

IgE antibody quantification and the probability of wheeze in preschool children

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Background: IgE-mediated sensitization is usually considered a dichotomous variable (either sensitized or not). Quantitative IgE antibody analysis may better predict the expression of wheeze.

Objective: Within the context of a population-based birth cohort, we investigated the association among wheeze, lung function, and specific IgE antibody levels.

Methods: Children (n = 521) were followed to age 5 years with repeated questionnaires, skin testing, and measurement of lung function (specific airway resistance) and specific serum IgE (ImmunoCAP).

Results: Using specific IgE as a continuous variable, the risk of current wheeze increased significantly with increasing IgE to mite, cat, and dog ($P < .0001$). When IgE levels to these 3 allergens were summed, the probability of current wheeze increased 1.33-fold (95% CI, 1.21-1.47; $P < .0001$) per logarithmic unit increase, corresponding to an odds ratio of 3.1 at 10 and 4.25 at 30 kU_A/L (kilo units of Allergen per liter). Similarly, increasing sum of mite-specific, cat-specific, and dog-specific IgE was associated with reduced lung function ($P = .004$). Among sensitized children (n = 184), the sum of mite, cat, and dog IgE was the strongest associate of current wheeze (odds ratio, 1.28; 95% CI, 1.13-1.46; $P < .001$), corresponding to an odds ratio of 2.56 at 10 and 3.32 at 30 kU_A/L. There was no association between current wheeze and the size of skin test wheal. Furthermore, the sum of IgE to mite, cat, and dog at age 3 years increased the risk of persistent wheeze by age 5 years (2.15-fold/logarithmic unit increase in the specific IgE).

Conclusion: IgE-mediated sensitization is not an all or nothing phenomenon. The probability of wheeze and reduced lung function increases with increasing specific IgE antibody levels. (J Allergy Clin Immunol 2005;116:744-9.)

Key words: IgE antibody, quantitative assay, wheeze, specific airway resistance

Sensitization to indoor allergens is a risk factor for asthma.¹ Most studies that investigated the relationship between allergy and respiratory symptoms considered IgE-mediated sensitization only as a dichotomous variable—that is, individuals were assigned as either sensitized or not (often on the basis of differing cutoff points).²⁻⁴

In food allergy, the level of specific serum IgE antibodies⁵⁻⁸ or the size of wheal diameter after skin testing⁹ may predict the likelihood of patients having a clinical reaction. In a group of 95 children with a history of clinical reactivity to peanut, mean wheal diameter ≥ 8 mm on skin prick testing predicted clinical reactivity with 100% certainty (however, 3 of 18 children with negative skin tests also reacted).⁹ When used as a dichotomous trait, skin tests are most valuable when they are negative (negative predictive value may be as high as 95%, whereas the positive predicted value is only 50%).¹⁰ Similarly, negative specific serum IgE result has a very high negative predictive value, with a positive result having a low specificity.^{10,11} However, quantitative measurement of specific IgE antibodies can improve the specificity (eg, for patients with peanut-specific IgE above 15 kU/L, there is a more than 95% probability for clinical reactivity¹¹). Similar prediction curves have been established for several other food allergens (eg, milk and egg).^{7,8,11}

It is as yet unknown whether a similar relationship between IgE or skin test quantification and clinical expression of symptoms exists in respiratory allergy. To test the hypothesis that quantitative analysis of IgE antibody responses predicts the presence and severity of childhood wheezing illness, we investigated the association between the level of IgE antibodies to common allergens and the occurrence and persistence of wheeze, and lung function in 5-year-old children.

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TABLE I. Association between IgE antibodies, SPTs, and current wheeze at age 5 years

	Quantitative IgE (n = 521)			Dichotomous IgE* (n = 521)			Size of skin test (n = 512)			Dichotomous skin test† (n = 512)		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Dust mite	1.29	1.16-1.45	<.0001	2.49	1.54-4.02	.0002	1.12	1.02-1.22	.01	1.91	1.12-3.25	.017
Cat	1.55	1.27-1.88	<.0001	4.64	2.52-8.55	<.0001	1.22	1.11-1.35	.0001	3.04	1.65-5.60	.004
Dog	1.68	1.32-2.15	<.0001	5.16	2.88-9.25	<.0001	1.23	1.09-1.39	.0007	2.76	1.31-5.83	.008
Grass	1.16	1.02-1.31	.02	1.86	1.13-3.04	.014	1.09	1.00-1.19	.05	1.68	0.99-2.8	.05
Milk	1.43	0.98-2.09	.06	1.52	0.84-2.74	.17	1.11	0.72-1.69	.64	1.77	0.16-19.76	.64
Egg	1.68	1.05-2.71	.03	2.1	1.12-3.96	.021	1.11	0.87-1.40	.40	2.15	0.51-9.15	.30
Peanut	1.38	1.06-1.80	.02	3.13	1.56-6.26	.001	‡			‡		
Mite + cat + dog	1.33	1.21-1.47	<.0001	NA			1.16	1.08-1.25	.0001	NA		
Sum of all	1.27	1.16-1.38	<.0001	NA			1.15	1.07-1.23	.0001	NA		

NA, Not applicable.

