Laboratory screening test with inhalant and food allergens in atopic Brazilian children and adolescents: a performance

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**Abstract**

The Phadiatop Infant® (PhInf) is a panel developed to assess allergic sensitization (immunoglobulin E [IgE]) in children aged <5 years and combines inhalant and food allergens. The test has not been evaluated outside Europe. This is a cross-sectional study conducted at 11 pediatric allergy centers to evaluate PhInf as an allergic disease screening method in Brazilian children. Children as controls and patients (aged 6 months–18 years) were grouped according to their primary disease and age group. PhInf and specific serum IgE (sIgE) screening was performed for *Dermatophagoides pteronyssinus* (DP), cat and dog epithelia, a mix of grasses and pollens, eggs, cow’s milk, peanuts, and shrimp. Values ≥ 0.35 kU/L (or PAU/L) were considered positive. A total of 470 children and adolescents, which included 385 patients and 85 controls, participated in the study (47.7% boys, average age: 6.3 years). In all, 72.6% of the

**Keywords**

child; hypersensitivity; immunoglobulin E; allergens; Phadiatop infant

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Introduction

Allergic diseases are examples of chronic noncommunicable diseases common in the modern world. Generally occurring in the early life, these significantly compromise with the quality of life of patients and their families along with the burden of treatment. Diagnosis of allergic diseases begins with a careful anamnesis and an extensive physical examination of patient. However, as the symptoms of allergic diseases are similar to those of other diseases, the medical history alone can cause diagnostic failure in more than 50% of cases. Thus, when the history is compatible with allergies, tests that detect serum immunoglobulin E (sIgE) specific to one or more suspected allergens can be used. This infers that allergic sensitization that is concomitant with allergic symptoms can help in the diagnosis.

Patients who do not have a typical history of allergy can be evaluated with multi-allergen screening tests. These tests were developed to detect sIgE in a panel of allergens in one analysis, with a lower cost, and a smaller amount of serum, presenting the highest negative predictive value available in allergy tests, since it is administered to the population sensitized to their constituent allergens.

Phadiatop Infant® (PhInf) is a qualitative and semi-quantitative panel that uses ImmunoCAP® system to detect sIgE in 11 inhalant and food allergens. Specifically meant for the 0-5-year-old age group, in which food allergens play an important role in allergic sensitization, it combines the most common allergens to which the Brazilian population is sensitized, according to studies conducted by our group. This study aims to evaluate PhInf as an allergic disease screening method in Brazilian children.

Materials and Methods:

This study was a part of Allergy Project II (Projeto Alergia II or PROAL II), which was aimed to estimate allergic sensitization profile in Brazilian children and adolescents using different methods. The sample was convenient and its calculation was based on the results obtained in our previous study, PROAL I. Hence, the following 11 Brazilian pediatric allergy centers participated in this study between August 2015 and November 2016: Goiás and Mato Grosso (Central-west); Pernambuco and Sergipe (Northeast); Rio de Janeiro, Santo André, and São Paulo (Southeast); and Paraná and Rio Grande do Sul (South). Each center selected 40 patients with allergic diseases divided in the following four groups: asthma and/or rhinitis (A+R), atopic dermatitis (AD), food allergy (FA), and wheezing infant (WI), in addition to 10 nonallergic controls (C), with age ranging from 6 months to 18 years. Those with allergen-specific immunotherapy, immunosuppressive treatment, or with underlying diseases that altered immunoglobulin production were excluded.

The participants’ serum PhInf levels were measured from peripheral blood samples. The sIgE was also measured on the following nine allergenic sources or a mixture of PhInf constituent: allergens, Dermatophagoides pteronyssinus (DP), cat epithelium, dog epithelium, grasses (combined from Cynodon dactyylon, Lolium perenne, Phleum pratense, Poa pratensis, Sorghum halepense, and Paspalum notatum), pollens (combined from Ambrosia artemisiifolia [Aspidistra elatior], Artemisia vulgaris, Plantago lanceolata, Chenopodium album, and Salsola kali), chicken egg, cow’s milk (CM), peanuts, and shrimp (ImmunoCAP®, ThermoScientific®, Uppsala, Sweden). All examinations were performed on the Phadia® 250 (ThermoScientific®, Uppsala, Sweden). The sIgE levels were expressed in kU/L and PhInf levels in Phadia Arbitrary Units (PAU/L). Values >0.35 indicated the individual as not sensitized.

Depending on the nature of the variables under study, parametric (Student’s t-test) or nonparametric tests (Kappa concordance coefficient and Spearman’s rank correlation coefficient [rs]) were used, setting the rejection level for the null hypothesis at 5%. Statistical analyses were performed using the IBM® SPSS 20.0 and Stata® 12.0 statistical software.

