

Common Components in Pollen Extracts

ASGER DIRKSEN and OLE ØSTERBALLE

Laboratory for Clinical Allergology, Rigshospitalet, State University Hospital, Copenhagen, Denmark

In order to evaluate the clinical relevance of testing with various pollen extracts in a multi-center investigation from Denmark, 5,052 patients were skin prick tested with the anamnestic relevant extract from a battery of 48. With the aid of a computer program comparing the extracts, two by two calculations were made over the percentage of patients reacting against allergens present in both extracts. Pollen extracts which frequently produce reaction were divided into three separate groups (tree pollen, grass pollen and herb pollen) within which common allergens were so dominant that it seemed advisable in routine examinations to use one extract and one extract only from each group.

Key words: allergens; cross-reactivity; pollen extracts; skin prick tests.

Accepted for publication 2 April 1980

Pollen allergy is traditionally divided into three groups: tree pollen allergy, grass pollen allergy and herb pollen allergy. Clinical experience has shown that patients who are allergic to one pollen are as a rule also allergic to other pollens in the same group, whereas reactions to pollen from the other two groups does not occur notably often. Biologically the plants are more or less closely related within each group, and a number of studies (2, 3, 4, 6) also suggest that the same allergen or the same allergens are dominant for the various extracts within one group. Through data processing it has now become possible to analyze test data from large patient materials, and to calculate the reaction frequency* for prospective common allergens.

MATERIAL AND METHOD

Data were collected through a multi-center investigation (1 April 1978 to 31 March 1979)

*The reaction frequency for an allergen is the percentage of patients tested who react against the allergen extract.

†Other results from this investigation will be published elsewhere.

comprising 5,052 patients from all over Denmark.† Forty-eight different standard allergen extracts from Allergologisk Laboratorium, Denmark, and Dome Laboratories, England, were used. The following report is based exclusively on results from prick tests.

The skin prick test was performed on the volar side of the forearm from approximately three fingers' breadth below the elbow flexure to a hand's breadth above the wrist. The prick in the skin was made with an ordinary blood lancet directed through the allergen drops at an angle of 60–70° to the skin, the lancet point only "catching" the skin and lifting it, thus making a canal through which the extract could penetrate intracutaneously. The lancet was then pulled out carefully, and after 30 to 60 sec, the drop was wiped off. Histamine HCl 1 mg/ml was used as a reference for recording the skin prick test results. The urticaria reactions were read after 10–20 min and designated as positive when equal to or higher than the histamine reference. Skin prick testing has been shown to correlate well with the clinical history and provocation test (1).

		extract X	
		neg.	pos.
extract Y	neg.	a	b
	pos.	c	d

Fig. 1. 2×2 contingency table: a. number of patients allergic to neither X or Y; b. number of patients allergic to X, but not to Y; c. number of patients allergic to Y, but not to X; d. number of patients allergic to both X and Y.

The allergen extracts were compared two at a time in 2×2 contingency tables (Fig. 1), and the reaction frequency f for common allergens was calculated according to the formula:

$$f = \frac{a \times d - b \times c}{a \times (a + b + c + d)}$$

Where x and y are the reaction frequencies for the specific allergens in the two extracts included in the 2×2 contingency table, on the basis of independence between reaction to different allergens the following can be drawn up:

$$\begin{cases} a = (a + b + c + d)(1 - f)(1 - x)(1 - y) \\ b = (a + b + c + d)(1 - f)x(1 - y) \\ c = (a + b + c + d)(1 - f)(1 - x)y \\ d = (a + b + c + d)(f + xy(1 - f)) \end{cases}$$

⇕

$$\begin{cases} \frac{a}{b} = \frac{1-x}{x} \Leftrightarrow ax = b - bx \Leftrightarrow x = \frac{b}{a+b} \\ \frac{a}{c} = \frac{1-y}{y} \Leftrightarrow ay = c - cy \Leftrightarrow y = \frac{c}{a+c} \\ \frac{d}{a+b+c+d} = f + \frac{bc}{(a+b)(a+c)}(1-f) \end{cases}$$

