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Running title : biological outcomes of infants from ORCA Study

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ABSTRACT

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BACKGROUND: Early-onset atopic dermatitis (AD) is a particular phenotype that may convey a risk of developing multiple sensitizations to allergens but little is known about the pathway of sensitization. The aims of this study were to describe the natural history of sensitization to allergens for this phenotype and to identify the most predictive marker associated with the risk of developing sensitization to inhaled allergens in a well-selected cohort of infants with AD. METHODS: Infants with active AD were enrolled and prospectively explored for biological markers of atopy every year until the age of 6 years. Allergic sensitization was defined as the presence of positive specific IgEs to allergens and multiple sensitizations as being sensitized to ≥2 allergens. Elevated blood eosinophilia was defined as an eosinophil blood count ≥470 eosinophils/mm^3 and elevated total IgE as a serum IgE level ≥45 kU/L. RESULTS: 229 infants were included. Elevated blood eosinophilia was observed at baseline in 60 children (26.2%) and elevated total IgE in 85 (37.1%). When elevated at baseline, eosinophilia and IgE levels remained significantly higher during the follow-up period. Sensitization to food allergens decreased from 58% to 34% whereas sensitization to inhaled allergens increased over time from 17% to 67%. Initial multiple sensitizations to food allergens were the most predictive factor for the risk of developing sensitization to inhaled allergens at 6 years (OR 3.72 [1.68-8.30] p<0.001). CONCLUSIONS: In the early-onset AD phenotype, multiple sensitization

to food allergens conveys a higher risk of sensitization to inhaled allergens than single sensitization.

Key words Atopic dermatitis, sensitization, food allergens, inhaled allergens, phenotypes, cohort

Introduction

Atopic dermatitis (AD), which often begins in infancy, is a chronic inflammatory disorder of the skin that affects 10 to 30% of children {1}. Prevalence of sensitization to inhaled allergens in the general population is between 16 to 25% [2]. It is suspected that there is a link between AD and the occurrence of sensitization to inhaled allergens during childhood. This could be because of percutaneous entry of the allergens through an impaired skin barrier due to inflamation. Moreover, early-onset AD, as well as the severity of AD, has been shown to be associated with a risk of sensitization to food allergens at 3 months of age [3]. Sensitization to food allergens in birth cohorts, particularly elevated egg-specific IgE, has also been shown to be a risk marker for sensitization to inhaled allergens later in life {4}. Furthermore, sensitization to inhaled allergens can predict the occurrence of respiratory disease which can start years before the first symptoms of allergic rhinitis or asthma [5]. All in all, AD could be the first step leading to asthma, particularly in children with severe [6] or early-onset AD [7]. However, the early-onset and severe phenotype of AD is quite rare; e.g., in Flohr *et al*.'s study {3} conducted in 619 infants from a population of breastfed infants, only 3.6% had severe AD and 5.4% were sensitized to at least one food allergen. This makes it relatively difficult to explore this phenotype. We therefore set out to explore a cohort of children suffering from early-onset AD from the prospective longitudinal ORCA (Observatory of Respiratory risks linked with Cutaneous Atopy) study to try to describe this phenotype more precisely. The objectives of the present analysis were to describe the natural history of sensitization in this cohort and then to identify the best marker associated with the risk of developing sensitization to inhaled allergens.

Methods

Design

Patients were part of the ten-year (2002-2012) Observatory of Respiratory risks linked with Cutaneous Atopy (ORCA) Study resulting from the collaboration between two tertiary care centers, the Allergology Department at the Armand Trousseau Children‘s Hospital and the Dermatology Department at the Saint-Louis Hospital,

both in Paris, France. The study prospectively included children with AD referred to the Saint-Louis Hospital by a primary care physician.

Ethics

Parents of each child provided written informed consent at inclusion. The protocol was endorsed by the Institutional Review Board of the Medical Ethics Committee on Research of the Saint-Louis Hospital. Data were collected for the study with respect to the confidentiality of patient records.

