

Allergy indices based on allergen dose-response curve in a randomly selected sample of schoolchildren

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Skin-test sensitivity was computed in 61 schoolchildren drawn from a stratified random sample in order to generate two allergy indices based on a quantitative skin-test assay: Individual Allergy Index (IAI) and Population Allergy Index (PAI). The former to quantitate IgE-mediated skin test sensitivity of each subject, the latter to describe the degree of skin reactivity of our sample to a single allergen. Five allergen extracts were tested at 4-fold dilutions. Sensitivity endpoint data were used to generate IAI. Frequency distribution of IAI seemed to be bimodal as asymptomatic subjects gathered at the lower part while symptomatic children showed a peak in the middle. By means of criteria based on the relation between IAI and the prevalence of symptoms we were able to classify atopy in five classes from normal (all asymptomatic subjects) to severe atopy (all symptomatic subjects). Each positive reaction was plotted as a function of the allergen concentration eliciting the weal, and linear regression with confidence limits was calculated for grass and *Dermatophagoides pteronyssinus* only. PAI was represented by the slope, which depends on the reactivity of the sample, and the intercept, which involves the potency of the allergen as well. Grass and *D. pteronyssinus* showed the same slope while the intercepts differed. The slope increased if we considered symptomatic subjects only. We propose both these indices for clinical and epidemiological studies.

Key words: allergic disorders; Allergy Indices; atopic status classification; epidemiology of allergy; quantitative skin test; skin prick test.

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CLINICAL ASPECTS

Skin prick testing has been used as a standard tool in the clinical evaluation of allergy for many years. However, positive skin tests may be found in individuals without allergic symptoms, and a positive reaction can be unrelated to clinical findings. Furthermore, up to now arbitrary grading systems have been used to discriminate both between positive and negative reactions and among different degrees of positive response. We studied 61 skin-test positive children drawn from a stratified random sample. To grade the total reaction of each child to the skin tests applied we tested five allergen extracts at four concentrations and computed an Individual Allergy Index based on sensitivity endpoint data. This index allowed us to classify atopy in five symptom classes and can be useful for following how skin sensitivity varies with age, immunotherapy, and time of the year in the case of pollen allergen. We recommend the use of this index in clinical practice.

Skin testing has been used as a diagnostic tool in the clinical evaluation of allergy and as a quantitative technique for measuring allergen potency. In recent years great effort has been made to standardize extracts and technique (1,

16, 19) in order to improve the reliability of this bioassay. While there is agreement about the technique of allergy skin testing (potent allergen, correct needles and technique, adequate distance between sites, proper controls

and carefully controlled time of reading), results and grading systems are arbitrary. It has been recommended that skin tests be interpreted in relation to the size of the reaction of histamine hydrochloride (1). The simplest grading system proposed (16) (+ + : weal 1/2 histamine; + + + : weal = histamine; + + + + : weal 2 × histamine) is of use to the clinician but cannot be used in epidemiological studies in which quantitative methods are required.

Apart from one study (3), arbitrary cutoff points have been used to discriminate between positive and negative reactions or among different degrees of positive response.

In a population sample skin test reactivity can be distinctly defined in two main aspects whether studying: 1) the distribution of positive skin tests within the population or 2) the degree of reactivity of a single allergen within the studied group. An allergy index which grades the total reaction of each individual to skin tests allows us to describe the distribution of skin reactivity in a sample. This index cannot be constructed by the simple addition of the size of the individual reactions (5, 6) because these tend to reflect primarily the individual results that produce the largest reactions (grass in Northern Italy), or by the number of positive skin tests (21). Up until now the degree of skin reactivity of a single allergen within a population has been defined either by weal ratios (allergen weal/histamine 1 mg/ml weal, HEP units) and results expressed as "median HEP", which is the median of individual HEP values in subjects with positive reactions to the allergen in question (10), or simply as the frequency distribution of prick test size in the sample (3).

The purpose of this study was to propose two allergy indices obtained by quantitative skin test assay: Individual Allergy Index (IAI) and Population Allergy Index (PAI). The former should be used to classify the subjects as having mild, moderate or marked allergy, the latter to describe the degree of skin reactivity of a sample group to a single allergen, thus allowing us to compare different populations and different allergens.

