIE	4/15	27 %
Risk of being allergic with equivocal test result:		
BPT	0/2	0 % (definition)
SPT	1/3	33 %
RAST	1/1	100 %
HIST	1/1	100 %
CRIE	1/1	100 %
Risk of being allergic with positive test result:		
BPT	22/25	89 %
SPT	21/23	91 %
RAST	16/16	100 %
HIST	18/20	90 %
CRIE	17/17	100 %

Table 4

The overall reliability of the examined diagnostic tests, i.e. the percentage of final diagnosis (clear-cut positive or negative) obtained by an unequivocal result of the test

Reliability of diagnostic tests		
BPT	22 + 6/33	85 %
SPT	21 + 5/33	79 %
RAST	16 + 6/33	67 %
HIST	18 + 5/33	69 %
CRIE	17 + 6/33	69 %

are evident (2, 14, 17, 21, 26). The value of using different allergological tests and especially the combination of tests in diagnostic work has been stated (30, 31). Almost all studies suffer from the error of assuming that a positive

provocation test (most often bronchial) is an expression of a "true diagnosis" (4, 8, 9, 11, 14, 22). In the present study the clinical relevance of a positive bronchial provocation test was evaluated, and the final diagnosis of respiratory

Table 5

Agreement between the clinical diagnosis and the sum of score obtained by the combination of diagnostic tests

SPT + RAST		Sum of score				
		0	1	2	3	4
Clinical diagnosis	pos	-	1	4	1	16
	incl	2	2	1	-	-
	neg	5	-	1	-	-

$$\chi^2 = 31.2, P = 0.0001.$$

SPT + HIST		Sum of score				
		0	1	2	3	4
Clinical diagnosis	pos	-	1	2	1	18
	incl	2	2	-	-	1
	neg	5	-	-	-	1

$$\chi^2 = 29.1, P = 0.0003.$$

SPT + RAST + HIST + CRIE		Sum of score							
		0	1	2	3	4	5	6	7
Clinical diagnosis	pos	-	1	2	1	-	-	2	1
	incl	2	2	-	-	1	-	-	-
	neg	5	-	-	-	1	-	-	-

$$\chi^2 = 15.3, P = 0.002.$$

Table 6

Result of stepwise combination of positive SPT (1st screening test) with RAST or HIST (2nd test) in relation to a final positive clinical diagnosis. The risk of being clinically allergic with test results positive, equivocal, and negative of the 2nd test is calculated

		Combination of diagnostic tests				
1st screening test		2nd test		Clinical diagnosis positive		
	No.		No.	No.	Percentage	
SPT positive	23 → RAST	positive	16	16	100	
		equivocal	1	1	100	
		negative	6	4	67	
1st screening test		2nd test		Clinical diagnosis positive		
	No.		No.	No.	Percentage	
SPT positive	23 → HIST	positive	20	18	90	
		equivocal	1	1	100	
		negative	2	2	100	

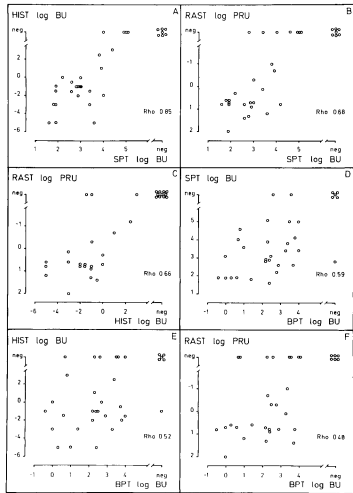


Fig. 2. The correlation (Spearman's Rho) between the degree of sensitivity of individual patients evaluated by the different diagnostic tests. The results of BPT, SPT and HST are given in log BU and for RAST in log PRU. The definitions of the sensitivity of the various tests are given in the text.

allergy to the mould *Cladosporium* was based on a combination of an unequivocal result of the BPT combined with clinical symptoms oscillating with the causative airborne spore concentration. Inversely, negative BPT and lack of respiratory symptoms in the peak *Cladosporium* season was interpreted as evidence of a clinically unimportant allergy (asthma) to *Cladosporium*. Thus we were able to differentiate between the demonstration of IgE reactivity (the diagnostic tests) and the specific diagnosis (clinically manifest allergy).

Using this approach an inevitable result will

be a small group of patients impossible to classify. However, from a clinical point of view this seems reasonable, as some patients at a given time cannot be classified as unequivocally negative or positive.