Inclusion criteria We considered for inclusion all the children meeting the following criteria: i. aged younger than 12 months, ii. with an active AD diagnosed by a dermatologist according to the United Kingdom Working Party criteria (UKWP) {8} and ISAAC questionnaire {9}, iii. without a history of wheezing.

Data collection at inclusion

Clinical data collected were:

1. Gender
2. Active AD defined by ISAAC questionnaire {9} and AD severity assessed by the SCORAD questionnaire [10]. We defined a low severity group for children when the SCORAD was under 15, a medium severity group when the SCORAD was between 15 and 40, and a high severity group when the SCORAD was above 40.
3. Any documented food allergy defined by relevant allergic symptoms following consumption of a food allergen associated with a sensitization to the same allergen.

Biological markers of atopy measured in peripheral blood included:

1. Specific IgEs for inhaled and food allergens (ImmunoCAP Phadiatop Infant; Uppsala, Sweden). Sensitization was defined as a specific IgE concentration

≥0.35 kU/L in serum against one of the following inhaled and food allergens: house dust mite (HDM), cat and dog dander, pollens (birch tree, timothy grass, mugwort), cockroaches; cow's milk, hen's egg, peanut, soy, fish and wheat. Multiple sensitizations were defined as at least two positive specific IgEs to allergens.

2. Other biological markers such as blood eosinophilia (cell counting by automated Sysmex; France), and total IgE (measured by ImmunoCAP; Uppsala, Sweden). Thresholds were used to define increased levels: increased blood eosinophilia was defined as a concentration of 470 eosinophils/mm^3 or more and increased total IgE as a concentration of 45 kU/L or more [11].

Prospective data collection

Children were followed up on biological parameters at the age of 6 months and then annually until the age of 6 years. Biological parameters assessed at each visit were: specific IgE levels against inhaled and food allergens; blood eosinophilia; and total IgE levels, as described above.

Statistical analysis

All the results were calculated from the export database. Statistical analysis was performed using the Open Source R software (> R 2.13.1) [R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.]. Observed distributions of variables are described as numbers and percentages for categorical variables and means, standard deviations and ranges for continuous variables. The baseline characteristics of the patients were compared by

the chi-square test or Fisher's exact test for categorical variables and Welsh's student test for continuous variables. The variables defining severity of AD were discretized in three classes according to the SCORAD questionnaire as described above. Clinical and biological features associated with the risk of developing sensitization to inhaled allergens by the end of the follow-up period were calculated with a logistic regression model. These prognostic factors ($p<0.2$) were then included in the multivariate analysis. Estimated OR are given with a 95% Confidence Interval. Survival analysis, describing time to sensitization (sensitization to inhaled allergens), was performed by Kaplan–Meier analysis. For Kaplan–Meier analysis, we analysed all clinical events by time to first event.

Results

Three hundred children were initially considered for inclusion. Twenty-nine were excluded (21 for lack of parental consent, 7 for a previous history of wheezing and one for gluten intolerance). 42 were lost to follow-up immediately after the inclusion visit and were not included in the analysis. The remaining 229 patients were included for baseline characteristics analysis. 190 completed the last visit and formed the final sample. The baseline characteristics of the children who were not included in the final analysis did not differ from those of the final sample (data not shown). Figure 1 summarizes the patients flowchart.

Descriptive data at baseline

Clinical parameters. There were 134 boys (58.5%) and the mean age was 6.5±2.7 months (mean ±SD). Mean SCORAD was 34.2±21.0 (mean±SD). Food allergy was present in 6% (13/229) of the children. 173 children (75.6%) had a parental history of atopy.

Biological markers

Blood eosinophilia. Increased levels of blood eosinophilia were observed in 26.2 % (59/225) with an average value of 356.2±548.7/mm^3 (mean ±SD).

Total IgE. Increased total IgE was observed in 36.9 % (83/225) with an average value of 111.2±316.1 KU/L (mean ±SD).