The study was approved by the Committee for Ethics in Research on Human Beings at the Federal University of São Paulo—UNIFESP-EPM (Technical Opinion No. 795.256) and by all research ethics committees of participating centers.

Results

A total of 470 individuals participated in the study, 385 allergic and 85 controls, with similar gender distribution (52.3% vs. 47.7%, respectively), with a mean age of 6.3 years (standard deviation [SD] = 4.5 years), and 45.3% were aged between 5 and 13 years. Distribution of patients according to age group and primary disease revealed that WI and those with FA had a mean age significantly lower than those in other groups. There was no statistically significant difference between the age of individuals with A+R, AD, and C (108 vs. 84 vs. 72 months, respectively). The mean age of patients with
A+R and AD was significantly higher than those with FA (36 months), WI (17.5 months), and C (72 months). The mean age of patients of WI was significantly lower than those in other groups (Figure 1); 72.6% of the participants tested had positive PhInf results (79% patients vs. 44% controls), with the highest proportion of positive tests being among patients aged >13 years and in groups FA (92.6%) and AD (91.9%) (Table 1).

Agreement between PhInf and any other specific allergen showed an almost perfect agreement among patients (Kappa = 0.94, P < 0.001) and controls (Kappa = 0.84, P < 0.001).

In case of separately analyzed specific allergens, we found substantial PhInf and DP agreement for all patients (Kappa = 0.665, P < 0.001) and in groups A+R (Kappa = 0.747, P < 0.001), AD (Kappa = 0.735, P < 0.001), C (Kappa = 0.706, P < 0.001), and in the following younger age groups: 3-4 years (Kappa = 0.764, P < 0.001), 4-5 years (Kappa = 0.677, P < 0.001), and 5-13 years (Kappa = 0.704, P < 0.001). An almost perfect PhInf and DP agreement (Kappa = 0.936, P < 0.001) was observed in patients aged ≥13 years.

The younger groups showed the highest concordance with food allergens: moderate for CM and egg in the age groups of up to 4 years, and only in the WI group in

![Figure 1](image.png)

**Figure 1** Age (median) according to main allergic disease.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total N</th>
<th>+ve PhInf</th>
<th>N (%)</th>
<th>Age (years)</th>
<th>Total N</th>
<th>+ve PhInf</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+R</td>
<td>111</td>
<td>95</td>
<td>85.6</td>
<td>&lt; 2</td>
<td>91</td>
<td>44</td>
<td>48.0</td>
</tr>
<tr>
<td>AD</td>
<td>99</td>
<td>91</td>
<td>91.9</td>
<td>2-5</td>
<td>113</td>
<td>82</td>
<td>73.0</td>
</tr>
<tr>
<td>FA</td>
<td>95</td>
<td>88</td>
<td>92.6</td>
<td>5-13</td>
<td>213</td>
<td>171</td>
<td>80.0</td>
</tr>
<tr>
<td>WI</td>
<td>80</td>
<td>30</td>
<td>37.5</td>
<td>&gt;13</td>
<td>53</td>
<td>44</td>
<td>83.0</td>
</tr>
<tr>
<td>Total</td>
<td>385</td>
<td>304</td>
<td>79.0</td>
<td>Total (PA+C)</td>
<td>470</td>
<td>341</td>
<td>72.6</td>
</tr>
<tr>
<td>Controls</td>
<td>85</td>
<td>37</td>
<td>43.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ve PhInf: Positive PhInf; A+R: Asthma and/or rhinitis; AD: Atopic dermatitis; FA: Food allergy; WI: Wheezing infant; PA: Patient; C: Control.
Laboratory screening for inhalant and food allergens in atopic Brazilian children and adolescents

specific disease distributions (Kappa coefficients from 0.429 to 0.554, all P-values < 0.001). The peanut agreement with PhInf was only moderate in children aged 3-4 years (Kappa = 0.497, P < 0.001).

Evaluation of correlation between PhInf quantitative values and those of specific allergens in the entire sample of 470 children revealed a strong and significant correlation for all constituent allergens and a moderate correlation for egg (rs = 0.629) and CM (rs = 0.564) (Table 2).

Regarding the diseases evaluated, PhInf showed a very strong correlation with DP in the A+R group (rs = 0.919) and strong for AD (rs = 0.861), FA (rs = 0.732), WI (rs = 0.721) and C (rs = 0.896) groups (Figure 2).

Cat and dog epithelium also showed strong correlations in group FA (rs = 0.824 and 0.848, respectively), AD (rs = 0.698 and 0.747, respectively), and control (rs = 0.751 and 0.818, respectively) (Table 2).

