

Value of a new screening test for respiratory allergy

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Phadiatop[™] (Pharmacia) is a new *in vitro* screening test for respiratory allergy which detects in serum the presence of specific IgE to a mixture of common inhalant allergens; results are either positive or negative. Among 100 patients suffering from rhinitis and/or asthma, investigated with 15 skin prick tests (SPT) and RAST for frequent allergens, the Phadiatop was positive in 63: all 63 had at least one positive SPT and RAST (specificity: 100%). Conversely, there were 67 patients who had a positive RAST for at least one allergen: among these 67 the total IgE concentration in serum was high (> 100 kU2l) in 43 (64%) and the Phadiatop was positive in 63 (94%). Phadiatop was negative in serum obtained from 10 cord bloods and 10 non-allergic subjects (disease-free, 15 negative SPT and RAST). Results of the radio-immunoassay originally proposed by the manufacturer were similar to those obtained with a modified enzyme assay using a fluorometric substrate. In the prediction of respiratory allergy, Phadiatop was found to be specific and more accurate than the determination of total IgE in serum.

Key words: allergy screening; Phadiatop.

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In the diagnosis of allergic disease, many primary-care physicians feel the need for a simple test indicative of allergy, before performing a complete, time-consuming allergy investigation. Determination of the total IgE concentration in serum, which is usually increased in atopic diseases, has so far been the most widely used for this purpose (12). However, total serum IgE can be increased in conditions other than allergic diseases and, conversely, has been found normal in up to 30% of patients known to be allergic (3, 6, 9, 10, 11).

It has been proposed therefore to use a radio-allergosorbent test instead which detects the simultaneous presence of specific IgE to a variety of frequent allergens (multiple antigen RAST) in serum (15, 17). We report here the results obtained with such a test, the Phadiatop[™] (Pharmacia, Uppsala, Sweden). In this assay, a balanced mixture of relevant inhalatory allergens is coupled to a cellulose disc: the solid phase is incubated with serum, then a

radio- or enzyme-labelled anti-IgE is used to detect any specific bound IgE.

MATERIAL AND METHODS

Phadiatop (Pharmacia) was performed in two ways:

- a) The radioimmunoassay originally proposed by the manufacturer: briefly, 50 μ l of the undiluted serum to be tested are incubated at room temperature for 2 h with a cellulose disc coated with the allergen mixture. After washing, the solid phase is incubated overnight with an immunosorbent purified ¹²⁵I labelled goat anti-IgE. After another wash, the bound radioactivity is measured in a gamma counter.
- b) An enzyme assay provided by the manufacturer (Phadiatop EIA, Pharmacia), adapted to microtechnique and fluorometric reading according to the technique described in detail elsewhere for IgE Phadezym RAST[®]

by Pécoud et al. (18). Briefly a 50 μ l aliquot of undiluted serum is added to the allergen-coated cellulose disc contained in the wells of the microtiter plates. After 1 h of incubation (while shaking) at room temperature the wells are automatically washed and a beta-galactosidase labelled goat anti-human IgE is added. After 2 h another wash is performed and allergen-specific IgE are revealed using a fluorometric substrate (4-methyl-umbelliperyl-galactoside 0.3 mM).

A reference serum is provided with the assay, which allows the determination of the cut-off level: the Phadiatop result is either positive (above the cut-off) or negative (below the cut-off).

Patients

Phadiatop and the determination of total IgE were performed in duplicate on sera obtained from three groups of subjects:

- 1) 100 patients (48 M, 52 F, mean age 33, range 16–60) suffering from rhinitis ($n = 46$) or bronchial asthma with or without rhinitis ($n = 54$) and referred to an allergy outpatient clinic. In all, 15 skin prick tests (SPT) and the 15 corresponding RAST were performed for the following allergens: house dust, *Dermatophagoides pteronyssinus* and *farinae*, cat horse, dog, a mixture in equal parts of 4-grass pollens (rye grass, timothy grass, cultivated rye, velvet grass), orchard grass, birch, hazel, oak, mugwort, plantain, *Cladosporium herbarum* and *Alternaria tenuis*. The frequency of the SPT and corresponding RAST positive for the 15 allergens in these 100 patients is given in Fig. 1.
- 2) 10 "non atopic healthy volunteers" (6 M, 4 F, mean age 31, range 21–37): none had any past or present history of respiratory, skin or gastric diseases in which allergy could be involved and all had negative SPT and RAST for the 15 allergens mentioned above.
- 3) 10 unselected newborns from whom approx. 15 ml of cord blood was collected at birth. The serum concentration of total IgE in these cord blood samples was lower than 0.5 kU/l.

Skin-prick test

The SPT were performed on the volar side of the forearms, with a disposable skin-prick tester (Allterprick, Scanmedica, Zwanenburg, Holland). With the exception of the extract "house dust" (Bencard Allergy Unit U.K.) all tests were performed using 100,000 BU/ml (biological units) of a standardized allergen preparation (Pharmalgen®, Pharmacia, Sweden). The results were graded according to the recommendations of the Northern Society for Allergology: a test was negative if the reaction was similar to that produced by the vehicle solution (negative control) and 3+ positive, when the magnitude of the wheal was identical to that produced by 0.1% histamine (positive control) (1). In our routine practice of allergy as in this study, all SPT with a negative or a 1+ result are considered negative, whereas results 2+ or above are considered positive.

Determination of total and specific IgE in serum

The determination of total IgE was performed by the Phadezym IgE PRIST® (Pharmacia). According to the recommendations of the manufacturer, and IgE concentration > 100 kU/l (approx. 200 ng/ml) is suggestive of atopy.

The presence of specific IgE antibodies for the 15 allergens mentioned above was tested with the standard EIA procedure (Phadezym IgE RAST Pharmacia). This was performed by strictly following the manufacturer's instructions. All RAST classes 1 to 4 were considered as positive.

Definition of allergy

Although there is no world-wide accepted definition of allergy, a patient with either positive SPT or positive RAST may reasonably be considered as allergic. In the present study three criteria will be considered: positive SPT, positive RAST and a combination of both; then the degree of correlation between Phadiatop and the allergic status will be established according to these criteria separately.

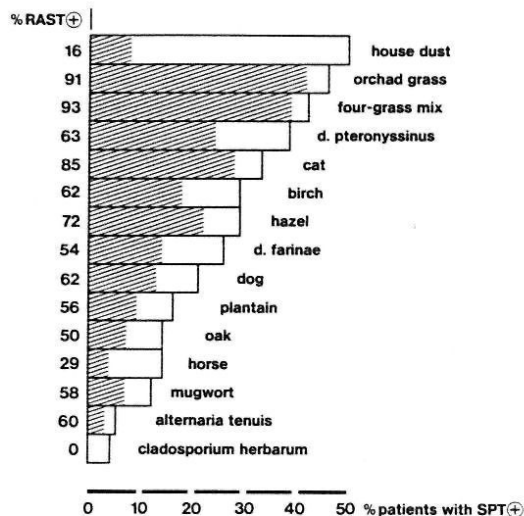


Fig. 1. Frequency of positive SPT and positive RAST (hatched zone) in the 100 patients with rhinitis and rhinitis/asthma. Figures on the ordinate indicate the % of positive RAST in patients with a SPT positive for the corresponding allergen.