Specific IgE. 58% (132/229) of the children had sensitization to food allergens and 37 % (86/229) multiple sensitizations. Hen's egg, cow's milk, peanut represented 95% (125/132) of sensitization to food allergens (hen's egg 44%, cow's milk 26%, peanut 25%, fish 4%, wheat 3%, soy 2.2%). Seventeen percent (40/229) of the children were sensitized to at least one inhaled allergen. Cat dander represented

52% (21/40) of sensitization to inhaled allergens, HDM and dog dander 17 % (7/40), all the pollens 12% (5/40) and cockroaches 2.5 % (1/40).

Descriptive atopic biological markers during the follow-up period

Changes in blood eosinophilia. Analysis by a linear mixed model taking the level of blood eosinophilia at baseline variable as an interaction with time showed that levels of eosinophilia remained on average significantly higher in children with an increased level of blood eosinophilia at baseline, compared to those with a low level at baseline (+705/mm^3 *vs* +76.1/mm^3 over the follow-up period, respectively, p<0.001).

***Changes in total IgE*.** Analysis based on a linear mixed model of the total IgE level variable considered as an interaction with time showed that children with an increased total IgE at baseline had significantly higher average values throughout follow-up than the children in whom the initial total IgE value was normal (+284.6 kU/L vs +39.2 kU/L over the follow-up period, p<0.001) (Figure 2).

Changes in specific IgEs.

Overall, sensitization to food allergens decreased from 58% (132/229) at inclusion to 34% (66/195) at the end of the follow-up period. In contrast, the percentage of children sensitized to inhaled allergens increased over time from 17% (39/229) at inclusion to 67% (130/195) at the end of follow up. 46% (90/195) of the children were sensitized to both food and inhaled allergens at the end of the follow-up compared to 17% (39/229) at baseline. More precisely, at the end of the follow-up period sensitization to inhaled allergens consisted of timothy grass pollens (30%), HDM (28%) while sensitization to cat dander decreased to 18%. Sensitization to dog dander remained stable at around 5%, as well as birch pollen that represented 18% of sensitizations. Hen’s egg, cow's milk and peanut together represented 86% of

sensitizations to food allergens, with 35%, 29% and 22% for peanut, egg and cow's milk respectively (Figure 3).

Factors associated with the risk of sensitization to inhaled allergens at the end of the follow-up period

In univariate analysis**,** clinical and biological markers were evaluated as risk factors for developing inhaled sensitization (Table 1). No clinical parameters (such as severity of atopic dermatitis or food allergy) were found to be risk factors. In contrast, elevated total IgE at baseline emerged as a risk factor for developing sensitization to inhaled allergens at the end of follow up (OR 2.94 {1.58-5.47} p< 0.001). One hundred infants (76.9%) sensitized to food allergens were sensitized to inhaled allergens at 6 years (OR 3.32 {1.90-5.84} p<0.001). More precisely, infants with multiple sensitizations to food allergens were more likely to be sensitized to inhaled allergens (OR 4.32 {2.22-8.40} p< 0.001) than infants with a single food sensitization (OR 2.20 {1.05-4.60} p=0.035).

In multivariate analysis, only sensitization to food allergen remained a determinant and multiple food sensitizations were the most predicitve marker associated with the risk of developing sensitization to inhaled allergens at shool age (OR 3.72 {1.68-8.30} p<0.001). This was almost double than that for children with one food sensitization (OR 2.20 {1.01- 4.72} p=0.05) (Table 2).

Discussion

The main result of this study is that multiple sensitizations to food allergens as opposed to a single sensitization, could be a predictor of sensitization to inhaled allergens in children suffering from early-onset AD. This finding leads to the emergence of a particular phenotype of sensitizations in early-onset AD.