⇕

$$f \left(1 - \frac{bc}{(a+b)(a+c)} \right) = \frac{d}{a+b+c+d} - \frac{bc}{(a+b)(a+c)}$$

⇕

$$\begin{aligned} f &= \frac{\frac{d}{a+b+c+d} - \frac{bc}{(a+b)(a+c)}}{1 - \frac{bc}{(a+b)(a+c)}} \\ &= \frac{d(a+b)(a+c) - bc(a+b+c+d)}{(a+b+c+d)(a+b)(a+c) - bc(a+b+c+d)} \\ &= \frac{a^2d + abd + acd + bcd - abc - b^2c - bc^2 - bcd}{(a+b+c+d)(a^2 + ab + ac)} \\ &= \frac{(ad - bc)(a+b+c)}{(a+b+c+d)a(a+b+c)} \\ &= \frac{ad - bc}{a(a+b+c+d)} \end{aligned}$$

RESULTS

The 48 allergen extracts are shown in Fig. 2. Indicated in the first column are the number of patients tested with the extract in question, and in the second column the percentage of patients reacting positively. In the Figure the greater the f -values the darker the fields so that when more than 10% of the patients included in the 2×2 contingency table react against common allergens the field is completely black. The black fields with one exception (dahlia-ryegrass) are found only where pollen from the same group are compared, and conversely with three exceptions (alder/beech, beech/hazel, reed/smooth meadow grass) there are always black fields where pollen extracts to which more than 7% of the patients react are compared. There is apparently a certain allergen interrelationship between pollen groups, although this interrelationship is not nearly so pronounced as within the groups. House dust extract, as expected, has allergens in common with a number of other extracts.

Sixty-five of the 85 black fields (76%) are found within the grass pollen group where 12 of the allergen extracts almost exclusively have black fields. In Table 1 the relations within the grass pollen group are more closely illustrated.

