

Evaluation of the overall degree of sensitization to airborne allergens by a single serologic test: Implications for epidemiologic studies of allergy

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In most epidemiologic studies of respiratory allergy, the overall degree of sensitization is usually measured by indices based on skin prick test reactions to a panel of relevant airborne allergens. In the present study we used a single assay (CAP-Phadiatop, Pharmacia, Uppsala, Sweden), which is based on the reaction between serum-specific IgE and a mixture of locally relevant inhalant allergens to measure the degree of sensitization to inhalants in the sera of 1815 young Italian men, in whom a complete diagnostic workup for respiratory allergy was performed in parallel. The intensity of reaction in the CAP-Phadiatop assay was highly correlated with: (1) an allergy index ($p < 0.005$), based on skin prick test reactivity to seven relevant inhalant allergens (Dermatophagoides pteronyssinus, mixed grass pollens, cat epithelium, Parietaria judaica, Olea europaea, Artemisia vulgaris, Alternaria tenuis); (2) the concentration of specific IgE to the same allergens, measured by CAP-RAST ($p < 0.001$), in both monosensitized and polysensitized subjects; (3) the rate of respiratory allergic diseases ($p < 0.0001$). Therefore CAP-Phadiatop intensity of reaction is a reliable indicator of the overall degree of sensitization to inhalant allergens. Furthermore, as demonstrated by relative operating characteristic analysis, it has an acceptable detectability power in identifying subjects with symptomatic expression of the atopy status [$P(A) = 0.948$]. The evaluation of the degree of sensitization to inhalants by a single and semiautomated assay represents a step forward in the standardization of procedures for epidemiologic studies of inhalant allergic diseases and for mass screening programs, which are aimed at identifying predictive markers of disease susceptibility. (J ALLERGY CLIN IMMUNOL 1994;93:68-79.)

Key words: Allergy, IgE, epidemiology, CAP-Phadiatop, screening

Studies of the epidemiology of atopy during the last decade have demonstrated considerable heterogeneity within the affected populations. Yet variations in definition, allergen source, and potency, as well as assay techniques and interpretation of test results, have often led to discordant conclusions and have demonstrated a need for a better standardization of study protocols.¹⁻⁴

The expression of atopy in the general popula-

Abbreviations used

AI:	Allergy index
FU:	Fluorescence unit
ln:	Natural log
lnFU:	Natural log-transformed fluorescence unit
ROC:	Relative operating characteristic
SPT:	Skin prick test

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tion cannot be completely measured by a single "yes/no" test. The frequency distribution of the degree of atopy in a given population is a more accurate representation of this phenomenon and might allow a better comparison among different studies. Discontinuous (multiple cutoff points)^{5, 6} or continuous^{7, 8} allergy index (AI), based on skin prick test (SPT), have been proposed to quantify the overall degree of sensitization to panels

of locally relevant airborne allergens. In vitro assays for allergen-specific IgE have been used far less than SPT in large epidemiologic surveys, presumably because of their high cost. Since the late 1970s, however, multiple antigen RASTs have been proposed to allow detection of IgE with different specifications in a single assay.^{9, 10} In particular, a commercially available assay (Phadiatop, Pharmacia, Uppsala, Sweden) for the screening of atopy has been introduced in the clinical setting.¹¹ This test is based on the reaction that occurs between serum IgE antibodies and a balanced mixture of allergens adsorbed on the solid phase. On the basis of the qualitative result of this test, several authors have demonstrated its usefulness in discriminating atopic from non-atopic subjects in populations of different age, sex, and nationality.¹¹⁻¹³ We¹⁴ and others¹⁵ have reported that this assay is also useful in screening for inhalant allergies in epidemiologic surveys in which the prevalence of atopic subjects is much lower than that reported in clinical studies. Recently, this test has been adapted to the Immuno-CAP system (CAP-Phadiatop, Pharmacia),¹⁶ which allows closer contact between the serum-specific IgE and the allergens adsorbed on the solid phase. With this modification a higher sensitivity and a broader range of IgE detection have been obtained. Indeed, the CAP-system can measure as high as 100 kU/L IgE antibodies.^{17, 18}

In the present study using the CAP Phadiatop, we analyzed the allergen-specific IgE in sera from a highly homogeneous population sample of 1815 young Italian men. The results were correlated with the degree of sensitization to inhalant allergens and the occurrence of respiratory allergic diseases carefully measured by standard diagnostic procedures. The data obtained suggest that CAP-Phadiatop represents a step forward in the methodology for measuring the overall serum sensitization to inhalant allergens in epidemiologic and genetic studies.

METHODS

Subjects

A group of 1815 Italian male subjects (age range, 17 to 24 years) attending the Italian Air Force Non-commissioned Officers Training Course at the Air Force Base of Caserta were examined. Informed consent was obtained from all subjects. This group was considered representative of the general population of the same age and sex, because the selection of candidates for the Non-commissioned Officers course is not based on allergy or lung function studies, so that

allergic candidates are usually not excluded. More than 90% of the examined subjects came from Central and Southern Italy. All subjects were clinically evaluated over a 3-month period (from March to May 1991) by two of the authors with the diagnostic procedures described below.

Diagnostic procedures and classification of subjects

A standard diagnostic procedure was used, and it consisted of a medical history, physical examination, and SPT.