MATERIAL AND METHODS

Subject selection

The study group comprised 61 children (age range 11–14, 41 M and 20 F), representing all skin test positive subjects of a larger randomly selected sample (240 schoolchildren) from a study on bronchial responsiveness being carried out in the Verbania (Novara, Italy) area.

History

Data on respiratory symptoms were obtained from a questionnaire which was completed by the parents. The questionnaire incorporated the American Thoracic Society children's questionnaire (7) expanded to include questions on rhinitis and allergies and further questions developed through a previous pilot study. Experienced interviewers collected the questionnaires and clarified any problems concerning the questions.

Allergens

All the allergen extracts in this trial were supplied by Bayropharm DHS, Milan. A screening prick test previously used in the entire population was performed with raw extracts (of grass, Dermatophagoides mix, Parietaria, birch and mugwort). Each of these allergen extracts used in endpoint titration underwent a mild aqueous extraction process, were partially purified by dialysis (hollon fibers cut-off 5000 daltons) and lyophilized to maintain allergenic activity. The dialyzed extract is termed "alpha fraction" and standardized for diagnostic purposes in AUR (Activity Units by RAST) (20). The chemical component is characterized by PNU, moisture content, isoelectric focusing, and the immunological activity by such methods as crossed immunoelectrophoresis (CIE), crossed radioimmunoelectrophoresis (CRIE) and radio allergosorbent assay (RAST).

Endpoint prick test solution

Lyophilized alpha-fractions were reconstituted in phosphate buffered saline (PBS): 0.85 %

NaCl, 0.087 % Na_2HPO_4 , 0.1 % NaN_3 , 0.012 % KH_2PO_4) diluted and dispensed into sterile vials at concentrations of 400, 100, 25, 6.25 AUR/ml, and freeze-dried. Each vial was reconstituted at the time of the prick test.

Skin prick test

All subjects were skin tested by the same investigator between 8.30 a.m. and 11 a.m. to avoid any possible circadian difference in cutaneous reactivity (10). Prick tests were performed with a disposable "Allergy pricker" on the volar surfaces of both forearms some distance from the wrists and antecubital area (9, 18). Care was taken to avoid pricking too deeply so as not to cause bleeding. After exactly 10 min (histamine) and 15 min (allergen extract) the weals were outlined by a soft ballpoint pen (Pentel) and the markings transferred to mm^2 paper by means of a tape (Boston). After 5-fold magnification by an overhead projector (Maliverno, Neo Solex 5000) each weal was measured in mm^2 using a planimeter (Salmoiraghi 261). Reproducibility of the double skin test determination was calculated as coefficient of variation = $\text{SD}/\text{mean area} \times 100\%$ (14). We accepted skin prick tests with a coefficient of variation of less than 40 % (area), equivalent to 20 % measured by diameter (1, 14).

a) *Screening prick tests*. Allergen drops plus histamine dihydrochloride (10 mg/ml) and diluent were applied in random order. Weals $\geq 3 \text{ mm}^2$ in area were arbitrarily termed positive in the absence of a reaction to the negative control solution.

b) *Endpoint titration*. Each skin test reactor was prick-tested in duplicate on both forearms. The allergen dilution and control were applied in a clockwise sequence, subject by subject, in order to reduce the site to site difference in skin sensitivity in the overall group. Histamine dihydrochloride (1 mg/ml) was used as control.

Computation of allergic sensitivity

The skin response at serial doses was used to obtain dose-response curves. As the allergen concentration increases, the dose-response

curve rapidly reaches a plateau. We therefore tested the doses which allowed us to explore the degree of skin reactivity from the plateau portion (400 AUR) to the endpoint (6.25 AUR). Each concentration was expressed as logarithm (base 4 since the dilutions were 4-fold (19)), and skin response was computed as the ratio between allergen weal area (SA) and histamine weal area (SH).

a) *Skin test threshold*. The mean and standard deviation of the duplicate of the histamine weal areas in all subjects was calculated. The weal equal to mean histamine area (14.2 mm^2 , range 4–31, $\text{SD} \pm 6.0$) minus 2 SD (12 mm^2) was considered as the weal threshold area. In other words, in our sample a weal area of 2.2 mm^2 was required to obtain the probability of 0.95 that the reaction was due to histamine release. Previous studies (15, 17) showed comparable values of mean histamine weal area and SD.

