

Use of the extended Phadebas RAST[®] panel in the diagnosis of mould allergy in asthmatic children

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The clinical significance of mould allergens in Phadebas RAST⁺ panel was investigated in 121 asthmatic children. They were selected from a total population of 1649 patients. The patients were distributed into four groups, based on the combination of positive or negative skin prick tests (SPT) together with symptoms suggestive or not of mould sensitivity. Mould-specific IgE antibodies were investigated using the original RAST panel (*Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Mucor* and *Penicillium*) and a set of 10 additional mould-allergen discs (*Aureobasidium*, *Botrytis*, *Curcularia*, *Epicoccum*, *Fusarium*, *Helminthosporium*, *Phoma*, *Rhizopus*, *Stemphylium* and *Trichoderma*). The set of additional RAST discs revealed patients with mould-reactive antibodies not found with the original RAST panel. This occurred in four of 49 (8.2%) RAST positive (class ≥ 2) patients. The allergens most frequently positive were *Cladosporium* (in 28% of the patients), *Candida* (28%) and *Helminthosporium* (26%). A remarkable degree of simultaneous reactivity to almost all moulds tested was observed. Patients with multiple (≥ 7) mould sensitization were effectively pinpointed using any duplicate combination of *Aureobasidium*, *Botrytis*, *Candida*, *Cladosporium*, *Helminthosporium*, *Penicillium* and *Stemphylium*.

Key words: allergy; asthma; mould; IgE antibodies

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CLINICAL ASPECTS

Increasing the number of mould allergens in the RAST panel increases the possibility to pinpoint a patient with mould allergy. Combinations of *Aureobasidium*, *Botrytis*, *Candida*, *Cladosporium*, *Helminthosporium*, *Penicillium* and *Stemphylium* in the test panel can effectively reveal patients with multiple mould allergy. This is often associated with multiple other allergies and many symptoms.

Mould allergy was first described by Charles Blackley (6) more than 100 years ago. Even before that Floyer (7) had drawn attention to the occurrence of asthma in damp environments. The clinical impact of airborne fungi on respiratory allergic disease is still largely unknown. This is due to many factors, among which are the following: 1. Diagnosis of mould allergy is still far from adequate. The optimal panel of mould allergens is not known. Relatively few moulds have been investigated in detail. Thus production of good diagnostic ex-

tracts for most fungi still awaits basic research. 2. Mould-allergic patients are usually also sensitive to many different allergen sources, such as pollens, epithelia, mites, etc. Thus analysis of the impact of moulds alone on symptoms is difficult. 3. There is no clear-cut mould season, which would enable easy observations on dose-response relationships between exposure and symptoms.

Substantial advances have been made in the past 10 years. It is now known that mould allergen extracts are extremely variable and

Table 1.

Primarily selected study groups (asthmatic children)

A. Symptoms	+	SPT	+	32
B. Symptoms	+	SPT	-	25
C. Symptoms	-	SPT	+	29
D. Symptoms	-	SPT	-	35

Symptoms + indicates asthmatic symptoms suggestive of mould allergy. Symptoms - indicates asthmatic symptoms not suggestive of mould allergy. As regards skin prick tests see Material and Methods.

complex, thus making it difficult to produce standardized mould allergen extracts.

The original Phadebas RAST® mould panel consisting of 6 mould genera has undergone many stages of development. This has been due to improvements in manufacturing mould allergen extracts and in RAST disc techniques and has led to more efficient diagnosis of mould allergy (16).

The aim of this study was to explore the diagnostic capacity of an extended Phadebas RAST mould panel and relationships between mould allergies.

MATERIAL AND METHODS

The patients for this study were selected from the Childrens' Asthma Register of Turku University (10). Asthmatic children are followed-up regularly until 17 years of age, and their data on symptoms, medication, allergy tests, etc. are entered into a computer. The total number of children in the register at the time of the study was 1649. For this study 121 children with a total of 785 follow-up years were selected.