The medical history was based on responses to a self-administered questionnaire followed by an interview. The questionnaire was derived from that of the Special Project on Chronic Obstructive Lung Disease of the Italian Research Council, a translated and modified version of the U. S. National Heart, Lung, and Blood Institute questionnaire.¹⁹ It included questions regarding the presence of sneezing, rhinorrhea, nasal congestion, or nasal itching; watering, itching, swelling or reddening of the eyes; coughing, dyspnea, wheezing, chest tightness, or whistling; eczema; and anaphylactic shock. The questionnaire also included questions on the frequency and periodicity of these symptoms, age of onset, and alleviation of symptoms, types of allergy testing performed, pharmacologic therapy and immunotherapy, and any evidence of allergic diseases among relatives. Questions related to food, drug, and insect venom allergy, as well as those related to exercise-induced asthma and physical urticaria, were also included. The questionnaire was used as a first-step tool to pretarget the interview. History was also supported by the case sheets, which may have been compiled during the Non-commissioned Officers course by the physicians on duty at the Air Force Base in Caserta; in particular, the occurrence of respiratory symptoms severe enough to require a medical intervention during the training course (10-month period) was always taken into consideration. Subjects were considered to have symptoms if: (1) they had history of rhinorrhea and sneezing and/or watery, itchy eyes (rhinitis and/or conjunctivitis) lasting for 3 weeks or more during allergy season or occurring nonseasonally but associated with exposure to specific triggers; (2) they presented a history of at least two episodes of coughing, dyspnea, wheezing, and chest tightness or whistling, not caused by any organic condition, occurring during season or recurrently (one or more times per month) if continuously exposed to specific triggers. Subjects who had had asthma attacks with a lower frequency (even a single attack), but which were documented by a physician at the base or clearly linked to a specific trigger factor, were also classified as having symptoms.

A panel of seven airborne allergens (*Dermatophagoides pteronyssinus*, mixed grass pollens, cat epithelium, *Parietaria judaica*, *Olea europea*, *Artemisia vulgaris*, *Alternaria tenuis*) was used for SPT. This panel has been

TABLE 1. Characteristics and classification of the population sample

	SPT reactivity*				Total (%)
	0 (%)	1+ (%)	2+ (%)	3+/4+ (%)	
Group 1†	7‡ (2.6)	5‡ (1.8)	86 (31.9)	172 (63.7)	270§ (100)
Group 2†	— —	96 (23.6)	170 (41.8)	141 (34.6)	407 (100)
Group 3†	1138 (100)	— —	— —	— —	1138 (100)
Total	1145 (63.1)	101 (5.6)	256 (14.1)	313 (17.2)	1815 (100)

The population consisted of 1815 male subjects with an age range of 17 to 24 years.

*Largest skin wheal reaction to one of the seven allergens included in the standard panel.

†Group 1: subjects with symptoms and SPT reactivity; group 2: subjects who were free of symptoms but had SPT reactivity; group 3: subjects without SPT reactivity.

‡Subjects with high SPT reactivity for allergens not included in the standard panel.

§Twenty-seven subjects had asthma, 41 had asthma and rhinitis, and 202 had rhinitis.

previously demonstrated to be adequate for detecting respiratory allergies in Central and Southern Italy.¹⁴

To achieve a clear diagnosis, less than 200 subjects required further testing. This included SPT for less common airborne allergens and specific IgE determinations. Bronchial provocation test with methacholine was occasionally performed to further support a diagnosis of asthma in the presence of a doubtful history.

On the basis of this diagnostic procedure, subjects were classified as follows: group 1 was composed of subjects with symptoms and SPT reactivity (subjects affected by allergic asthma and/or rhinitis and/or conjunctivitis whose symptom occurrence was clearly related to their sensitization to airborne allergens; group 2 was composed of subjects who were free of symptoms with SPT reactivity (subjects with no symptoms but were sensitized to at least one of the seven allergens included in the standard panel); group 3 was composed of subjects without SPT reactivity (both with and without symptoms, the cause of which was clearly not related to airborne allergen exposure, thus including, for example, intrinsic asthma).

Allergy SPT index

The allergens were titrated in histamine equivalent prick units (Standard Quality line, Pharmacia) and were used at a concentration of 10 histamine equivalent prick units per milliliter; all came from a single lot and were preserved in a 50% glycerol-saline solution. For *O. europea* and *Alternaria tenuis* a Phazet test (Pharmacia) was used, because HEP-titrated allergens were not available. The volar surface(s) of both forearms were used as test sites, and both positive (histamine, 10 mg/ml) and negative (50% glycerol) controls were included for each subject. A needle of the osterballe type was used. All participants were questioned regarding recent ingestion of any medications that might interfere with the results of the test. Test results were measured by two of the authors after 15 minutes. Interobserver variability in the interpretation of the results was controlled before initiation of the study. The scoring of

SPT was performed according to the method of Eriksen¹⁶ with minor modifications. Briefly, the mean of the largest wheal diameter and that of its orthogonal dimension were measured for each reaction, and a value from 0 to 4 was assigned as follows: 0 = negative control, 1+ = 1 mm larger than that of negative glycerol/saline control but smaller than half the histamine wheal, 2+ greater than or equal to the histamine wheal, 3+ greater than or equal to histamine wheal, 4+ greater than 20 mm or with many pseudopods. To grade the total reaction of each subject to the skin test applied, an AI was calculated. This was derived by summing the scores of individual tests. For example, a subject with a 3+ positive reaction to *D. pteronyssinus* and a 2+ positive reaction to grass pollen was assigned an AI of 5; thus, the maximum possible AI value was equal to 28. Positive reactions to other allergens, when observed, were not taken into consideration in the calculation of the AI.

RAST

Blood was allowed to clot, and the serum was separated by centrifugation and stored in 1 ml aliquots at -70° C until tested. RAST (Immuno-CAP, Pharmacia) determinations were performed in 65 subjects with monosensitizations and 51 subjects with polysensitizations. In these cases the airborne allergens examined were the seven already described (for grass pollen both g2 and g5 allergens were used). The characteristics of this assay have already been reported.¹⁷ The natural log (ln)-converted titers in kilounits per liter obtained were compared with the results of CAP-Phadiatop. In the case of polysensitized subjects, the sum of the single results was calculated and converted to ln before comparison with the corresponding ln fluorescence unit (FU) value of CAP-Phadiatop.