During recent decades several *in vitro* techniques have been introduced in clinical allergology with the aim of improving the diagnosis of reagin allergy (3, 7, 13, 16). However, some important factors must be borne in mind. As discussed above, all tests estimate IgE in different compartments, but are not able to evaluate the clinical importance of the detected IgE.

Furthermore, the capability of determining IgE is highly dependent on the quality of the allergen extract used (13, 17). Both in terms of potency and antigenic composition the extract must be of a high quality in order to give optimum information. Several studies have documented the advantages of the *Cladosporium* extract employed in this study compared to other commercial extracts (2, 17). The use of the same extract for both *in vivo* and *in vitro* tests further strengthens the validity of our findings.

Diagnostic value of individual tests

Concerning the group of patients classified as positive (clinically manifest asthma caused by *Cladosporium*) no false negative tests were found using BPT (due to the chosen definition of positive allergy). Furthermore, no patients with a negative BPT had clinical symptoms, i.e. clinical score 0 in all patients with a negative BPT. "False negative" tests were found in increasing order (5%–27%) with the use of SPT, HIST and RAST. The problem is well-known with regard to RAST (15) and is probably a question of inability to bind some of the allergens to the solid phase (3, 6). Concerning HIST, "false negative" tests might partly be caused by the observation that up to 15% of patients with IgE-mediated disease do not liberate histamine by challenge with the causative allergen (15). The only patient with "false negative" SPT was not exclusively negative, but the SPT reaction is classified as equivocally positive, which may be a result of the chosen limits between positive and negative reactions. Reactions of 100,000 BU probably express a low grade, but still specific reactivity.

"False positive" BPT was found in 27% of the positive tests. As earlier discussed the patients did not have asthma symptoms in the *Cladosporium* spore peak season. Two of these patients had a low threshold dose in histamine BPT, indicating non-specific hyperreactivity (21). The third patient was positive in BPT to other inhalant allergens without clinical relevance. "False positive" SPT was found in one patient classified as inconclusive (latent allergy?) and in one patient without asthma, but

with clear-cut rhinoconjunctivitis symptoms in the *Cladosporium* season. No "false positive" RAST was found, indicating that the registration of circulating IgE in *Cladosporium* allergic patients is evidence of clinical disease. This may be due to low binding of *Cladosporium* extract to the discs, and consequently only patients with high values of circulating specific IgE are detected by the RAST (5, 13). Using HIST, "false positive" tests were found in one inconclusive patient (the one with a "false positive" SPT) and in the rhinitis patient with "false positive" SPT too.

The problem of "false positive" test is rather complex and the positive reaction might represent a) a true "false positive", b) compartmentalization, c) latent allergy or d) allergy in another shock organ than the one used to evaluate the true diagnosis.

As regards a), this phenomenon might be caused by irritants in the extract (BPT, SPT, HIST) (1, 20) or by non-specific binding to the discs (RAST). Compartmentalization and latent allergy might represent similar findings, i.e. specific IgE localized in the skin for instance, but not in other shock organs, or circulating (1, 20). As for d) it implies positive tests in patients with rhinitis caused by the examined allergen, but without asthma, i.e. negative BPT and asthma symptom score.

In the present study the highest correlation between the score obtained in the individual tests and the clinical diagnosis was found for BPT (Table 2). SPT, CRIE, RAST, HIST followed in this order. This is in contrast to other studies that have found RAST (4, 9) or HIST (31) superior to SPT. The high validity of SPT (10) is probably due to the use of quantitative SPT in the present study, which is further strengthened by the data in Table 3, showing that only a negative BPT and SPT are useful in ruling out clinical allergy. No significant difference in the capacity to predict allergy between the examined tests was found (10, 25). The number of tests classified as equivocal is too small to act as a basis for conclusions, but is of interest when evaluating the overall reliability of tests (Table 4). The highest reliability was found with BPT and then decreasing value

with SPT, HIST, CRIE and RAST, respectively.

Diagnostic value of combination of tests

To reduce the number of BPT, combinations of tests are claimed to increase the diagnostic reliability (31). However, in the case of mould allergy the results of combining various tests (Table 5) do not support this theory, which is not surprising, as combination of a highly specific and sensitive test with less reliable tests inevitably invalidates the reliable tests. The discrepancy in relation to the clinical diagnosis of individual tests is reinforced by the combination of tests. On the other hand, combination of tests might be useful in the stepwise approach. Due to the relative advantages of SPT as inexpensive, fast and safe, it is often used as the first screening test in clinical practice (20). Negative SPT excludes allergy to the allergen in question on condition that an extract with optimum potency and antigenic composition is employed. The combination of a positive SPT and RAST indicates a clinical relevance of the allergen and makes BPT unnecessary. Positive SPT and negative RAST are often found in patients with clinical symptoms, and an additional BPT has to be performed.