b) *Individual Allergy Index*. Skin test sensitivity toward each allergen was estimated as endpoint (the lowest positive concentration) and this value was used to compute the IAI. Computation proceeded as follows: – Results were scored as $1:\log$ (base 4) of the lowest concentration (inverse endpoint concentration), so we obtained a positive relation with skin reactivity (as skin response at lower concentrations means a high degree of skin reactivity). – For each subject the sum of scores for each allergen was called IAI. In other words, in a subject with two positive reactions (Grass: endpoint = 400 AUR, $1:\log_4 400 = 0.23$; *D. pteronyssinus*: endpoint = 100 AUR, $1:\log_4 100 = 0.30$) the IAI was 0.53.

c) *Population Allergy Index*. PAI was based on linear regression on dose-response curves from the total number of subjects. In all subjects the ratio between SA and SH was calculated for each concentration causing a positive reaction. This value was considered as a "statistic unit" and was plotted as a function of allergen concentration ($\log$) eliciting the weal. The best fit regression function was found by using a set of regression functions (family regression Hewlett Packard 9825 A software vol. 1). The regression was computed for grass and *D. pteronyssinus*. Confidence limits (95 %) were also computed.

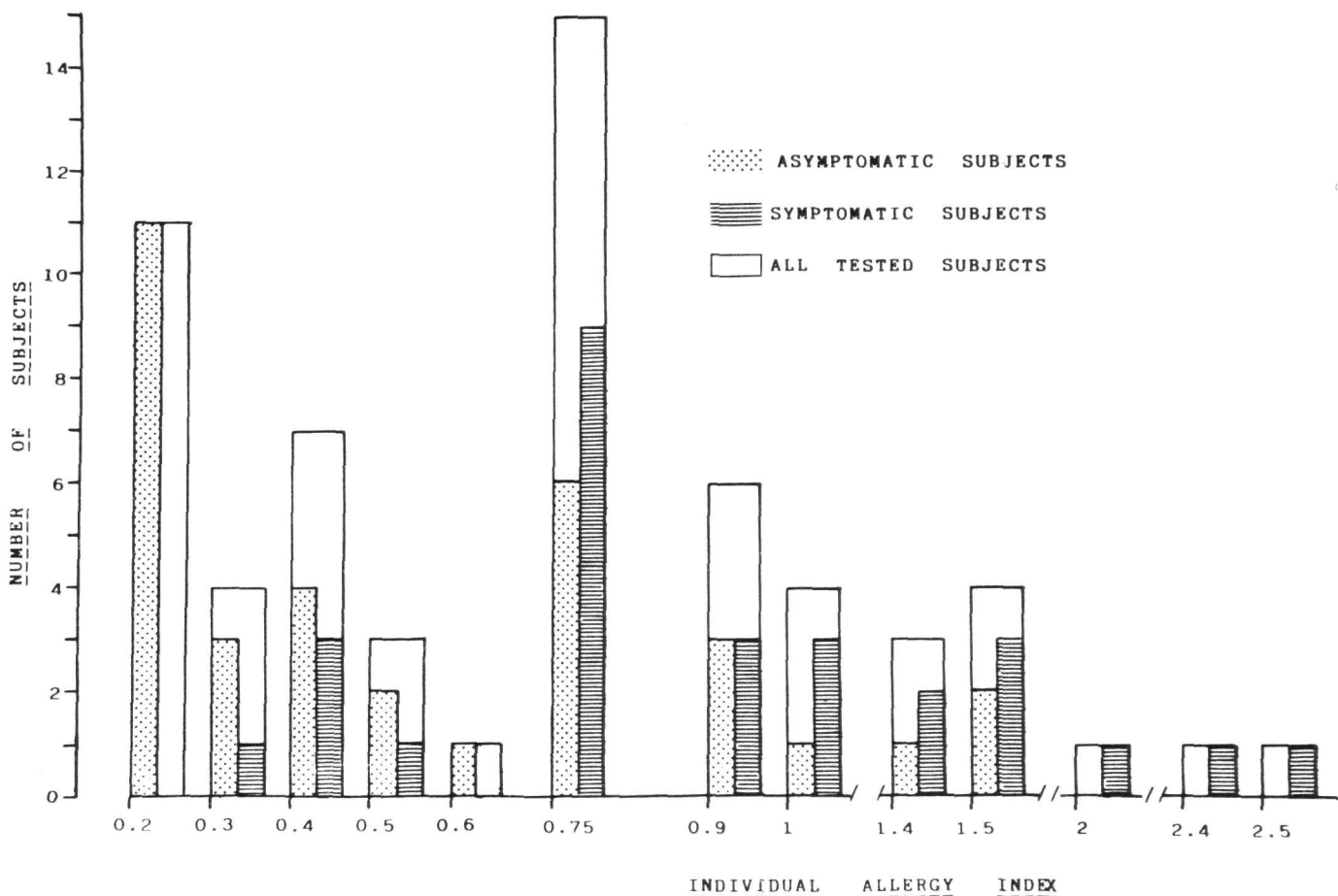


Fig. 1. Prevalence of allergic disease (asthma, rhinitis) and absence of symptoms compared to IAI data.

RESULTS