CAP-Phadiatop

The Phadiatop assay is based on the allergosorbent principle and uses a balanced mixture of relevant allergens causing common inhalant allergy (the exact for-

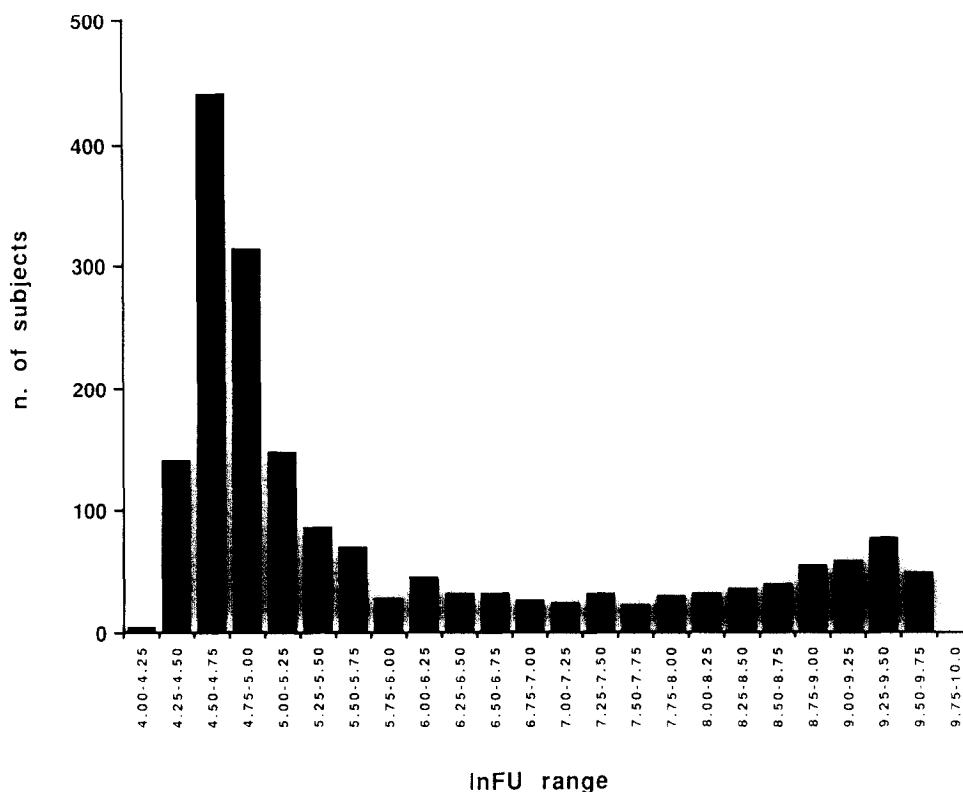


FIG. 1. Frequency distribution of CAP-Phadiatop lnFU in 1815 subjects. Each bar represents the number of subjects with an lnFU value included in the range reported on the abscissa.

mulation of Phadiatop has not been disclosed by the producer). CAP-Phadiatop is a modified version of Phadiatop that has been adapted to a new type of solid-phase radioimmunoassay or fluorimetric assay (CAP-system). Characteristics of the CAP-system have already been reported.¹⁷ Briefly, it consists of a solid-phase immunoassay, instrumentation, and information-management software for the automated measurement of total and specific IgE in undiluted serum or plasma. In the present study the fluorimetric assay was used. The system uses a new type of solid phase, which consists of flexible hydrophilic carrier polymer encased in a capsule (CAP). The carrier consists of a CNBr-activated cellulose derivative. The solid phase has a three-dimensional structure, which increases the surface and allows a closer contact between allergen and serum IgE antibodies. Polyclonal and monoclonal β -galactosidase antibodies, which generate fluorescence by splitting the fluorogenic substrate 4-methylumbrelliferyl- β -D-galactoside, are used. Results are expressed as fluorescence units. (For statistical analysis the natural logarithm transformed, lnFU, was used.) As with the former Phadiatop, positivity or negativity is determined when sample fluorescence emission results are above or below the reference serum value, respectively. The reference serum is chosen by the producer to discriminate as positive more than 90% of the sera from well-characterized atopic subjects from many different

reference clinical centers (L. Yman, oral communication, 1992). In this study the cutoff identified by this serum was 310 ± 21.35 FU (5.74 ± 0.07 lnFU). Results can also be expressed as Phadiatop ratio units, obtained by the following formula: (FU sample $\div$ FU reference serum) = ratio unit.

A standard procedure was followed according to the manufacturer's instructions. All sera were analyzed in five consecutive assays performed in the same week by two experienced technicians. Each assay included more than 360 samples. Reagents from several packages of the same lot were pooled and mixed before use. Particular attention was directed toward having similar conditions of time and temperature among the diverse assays and in the same assay for standards and each subject sample. The fluorescence was measured immediately after the last step in the test procedure. Intraassay and interassay reproducibility were always below 10%.

Statistical analysis

The correlation between CAP-Phadiatop and SPT-AI values was analyzed by the Spearman rank correlation coefficient, and the correlation between CAP-Phadiatop and Immuno-CAP was analyzed by Pearson's product-moment correlation coefficient. The correlations between CAP-Phadiatop values and the rate of (1) group 1 versus total or (2) the sum of group