However, as pointed out earlier the final diagnosis has to be based on a combination of symptoms (score system) and BPT. In the present study HIST was of less value than RAST in the capacity of predicting clinical allergy.

Correlation between diagnostic tests

The correlation between the degree of sensitivity estimated by the different tests was statistically significant, but with a lower coefficient of correlation than could be expected based on the optimization of the quantitative tests. The results, however, are in agreement with other reports (10, 19, 23, 27). Based on the coefficient of correlation the interpretation of the data might be that the different tests to a high degree measure the same phenomenon. The definition of the degree of sensitivity, however, is arbitrary

and reevaluation of the definition might give rise to other results.

Furthermore, the data suggest that in their response to allergens, bronchial and skin mast cells and circulating basophils behave as if they were a single population of reactive cells in equilibrium with circulating specific IgE (10, 23, 25, 28). The possibility of compartmentalization is partly ruled out by the significant correlation. "False positive and negative" tests are well-known phenomena invalidating the correlation, and the present study confirms this problem (1, 13, 20, 21, 23).

Conclusion

Based on our data we can state that the diagnosis of respiratory allergy to moulds probably involves more problems than the diagnosis of pollen allergy (2, 21, 26). Even if a potent *Cladosporium* extract including a representative composition of epitopes (2) is employed in optimum diagnostic tests, a final diagnosis, i.e. clinically relevant allergy, can only be established by completion of daily symptom score in the peak mould season. The occurrence of "false positive" BPT is extremely important in this context. In daily practice negative SPT rules out clinically important allergy and no further tests are indicated. A combination of positive SPT and RAST was found consistently in clinically positive patients, and on condition that the patients have symptoms from exposure to moulds a BPT might be omitted. The combination of positive SPT and negative RAST called for a BPT. The relative value of HIST and CRIE in relation to confirming and ruling out, respectively, a specific diagnosis of mould allergy is inferior to the above mentioned tests. The data refer to a highly selected group of patients, and indiscriminate use of the diagnostic tests in unselected patients might invalidate the specificity and sensitivity found in the present study.