High T-helper cell 2 (Th2) dominant lymphocyte pattern exists in early-life and persists during preschool age

We have shown that both elevated blood eosinophilia and elevated total IgE seem to follow a track during childhood for infants with a particular phenotype of AD. This corresponds to what is known as the extrinsic form of AD as opposed to the intrinsic form. While both forms are clinically identical, the former is characterized by high levels of specific IgEs. Yamamoto *et al.* {12} reported significant differences in terms of heterogeneity of the interleukin 5 gene between AD with high and low blood eosinophil levels. Thus AD can present different clinical phenotypes-genotypes. There is considerable evidence that some individuals with AD present immune dysregulation, including increased serum IgE and allergen sensitization, and increased Th2 cytokine expression in eczematous lesions {13}. Genetic factors predispose atopic subjects to mount exaggerated Th2 responses {14} and to exaggerated abnormality of the epidermal barrier {15}, which may favor allergic sensitization. Recently, Suárez-Fariñas *et al.* {16} demonstrated a significant correlation between IgE levels and SCORAD scores ($r=0.76$, $p<10^{-5}$) only in patients with extrinsic AD.

Sensitizations to food allergens move towards sensitizations to inhaled allergens in some children suffering from early-onset AD Extrinsic AD implies the presence of a Th2 lymphocyte pattern with a cytokine profile facilitating an IgE response to environmental antigens. It is therefore hardly surprising that early expression of IgE-mediated sensitization to food is accompanied by a high risk of sensitization to inhaled allergens. This fact is in accordance to results published from the DARC cohort, in which predominately sensitization to foods, however shifting toward inhalant allergens with age (Eller E, Kjaer HF, Høst A, Andersen KE, Bindslev-Jensen C.Development of atopic dermatitis in the DARC birth cohort. Pediatr Allergy Immunol. 2010 Mar;21(2 Pt 1):307-14.)

In the case of early-onset eczema, IgE sensitization often occurs weeks or months after the eczema lesions first appear, suggesting the allergens are first introduced through the skin. Once allergens have penetrated the skin barrier, they interface with antigen-presenting cells, which can then initiate a Th2 response by dendritic cells {17}. The ensuing cascade can result in a long-lasting response with sensitization of the host. Subsequent exposures can then lead to allergic rhinitis and asthma {18}.

A recent larger birth cohort study demonstrated a strong association between food allergen sensitization, especially hen's egg, and asthma development by age 6 years {19}. Our study therefore confirms that early-onset AD is often associated with sensitization to food allergens and more precisely multiple food sensitizations.

Multiple food sensitizations are the most predictive biomarker of early sensitization to inhaled allergens

These results validate our previous findings that there are multiple atopic phenotypes {20}. In the same way, Lazic *et al.* {21} recently validated his previous study

suggesting that allergic phenotypes change little over time, and that one phenotype with sensitization to a wide variety of allergens was much more likely to give rise to asthma during childhood. This class is relatively unfrequent, comprising approximately one third of the children who would be considered atopic by conventional criteria. In the same manner, in a particular phenotype with early-onset AD, we have shown here that sensitization to food allergens conveys a high risk of sensitization to inhaled allergens rather when multiple than when unique. This finding supports the hypothesis that the clinical expression of allergic diseases does not merely depend on the presence of specific IgE antibodies, but rather on patterns of IgE responses over time.

Strength and limitations of our study

The strength of the study resides in the fact that it was a longitudinal prospective cohort in a highly selected population of infants with early-onset AD explored annually in a standardized manner. However, one limitation could be the rather small size of the cohort and the absence of a control group. However, as mentioned in the introduction, we selected a rare but potentially severe phenotype i.e., early-onset AD. In this context, the size of this selected population was greater than the number of patients suffering from this phenotype if selected from a large birth cohort. It would have been of interest to know if multiple food sensitizations could predict not only sensitization to inhaled allergens but also to severe allergic diseases such as persistent AD and mainly asthma. Nevertheless, inhaled sensitization has been found to be a strong predictor of asthma development and airway hyperresponsiveness up to school age, which is a strong risk factor for respiratory allergies {22. In the same manner, Kjaer HF et al showed that children with atopic

dermatitis, asthma, or rhinoconjunctivitis, and sensitization at 6 yr, were sensitized to food allergens to a large extent (53%, 42%, and 47%, respectively) already at 6 months. This relationship will constitute our future research on this cohort.

