

ORIGINAL PAPER

Lack of eosinophilia can predict remission in wheezy infants?

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Summary

Background Early wheezing in infants is a potential risk factor for persistence of asthma into adulthood. Moreover, a personal or familial history of atopy are risk factors associated with persistence of pre-existing wheezing during childhood. However, their relative importance remains unclear.

Objectives Firstly to determine the critical thresholds of common biological markers of atopy in wheezy infants associated with persistence of wheezing into childhood and secondly to rank these biological markers together with clinical parameters according to the strength of their association with wheezing persistence.

Methods A cohort of infants less than 30 months old with recurrent wheezing was established in order to assess severity of respiratory symptoms and to look for the presence of atopy. At the age of 6 years, they were re-evaluated regarding remission of wheezing over the previous 12-months period.

Results Data were available for 219 subjects. In 27% of them, wheezing persisted at 6 years of age. Critical biological thresholds associated with the risk of wheezing persistence were: (1) a blood eosinophilia count $\geq 470/\text{mm}^3$ (defining eosinophilia), and (2) a total serum IgE level $\geq 45 \text{ IU/mL}$ (defining elevated IgE) during infancy. A multiple component factorial analysis identified a dimension associating eosinophilia, elevated IgE and allergic sensitization on the one hand with persistent wheezing at 6 years of age on the other ($\lambda = 0.15$). According to a segmentation analysis, the main discriminative parameter of wheezing persistence was eosinophilia. Thus a lack of eosinophilia alone could account for 91% of infants in remission, and when combined with absence of allergic sensitization, remission was correctly discriminated in 96.9% of the study population.

Conclusion Our data strongly suggest that the lack of eosinophilia in wheezy infants without ongoing infection could predict future remission of wheezing in a large majority of cases.

Keywords allergic sensitization, eosinophilia, wheezy infants

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Introduction

The term 'asthma syndrome' includes a range of respiratory disorders, all characterized clinically by a combination of cough, wheezing and breathlessness. Despite increasing evidence that both genetic and environmental factors have significant effects on airway development and function early in life, our understanding of the natural history of the disease is limited. Early onset of persistent wheezing is associated with impairment of respiratory function leading to a risk of chronic asthma in adulthood [1]. However, in a cohort of neonates taken

from the general population, the Tucson cohort, Martinez et al. [2] have shown that, in the majority of infants presenting wheezing in the first months of life, wheezing was transient and had disappeared at 6 years of age. Nevertheless, identification of clinical and biological markers early in life could help distinguish between infants with transient wheezing and those at risk of progressing to persistent wheezing, and thus allow specific health care to be targeted to the latter group. In the Tucson cohort, a 'wheezy infant' phenotype was identified in which atopy is a risk factor for persistence of wheezing into later childhood [2]. The main

biological marker of atopy was proposed to be allergic sensitization [3].

In the present study, we have used a mathematical approach without *a priori* hypotheses to attempt to classify the various clinical and biological markers of atopy with respect to their association with wheezing persistence in wheezy infants explored in a stable condition without concomitant respiratory tract infection. The aims of this study were (1) to determine critical thresholds of common biological markers of atopy in wheezy infants associated with persistence of wheezing later on in childhood and (2) to rank these biological markers together with clinical parameters according to the strength of their association with persistence of wheezing.

Methods

Design

In this prospective cohort study, wheezy infants were enrolled before 30 months of age. Clinical and biological data were collected when they entered the study. Infants were then followed up prospectively until the age of 6 years in order to evaluate their wheezing outcome.

Subjects

Infants included in the study were referred to the Paediatric Pulmonology and Allergy Center in Paris by their primary care physician because of recurrent wheezing (at least three episodes) before 30 months of age in order to evaluate the need for a preventive treatment. All infants were explored when they were in a stable condition without concomitant respiratory tract infections. Severity of respiratory symptoms was assessed and the presence of atopy investigated.