In relation to the age groups studied, strong and very strong correlations to food allergens tended to be concentrated in patients aged <5 years, while inhalant allergens in 3-4 years old group, with a very strong correlation with the level of dog epithelium in 3-4 years old (rs = 0.913) and DP in 4-5 years old (rs = 0.936) groups (Table 3).

PhInf showed low diagnostic accuracy (sensitivity [Se] = 79%, specificity [Sp] = 56%) with 70% false-negative results. However, it had a high performance in relation to

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Table 2  Spearman’s rank correlation coefficient (rs) between Phadiatop Infant® and specific allergens, according to the total group and by specific disease.

<table>
<thead>
<tr>
<th>Main allergic disease</th>
<th>Total</th>
<th>A+R</th>
<th>AD</th>
<th>FA</th>
<th>WI</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td></td>
<td>0.481</td>
<td>0.485</td>
<td>0.482</td>
<td>0.632</td>
<td>0.656</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td></td>
<td>0.347</td>
<td>0.453</td>
<td>0.565</td>
<td>0.574</td>
<td>0.686</td>
</tr>
<tr>
<td>Peanut</td>
<td></td>
<td>0.624</td>
<td>0.699</td>
<td>0.560</td>
<td>0.426</td>
<td>0.708</td>
</tr>
<tr>
<td>Shrimp</td>
<td></td>
<td>0.596</td>
<td>0.658</td>
<td>0.677</td>
<td>0.508</td>
<td>0.741</td>
</tr>
<tr>
<td>D. pteronyssinus</td>
<td></td>
<td>0.919</td>
<td>0.861</td>
<td>0.732</td>
<td>0.721</td>
<td>0.896</td>
</tr>
<tr>
<td>Dog</td>
<td></td>
<td>0.688</td>
<td>0.698</td>
<td>0.824</td>
<td>0.475</td>
<td>0.751</td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td>0.690</td>
<td>0.747</td>
<td>0.848</td>
<td>0.676</td>
<td>0.818</td>
</tr>
<tr>
<td>Mixed grasses</td>
<td></td>
<td>−0.035</td>
<td>−0.265*</td>
<td>−0.058</td>
<td>0.299*</td>
<td>0.230*</td>
</tr>
<tr>
<td>Mixed pollens</td>
<td></td>
<td>0.659</td>
<td>0.683</td>
<td>0.632</td>
<td>0.546</td>
<td>0.666</td>
</tr>
</tbody>
</table>

All rs: P < 0.001 except those with *P < 0.05.
A+R: Asthma and/or rhinitis, AD: Atopic dermatitis, FA: Food allergy, WI: Wheezing infants.

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Figure 2  Scatter plot of Phadiatop Infant® levels in relation to D. pteronyssinus in each atopic disease. A+R: Asthma and/or rhinitis; AD: Atopic dermatitis; FA: Food allergy; WI: Wheezing infants.
the dosage of nine isolated specific allergens: Se = 97%, Sp = 94%, positive predictive value (PPV) = 98%, and negative predictive value (NPV) = 93%.

**Discussion**

Global statistics point to an increase in the prevalence of allergic diseases. Although genetic predisposition is increasingly established, environmental influences seem to play a major role in the etiology and prognosis of these diseases (epigenetics).7

Along with the real increase in prevalence, there is also tremendous suspicion and “overdiagnosis,” with an increase in spending on unnecessary investigations, and expensive and ineffective treatments, in addition to hampering the quality of life. At the same time, it is common knowledge that the symptoms of allergic diseases are common to numerous other diseases, especially infectious diseases and that differentiating them only by history and physical examination becomes a very difficult task, especially among younger people.8

Previous PhInf studies have demonstrated that it was a flaw to define patients as allergic or nonallergic on the basis of clinical history and physical examination. Fiocchi et al. documented that in 51.2% of children they had assessed, atopy was not determined with clinical data only.9 The figure reduced to 0.5% when they linked with sIgE detection tests.9 Duran-Tauleria et al. published a study in which primary care physicians were able to correctly diagnose allergic diseases in less than 60% of patients without using sIgE or atopic, and 305/385 could be atopic if we considered the presence of sIgE ≥ 0.35 for any of the tested allergens (Table 2). This result was performed on a population of children in Olso, Norway and had a more severe phenotype, and found a similar result with 72% PhInf positivity.7

Nilsson et al. found PhInf positivity to be 30% on evaluating the children (up to 5 years old) of allergic parents in a prospective birth cohort.4 Given that the children were not chosen because they were allergic, the results are comparable to the PhInf positivity of 44% in our control group (Table 1).

