

DR CAROLINA SANCHEZ ARANDA (Orcid ID : 0000-0002-2187-8192)

DR MAGNUS P BORRES (Orcid ID : 0000-0002-9045-2304)

Article type : Letter to the Editor

Increased sensitization to several allergens over a 12-year period in Brazilian children

To the Editor,

The prevalence of allergic sensitization and diseases, especially in the last decades, appears to have increased worldwide ^(1,2). Change of habits and climate has been discussed as major contributing factors ^(1,3). The first Brazilian Allergy Project (PROAL I) study was carried out in 2004 with the objective of documenting the sensitization rates to the major allergens in Brazilian allergic children ⁽⁴⁾. This was the first study of its kind, which contributed to better understanding of regional differences and enabled comparison with other countries. During the past decade, the prevalence of asthma and allergic diseases in children and adolescents has increased ⁽²⁾. Due to this increase, we wanted to repeat the PROAL study with similar design. The objective of the study was to evaluate changes of sensitization over a period of twelve years for major allergens in Brazil.

The national PROAL study I and II were carried out in 2004 and 2016, respectively, had a multicenter and cross-sectional design and covered most regions of Brazil. Included subjects presented with physician's diagnosis of an allergic disease (asthma, allergic rhino-conjunctivitis, eczema and/or food allergy). Children

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.12860

This article is protected by copyright. All rights reserved.

who did not present clinical symptoms of allergy were invited to participate and to compose the control group. Recruitment was done by doctors. Allergic patients were collected consecutively at Pediatric allergy clinics and for control subjects, only those without any clinical characteristic of allergic disease was asked to participate. For this, patients in pediatric outpatient clinics were sought to avoid selection bias. The sample size was calculated according to the results observed in the first study ⁽⁴⁾. Thus, a sample of 430 children and adolescents was recruited to estimate the frequency of sensitization, with a sampling error of 4.0% and a 95% confidence level, using an estimated dimensioning frequency variable in 73.0% ⁽⁴⁾. To compare allergic patients to the non-allergic group, it was necessary to evaluate, in the previous study, the percentage of sensitization of an allergen that was prevalent and in similar magnitudes between allergy and non-allergy groups. A two-way comparison test was used to estimate the difference in allergic sensitization. Calculations defined a sample composed of 360 patients with allergy and 73 participants without allergy (called the control group), assuming a sensitization ratio of 20% and 5%, respectively. Considering possible losses, the sample size was enlarged.

Eleven Brazilian Centers of Pediatric Allergy participated in the study ensuring geographical representation. Each center was requested to include 40 patients with allergic disease and 10 participants without allergy symptoms, with ages between six months and 18 years. Individuals with immunotherapy or immunosuppressive treatment were excluded. Recruitment for the study took place between August 2015 and November 2016, covering all allergen seasons.

A peripheral blood sample was collected from all participants for determination of serum IgE and analyzed according to the manufacturer (ImmunoCAP® Thermo Scientific) for the following allergens: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blomia tropicalis*, *Periplaneta americana*, cat, dog, cow and horse epithelium, cow's milk, egg, corn, peanut, soy and wheat. Serum IgE equal to or greater than 0,35 kU_A/L were considered positive and these subjects were classified as sensitized in accordance with study protocol of PROAL I ⁽⁴⁾.

For statistical analysis, the rejection level was set at 5% for the null hypothesis. This study was approved by the Research Ethics Committee of all participating centers.

In PROAL II, 470 individuals, 385 allergic patients and 85 controls were included, whereas PROAL I included 519 participants, 457 allergy patients and 62 controls ⁽⁴⁾ (n.s.). The mean age (6.2 vs 5.7 years) and gender distribution (male = 52.3% vs 57.8%) were similar in both studies (n.s.).

Three out of four regions recruited similar percentage of individuals (Table 1). South region recruited an increased number of subjects (11.2% vs 20,3%, p<0.001) due to an increased number of participating study investigators.

There was a significant increase of sensitization between the two-time points for cat, dog and cow epithelia, grass and cow's milk (Table 2). This increase was observed both in the allergy group as well in the control group. In the allergy group by itself, a rate increase was observed for sensitization to maize and peanut. A significant increase of sensitization to all three mite allergens was noted in the control group.