Data collection

At inclusion, clinical and biological data were collected using the ISAAC questionnaire [4] and blood tests. The questionnaire was given to the family for completion and subsequently validated by the referent medical physician of the centre. Several parameters were evaluated. (i) Demographic characteristics: age and gender of infants. (ii) Familial history of allergy (i.e. asthma, atopic dermatitis and/or allergic rhinitis in the mother or father) and personal history of atopic dermatitis. (iii) The severity of respiratory symptoms: a severity score, derived from the EGEA severity score [5], was used. This took into account four parameters, namely the frequency of attacks, symptoms observed during the intervals between attacks, hospitalization following an attack, and use of inhaled corticosteroids, all with respect to the previous 3 months (Table 1). (iv) Biological data. Blood eosinophilia was

Table 1. Severity score for wheezy infants

Item	Score	Definition
Attack frequency	0	Less than once a month
	1	≥1 per month but <1 per week
	2	≥1 per week
Symptoms between attacks	0	Asymptomatic
	1	Wheezing without restriction of activity
	2	Wheezing which restricts activity
Hospitalization for wheezing attack in the previous year	0	No
	1	Yes
Inhaled corticosteroid treatment at time of assessment	0	None
	1	For at least once a month

If the sum of the parameters was ≤2, infant wheezing was considered as being mild. A score >2 categorized infant wheezing as being severe.

expressed as the absolute value per cubic meter. Total serum IgE level was expressed in IU/mL. Allergic sensitization was defined by the presence of specific IgE (≥0.35 kU/L) (CAP System; Pharmacia & UpJohn, Saint-Quentin-en-Yvelines, France) against at least one common aeroallergen (house dust mites, dog and cat danders, grass pollen, birch pollen) or food allergens (cow's milk, hen's egg, peanut, soya, wheat, fish).

At the age of 6 years, the families of 300 children were re-contacted in order to determine the clinical status of their child. Outcome was determined using the ISAAC questionnaire [4], completed by the family. Remission of wheezing over the previous 12-month period was defined as the absence of obstructive respiratory symptoms, i.e. asthma attacks, wheezing or nocturnal cough (not related to a cold) without any bronchodilator or anti-inflammatory treatment.

Statistical analysis

Quantitative variables were recoded as categorical variables using an information theory-based algorithm. This mathematical approach enables thresholds for continuous variables to be identified that most accurately discriminate between subgroups in a given source population [6]. In the current application, thresholds were determined as they were able to distinguish between persistent wheezing and remission.

Three different procedures were used to assess the degree of association between clinical and biological parameters on the one hand and persistent wheezing or remission at 6 years of age on the other.

In the first step, each variable was tested for association with persistent wheezing or remission using univariate analysis (χ^2 test). In the second step, a multiple component factorial analysis was performed in order to assess

potential interactions between all study variables. The results were displayed as a two-dimensional factorial map, representing the inertia in the data explained by the analysis. The two dimensions of the map represent two artificial variables that explain the maximum amount of variance in the data. The distribution of the centres of gravity of subpopulations defined by the presence or absence of the variable of interest was then represented on the factorial map. The proximity of the centres of gravity of two subpopulations on a factorial map is a measure of their degree of association. The separation of the centres of gravity of the two modes of the same variable (presence or absence) is an indication of how well the variable is discriminated in the model.

Finally, segmentation analysis [7] was used to identify determinants of persistent wheezing or remission at 6 years of age. This consisted of an iterative procedure in which individual variables, identified as discriminant in the multiple component factorial analysis, were screened for their ability to discriminate between the two categories of persistence and remission. Each variable was evaluated in turn and the most discriminating item was identified. The procedure was repeated in order to select the item that would identify correctly the discordant cases from the previous round. This procedure was repeated until the cell size became too small for further iterations to be informative (20 subjects per cell). Segmentation analysis using decision tree methods are designed for classifying categorical variables. In order to evaluate quantitative variables using this approach, these have to be first transformed into categorical variables by the application of appropriate threshold values. Optimization of these thresholds is obviously critical for the quality of the resulting segmentation model.

Ethics

The study was performed according to International and French regulatory guidelines and current codes of Good Epidemiological Practice. Parents of each subject provided written informed consent before the questionnaire was distributed. Since patient care was not altered by inclusion in the study, and since all procedures reflect standard patient care at the study centre, ethics committee approval was not considered to be necessary by the Institutional Review Board of the Medical Ethics Committee on Research of the Hôpital Saint Antoine. With respect to confidentiality of patient records, data handling for the study was authorized by the 'Commission Nationale d'Informatique et Libertés'.