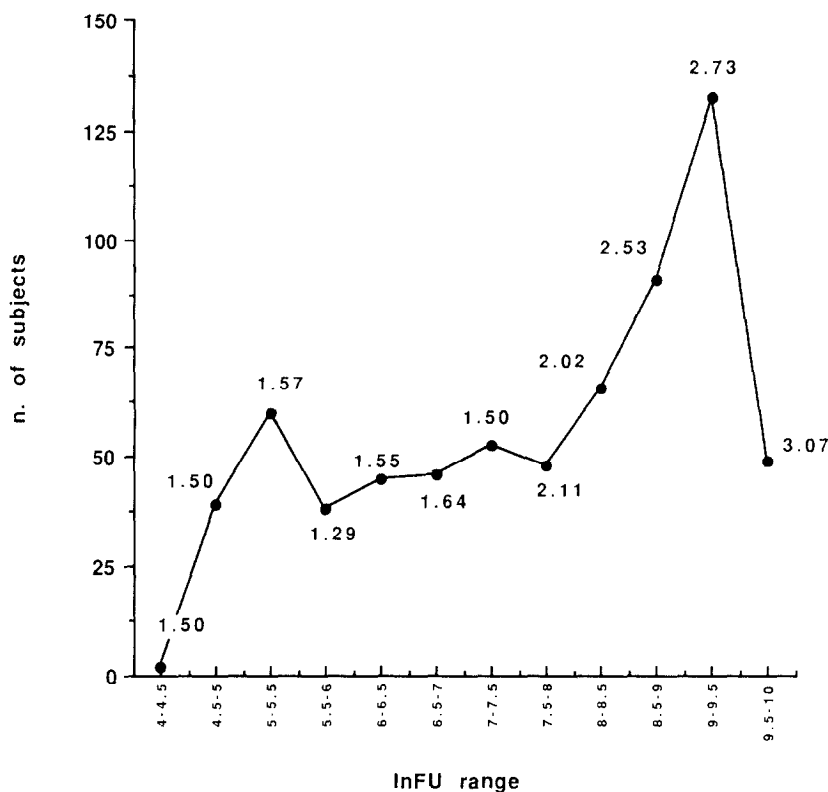


FIG. 2. Frequency distribution of CAP-Phadiatop InFU in 670 SPT-positive subjects. The values along the curve indicate the mean number of allergens to which the subjects are sensitized. A bimodal curve is observed, the lower peak including most asymptomatic, low-grade-monosensitized subjects, and the higher peak most symptomatic, high-grade-polysensitized subjects.

1 + group 2 versus total, were examined by the chi square method for linear trend, and the *p* values were adjusted with Bonferroni's inequality correction. Relative operating characteristic (ROC) analysis to evaluate the detectability power of the CAP-Phadiatop in discriminating group 1 from the other groups was performed according to the method of Erdreich and Lee.²⁰

RESULTS

Clinical data

Respiratory allergy was observed in 270 subjects. We diagnosed allergic asthma in 27 subjects (10%), allergic rhinitis and/or conjunctivitis in 202 (74.8%), and an association of asthma and rhinitis and/or conjunctivitis in 41 (15.2%). Allergy symptoms were seasonal in 111 subjects (41.1%), non-seasonal in 127 (47.0%), and both seasonal and nonseasonal in 32 (11.8%). The overall rates of group 1 (subjects affected by respiratory allergy caused by inhalants), group 2 (subjects who were free of symptoms but had SPT reactivity), and group 3 (subjects without SPT reactivity) are shown in Table I, in relation to the distribution of the SPT reaction (only the largest skin wheal reaction is reported). Group 1 also included seven

subjects with allergic symptoms related to SPT reactivity only to allergens (birch, *D. farinae*, storage mites, etc.) not included in the standard panel of seven allergens tested in the overall population sample. Group 3 included 17 subjects affected by exercise-induced or intrinsic asthma.

Skin sensitivity to *D. pteronyssinus* (62.2% among SPT-reactive subjects, 23% of all subjects), grass pollen (48% among SPT-reactive subjects, 17.7% of all subjects), cat epithelium (34.5% among SPT-reactive subjects, 12.7% of all subjects) and *P. judaica* (25.1% among SPT-reactive subjects, 9.3% of all subjects) accounted for the majority of positive reactions.

CAP-Phadiatop testing

CAP-Phadiatop InFU values had a bimodal distribution in the population sample studied (Fig. 1). A major peak (range = 4.25 to 5.75, mode = 4.5 to 4.75 InFU), in fact, included all of the 1193 subjects (65.73%) with InFU values below the cutoff identified by the reference serum. Subjects with results above this cutoff (*n* = 622; 34.27%) were distributed over a broad range of

values varying from 5.75 lnFU to about 9.75 lnFU. A second, smaller peak could be observed at the highest end of the scale.

A bimodal distribution is observed even in the frequency of lnFU values in SPT-reactive subjects (Fig. 2); the lower peak mainly includes subjects free of symptoms with low-grade sensitization generally to only one allergen, and the higher peak mainly includes subjects with symptoms with high-grade sensitization, often to more allergens.

Relationship between the degree of atopy and CAP-Phadiatop lnFU values

The solid phase of the CAP-Phadiatop is a mixture of common inhalant allergens; thus, it could be expected that the intensity of the reaction of CAP-Phadiatop would be a function of the degree of the subject's sensitization to airborne allergens.

To test this hypothesis, we compared the CAP-Phadiatop results with the semiquantitative AI and the concentrations of serum IgE specific for the seven allergens measured by RAST.

Relationship between the semiquantitative AI and the lnFU values. The relationship between the CAP-Phadiatop lnFU values and the SPT-based AI score is shown in Fig. 3. With the use of Spearman's rank correlation coefficient, a significant correlation ($r = 0.71$, $p < 0.005$) was observed between the AI and the lnFU means.

Relationship between lnFU and serum IgE concentration for inhalants. In the sera of 65 monosensitized subjects (*D. pteronyssinus* = 31, grass pollen = 15, *P. judaica*, = 9, cat epithelium = 4, *Artemisia* spp. = 3, *Alternaria* spp. = 2, *O. europea* = 1) the correlation between CAP-Phadiatop lnFU and ln-converted kilounits per liter of IgE was highly significant ($r = 0.91$; $p < 0.001$) (Fig. 4, A). Of interest was the observation that the linear correlation was also present at very high concentrations of specific IgE for different allergens. Indeed, CAP-Phadiatop could measure more than 50 KU/L (ln = 3.912) of IgE antibodies in sera monospecific for grass pollen, as well as for *Parietaria* spp. or *D. pteronyssinus*, indicating that all of these allergens were adsorbed in a sufficient amount on the solid phase. A good correlation between CAP-Phadiatop lnFU and CAP-RAST kilounits per liter could also be observed in sera monospecific for other allergens, such as *Artemisia* spp., cat dander, and olive pollen. Two monospecific sera for *Alternaria* spp., on the contrary, resulted in negative reaction in the Phadiatop assay, indicating that this allergen is not sufficiently adsorbed in the solid phase.