Prevalence of endpoint (lowest concentration at which a positive reaction was obtained) to the five allergens is shown in Table 1. All subjects were reactors to either grass only (24/39 %), *Dermatophagoides pteronyssinus* only (20/32 %) or to both allergens (17/28 %). Two of these 17 had an equal endpoint to both allergens. The prevalence of grass and *D. pteronyssinus* reactions was about equal (67 % and 61 % respectively). Eleven and nine subjects were skin-test positive to birch and mugwort, while positivity to Parietaria was the least prevalent (2 subjects). In the overall group we found no differences in reaction prevalence between raw extract and AUR as regards grass and *D. pteronyssinus*, while five of 14 mugwort responders to raw extract showed no reaction to endpoint titration up to 400 AUR. In Fig. 1 the prevalence of allergic disease (asthma, rhinitis) and absence of symptoms was compared to IAI data. Asymp-

tomatic subjects gathered at the lower end (IAI = 0.23, endpoint 400 AUR), but peaked also at mean IAI = 0.75. Symptomatic subjects showed a peak at 0.75. A close relationship ($r = 0.80$) was found between IAI and the number of positive reactions. Atopy could be categorized with respect to severity by means of criteria based on the relation between IAI and prevalence of symptoms (Table 2). We obtained a score index for each

Table 1
Prevalence of endpoint to the five allergens

Lowest concentration to obtain a positive reaction	Number of individuals = 61				
	Grass	<i>D. pt.</i>	Mugwort	Parietaria	Birch
400 AUR	10	7	2	2	3
100 AUR	7	6	-	-	1
25 AUR	8	8	2	-	3
6.25 AUR	16	16	5	-	4