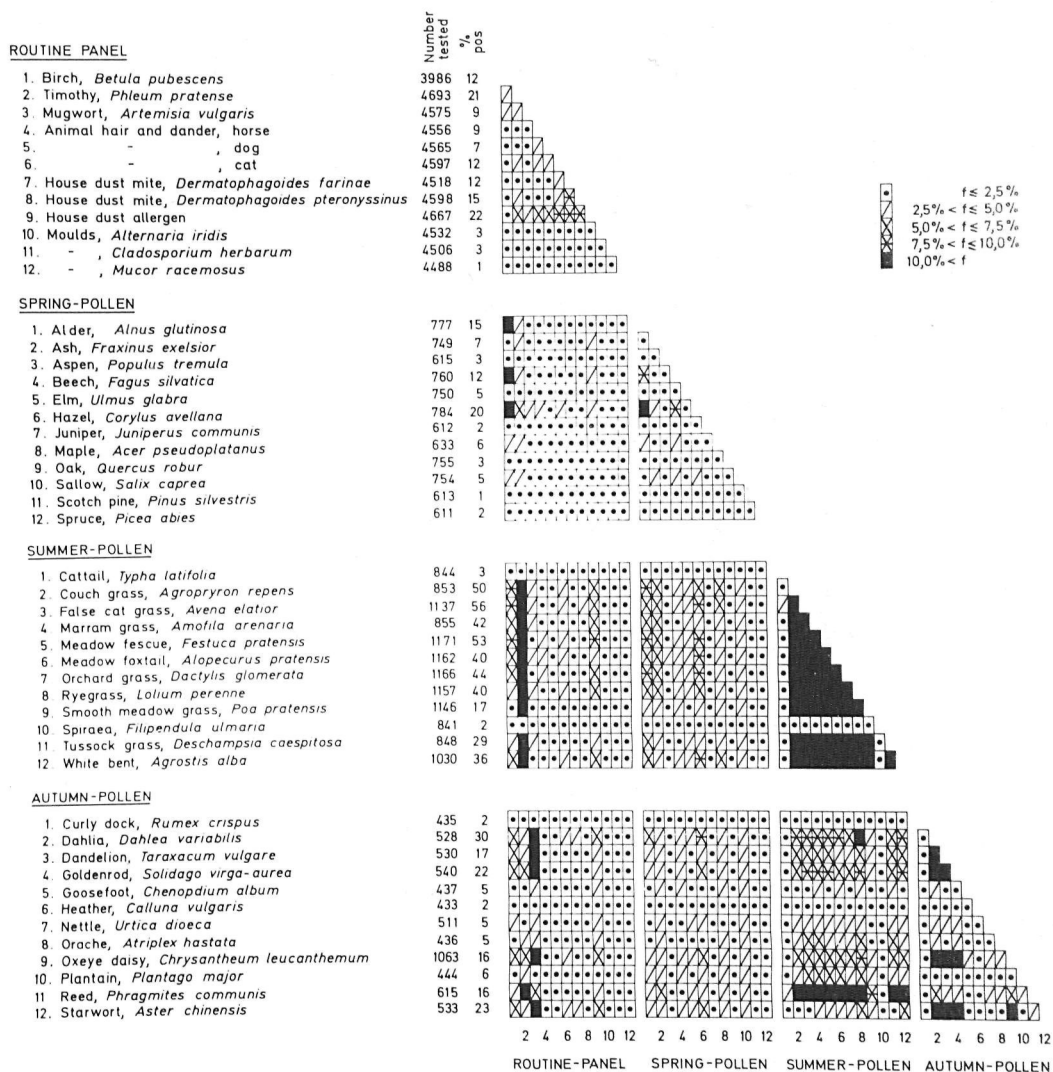


Fig. 2. 48 allergens and their common components expressed in f which is the percentage of patients who react to allergens common for both extracts.

The upper righthand triangle of the table gives the 2×2 contingency tables for the grass pollen extracts. The table includes only those 802 patients tested with all 11 grass pollens*, i.e. the same patients are included in all of the 2×2 contingency tables. The lower

lefthand triangle gives the f-values for the 2×2 contingency tables. The grass pollen extracts are listed according to reaction frequency, which appears in the first column of the table. It is noted that common allergens throughout are included in more than 80% of the observed reactions. In addition it is seen that f varies considerably even though these same patients are included in the calculation of all of the f-values.

*Reed is not included since it would have reduced the patient number to 258 if all patients were to have been tested with reed extract.

Table 1.
2 × 2 contingency tables (upper righthand triangle) and f-values (lower lefthand triangle) for 11 grass pollen extracts

	Reaction frequency	False cat grass	Meadow fescue	Timothy	Couch grass	Orchard grass	Ryegrass	Marram grass	Meadow foxtail	White bent	Tussock grass	Smooth meadow grass
False cat grass	56		293 59 56 394	293 59 85 365	312 40 94 356	328 24 102 348	314 38 142 308	340 12 130 320	333 19 139 311	331 21 159 291	341 11 236 214	349 3 305 145
Meadow fescue	56	48		280 69 98 355	312 37 94 359	317 32 113 340	324 25 132 321	336 13 134 319	326 23 146 307	323 26 167 286	335 14 242 211	344 5 310 143
Timothy	53	43	41		301 77 105 319	323 55 107 317	317 61 139 285	333 45 137 287	336 42 136 288	331 47 159 265	350 28 227 197	367 11 287 137
Couch grass	49	43	43	36		354 52 76 320	358 48 98 298	365 41 105 291	356 50 116 280	377 29 113 283	385 21 192 204	395 11 259 137
Orchard grass	46	42	41	37	39		363 67 93 279	393 37 77 295	388 42 84 288	394 36 96 276	408 22 169 203	424 6 230 142
Ryegrass	43	36	39	32	36	33		399 67 81 265	374 82 98 248	391 65 99 247	435 21 142 204	437 19 217 129
Marram grass	41	39	39	33	35	36	31		405 65 67 265	415 55 75 257	446 24 131 201	458 12 196 136
Meadow foxtail	41	38	37	34	33	35	28	32		406 66 84 246	436 36 141 189	465 7 189 141
White bent	39	35	34	30	34	33	29	31	29		452 38 125 187	475 15 179 133
Tussock grass	28	26	25	22	24	24	25	24	22	22		543 34 111 114
Smooth meadow grass	18	18	17	16	16	17	15	16	17	16	13	

Table 2.