PhInf performance was separately compared with sIgE dosages. Among the 385 patients evaluated, 304 were evaluated as atopic, and 305/385 could be atopic if we considered the presence of sIgE ≥ 0.35 for any of the tested allergens (Table 2). A similar result was observed with the controls (n = 85): 37/85 were identified as sensitized by PhInf and 38/85 by sIgE dosage. In both groups, the agreement level was very high (Kappa = 0.8) between the two tests (Table 2). As such, we verified in the studied population that PhInf proved to be a screening test that combines good characteristics: Se, Sp, PPV, and NPV, when the sIgE is compared to at least one of the allergens separately. Nilsson et al. also found similar values, except for a slight decrease in sIgE-Se: 84% versus 97% in our sample.4

## Table 3
Spearman’s rank correlation coefficient (rs) between Phadiatop Infant® and specific allergens according to age group.

<table>
<thead>
<tr>
<th></th>
<th>Age group (years)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;2</td>
<td>5–13</td>
</tr>
<tr>
<td>Egg</td>
<td>0.806</td>
<td>0.722</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>0.738</td>
<td>0.590*</td>
</tr>
<tr>
<td>Peanut</td>
<td>0.614</td>
<td>0.725</td>
</tr>
<tr>
<td>Shrimp</td>
<td>0.557</td>
<td>0.652</td>
</tr>
<tr>
<td>D. pteronyssinus</td>
<td>0.671</td>
<td>0.936</td>
</tr>
<tr>
<td>Cat</td>
<td>0.620</td>
<td>0.842</td>
</tr>
<tr>
<td>Dog</td>
<td>0.827</td>
<td>0.913</td>
</tr>
<tr>
<td>Mixed grasses</td>
<td>0.136</td>
<td>-0.156</td>
</tr>
<tr>
<td>Mixed pollens</td>
<td>0.615</td>
<td>0.702</td>
</tr>
</tbody>
</table>

All rs: P < 0.001.
The data observed herein indicated that the allergen panel was simpler to order, less expensive, and required a smaller volume of serum to function (an important detail in the pediatric age group). The panel had an excellent correlation between sIgE and the nine constituent allergens, and was quantified by ImmunoCAP®.

However, 20% (n = 76) of the patients showed no signs of sensitization to the allergens in either test. Among the 81 patients with negative PhInf results, 50 were from the WI group, a group known to have a lower proportion of sensitization, with only 38% positive PhInf results, a value similar to 33% found in the WI sample of Fiocchi et al.⁹

In 2004, the performance of Phadiatop® was assessed (a screening test created by the manufacturer of PhInf), which at that time comprised only D. pteronyssinus and D. Farinaemites.¹⁰ In all, 457 allergic children (1-12 years old) were tested and the percentage of positive tests (since the total number of children in each age group was different) was much more favorable to the greater positivity of PhInf—with the addition of four food allergens and six types of inhalant allergens—in the target age range of the test (up to 5 years old).

According to the “atopic march,” proved by numerous studies for more than two decades, sensitization to food allergens and the presence of AD were the risk factors for developing a persistent allergic disease during childhood, most notably asthma.¹¹,¹² In our sample, 93% of children with FA and 92% of those with AD had positive PhInf results, and in all age groups aged <5 years, there was moderate agreement between PhInf and sIgE for CM and egg. If we consider that AD and FA are the two most common allergic morbidities with a high prevalence of sensitization in the target age of the test, it is one more reason to corroborate their importance and practical usefulness.

The Brazilian population is most sensitive to domestic mites, a common allergen, with DP being the most prevalent of these.⁴ Hence, as expected, 60% of the individuals tested were sensitized to DP, with rates lower than this in only WI and control groups and, consequently, with moderate PhInf agreement for the total sample, A+R and AD, the age group aged >13 years, and the agreement was perfect (Table 3).

Therefore, we observed an excellent correlation between PhInf levels and sIgE concentrations to separately quantified allergens. However, it is important to note that PhInf is a screening test and should not per se serve as an end to the investigation of allergic diseases. Once positivity is established, a specialist should be referred and requested to target likely allergens to complete the investigation.

Conclusions

We conclude that frequency of positive PhInf tests in allergic Brazilian children and adolescents is high and has a significant correlation with their separately dosed constituent allergens. This is the first evaluation of the performance of PhInf outside Europe. Thus, it is observed that this is a method with good performance in screening non-European children with a low probability of sensitization or when little is known about involved allergens, when compared with the most widely used serological method, ImmunoCAP®. Correct diagnosis is required for individualized treatment with fewer symptoms and reduced medication.

References