To ensure that the study comprised all types of mould allergy-related asthmatics, 121 children were distributed into four primary groups according to their symptoms and previous skin prick test (SPT) results (Table 1). As asthmatic symptoms suggestive of mould allergy are supposed to be perennial, we selected only children with perennial asthma for Groups A and B (symptoms +) and specifically those with aggravating symptoms during the snow-free period (April through November in the Turku area of Finland). Mould SPT-positive patients were defined as those showing multiple mould

reactions, some of at least magnitude +++ according to the Nordic norm (1). Mould SPT-negative patients were not only those who were totally negative on repeated test occasions but also those who had occasionally shown a positive SPT, i.e. the same SPT was negative on the majority of test occasions.

Estimation of mould-specific IgE antibodies was done by means of Phadebas RAST, Pharmacia Diagnostics AB, Uppsala, Sweden. The original panel (m1-m6) was extended with 10 other experimental moulds (m7-m16), which are all now included in the Phadebas RAST panel. The original panel consisted of *Alternaria alternata* (tenuis) (m6), *Aspergillus fumigatus* (m3), *Candida albicans* (m5), *Cladosporium herbarum* (m2), *Mucor racemosus* (m4) and *Penicillium notatum* (m1). The experimental set of new mould RASTs consisted of *Aureobasidium pullulans* (m12), *Botrytis cinerea* (m7), *Curcularia lunata* (m16), *Epicoecum purpurascens* (m14), *Fusarium moniliforme* (m9), *Helminthosporium halodes* (m8), *Phoma betae* (m13), *Rhizopus nigricans* (m11), *Stemphylium botryosum* (m10) and *Trichoderma viride* (m15). These moulds were selected on the basis of a current analysis of the moulds implicated in allergy, e.g. extracts available for skin testing, requests for RAST discs, and discussions with experts. In this study RAST class 2 or higher was considered as positive.

The SPT results used for scoring (see below) were gathered from those done during the years 1974-1984. No new SPT were performed especially for this study. All SPT were evaluated according to the Nordic norm (1).

To grade the patients' total sensitivity a SPT score was constructed. The scores were chosen from five allergen groups. When several allergens from one group were tested the one with the strongest reaction (from - to 5+ in the Nordic histamine reference system) was chosen to represent the group. Consequently, a scale of 0-5 was used as SPT scores. The five allergen groups selected for SPT scoring were: 1) tree pollens (alder, birch, willow), 2) grasses (mix), 3) mugwort, 4) epithelium (cat, dog, horse, cow and house dust mixture) and 5) moulds (6 to 16 different moulds have been tested at various occasions). The highest score in each group

represented the group and the total score from all 5 groups (maximum 25) was calculated.

The patient's mould RAST score (maximum 64) was calculated as the sum of the 16 RAST class results (classes 0-4). Sera for RAST analyses had been taken during patients' visits to the asthma clinic and stored at -20°C until analysis. The mean age of the samples stored was 4 years.

RESULTS

Patient characteristics

The characteristics of the primarily selected patient groups are presented in Table 2. The mean age of the patients was almost the same in the 4 groups (Table 2). There was a clear tendency to higher SPT scores to inhalant allergens in Groups A and C (the patients selected on the basis of positive skin tests to moulds). The mould SPT scores of Groups B and D were higher than zero because patients with occasional positive reactions to single moulds had not been excluded. The total SPT score was lowest in Group D. The mould RAST score of Group A was higher than in the other groups. An almost identical mould RAST score was seen in Groups B and C. Asthmatic symptoms at the age of 12 years were clearly highest in Groups A and B due to selection criteria.

Table 2.
Characteristics of study Groups A to D

	A	B	C	D
Mean age at entering follow-up	6.4	5.6	7.9	5.2
age range	2-14	1-14	2-15	1-11
Mean SPT scores				
tree pollen	2.7	2.4	1.8	1.0
grass pollen	2.3	1.8	2.0	1.5
mugwort pollen	2.5	0.6	1.2	0.5
animals & house dust	3.1	2.7	2.9	1.8
moulds	3.7	1.1	2.5	0.5
Total SPT score	14.3	8.6	10.4	5.3
Mould RAST score	22.2	4.2	4.45	0.09
Symptoms of asthma (days/year) at 12 years	47.0	89.3	12.1	12.7

RAST-results

Table 3 shows the number of patients with positive (class ≥ 2) mould RAST responses distributed between the original Phadebas RAST panel (m1-m6) and the extended (m7-m16). Forty-nine patients (40.5%) had RAST class ≥ 2 to one or more mould allergens. Group A patients are more frequently sensitive to moulds belonging to either the original or extended RAST panels (94%) than those in the B, C and D the Groups (36, 31 and 3%, respectively).