In conclusion, Our data have showed longitudinal changes in sensitization patterns of children with early-onset AD and more precisely that multiple food sensitizations, rather than single food sensitization, conveys a high risk of sensitization to inhaled allergens at school age. It is thus important to identify this phenotype during infancy to optimize patient management.

Acknowledgements

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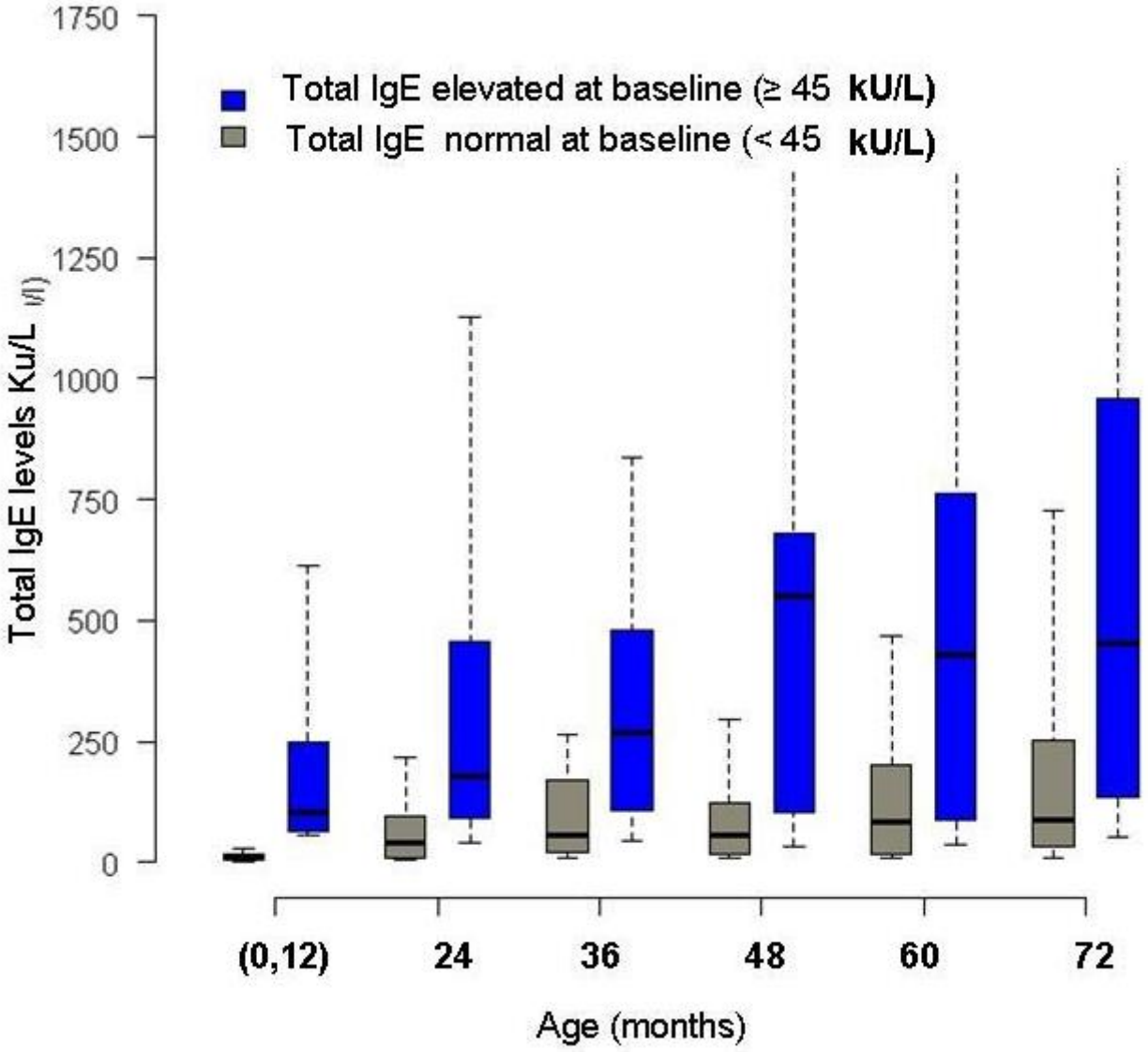
Figure 1. Flow-chart of the study.