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REFERENCES

- Aas, K.: Bronchoprovocative tests (BPT) in clinical and experimental allergy. *Ann. Allergy* 33, 320-324, 1974.
- Aas, K., Leegaard, J., Aukrust, L. & Grønner, Ø.: Immediate type hypersensitivity to common moulds. *Allergy* 35, 443-451, 1980.
- Adkinson, N. F.: The radioallergosorbent test: Uses and abuses. *J. Allergy Clin. Immunol.* 65, 1-4, 1980.
- Akiyama, K., Yui, Y., Shida, T. & Miyamoto, T.: Relationship between the results of skin, conjunctival and bronchial tests and RAST with *Candida albicans* in patients with asthma. *Clin. Allergy* 11, 343-351, 1981.
- Ali, M. & Ramanarayanan, M. P.: Correlation of a micro-ELISA assay with the skin test and the RAST assay in inhalant allergy. *Ann. Allergy* 53, 143-150, 1984.
- Aukrust, L. & Aas, K.: The diagnosis of immediate type allergy to *Cladosporium herbarum*. *Allergy* 33, 24-29, 1978.
- Aukrust, L.: Crossed radioimmuno-electrophoretic studies of distinct allergens in two extracts of *Cladosporium herbarum*. *Int. Arch. Allergy Appl. Immunol.* 58, 375-390, 1979.
- Berg, T., Bennich, H. & Johansson, S. G. O.: In vitro diagnosis of atopic allergy. I. A comparison between provocation tests and the radioallergosorbent test. *Int. Arch. Allergy* 40, 770-778, 1971.
- Berg, T. L. & Johansson, S. G. O.: Allergy diagnosis with the radioallergosorbent test. *J. Allergy Clin. Immunol.* 54, 209-221, 1974.
- Bruce, C. A., Rosenthal, R. R., Lichtenstein, L. M. & Norman, P. S.: Diagnostic tests in ragweed-allergic asthma. *J. Allergy Clin. Immunol.* 53, 230-239, 1974.
- Bryant, D. H., Burns, M. W. & Lazarus, L.: The correlation between skin tests, bronchial provocation tests and the serum level of IgE specific for common allergens in patients with asthma. *Clin. Allergy* 5, 145-157, 1975.
- Ceska, M., Eriksson, R. & Vanga, J. M.: Radio-immuno-sorbent assay of allergens. *J. Allergy Clin. Immunol.* 49, 1-9, 1972.
- Johansson, S. G. O.: In vitro diagnosis of reagin-mediated allergic diseases. *Allergy* 33, 292-298, 1978.
- Kersten, W. & Hock, G. T.: Schimmelpilzallergie. *Wiener Med. Wochenschr.* 8, 275-282, 1980.
- Lichtenstein, L. M., Valentine, M. D. & Sobotka, A. K.: Insect allergy: The state of the art. *J. Allergy Clin. Immunol.* 64, 5-12, 1979.
- Malling, H.-J.: Histaminfrigørelse fra basofile granulocytter. En sammenlignende undersøgelse mellem bronkialprovokation og histaminfrigørelse hos astmapatienter. *Ugeskr. Læg.* 139, 2312-2316, 1977.
- Malling, H. J., Agrell, B., Groner, S., Dreborg, S., et al.: Diagnosis and immunotherapy of mould allergy. I. Screening for mould allergy. *Allergy* 40, 108-114, 1985.
- Malling, H.-J.: Diagnosis and immunotherapy of mould allergy. II. Reproducibility and relationship between skin sensitivity estimated by end-point titration and histamine equivalent reaction using skin prick test and intradermal test. *Allergy* 40, 354-362, 1985.
- Mijamoto, T., Akiyama, K., Ohta, K., Urata, C. & Horiuchi, Y.: Studies in atopic asthma with emphasis on correlation among various tests and various antibodies. *J. Allergy Clin. Immunol.* 67, 279-284, 1981.
- Nelson, H. S.: Diagnostic procedures in allergy. I. Allergy skin testing. *Ann. Allergy* 51, 411-417, 1983.
- Nelson, H. S.: Diagnostic procedures in allergy. II. Bronchial provocation testing. *Ann. Allergy* 51, 488-492, 1983.
- Nitsson, D. & Aas, K.: Immunological specific and correlation of diagnostic tests for bronchial allergy to *Cladosporium herbarum*. *Acta Paediatr. Scand.* 65, 33-38, 1976.
- Norman, P. S., Lichtenstein, L. M. & Ischizaka, K.: Diagnostic tests in ragweed hay fever. A comparison of direct skin tests, IgE antibody measurements, and basophil histamine release. *J. Allergy Clin. Immunol.* 52, 210-224, 1973.
- Norman, P. S.: In vivo methods of study of allergy. Skin and mucosal tests, techniques, and interpretation. In Middleton, E., Reed, C. E. & Ellis, E. F. (eds.): *Allergy. Principles and practice*, pp. 295-302. C. V. Mosby, St. Louis, Toronto, 1983.
- Rosenthal, R. R., Bruce, C. A., Lichtenstein, L. M. & Norman, P. S.: The role of inhalation challenge. *Int. Arch. Allergy Appl. Immunol.* 49, 89-94, 1975.
- Salvaggio, J. & Aukrust, L.: Mold-induced asthma. *J. Allergy Clin. Immunol.* 68, 327-346, 1981.
- Schulze-Werlinghaus, G.: Comparison of standardized skin and provocation tests with RAST and histamine release from leukocytes in bronchial asthma. *Allergy* 35, 259-261, 1980.
- Skov, P. S., Permin, H. & Malling, H.-J.: Quantitative and qualitative estimations of IgE bound to basophil leukocytes from hay fever patients. *Scand. J. Immunol.* 6, 1021-1028, 1977.
- Thompson, R. A. & Bird, A. G.: How necessary are specific IgE antibody tests in allergy diagnosis? *Lancet* i, 169-173, 1983.
- Vanto, T.: Immediate hypersensitivity to dog in asthmatic children. Thesis, Turku, Finland, 1982.
- Osterballe, O., Malling, H.-J. & Weeke, B.: Specific diagnosis of exogenous bronchial asthma in adults. *Allergy* 34, 175-185, 1979.
- The Danish Spore Bulletin, The Aerobiological Group, Danmarks Astma-Allergiforbund.

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