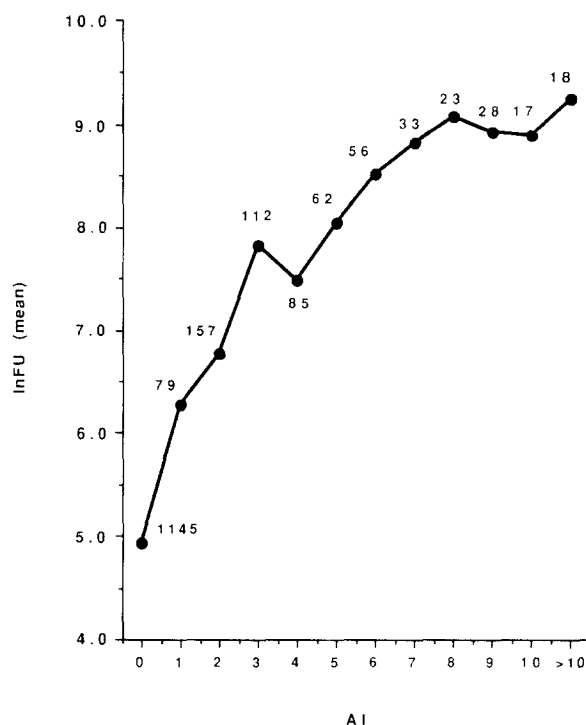


FIG. 3. Relationship between CAP-Phadiatop lnFU value and AI in 1815 subjects. Each point represents the mean lnFU value of all the subjects having the AI reported on the abscissa. The number of subjects for each subgroup is reported along the curve. The Spearman rank correlation coefficient of AI against lnFU means is: $r = 0.71$; $p < 0.005$.

The relationship between CAP-Phadiatop lnFU and the immuno-CAP specific IgE concentration was also examined in sera from 51 polysensitized subjects. IgE titers for each allergen were summed in order to obtain a value of kilounits per liter that could measure the subject's overall concentration of specific IgE for these allergens. The relationship between the ln conversion of this value and the result of CAP-Phadiatop is shown in Fig. 4, B. A highly significant correlation ($r = 0.94$; $p < 0.001$) was observed in the range between 0.35 and 100 kU/L (3.555 to 4.605 ln). A plateau was observed in the CAP-Phadiatop lnFU values at very high concentrations of specific IgE.

Collectively, these data demonstrate that CAP-Phadiatop is a good indicator of the degree of sensitization to six of the seven allergens studied.

Relationship between CAP-Phadiatop lnFU value and respiratory allergy to inhalants

The good correlation between CAP-Phadiatop values and the degree of sensitization to inhalants observed prompted us to look for a possible