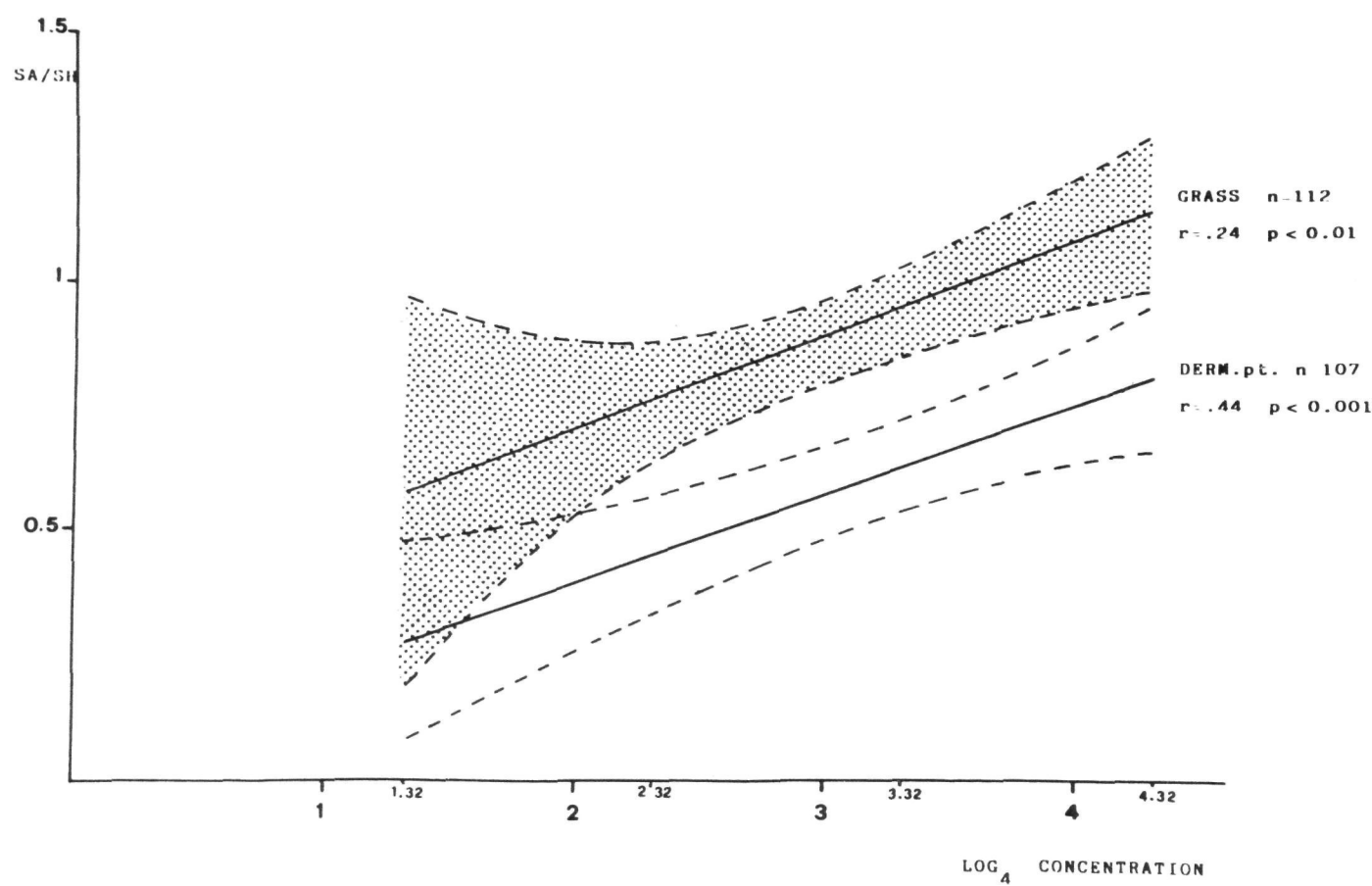


Fig. 2. Regression of (log₄) concentration of allergen extract in AUR (horizontal axis) and ratio between allergen weal area and histamine (1 mg/ml) weal area (vertical axis) in the overall group. 95 % confidence limits are shown.

Table 2				
Classification of atopic status based on the relation between Individual Allergy Index (IAI) and prevalence of symptoms				
Grade		IAI	Criteria	Our sample
I.	"normal"	= 0.23	all asymptomatic subjects	only one allergen positive at highest concentration (400 AUR)
II.	mild	> 0.23 ≤ 0.53	"normal" subjects 66 % (2/3) symptomatic subjects 34 % (1/3)	two allergens positive at highest concentration (400 AUR, 100 AUR) or one allergen at 25 AUR
III.	moderate	> 0.53 ≤ 0.75	prevalence of symptomatic and asymptomatic subjects is equal	one allergen positive at lowest concentration (6.25) or more allergens at higher (400 AUR, 100 AUR).
IV.	marked	> 0.75 ≤ 1.50	asymptomatic subjects 34 % (1/3), symptomatic subjects 66 % (2/3)	up to two allergens at lowest concentration (6.25)
V.	severe	> 1.50	all symptomatic subjects	more than two allergens at lowest concentration (6.25)