Eight patients were RAST-positive (class ≥ 2) to one or more moulds of the original panel but negative to all of the extended one and the reverse pattern was seen in four patients. One of these four had no symptoms suggestive of mould allergy and was mould SPT negative (Group D). Ten of 60 patients (Group B, 9 patients and D, 1 patient) with previously negative SPT to moulds were mould RAST-positive. This mould RAST panel detected nine of the 29 patients (Group C) with previously positive SPT to moulds.

In addition to the above mentioned mould-positive patients (RAST class ≥ 2), there were 13 patients showing class 1 RAST results (1, 4, 7 and 1 in Groups A to D, respectively).

Table 4 gives the frequency of positive mould RAST responses to various moulds. The highest frequency of positive RAST results was seen for *Cladosporium* and *Candida* (each positive in 28% of all patients) followed by *Helminthosporium* (26%), *Botrytis* (24%) and *Aureobasidium*, *Fusarium*, and *Stemphylium* (each 22%). For 12 moulds the frequency was 15% or higher. Seven of these 12 moulds belonged to the 10 new experimental moulds added to the original panel. As expected, the highest frequency of positive RAST was seen in Group A compared with Groups B-D. Sensitization to seven or more moulds occurred frequently.

Positive mould RAST responses generally showed a high correlation between the 16 moulds (Table 5). The highest correlation 0.97 ($= 97\%$) was between *Aureobasidium* (m12) and *Stemphylium* (m10). In 38 of the 240 possible

Table 3.

Occurrence of the original / extended mould RAST positive (class ≥ 2) and negative (class < 2) cases in Groups A to D

Mould RAST panel	Study group				Total	
	A	B	C	D	n	%
	n = 32	n = 25	n = 29	n = 35		
Original + / Extended +	25	6	6	0	37	30.6
Original + / Extended-	4	3	1	0	8	6.6
Original- / Extended +	1	0	2	1	4	3.3
Original- / Extended-	2	16	20	34	72	59.5
RAST positive	30	9	9	1	49	40.5
RAST positive (%)	93.6	36.0	31.0	2.9		

correlations the r-values were ≥ 0.80 and in 98 ≥ 0.70 . In only three cases were they < 0.40 . In five of the seven highest correlations *Aureobasidium* was involved. There was no outstandingly high correlation between the two moulds *Alternaria* and *Stemphylium* ($r = 0.66$), both of which contain the antigen Alt 1.

Altogether, 28 patients had multiple (≥ 7) positive mould RAST responses. Of these, 25 belonged to Groups A and B with many asthmatic symptoms. No single mould allergen RAST indicated which patients were most likely to have multiple mould sensitization. However, many

two-mould combinations (Table 6) effectively detected these patients, particularly combinations of *Aureobasidium*, *Botrytis*, *Candida*, *Cladosporium*, *Helminthosporium*, *Penicillium* and *Stemphylium* with each other, or with most other moulds, are nearly 100% effective in detecting these highly mould-sensitive patients.

DISCUSSION

The patient selection used can be considered to have yielded a material representative of all

Table 4.

Frequency of positive RAST (classes 2-4) to the different moulds in Groups A - D. The genera are presented in decreasing total frequency of positive RAST responses