Accepted Article

Those are first results from PROAL II study and we document a significant increase for both perennial, seasonal and food allergens. Our findings are in line with similar European studies, which focus on sensitization rate over time using the same study design and test technology. Rönmark *et al.* found recently in two Swedish pediatric cohorts 10 years apart, with identical methods, that both prevalence and incidence of allergic sensitization to airborne allergens had increased significantly ⁽⁵⁾. However, Venter *et al.* could not document a significant difference in peanut sensitization or clinical peanut allergy between 1989 and 2001 in two pediatric cohorts at the Isle of Wight, UK ⁽⁶⁾. The most striking increase in our study was for the sensitization to furry animals (cat, dog, horse and cow). The Brazilian Institute of Geography and Statistics recently reported that 44.3% of households in Brazil have at least one dog and 17.7% have one cat ⁽⁷⁾, which supports our findings that dog seems to be the most common animal sensitizer in Brazil. Heinzerling *et al.* have also documented a nearly 20% increase in cat sensitization in Europe during a seventeen-year period ⁽⁸⁾. This is alarming, as allergic sensitization to cats and dogs appears to be a determinant of asthma and rhinitis. Allergens of domestic animals are smaller and have lower molecular weight than mites and pollens, which enables their dispersion. Therefore, even people without pets at home may often be sensitized to their allergens ⁽⁹⁾.

Another robust result was the increase in sensitization to grass, both among allergic individuals and in the control group. Pollens appear as of great importance in the etiology of allergies. Some studies show that exposure to pollen, under high levels of ozone, due to atmospheric pollution, increases its pro-inflammatory action. In the last decade, there has been an increase in the rate of urbanization in Brazil (from 75% to 91.2%), with warmer temperatures and some higher ambient carbon

dioxide concentrations resulting in incremented pollen production, particularly in areas of elevated temperature, as large cities. Consequently, the high rates of sensitization to pollen in this study may be the result of urbanization and exposure to pollutants ⁽³⁾.

We could also document increased sensitization to food allergens, such as cow's milk, peanut and maize. One explanation for the increasing cow's milk sensitization could be the cross reactions with furry animals, through serum albumins. Modifications on feeding pattern could be another explanatory factor ⁽¹⁾. Rising sensitization to peanut was observed in our study and the most likely explanation would be increased consumption of peanut or cross-reaction with grass pollen. The increase of maize sensitization has not been documented elsewhere and needs further data analysis. Some changes have occurred in the production of maize as climate change, which might have altered the protein allergenicity ⁽³⁾.

The increased mite sensitization over time was only observed in the control group. Most of the allergic children in PROAL I was already sensitized to a high degree (*D. pteronyssinus*: 68%) in 2004. The increase in the control group observed in 2016 might be a result of a warmer and more humid climate ⁽³⁾. Further studies with larger cohorts are needed to support this hypothesis.

The Hygiene hypothesis and changes in the manufacture of agricultural products were the most probable explanations for the raising of allergies in the twentieth century. However, a new lifestyle revolution has been observed since 1990s in industrialized countries: the so-called "*indoor lifestyle*" ^(1,9). Based on this concept, an important growth in allergic diseases rates, especially respiratory allergies, is being reported in different studies ^(5,9). Developing countries, such as

Brazil, could experience these changes with a delay of a few years, which would explain the increase in the sensitization within the evaluated children.

This is the first cross-sectional study in South America to evaluate allergic diseases throughout a decade and the only one that demonstrate such high levels of sensitization for perennial, seasonal and food allergens. The subjects were all diagnosed by a doctor and not only based on questionnaire data, which is a strength in our study. Both PROAL I and II were performed with identical design, inclusion criteria, study protocol and test technology. Changes of sensitization over the years could not be explained by improved sensitivity of the laboratorial method, since ImmunoCAP® has been shown to be reproducible⁽¹⁰⁾.

Individuals from the South region was overrepresented in PROAL II, which is a drawback in our study. The increased rates of sensitization to grasses might therefore be statistical artefacts because pollen sensitization is more common in this region. Our study has other limitations regarding demographic background data. We were not able to collect data about type of delivery, breastfeeding, complementary feeding which might obscure the analysis of some results, especially for food allergens.

We conclude that sensitization to three allergen groups, perennial, seasonal and food allergens have increased over a 12-year observational period in Brazil. The most striking differences was found for furry animals, especially dog, and seem to reflect a change in lifestyle. Higher grass sensitization was observed, as well as for cow's milk, peanut and maize. The analysis of allergen components in this study is ongoing and may help us to differentiate genuine allergies versus cross reactivity and better describe our regional pattern of sensitization.