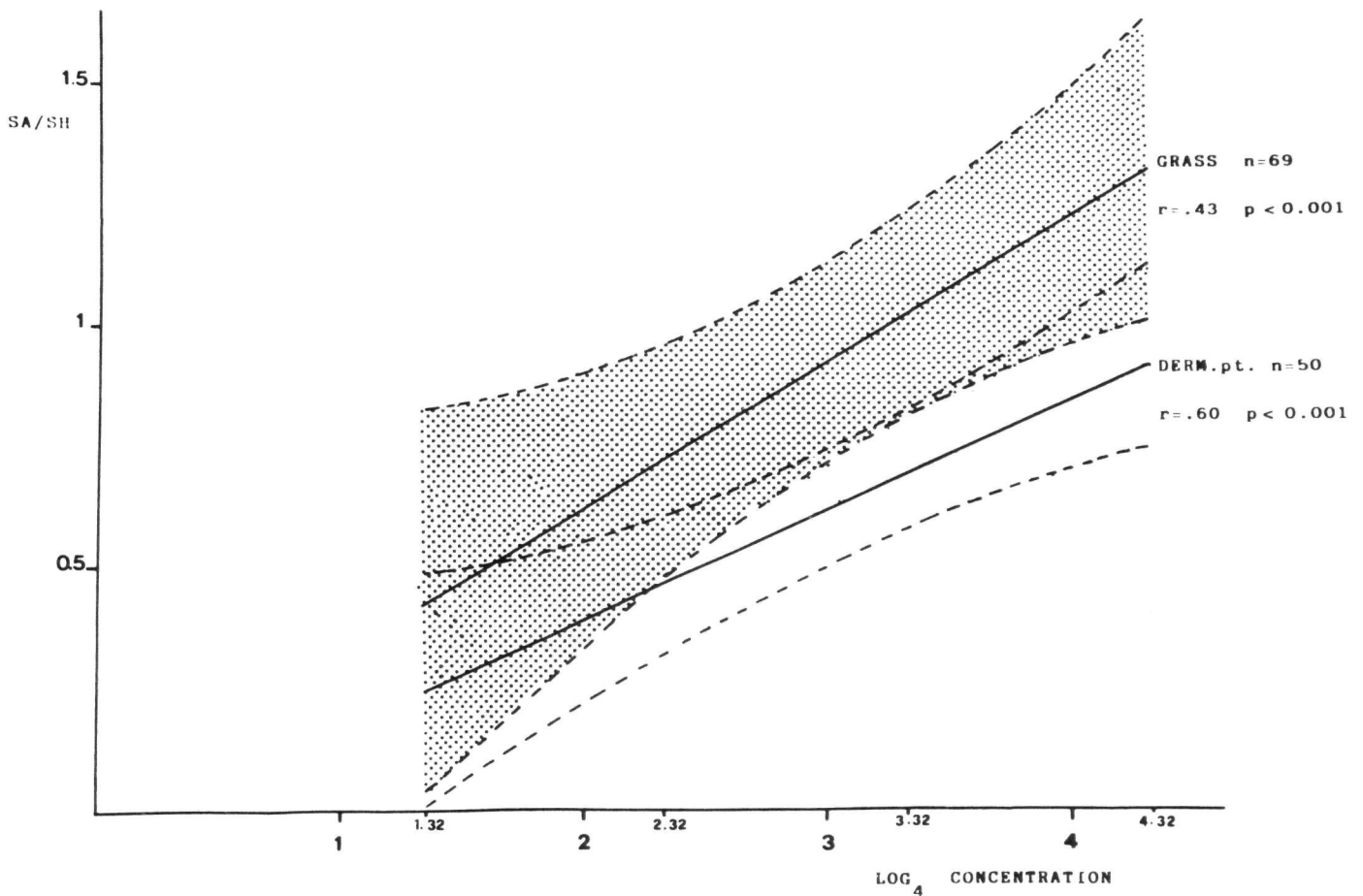


Fig. 3. Regression of ($\log_4$) concentration of allergen extract and ratio between allergen weal area and histamine (1 mg/ml) weal area in symptomatic subjects only.

class by determining the prevalence of symptomatic subjects along the distribution curve of IAI (Fig. 1).

To determine the PAI we excluded skin test data for birch, mugwort and *Parietaria* since the prevalence of positive reactions to these allergens was low. The best regression function was linear. Linear regression with confidence limits was calculated for grass (112 statistic units) and *D. pteronyssinus* (107 statistic units) (Fig. 2). The slopes of regression lines ($y = ax + b$) were almost identical (grass: $a = 0.189$, *D. pteronyssinus*: $a = 0.181$) while intercepts differed (grass: $b = 0.326$, *D. pteronyssinus*: $b = 0.033$). Ninety-five percent confidence limits of the two allergens did not overlap except at the lowest concentration. If we consider symptomatic subjects only (Fig. 3), slopes became steeper for both allergens (grass: $a = 0.30$, *D. pteronyssinus*: $a = 0.23$) than in the overall population.