Mould	Groups										Total	
	A		B		C		D					
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)		
m2	<i>Cladosporium</i>	24	(80)	6	(24)	4	(14)	0		34	(28)	27
m5	<i>Candida</i>	23	(77)	7	(28)	4	(14)	0		34	(28)	26
m8	<i>Helminthosporium</i>	23	(77)	3	(12)	6	(21)	0		32	(26)	26
m7	<i>Botrytis</i>	24	(80)	3	(12)	2	(7)	0		29	(24)	26
m12	<i>Aureobasidium</i>	22	(73)	2	(8)	3	(10)	0		27	(22)	25
m9	<i>Fusarium</i>	18	(60)	6	(24)	2	(7)	1	(3)	27	(22)	22
m10	<i>Stemphylium</i>	22	(73)	1	(4)	4	(14)	0		27	(22)	25
m1	<i>Penicillium</i>	20	(67)	3	(12)	3	(10)	0		26	(21)	24
m4	<i>Mucor</i>	18	(60)	3	(12)	2	(7)	0		23	(19)	17
m6	<i>Alternaria</i>	19	(63)	0		3	(10)	0		22	(18)	20
m13	<i>Phoma</i>	18	(60)	1	(4)	3	(10)	0		22	(18)	19
m16	<i>Curvularia</i>	16	(53)	0		2	(7)	0		18	(15)	18
m3	<i>Aspergillus</i>	14	(47)	1	(4)	2	(7)	0		17	(14)	17
m15	<i>Trichoderma</i>	12	(40)	2	(8)	1	(3)	0		15	(12)	14
m11	<i>Rhizopus</i>	9	(30)	0		1	(3)	0		10	(8)	10
m14	<i>Epicoccum</i>	7	(23)	0		2	(7)	0		9	(7)	9

The last column on the right indicates the number of patients associated with multiple (≥ 7) mould sensitivity.

types of mould-related asthmatics in our asthma register. However, mould-allergic patients are overrepresented, because the study groups A, B and C (many asthmatic symptoms and/or previously positive skin tests to moulds) were purposely required.

The results of the present study are in line with previous ones (2, 4, 5, 13), showing the diagnostic value of the RAST in mould allergy. Compared with the original panel the extended panel detected four new positive patients with mould allergy, and up to 15% of new cases of mould allergy in Scandinavian patients (12) and up to 27% in sera from USA (9). One of the new positive mould RAST results was found in Group D where neither symptoms nor SPT indicated mould allergy.

Comparing the results with the findings by Jonsson et al. (9) and Foucard et al. (8) showed roughly the same most frequently occurring positive responses: *Cladosporium*, *Candida*, *Helminthosporium* and *Botrytis*, indicating a similar pattern of sensitization in Finland and other Scandinavian countries. Two middle-European materials (12, 17) suggest *Alternaria* sensitivity to be more common there than the above mentioned.

Concordances between the positive mould RAST were generally high. The highest were between *Aureobasidium-Stemphylium* (97%) and *Botrytis-Helminthosporium* (89%). Five of seven concordances of 85% or more were between *Aureobasidium* and other moulds. A high concordance between two genera may indicate that the two genera combined share the same (major) allergen(s), or that patients are sensitized separately to two genera not cross-reacting with each other. In two of the three cases with a low concordance ($\leq 40\%$) *Epicoecum* was involved. This may indicate that *Epicoecum* does not share any antigens with the other genera or, that if *Epicoecum* does, they are only minor ones. The antigen ALT 1 has been found to occur in *Alternaria*, and *Stemphylium* (3). The concordance rate between these two genera was not, however, very high (66%), indicating that the patients studied were not often sensitized to that antigen.

Some of the high and low concordances are similar to those observed by Foucard et al. (8), but many differences exist. This may depend on the patient selection or reflect different sensitization patterns in these two neighboring Scandinavian populations.

Table 5.

Positive mould RAST correlations (%) between the 16 moulds tested. Two lowermost rows indicate the number of $\geq 80\%$ and $\geq 70\%$ correlations of the mould in question