Results

Out of the 300 families re-contacted, 240 (80%) replied to the questionnaire. Responders and non-responders were very similar with respect to the available clinical and biological parameters that had been collected previously (data not shown). At inclusion, all clinical and biological parameters were available for 219 children representing the final study population.

Study population

The demographical and clinical characteristics of the study population at the time of initial diagnosis are presented in Table 2. Seventy percent of infants were less than 24 months of age at the time of diagnosis of wheezy infant status and 69% were male. A family history of allergy was present in 52% of cases. Thirty percent of

Table 2. Univariate analysis to determine predictive factors for persistent wheezing at 6 years of age

	All subjects (%) (n = 219)	Persistent wheezing at age (%) 6 (n = 60)	Remission of wheezing at age (%) 6 (n = 159)	P-Value
Gender (male)	69 (152)	72 (43)	69 (109)	0.085
Paternal asthma	16 (36)	25 (15)	13 (21)	0.032
Maternal asthma	17 (37)	18 (11)	16 (26)	0.434
Paternal atopic dermatitis	7 (15)	3 (2)	8 (13)	0.168
Maternal atopic dermatitis	11 (25)	15 (9)	10 (16)	0.213
Paternal allergic rhinitis	9 (19)	12 (7)	7 (12)	0.238
Maternal allergic rhinitis	12 (26)	13 (8)	11 (18)	0.420
Severe infant wheezing*	31 (67)	38 (23)	28 (44)	0.088
Infant atopic dermatitis	30 (66)	37 (22)	28 (44)	0.130
Eosinophilia [†]	15 (34)	32 (19)	9 (15)	<0.001
Elevated IgE [‡]	30 (67)	42 (25)	26 (42)	0.023
Allergic sensitization	24 (53)	42 (25)	18 (28)	<0.001

The P-values correspond to differences between the persistence and remission groups and were generated with the χ^2 test.

*Severity score >2.

[†] $\geq 470/\text{mm}^3$.

[‡] $\geq 45 \text{ IU/mL}$.

infants presented atopic dermatitis. At the age of 6 years, persistent wheezing was found in 60 cases (27%).

Critical thresholds associated with a risk of persistent wheezing

Critical thresholds associated with a risk of persistent wheezing at 6 years of age were determined by a mathematical approach previously described from two quantitative biological variables: eosinophil count and total serum IgE level. These discriminant thresholds were as follows: an absolute blood eosinophilia value $\geq 470/\text{mm}^3$ (henceforward 'eosinophilia' refers to values above this threshold) and a total serum IgE level $\geq 45 \text{ IU/mL}$ (henceforward 'elevated IgE' refers to values above this threshold).

Factors associated with evolution of wheezing at 6 years of age

Univariate analysis. All clinical and biological variables determined at inclusion were also assessed individually for investigating a potential association with persistent wheezing or remission at 6 years of age (Table 2). The variables associated with persistent wheezing at 6 years of age in the univariate analysis were eosinophilia ($P < 0.001$), allergic sensitization ($P < 0.001$), elevated IgE ($P < 0.023$), and asthma in the father ($P < 0.032$).

Multiple component factorial analysis. The factorial map generated by the multiple component factorial analysis is presented in Fig. 1. The different subpopulations were distributed across two axes. The first principal axis ex-

plained 15% of the inertia (λ) in the dataset and discriminated well between persistent wheezing and remission at the age of 6 years. Elevated IgE, existence of allergic sensitization and eosinophilia were closely grouped with persistence along this axis. In contrast, the severity of symptoms did not appear to be associated with the persistence of wheezing in this analysis.

Segmentation analysis. All the clinical and biological variables identified as discriminating in the multiple component analysis were assessed to determine to what extent they could discriminate persistent wheezing from remission at the age of 6 years. The most discriminating items were identified by segmentation analysis. The variable that discriminated persistent wheezing best was eosinophilia, the next most discriminating variable being the presence of allergic sensitization. Absence of eosinophilia alone could account for 91% of infants in remission, and when combined with absence of allergic sensitization, remission was correctly discriminated in all but five infants, i.e. 96.9% of cases in the study population. In contrast, persistent wheezing at the age of 6 years was less sensitively discriminated. Thus eosinophilia and allergic sensitization alone were only associated with persistent wheezing in 32% and 23% of cases, respectively. Combination of the two variables could allow prediction of persistent wheezing in 55% of cases. The segmentation tree is presented in Fig. 2.