RESULTS

Technical data

Using the original Phadiatop radioimmunoassay (2-day procedure) results of duplicates were identical in 119/120 sera tested; 1/120 was borderline positive in one duplicate and negative in the other (patient with 15 negative RAST). With the modified technique using microtiter plates and fluorescence reading, results were obtained in 1 day. Results were identical (positive or negative) in the 120 sera which were tested both with the original RIA

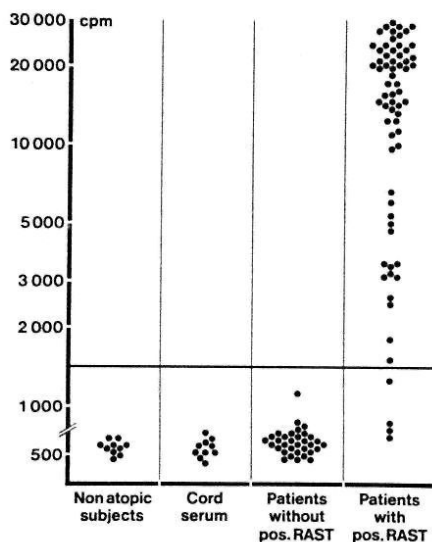


Fig. 2. Results of the 120 PhadiatopTM tests performed in nonatopic subjects, cord sera and patients with rhinitis and/or asthma. Value of the cut-off for this assay: 1425 cpm (horizontal line).

and the modified fluorometric assay: the coefficient of correlation of bound radioactivity to fluorometric intensity was 0.83 ($n = 120$, $P < 0.001$). All results reported thereafter are those obtained with the radioimmunoassay.

Comparison of Phadiatop with RAST results

As shown in Fig. 2, no positive Phadiatop was found in sera from non-atopic subjects or in cord blood. The 100 patients were divided into two subgroups: one group comprised 33 patients who had 15 negative RAST and one 67 who had at least one out of 15 positive RAST.

Table 1
Value of total IgE and PhadiatopTM to predict allergy

	n	% of patients with	
		IgE > 100 kU/l	Phadiatop TM pos.
Patients with at least 1 pos. SPT	74	61	85
Patients with at least 1 pos. RAST	67	64	94
Patients with at least 1 pos. SPT and RAST*	65	66	94

* for the same allergen

Whereas all Phadiatop were negative in the first subgroup, 4/67 (6%) patients who had a positive RAST had a negative Phadiatop: the four patients were found to have only one out of 15 positive RAST (*D. pteronyssinus* in 2, cat in 1, *Alternaria tenuis* in 1; 2 of these positive RAST were class 2 and 2 were class 1). Conversely, all 63 positive Phadiatop were found in sera of patients who had at least one positive RAST. The correlation between Phadiatop and RAST results is summarized in Table 2.

There was a positive correlation between the intensity of the Phadiatop signal (in cpm) and a RAST score consisting of the sum of the RAST class for each allergen ($r = 0.55$, $P < 0.001$, $n = 67$).

Comparison of Phadiatop with SPT results

All patients with positive Phadiatop (63) had a positive SPT for at least one of the 15 allergens tested. Conversely, the Phadiatop result was found negative in 11 of 74 patients (15%) who had at least one positive SPT. In these 11 patients, 4 had a positive RAST (4 "false negative" Phadiatop mentioned above) and 7 had a negative RAST for corresponding allergen. A decision matrix with these correlations is shown in Table 3.

Table 2

Correlation between Phadiatop and RAST results

Patients with:	Phadiatop result	
	positive	negative
at least 1 pos. RAST	63	4
15 neg. RAST	0	33

Sensitivity: 94% (63/67) Specificity 100% (33/33)

Table 3

Correlation between Phadiatop and SPT results

Patients with:	Phadiatop result	
	positive	negative
at least 1 pos. SPT	63	11
15 neg. SPT	0	26

Sensitivity: 85% (63/74) Specificity 100% (26/26)

Comparison of Phadiatop with total IgE determination (PRIST)

As shown in Table 1, the value of the Phadiatop to predict atopy was superior to that of the total IgE concentration, with all the criteria chosen to define allergy (SPT, RAST or association of both). Conversely, among the 33 patients who had 15 negative RAST, six had a total IgE over 100 kU/l, whereas none had a positive Phadiatop.

DISCUSSION

In patients suffering from diseases with a possible allergic cause, such as asthma or rhinitis, it would be helpful to have a rapid and easy screening test to predict allergy. Taking the medical history has not proved to have sufficient predictive value, except in patients suffering from seasonal symptoms (19). If reliable, a good screening test would be cost and time effective since it would help select patients requiring a detailed allergy investigation.