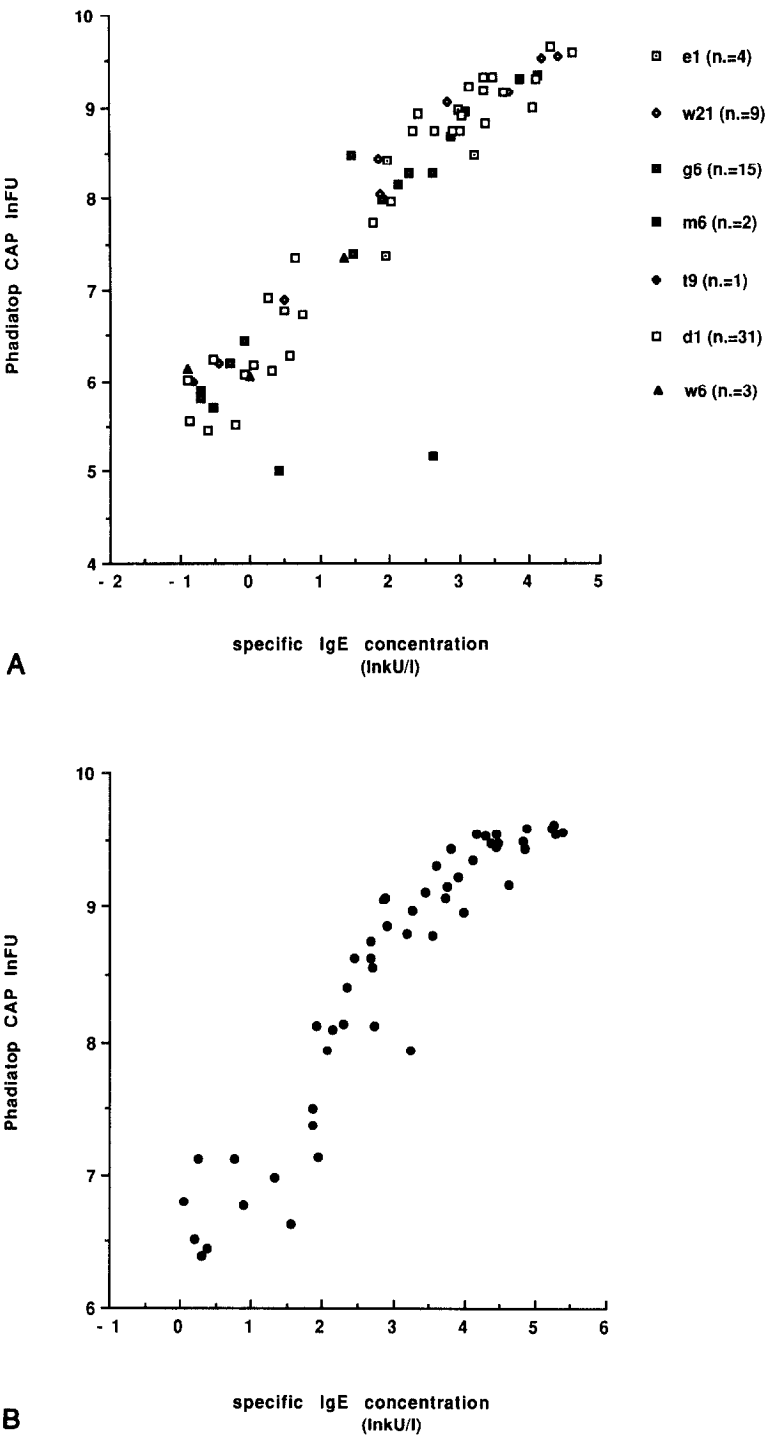


FIG. 4. Relationship between CAP-Phadiatop and Immuno-CAP. **A**, Correlation between CAP-Phadiatop InFU and immuno-CAP In kilounits per liter in 65 monospecific sera. The number of sera specific for each allergen are reported (d1, *D. pteronyssinus*; g6, *Phleum pratense*; w21, *P. judaica*; e1, cat epithelium; w6, *Artemisia*; m6, *Alternaria*; t9, *O. europea*). The product-moment correlation coefficient ($r = 0.91$; $p < 0.001$) was calculated, taking into account all the sera, independently of the specificity expressed. **B**, Correlation between CAP-Phadiatop InFU and Immuno-CAP In kilounits per liter in 51 polyspecific sera ($r = 0.94$; $p < 0.001$). In this case the concentration of specific IgE against seven different allergens was evaluated by immuno-CAP and expressed in kilounits per liter. For a single serum positive results for each determination were summed, and the value obtained was converted to ln. Sera included in the figure showed positive results to at least two different allergens.

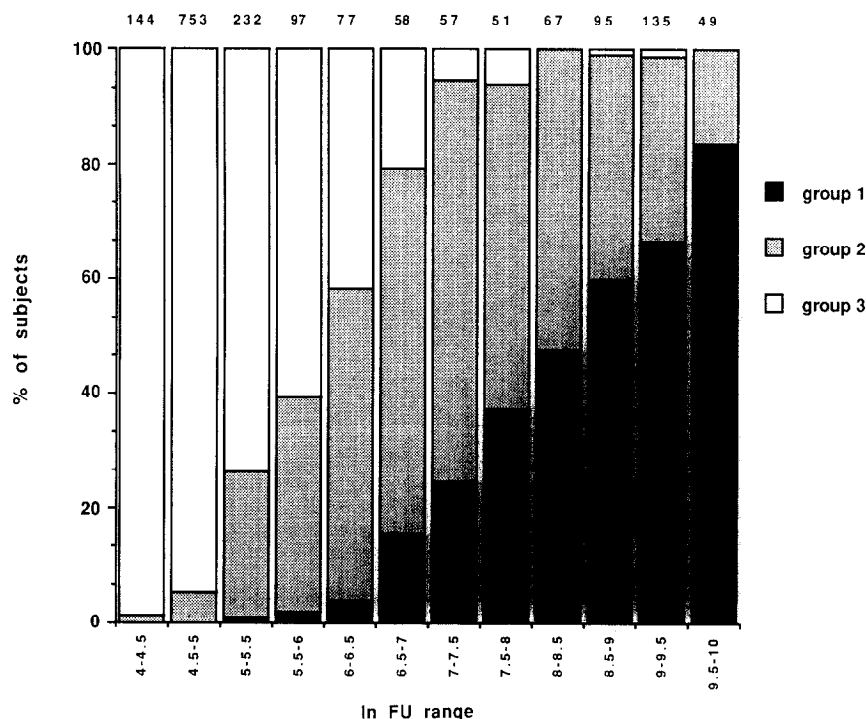


FIG. 5. Relationship between Phadiatop-CAP InFU values and the subjects' classification. Each bar represents a subgroup of subjects with an InFU value included in the range reported on the abscissa. The number of subjects included in each subgroup is reported at the top of the bar. For each range the prevalence of group 1 (subjects with symptoms and SPT reactivity), group 2 (subjects without symptoms with SPT reactivity), and group 3 (subjects without SPT reactivity) is reported. Starting from 6.0 InFU value, a 0.5 increase in the InFU value was associated with an increase of about 10% in the prevalence of group 1 subjects. Group 1 subjects are significantly different from the total ($\chi^2 = 856.4$; $p < 0.0002$); the sum of group 1 and group 2 (SPT-reactive subjects) is significantly different from the total ($\chi^2 = 1085.8$; $p < 0.0002$) (χ^2 for linear trend, p values are adjusted by Bonferroni's inequality correction).

clinical relevance of the quantitative result of the test. In Fig. 5, each bar represents the subjects with a given range of InFU value. The number of subjects is reported at the top of the bar. The prevalence of group 1, group 2, and group 3 subjects is also reported for each range. The rate of SPT-positive subjects is directly related to the InFU value ($\chi^2 = 1085.8$, $p < 0.0002$, SPT-reactive vs all subjects).

Interestingly, the rate of respiratory allergic disease showed a highly significant progressive linear increase with the increase in CAP-Phadiatop InFU values ($\chi^2 = 856.4$, $p < 0.0002$, subjects with symptoms vs all subjects), reaching 85% among the subjects with an InFU value higher than 9.5. Actually, increasing intervals of 0.5 InFU starting from 6 InFU regularly corresponded to about 10% of increase in the prevalence of subjects affected by allergy to inhalants (group 1).

Collectively, these data suggest that the quantitative results of CAP-Phadiatop may provide a

good predictive marker not only of positivity for specific IgE but also of the risk of respiratory disease caused by inhalant allergens in the study population.