	ALT	ASP	AUR	BOT	CAN	CLA	CUR	EPI	FUS	HEL	MUC	PEN	PHO	RHI	STE	TRI
ALT	100															
ASP	62	100														
AUR	64	73	100													
BOT	63	80	85	100												
CAN	47	44	69	78	100											
CLA	60	73	85	73	56	100										
CUR	53	52	75	74	60	60	100									
EPI	56	42	58	48	47	62	45	100								
FUS	56	56	71	78	86	61	59	45	100							
HEL	67	70	86	89	81	80	67	61	75	100						
MUC	46	64	76	80	77	58	54	33	76	75	100					
PEN	63	63	75	76	58	68	70	30	55	69	67	100				
PHO	61	82	85	74	48	83	66	59	52	77	54	60	100			
RHI	39	40	65	59	60	41	65	42	60	54	68	45	44	100		
STE	66	73	97	84	70	83	78	61	71	84	77	77	83	62	100	
TRI	59	50	75	76	82	64	70	68	84	75	67	51	56	59	77	100
$\Sigma \geq 80\%$	0	2	5	5	3	4	0	0	2	5	1	0	4	0	5	2
$\Sigma \geq 70\%$	0	6	11	12	6	6	5	0	7	10	6	4	6	0	12	7

Table 6.

Efficacy (%) of duplicate combinations of positive mould RAST results in detecting multiple (≥ 7 positive) mould sensitizations

	ALT	ASP	AUR	BOT	CAN	CLA	CUR	EPI	FUS	HEL	MUC	PEN	PHO	RHI	STE	TRI
ALT	71															
ASP	86	61														
AUR	93	93	89													
BOT	100	93	100	93												
CAN	100	100	96	100	93											
CLA	96	96	100	100	100	96										
CUR	79	86	93	96	93	96	61									
EPI	79	75	93	100	93	96	68	32								
FUS	89	86	96	93	100	100	93	89	79							
HEL	96	93	100	96	100	100	96	96	96	93						
MUC	82	86	96	96	96	100	82	79	86	93	61					
PEN	93	96	100	100	96	96	86	86	96	100	93	86				
PHO	82	79	96	100	100	96	86	71	93	96	89	96	68			
RHI	79	71	89	93	93	96	68	54	82	93	64	86	82	36		
STE	89	89	96	100	100	96	89	89	100	96	96	96	89	89	89	
TRI	82	82	93	96	93	100	71	61	82	100	75	89	82	64	100	50
$\Sigma 100\%$	2	1	4	8	8	7	0	1	3	5	1	3	2	0	4	3

Explanation: The lowermost row indicates the number of pairs of 100 % detections of the mould RAST in question. The last figure of each row indicates the detection of multiple mould sensitization by the single mould RAST in question from the whole material.

Only slight exposure appears sufficient to sensitize. Examples of frequent sensitization without high general atmospheric exposure (11) are *Aureobasidium* (22 %), *Botrytis* (24 %), *Helminthosporium* (26 %) and *Stemphylium* (22 %). Exposure to these genera is likely as human beings spread mould spores into the air by their own outdoor activities. This is exemplified by the finding that in Finland *Botrytis* and *Helminthosporium* occur almost only in the air of childrens' playgrounds (15), but not 10 meters distant. *Helminthosporium* and *Epicoccum* are both moulds with only a small atmospheric occurrence in Finland (11). The observed frequencies of positive RAST results were 26 % and 7 %, respectively. Such a large difference cannot be explained only by the amount of exposure.

It is noteworthy that, due to selection criteria, Group A patients have many symptoms and a high incidence of mould allergy. These patients tended to have multiple mould allergies as well as high SPT scores. They could be effectively pinpointed, using pairs of RAST for *Aureobasidium*, *Botrytis*, *Candida*, *Cladosporium*, *Helminthosporium*, *Penicillium* and *Stemphylium*. With the ex-

ception of *Cladosporium* these moulds occur in Finland only in small quantities. This may indicate that high capacity for developing mould allergy (= multiple sensitization) is best diagnosed by using RAST to moulds which seldom occur in the environment in question.

Malling et al. (13) found RAST had higher specificity but less sensitivity than SPT in the diagnosis of *Cladosporium* allergy in asthmatics. Also in this study the mould RAST panel did not detect all the patients with previously positive SPT to moulds. In clinical practice this means that RAST can give "false-negative" results. One possible cause is the inability to bind some of the mould allergens to a solid phase. The binding capacity of Phadebas RAST discs has since been improved and the sensitivity and specificity of mould RAST are now better (14, 16). In keeping with this we found three patients of 60 with previously negative SPT to moulds to have more than six positive mould RAST results. The question of overall test efficiency for each mould RAST must finally be investigated by comparing with provocation test results.

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