Legend: AD atopic dermatitis, n number of patients, mean age at each visit is under brackets.


Total IgE elevated at baseline (≥ 45 kU/L)
Total IgE normal at baseline (< 45 kU/L)
Total IgE levels Ku/L (l/l)
1750
1500
1250
1000
750
500
250
0
(0,12)
24
36
48
60
72
Age (months)

344

Figure 2: Phenotypes of total IgE levels during the 72 months of follow up: changes in the low (<45 kU/L) and high (≥45 kU/L) total IgE levels at inclusion. The horizontal lines of the box represent the lower, median and upper quartile, the hatched traits represent the values outside of the whiskers (the ends of the whiskers represent the lowest datum still within 1.5 interquartile range (IQR) of the lower quartile, and the highest datum still within 1.5 IQR of the upper quartile).

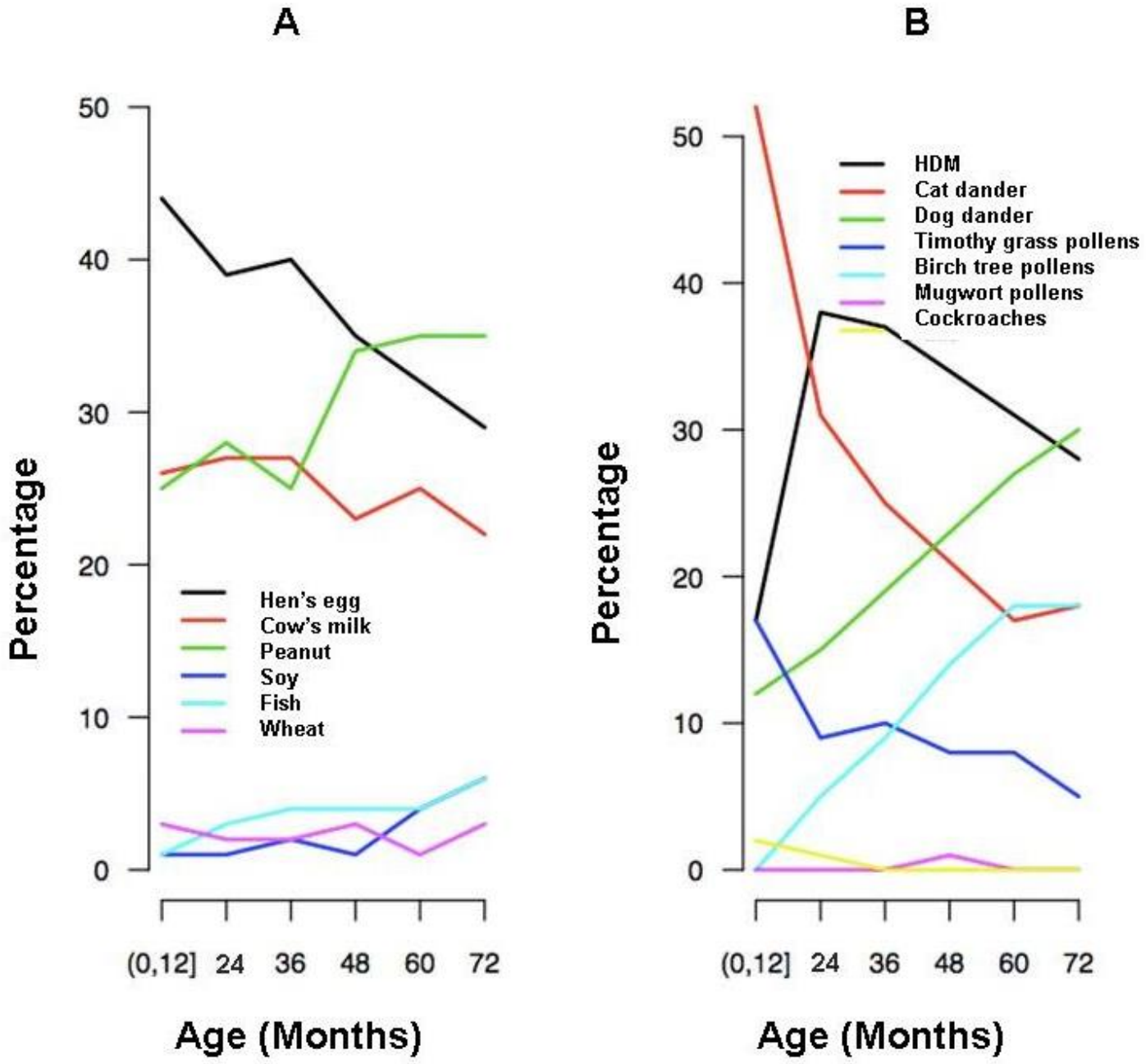

A
B
Percentage
Age (Months)
0
10
20
30
40
50
(0,12]
24
36
48
60
72
Hen's egg
Cow's milk
Peanut
Soy
Fish
Wheat
HDM
Cat dander
Dog dander
Timothy grass pollens
Birch tree pollens
Mugwort pollens
Cockroaches

Figure 3: Changes in each sensitization to food (A) and inhaled allergen (B) during the 72 months of the follow-up