Discussion

We have analysed clinical and biological parameters associated with the risk of persistence or remission of

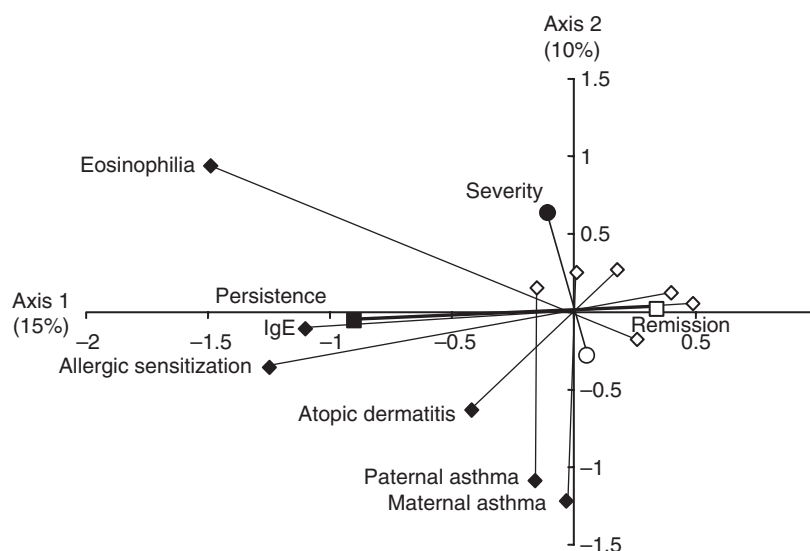


Fig. 1. Multiple component factorial analysis. The bold line indicates the separation between persistence or remission of wheezing along axis I. Filled symbols indicate the subgroup with the variable and open symbols the subgroup without the variable. For clarity, only variables which are well discriminated by the factorial map are presented.

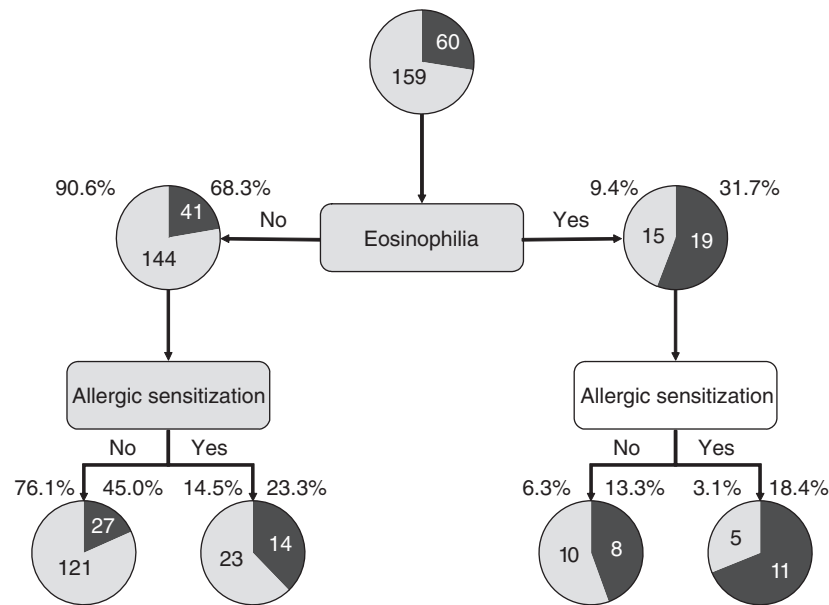


Fig. 2. Segmentation analysis of determinants of persistence or remission of wheezing at 6 years of age. The upper circle represents the total population and the circles below the sets generated at each stage of the iteration. Pale segments and black segments represent remission or persistence of wheezing, respectively. Percentages are calculated with respect to the source population.

wheezing at 6 years of age in a cohort of 219 wheezy infants.

In our work, factors associated with persistence of wheezing were: (i) eosinophilia, allergic sensitization, elevated IgE, and paternal asthma in the univariate analysis; (ii) eosinophilia, allergic sensitization and elevated IgE level in the multiple component factorial analysis; (iii) eosinophilia and allergic sensitization in the segmentation analysis. Thus all these three complementary statistical approaches identified these latter two factors as critical determinants of persistence of wheezing.