2 × 2 contingency tables (upper righthand triangle) and f-values (lower lefthand triangle) for four tree pollen extracts

	Reaction frequency	Birch	Hazel	Alder	Beech
Birch	45		303 26 157 110	316 13 180 87	321 8 197 70
Hazel	23	16		436 24 60 76	446 14 72 64
Alder	17	13	12		470 26 48 52
Beech	13	11	10	8	

DISCUSSION

The variation in f-values in Table 1 may be attributable either to a varying number of common allergens or a varying concentration of common allergens in the different extracts.

The most thought-provoking finding in Table 1 is the very high f-values. In the calculation of f we have assumed independence between reactions against different allergens and therein failed to take into consideration that allergy against one agent disposes to allergy against other agents (multi-allergy). That this phenomenon is not especially pronounced in our findings can be seen in

Fig. 2 where, for example, *Dermatophagoides pteronyssinus* extract, which 15% of the patients reacted positively against, shows f-values of less than 5% against other extracts. The only exception here being against the other mite extract and against house dust, and *D. pteronyssinus* clearly has allergens in common with both of these. It can thus be concluded that the majority of the f-values in Table 1 are exponents of allergens common to the extracts. The uncertainty with the prick test is empirically considerable, and this uncertainty tends to give a lower f-value than one corresponding to the actual common component.

We believe that the f-values in Table 1, in agreement with biochemical findings (5), are an indication that over 90% of the reactions against grass pollen are attributable to reactions against allergens common for the various grass pollen extracts, and it seems therefore advisable in routine examinations to utilize one grass pollen extract and one grass pollen extract only, and it should be one against which relatively many patients react.

Tables 2 and 3 demonstrate the corresponding relations for tree and herb pollen, and here it would seem sufficient, in routine examinations, to test with birch and mugwort extract.

Table 3.

2 × 2 contingency tables (upper righthand triangle) and f-values (lower lefthand triangle) for six herb pollen extracts

	Reaction frequency	Mugwort	Dahlia	Oxeye daisy	Starwort	Goldenrod	Dandelion
Mugwort	38		288 23 57 130	294 17 68 119	302 9 81 106	299 12 84 103	308 3 108 79
Dahlia	31	25		314 31 48 105	333 12 50 103	323 22 60 93	338 7 78 75
Oxeye daisy	27	23	20		340 22 43 93	336 26 47 89	348 14 68 68
Starwort	23	21	20	18		351 32 32 83	367 16 49 66
Goldenrod	23	20	18	17	16		374 9 42 73
Dandelion	16	16	15	13	13	14	

ACKNOWLEDGEMENT

We are indebted to statistician Jørgen Nyboe for inspiring discussions.

REFERENCES

1. Aas, K., Backman, A., Belin, L. & Weeke, B.: Standardization of allergen extracts with appropriate methods. The combined use of skin prick testing and radio-allergosorbent tests. *Allergy* 33, 130–137, 1978.
2. Bernstein, I. L., Perera, M., Gallagher, J., Michael, G. & Johansson, S. G. O.: In vitro cross-allergenicity of major aeroallergenic pollens by the radioallergosorbent technique. *J. Allergy Clin. Immunol.* 57, 141–152, 1976.
3. Leiferman, K. M. & Gleich, G. J.: The cross-reactivity of IgE antibodies with pollen allergens. I. Analysis of various species of grass pollens. *J. Allergy Clin. Immunol.* 58, 129–139, 1976.
4. Löwenstein, H.: Immunological partial identity and in vitro inhibitory effect of two major timothy pollen allergens to whole pollen extract of four grasses. *Int. Arch. Allergy Appl. Immunol.* 57, 379–383, 1978.
5. Löwenstein, H.: Cross reactions among pollen antigens. *Allergy* 35, 198–200, 1980.
6. Tangen, O. & Nilsson, B. E.: Grass allergens: A preliminary characterization by means of RAST-based allergen assay methods. *Dev. Biol. Stand.* 29, 175–187, 1975.

Address:

Asgar Dirksen
 Laboratory for Clinical Allergology
 Rigshospitalet
 Blegdamsvej 9
 DK-2100 Copenhagen Ø
 Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.