References

1. Platts-Mills TA. The allergy epidemics: 1870-2010. *J Allergy Clin Immunol*. 2015;136(1):3-13.
2. Rosario-Filho NA, Jacob CM, Sole D, Condino-Neto A, Arruda LK, Costa-Carvalho B, Cocco RR, Camelo-Nunes I, Chong-Neto HJ, Wandalsen GF, Castro AP, Yang AC, Pastorino AC, Sarinho ES. Pediatric allergy and immunology in Brazil. *Pediatr Allergy Immunol*. 2013;24:402-9
3. D'Amato G, Vitale C, Rosario N, Neto HJC, Chong-Silva DC, Mendonça F, et al. Climate change, allergy and asthma, and the role of tropical forests. *World Allergy Organ J*. 2017;10(1):11.
4. Naspitz CK, Solé D, Jacob CA, Sarinho E, Soares FJ, Dantas V, et al. Sensitization to inhalant and food allergens in Brazilian atopic children by in vitro total and specific IgE assay. *Allergy Project - PROAL. J Pediatr (Rio J)* 2004;80(3):203-10.
5. Bunne J, Moberg H, Hedman L, Andersson M, Bjerg A, Lundbäck B, Rönmark E. Increase in Allergic Sensitization in Schoolchildren: Two Cohorts Compared 10 Years Apart. *J Allergy Clin Immunol Pract*. 2017;5:457-463.
6. Venter C, Maslin K, Patil V, Kurukulaaratchy R, Grundy J, Glasbey G, Twiselton R, Dean T, Arshad SH. The prevalence, natural history and time trends of peanut allergy over the first 10 years of life in two cohorts born in the same geographical location 12 years apart. *Pediatr Allergy Immunol*. 2016;27:804-811.
7. Pesquisa Nacional por Amostra de Domicílios 2013. Animais de estimação. Disponível em: <<http://www.ibge.gov.br/pub>>. Access in April. 2017.

8. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy*. 2009;64(10):1498-506.
9. Patelis A, Dosanjh A, Gunnbjörnsdottir M, Borres MP, Högman M, Alving K, et al. A. New data analysis in a population study raises the hypothesis that particle size contributes to the pro-asthmatic potential of small pet animal allergens. *Ups J Med Sci*. 2016;121(1):25-32.
10. Linneberg A, Nielsen NH, Madsen F, Frølund L, Dirksen A, Jørgensen T. Increasing prevalence of specific IgE to aeroallergens in an adult population: two cross-sectional surveys 8 years apart: the Copenhagen Allergy Study. *J Allergy Clin Immunol*. 2000;106:247-52.

Table 1: Number of allergic participants according to Brazilian regions - comparison between data from PROAL I (2004) * and PROAL II (2017).

Regions	Participants		<i>p</i>
	PROAL I* n (%)	PROAL II n (%)	
<i>North/Northeast</i>	129 (28,2)	76 (19,7)	0,077
<i>Midwest</i>	52 (11,4)	54 (14,0)	0,425
<i>Southeast</i>	225 (49,2)	177 (46,0)	0,844
<i>South</i>	51 (11,2)	78 (20,3)	<0,001
<i>Total</i>	457 (100)	385 (100)	0,831

**Naspitz, 2004.*

Table 2: Prevalence of sensitization to inhalation and food allergens in patients with a history of allergy (allergic) and non-allergic participants (controls) - comparison between PROAL I (2004) and PROAL II (2017) [§].

Allergens	Allergic		<i>p</i> [*]	Controls		<i>p</i> [*]
	PROAL I N=457 (%)	PROAL II N=385 (%)		PROAL I N=62 (%)	PROAL II N=85 (%)	
<i>D.pteronyssinus</i>	310 (67,8)	246 (63,9)	0,229	9 (14,5)	28 (32,9)	0,011
<i>D.fariane</i>	304 (66,5)	246 (63,9)	0,425	11 (17,8)	35 (41,2)	0,002
<i>Blomia tropicalis</i>	261 (57,1)	221 (57,4)	0,932	12 (19,4)	32 (38,1)	0,017
Cat	56 (12,3)	115 (29,9)	<0,001	5 (8,1)	13 (15,3)	<0,001
Horse	21 (4,6)	51 (13,2)	<0,001	0 (0,0)	1 (1,2)	1,000
Cow	52 (11,4)	115 (29,9)	<0,001	4 (6,5)	21 (24,7)	<0,001
Dog	37 (8,1)	155 (40,3)	<0,001	2 (3,2)	24 (28,4)	<0,001
Grasses	49 (10,7)	87 (22,6)	<0,001	3 (4,8)	15 (17,6)	0,021
<i>P. americana</i> [†]	157 (34,4)	119 (31,2)	0,325	6 (9,7)	14 (16,5)	0,235
Egg	112 (24,5)	113 (29,4)	0,114	3 (4,8)	8 (9,4)	0,357
Cow's milk	93 (20,4)	122 (31,9)	<0,001	2 (3,2)	11 (12,9)	0,033
Wheat	92 (20,1)	90 (23,4)	0,254	5 (8,1)	8 (9,4)	0,776
Maize	50 (10,9)	65 (16,9)	0,012	3 (4,8)	6 (7,1)	0,734
Peanut	67 (14,7)	79 (20,5)	0,025	3 (4,8)	8 (9,4)	0,357
Soy	56 (12,3)	61 (15,8)	0,134	3 (4,8)	5 (5,9)	1,000

^{*}Descriptive level Chi-square test or Fisher's exact test; [§] - positive measurement >0.35 kU_A/L; [†]*P=Periplaneta*