When sensitization to aeroallergens and food allergens is considered, 24% of our population of wheezy infants was found to be sensitized. Sensitization to either inhaled or ingested allergens has been reported to be a strong risk factor for asthma in the literature. Two previous studies have found that asthma in 7–22-year-old subjects was associated with allergic sensitization during the first 2 years of life [8, 9]. Another study [10] reported that the presence of early and long-lasting sensitization (persisting up to the age of 7 years) to both food allergens and aeroallergens was associated with an increased prevalence of asthma and bronchial hyperactivity. Allergic sensitization was not retained in the original asthma persistence risk index generated from the Tucson study [11], but the same authors [12] added this parameter to the modified index used for the PEAK study.

In the PEAK study, the authors considered that the contribution of aeroallergens to the asthma predictive index was more important than that of food allergens. In our univariate analysis, we did not find a significant correlation between each allergen type taken alone and

the persistence of wheezing, although a significant correlation was observed when the two types of allergen were combined. This discrepancy might be explained by a lack of power in our statistical analysis due to the relatively low number of observations for each type of allergen.

Our segmentation analysis showed that eosinophilia was the strongest determinant of persistent wheezing. In the Tucson study [11], this parameter was considered as having only a minor influence on the persistence of wheezing during childhood. The divergence between the two studies may be explained by methodological differences. In the Tucson cohort [11], eosinophilia was defined using an arbitrary 4% threshold, whereas in our study it was defined operationally according to its discriminant power, perhaps thus allowing a greater predictivity. Moreover, a subsequent analysis of the Tucson data reported that persistent eosinophilia (> 5%) throughout childhood (up to the age of 11) was associated with the presence of chronic asthma [13]. It should be noted that blood eosinophilia is also a predictive factor of asthma persistence [14, 18] in infants with severe lower respiratory tract infections such as bronchiolitis and pneumonia. Moreover, in these and other studies, atopic markers such as total IgE, allergen-specific IgE [14, 15] and markers of eosinophilia such as ECP [15, 19, 20] were found to be associated with recurrent wheezing following a severe lower respiratory tract infection. However, these studies were conducted mostly in infants without recurrent wheezing, in contrast to the infant population which we have analysed. In our study, critical thresholds of blood eosinophilia associated with a risk of persistent wheezing emerged from an *ab initio* mathematical approach,

whereas these thresholds were either pre-defined [14–17] or observed [18] in previous reports. These different methods yielded similar results, supporting the external validity of our mathematical model.

Most previous studies which have assessed blood eosinophilia during acute lower respiratory tract infections as a potential risk factor for wheezing persistence in infants [15, 16, 18] have identified a lack of an eosinophilia as a possible pathological factor. Less frequently, eosinophilia itself [14–17] or markers of eosinophil activation such as ECP [19, 20] have been studied outside the acute infectious episode. These studies suggested a possible association between sustained eosinophilia or elevated ECP on the one hand and atopy on the other.

Allergic sensitization is associated with proliferation of eosinophils especially in children. However, the level of these white blood cells is increased in both asthma and atopy. In a previous study involving infants and children with asthma, we have shown that alveolar eosinophilia was strongly related to allergic sensitization rather than to asthma activity itself [21]. However, in the latter study, alveolar eosinophilic inflammation was restricted to older children and not identified in infants. Even though alveolar eosinophilia may not be demonstrated in infants, bronchiolar eosinophilia has been described in some [22] but not all studies [23]. Our present results strongly suggest that blood eosinophilia is an early sign of allergic inflammation before eosinophilia can be detected in alveolar or bronchiolar tissues. This hypothesis is consistent with the idea that bronchoalveolar inflammation may be triggered by certain populations of haematopoietic stem cells [24].

A limitation of the present study may be possible bias related to incomplete data sampling, because only 240 (80%) of potential participants of the study replied to the questionnaire and only 91% provided complete data. Moreover, the analytical approach used did not allow the relative risk of asthma persistence associated with the identified variables to be determined. Future development of this work will necessitate the use of multivariate models to quantify the relative importance of these variables, as well as of environmental variables, which were not taken into account in the present analysis.

In conclusion, our study strongly suggests that the lack of eosinophilia (blood eosinophilia $\geq 470/\text{mm}^3$) in wheezy infants in a stable condition without ongoing respiratory infection could predict a future remission of wheezing.

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