ROC analysis of the performance of CAP-Phadiatop as a "yes/no" test in the diagnosis of allergy to inhalants at varying cutoff levels

Phadiatop has been so far proposed as a "yes/no" assay for identification of serum-specific IgE to airborne allergens on the basis of the cutoff point provided by the reference serum. Data reported in the previous paragraph (Fig. 5) prompted us to hypothesize whether a higher cutoff point would have been useful also in identifying as positive only subjects who were SPT-reactive and had symptoms (group 1). To this end, the cutoff points for positivity were set at 0.2 InFU intervals, starting from 4.6 and going up to 9.6 InFU. For each cutoff point, the performance

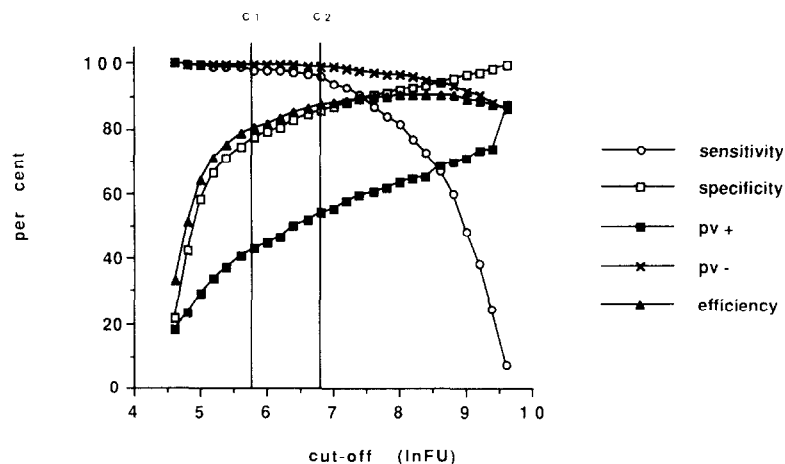


FIG. 6. Analysis of the performance of the CAP-Phadiatop InFU in identifying only group 1 subjects as positive by varying the cutoff from 4.6 up to 9.6 InFU. Sensitivity, specificity, positive and negative predictive value and efficiency were calculated at each cutoff point; the results are shown on the graph. Two lines indicate the cutoff points provided by the reference serum (C1 = 5.74 InFU) and the cutoff chosen on the basis of the ROC analysis (C2 = 6.80 InFU).

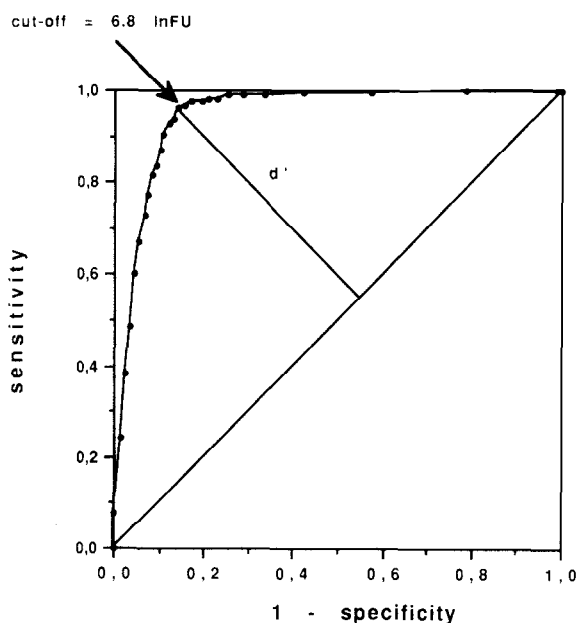


FIG. 7. ROC curve depicting the relationship between CAP-Phadiatop true-positive and false-positive probabilities for various cutoff points. Group 1 classification has been used as the standard. Cutoff is varied from the highest value (9.8 InFU, corresponding to an x,y value of 0,0) to the lowest value (4.0 InFU, corresponding to an x,y value of 1,1). The area under the ROC curve represents a measure of the detectability of the assay $P(A) = 0.948$. The highest point of the curve above the chance line corresponds to a cutoff of 6.80 InFU.

of the assay in identifying only group 1 subjects as positive was calculated in terms of sensitivity, specificity, efficiency, and positive and negative predictive values. The results obtained are shown in Fig. 6. Interestingly, in the range between 5.74

and 7 InFU, the sensitivity of the assay decreases very little, whereas specificity and positive predictive value consistently increase. An ROC curve depicting the relationship between true-positive and false-positive probabilities is shown in Fig. 7. A good detectability power, expressed as the area under the ROC curve,²⁰ was observed [$P(A) = 0.948$]. The highest point above the chance line corresponded to a cutoff point of 6.8 InFU where $[P(D/d) - P(D/n)] = 0.82$ is at maximum.

These data indicate that in addition to the preestablished cutoff provided by the reference serum, another cutoff point can be identified, which better distinguishes subjects with symptomatic expression of atopy (Fig. 6, Table II).

DISCUSSION

The results of the present study demonstrate that the quantitative results of CAP-Phadiatop assay may provide a valuable, reproducible, and time-saving method to measure the sensitization to inhalants in a large population sample. This conclusion is supported by the significant correlation of CAP-Phadiatop InFU values in 1815 young Italian men to: (1) the cutaneous sensitivity to inhalants, expressed as a semiquantitative AI, based on SPT reactivity to a panel of seven relevant allergens; (2) the overall concentration of IgE antibodies against those allergens, measured by RAST, both in monospecific and in polyspecific sera; (3) the prevalence of respiratory allergy to airborne allergens.

The good correlation between CAP-Phadiatop and RAST results, also in polyspecific sera, indicates that in the CAP-Phadiatop assay the binding

TABLE II. Performance of the CAP Phadiatop in identifying as positive subjects affected by respiratory allergy (group 1): Comparison between the cutoff given by the reference serum and a cutoff chosen by ROC analysis.