The determination of total IgE concentration in serum has often been used for this purpose. However, many factors, in addition to atopy, affect the serum level of total IgE, such as race (8), sex (2, 3), age (13, 22) or extrinsic factors such as parasitic infection (14), or season (2, 5). Moreover, 20–30% of patients known to be allergic by all other criteria, have a normal or low IgE level (6, 9, 11).

There is no accepted definition of allergy. We, like others, assume that a patient who is suffering from asthma or rhinitis and has positive SPT or RAST, or both, for some well-characterized allergens, can be considered allergic (16). The 15 allergens chosen in this study represent the most frequently detected allergens in our area. The SPT were performed in drug-free patients, using standardized preparations, and compared with positive and negative controls. The proportion of positive RAST corresponding to a positive SPT with the allergen tested (62%) was similar to that found by numerous authors (7, 20). We therefore based the diagnosis of allergy on the presence of SPT and RAST in order to investigate the predictive value of a new screening test.

Our data indicate first that the Phadiatop did not disclose any false positive result in cord blood, known to contain only trace levels of IgE (4) and in clearly non-allergic individuals (subjects without any disease, with 15 negative SPT and RAST). Technically, the test was easy to perform and highly reproducible whatever the method used. Results of the radioimmunoassay and those of the EIA adapted to microtiter plates and fluorometric reading were identical, the latter method allowing a 1-day procedure.

The specificity of the Phadiatop was excellent in patients with asthma and rhinitis, since we found at least one positive SPT and RAST in all the patients who had a positive Phadiatop: there was therefore no false positive result. Since many studies have shown that, in general, RAST is less sensitive than SPT, a positive Phadiatop will indicate to the primary care physician that a more complete allergy work-up will most probably result in some positive findings.

For inhalant allergies this test is better than the determination of total IgE in serum, since in this study as in many others, an allergy investigation was negative in 15–25% of patients who had a total IgE concentration over 100 kU/l, which is considered suggestive of atopy (6, 9).

In our study, the sensitivity of the Phadiatop, i.e. the proportion of allergic patients detected by the test, was found to be 94% when compared with RAST results and 85% when compared with SPT results; this difference again reflects the difference in sensitivity between SPT and RAST. These figures are in fact better than those well known for total IgE, which has been found in the normal range in 20–30% of patients who had specific IgE in serum, as judged by RAST (6, 23).

Based on our data, it is clear, however, that a negative Phadiatop does not exclude an allergy in respiratory disease. Indeed, it must be emphasized that the Phadiatop uses a disc coated with a selected panel of allergens. In the design of our study, we also selected only 15 allergens, i.e. a small number of allergens, even though they probably cover the majority of allergens in our region. This implies that the

occasional patient who has an isolated hypersensitivity to rare allergens will be missed by the Phadiatop: for instance in patients with occupational allergies or those who are allergic to a very narrow spectrum of infrequently diagnosed pollens.

Similar conclusions have been reached by Merret et al. who used a multi-RAST with 3 of the most frequently diagnosed allergens in the UK (*D. pteronyssinus*, grass pollen, cat dander), and compared it with the traditional RAST (15). Ownby et al. using a multiple antigen RAST (*D. pteronyssinus*, grass pollen, short ragweed pollen) compared the multi-RAST results with the results of other screening tests, such as combination of RAST, total IgE determination, nasal eosinophilia as well as the clinician's impression of the likelihood of allergy (17). Using PST only as a positive criteria for allergy, the multi-RAST was a better screening test than the others for allergy to inhalant allergens. The sensitivity of their multi-RAST, however, was lower (63%) than the test described in the present study.

We conclude therefore that the Phadiatop is a valuable adjunct to the routine practice of the primary care physician. This assay is highly specific and more sensitive than other available means for screening respiratory allergic disease.

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