DISCUSSION

This study was planned to check the reliability of an innovative technique for quantifying atopy and to describe the degree of reactivity to a single allergen in a sample. We were able to assess the distribution and degrees of atopy on the basis of the prevalence of the asymptomatic subjects in each class. Due to differences in methods (number, type and activity of allergen extracts) and in sample selection criteria, skin test reaction rates have varied (12, 21). However, the prevalence of positive skin test reactions in our group differed only slightly from that found by others (3, 10). We limited the panel of skin test reagents to the most common inhalant allergens without cross-reactivity. The high prevalence of grass and *D. pteronyssinus* confirms that the allergens selected were the most relevant in the studied area.

Important features of our study were the use

of high-quality extracts, an experienced team of the same operators throughout the study, and a carefully standardized technique. Measuring the weal by planimetry after magnifying its circumference five-fold added precision to our method. We found a difference in the prevalence of positive reactions obtained by the screening tests with raw extracts and the end-point titration with AUR standardized extracts. This was noteworthy for mugwort (14 vs 5) and Parietaria (4 vs 2). In agreement with Freed et al. (8), this could mean that the skin responses obtained by screening tests were due to non-allergenic components of raw extracts.

CONCLUSION

Individual Allergy Index

We found it useful to describe the distribution of atopy both as positive skin tests and with regard to allergic disease. It is noteworthy, but not surprising (11), that some subjects showed a high index without symptoms.

The distribution of the IAI seemed to be bimodal (Fig. 1). The largest class comprised those with only one positive reaction and a high sensitivity. The next peak comprised the asymptomatic subjects with one positive reaction but with low sensitivity. This was not unexpected as it was observed by Barbee et al. (2), even if only the frequency distribution of positive allergen skin tests was taken into account. In addition, distribution of weal ratios (SA/SH) in sensitive subjects could be bimodal (10) depending on human skin test responsiveness (4) and on the allergen tested (10). Bimodal distribution has to be confirmed by using the IAI in larger population studies.

In the classification of atopic status (Table 2) Grade I represents preclinical atopy as there were no symptomatic subjects. The prevalence of symptoms increased from 34 % (mild) to 50 % (moderate), 66 % (marked), up to 100 % in the highly allergic group which included symptomatic subjects only. In the mild and marked classes asymptomatic and symptomatic subjects showed identical prevalence, while moderate atopy represented the middle class

(equal prevalence). Such a classification would assist in determining the grading of atopy in the individual. This is of importance when studying the relationship between atopy and bronchial responsiveness; up until now arbitrary grading systems have been used (6). Furthermore, IAI can be useful in clinical practice to study changes in skin sensitivity according to immunotherapy, age, and seasonal change in IgE-mediated sensitivity in the case of pollen allergens.

Population Allergy Index

Skin response as a function of the concentration of allergen would enable us to use a more rigorous statistical approach in terms of skin test reactivity (slope and intercept of regression line) and distribution (confidence limits) in the studied sample.

The slope of the regression line is an expression of the weal increase as a function of the allergen concentration and depends on the reactivity of the sample. Grass and *D. pteronyssinus* regression lines were parallel in the overall sample (Fig. 2). However, if we consider symptomatic subjects only (Fig. 3), the slope for grass increased more than the slope for *D. pteronyssinus* as symptomatic grass reactors showed a significantly lower mean endpoint than *Dermatophagoides* reactors. We observed that grass elicited larger weals than *D. pteronyssinus* (3); since extract concentration and reactivity (slope) were equal, we can assume that intercept (b) was mainly related to the potency of the allergen. Confidence limits of linear regression describe the distribution of reactivity and depend on the number and sensitivity of the subjects investigated. Grass and *D. pteronyssinus* did not show any overlap except at the lowest concentration, i.e. in the most sensitive subjects (Fig. 2). In symptomatic subjects only, we observed an overlap over the complete range of concentrations (Fig. 3).

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