Cutoff = reference serum (5.74 lnFU, 310 FU)			Cutoff = ROC analysis derived (6.80 lnFU, 900 FU)*	
Positive	Negative		Positive	Negative
265	5	Group 1	260	10
357	1188	Group 2 + Group 3	219	1326
98.14		Sensitivity (%)	96.29	
76.89		Specificity (%)	85.82	
80.05		Efficiency (%)	87.38	
42.60		Positive predictive value (%)	56.16	
99.58		Negative predictive value (%)	99.25	
4.25		Likelihood ratio ²⁰	6.8	

*Phadiatop ratio: 900/310 FU = 2.9 ratio units.

of IgE to one allergen is not affected by the presence of IgE with other specificities, at least in the linear range of the correlation curve. CAP-Phadiatop assay therefore may obviate the need for multiple determinations in the measurement of the overall amount of IgE antibodies to inhalants. The correlation between RAST (ImmunoCAP) and CAP-Phadiatop is obviously favored by the fact that the two assays use allergens obtained from the same source (L. Yman, oral communication, 1992) and employ the same methodology. The analysis of monospecific sera, furthermore, provided further insights into the composition of the allergen mixture adsorbed on the solid phase of CAP-Phadiatop. The amount of grass pollen, *Parietaria* spp., *D. pteronyssinus*, as well as that of cat epithelium, *Artemisia* spp., and olive pollen allergens adsorbed on the solid phase were found to be adequate in detecting specific sensitization. This was not the case for *Alternaria tenuis*; two sera monospecific for *Alternaria* spp., in fact, had background values in the CAP-Phadiatop assay. This result is in line with previously reported observations.^{11, 14} In this study, however, the apparent absence of antigenic determinants of *Alternaria* in the CAP-Phadiatop assay did not significantly affect the correlations we observed. In our population sample, in fact, *Alternaria* sensitization was only observed by SPT in 4.5% of subjects with usually mild reactions, generally associated with sensitizations to other allergens. A low sensitivity for mold sensitization is more relevant in a clinical setting or in epidemiologic studies dealing specifically with allergy to molds.

The analysis of the lnFU distribution of SPT-positive subjects showed a bimodal frequency

curve. The first peak, which corresponded to lnFU values of 5 to 5.5, was mainly representative of subjects who were free of symptoms with low-grade monosensitizations; the second peak, which corresponded to lnFU values of 9 to 9.5, was mainly representative of subjects with symptoms and high-grade sensitizations to many allergens. The fact that among SPT-reactive subjects those with high-grade polysensitization have a higher risk of showing allergic symptoms has already been reported in previously published cross-sectional studies on the general population.^{5, 21} Our observations indicate that CAP-Phadiatop testing might not only represent a good indicator of the degree of sensitization to inhalants but might also provide a predictor of mono- or polysensitization state. Furthermore, the observation of two discrete peaks of frequency distribution may identify subpopulations genetically different in their responsiveness to inhalant allergens.

Our data may have implications both for scientific studies concerning epidemiology and genetics of allergic diseases and for screening programs aimed at identifying individuals at risk for allergic diseases for purposes of selection or prevention.

The CAP-Phadiatop lnFU results of the present study measure the whole continuum of sensitization, rather than the discrete categorization of negative and positive reactions, which has been widely used in previous studies on this test.¹¹⁻¹⁵ Therefore CAP-Phadiatop could be applied in studies that investigate the genetic relationship(s) between the frequency distribution of atopy and HLA aplotypes in the general population or in large families or small communities. Investigations on the influence of environmental exposure, with particular regard to the month of

birth, urbanization, air pollution, house dust contamination, smoking habits, etc., on atopic status, which are of exceeding interest now,²² could be another application of this system of screening.

Furthermore, we showed that ROC analysis could be usefully applied to CAP-Phadiatop numerical data to detect a cutoff point with the best performance in identifying subjects affected by respiratory allergy in our study population. The same approach might be used to identify proper cutoff points for single allergen-specific IgE by examining, for each allergen, high numbers of monosensitized subjects selected in the general population.

Practical implications of our study concern the use of the assay as a tool in mass screening programs for allergic diseases. A complete diagnostic procedure, correlating history, physical examination, SPT and/or in vitro testing for specific IgE is mandatory in this field and cannot be replaced by a single assay. It would be useful, however, to restrict the above-mentioned, time-consuming procedure only to subjects in which a significant risk of allergy to inhalants has been predetermined by CAP-Phadiatop. This test might therefore represent a first step of an algorithm aimed at identification of allergic subjects during mass screening programs, especially when history is not immediately available or is not reliable. Our data show that instead of using the "rigid" cutoff included in the kit, a more suitable cutoff point might be chosen on the basis of the desired level of risk and also after a careful cost-benefit analysis.²⁰ In this respect, the cost of the assay itself (about 10 US dollars per sample in Italy, April 1993) should obviously be taken into consideration. We are currently using this approach, for example, during selections of candidates for military aircraft piloting, who are matched for age and sex with the population examined in this study (Matricardi et al, manuscript in preparation).

Epidemiologic studies on population samples more heterogenous for age, sex, race, and geographic area are necessary to confirm the high significance of the correlations we found, which were obviously favored by the extreme homogeneity and the large number of the subjects examined. Keeping this in mind, we conclude that the availability of a single semiautomated and reproducible assay for serologic quantitative assessment of overall serum IgE sensitization represents a significant diagnostic methodology, which could be useful in epidemiologic studies of atopy. Total

IgE determination, although sharing with CAP-Phadiatop the simplicity and rapidity of execution, reproducibility and precision, safety and freedom from trauma, does not reach the diagnostic accuracy of the Phadiatop assay.^{15, 23} On the other hand, quantitative AIs based on SPT, although fulfilling the needs for epidemiologic study of allergic populations, require much more time, measurement, and calculations, as well as subject compliance and have considerably greater inter-study variability. The use of CAP-Phadiatop as a possible substitute for SPT-AI, would provide a standardized technique for investigation. The inclusion of an IgE standard curve in the assay would further improve the technique, providing the possibility of expressing the results as kilo-units per liter of specific IgE, as is already possible in the CAP-RAST assay. Such an improvement of the CAP-Phadiatop kit would assure the best interstudy reproducibility.

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