Changes are expressed in percentage of sensitized children. HDM house dust mite

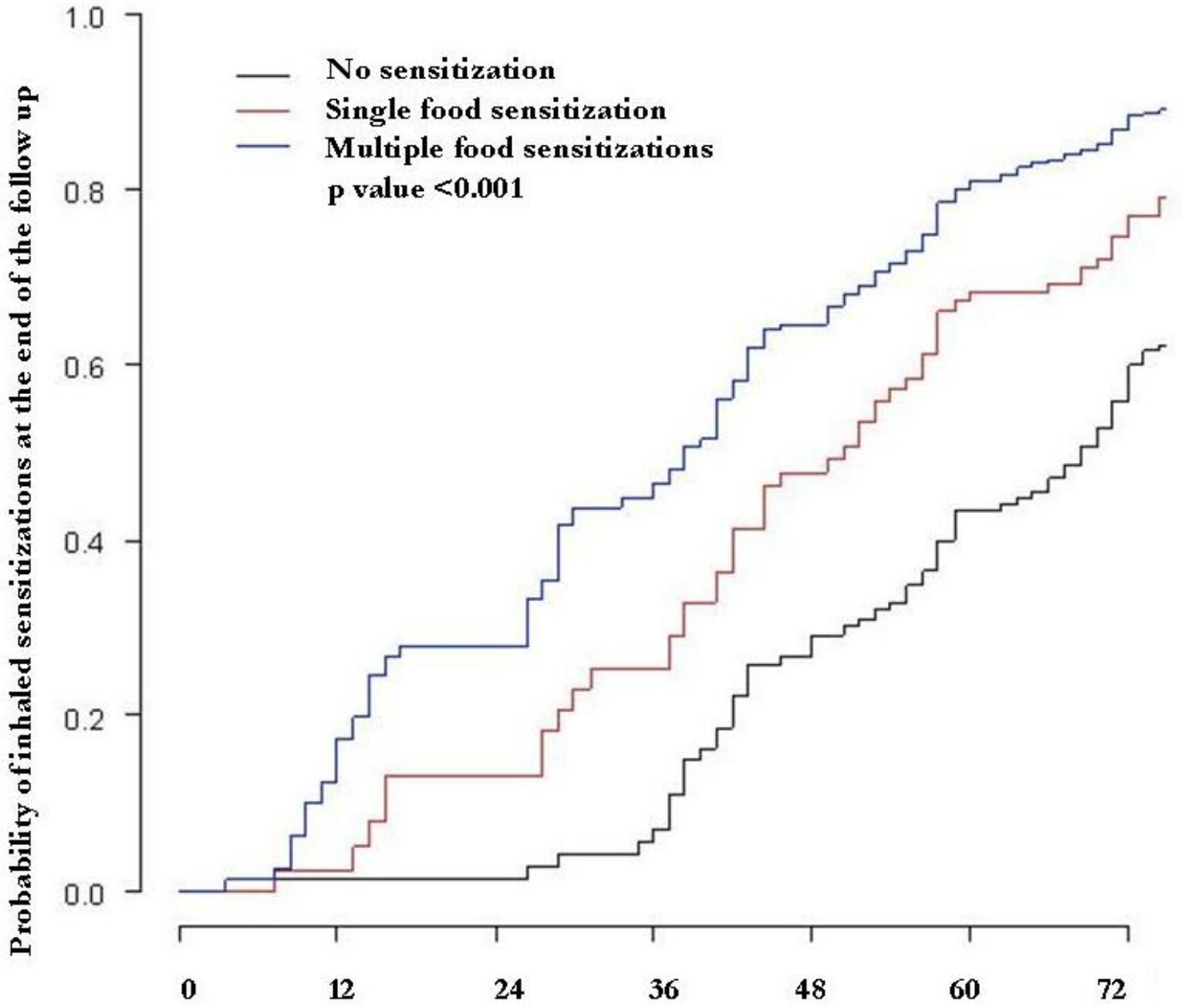

No sensitization
Single food sensitization
Multiple food sensitizations
p value <0.001
Probability of inhaled sensitizations at the end of the follow up
1.0
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72

Table 1: Estimated univariate OR of variables at baseline associated to sensitization to inhaled allergens at the end of follow up

Covariables at baseline		Univariate models	
	Sensitization to inhaled allergens at 5 or 6 years N / N total	OR (CI 95%)	p-value
Eosinophilia level			
Low	94/150	1.00	
High	39/59	1.16 (0.62; 2.18)	0.64
Total IgE level			
Low	75/136	1.00	
High	65/83	**2.94 (1.58; 5.47)**	**0.0007**
Atopic Dermatitis score			
SCORAD ≤15	24/45	1.00	
15<SCORAD ≤40	69/104	1.72 (0.84; 3.51)	0.13
SCORAD >40	54/80	1.82 (0.86; 3.84)	0.12
Food allergy			
No	123/196	1.00	
Yes	10/13	1.98 (0.53; 7.42)	0.31
Sensitization to food allergens			
No	47/97	1.00	
Yes	**100/132**	**3.32 (1.90; 5.84)**	**<0.001**
Sensitization to food allergens			
No	47/97	1.00	
Single food sentization	31/46	2.20 (1.05; 4.60)	0.035
Multiple food sensitizations	**69/86**	**4.32 (2.22; 8.40)**	**< 0.001**
Sensitization to inhaled allergens			
No	117/189	1.00	
Yes	30/40	1.85 (0.85; 4.00)	0.12

Specifying those for the first group awareness / those for all patients. Boldfaced text indicates statistical significance.
Allergen sensitization: specific IgE ≥0.35kU/L. multiple senstizations to food allergen is defined as two or more specific allergen sensitizations.

Table 2: Estimated multivariate OR of variables at baseline associated to sensitization to inhaled allergens at the end of follow up

Covariables at baseline	Multivariate models	
	OR (CI 95%)	p-value
Total IgE level		
Low	1.00	0.27
High	1.52 (0.72; 3.21)	
Sensitization to food allergens		
No	1.00	
Single food sensitization	**2.20 (1.01; 4.72)**	**0.05**
Multiple food sensitizations	**3.72 (1.68; 8.30)**	**0.0012**

Allergen sensitization: specific IgE≥0.35kU/L. Multiple senstization to food allergen is defined as sensitization to two or more specific allergens. Risk factors associated with allergic sensitization to inhaled allergens in the univariate analysis ($p<0.2$) are included in the multivariate analysis. Boldface values indicate statistical significance.

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