*Sensitization defined as specific IgE values >0.2 kU_A/L.

†Sensitization defined as a mean wheal diameter 3 mm greater than negative control.

‡Children were not skin tested to peanut at the age of 5 years.

Abbreviations used

OR: Odds ratio

SPT: Skin prick test

sR_{aw}: Specific airway resistance

METHODS

Study population

The Manchester Asthma and Allergy Study is an unselected population-based birth cohort study.^{12,13} Subjects were recruited from the antenatal clinics within the first trimester of the pregnancy when all pregnant women were screened for eligibility. Both parents completed a questionnaire and skin prick testing to common inhalant allergens. Multiple births were excluded. Children were followed prospectively and attended review clinics at ages 3 and 5 years (± 4 weeks). The study was approved by the Local Research Ethics Committee. Written informed consent was obtained from subjects' families, and children gave their assent.

Outcomes

Symptoms were as follows. A validated questionnaire¹⁴ was interviewer-administered to collect information on parentally reported symptoms, physician-diagnosed illnesses, and treatments received. Children were defined as having *current wheeze* if they had wheezing in the previous 12 months at age 5 years. In addition, we assigned children into different wheeze phenotypes according to history of wheeze at the 2 follow-up visits.¹⁵ *No wheezing* was identified as no wheeze during the first 3 years of life, and no wheezing ever by age 5 years. *Transient early wheeze* was identified as wheezing during the first 3 years, and no wheezing in the previous 12 months at age 5 years. *Late-onset wheezing* was identified as no wheeze during the first 3 years, and wheezing in the previous 12 months at age 5 years. *Persistent wheezing* was identified as wheezing during the first 3 years, and wheezing in the previous 12 months at age 5 years.

Specific airway resistance (sR_{aw}) was measured at age 5 years by using whole-body plethysmography as previously described.^{13,16,17} sR_{aw} was calculated from a simple algebraic manipulation of the known formulas for airway resistance and total gas volume,¹⁶ which precludes the need for shutter occlusion. Three measurements of sR_{aw} were performed, and each was calculated from the medians of 5 consecutively measured technically acceptable loops.¹³ The mean

of these 3 measurements of effective sR_{aw} was used in the analysis. Children were asymptomatic at the time of assessment of lung function.

Sensitization status was ascertained by the measurement of specific IgE to mite, cat, dog, grasses, milk, egg, and peanut by ImmunoCAP (Pharmacia Diagnostics AB, Uppsala, Sweden) in serum collected at age 5 years. The detection limit of the assay was 0.2 kU_A/L (kilo units of Allergen per liter). Consistency, reproducibility, and precision is reported to be very good.¹⁸ When IgE was used as a dichotomous variable, sensitization was defined as values >0.2 kU_A/L. Specific IgE was also measured to mite, cat, dog, milk, and egg in a subsample of children at age 3 years. We also skin prick tested the children (*Dermaphagoides pteronyssinus*, cat, dog, grasses, molds, milk, egg; Bayer, Elkhart, Ind) and defined sensitization as a mean wheal diameter 3 mm greater than negative control.

Statistical methods

The primary outcome measures were current wheeze and lung function (sR_{aw}) at age 5 years (see results for persistent wheeze in the Online Repository in the online version of this article at www.jacionline.org). The relationship between sensitization status and outcome measures was analyzed by using logistic regression. Odds ratios (ORs) were estimated by using the regression models, and 95% CIs were generated according to Wald, using a *P* value of .05 as significant. Fitted predicted probability curves were plotted using the results from the logistic regression.

The levels of specific IgEs, mean wheal diameters, and sR_{aw} measurements were subject to a logarithmic transformation before analysis. For the quantitative evaluations, the ORs are presented for different IgE antibody levels expressing the increased risk associated with increasing antibody levels. Because a logarithmic transformation was used, all calculations were performed on the logarithmic scale. The relationship between sR_{aw} and IgE antibody levels was ascertained by using linear regression analysis. Computerized statistical analysis was carried out by using SAS System V8.01 (SAS Institute Inc, Cary, NC).

RESULTS

Of 1211 couples who initially agreed to take part, 1085 had a successful full-term pregnancy (>36 weeks of gestation; child did not need intensive care) and gave consent to a further follow-up. Of those, 128 were prenatally randomized to environmental control^{19,20}

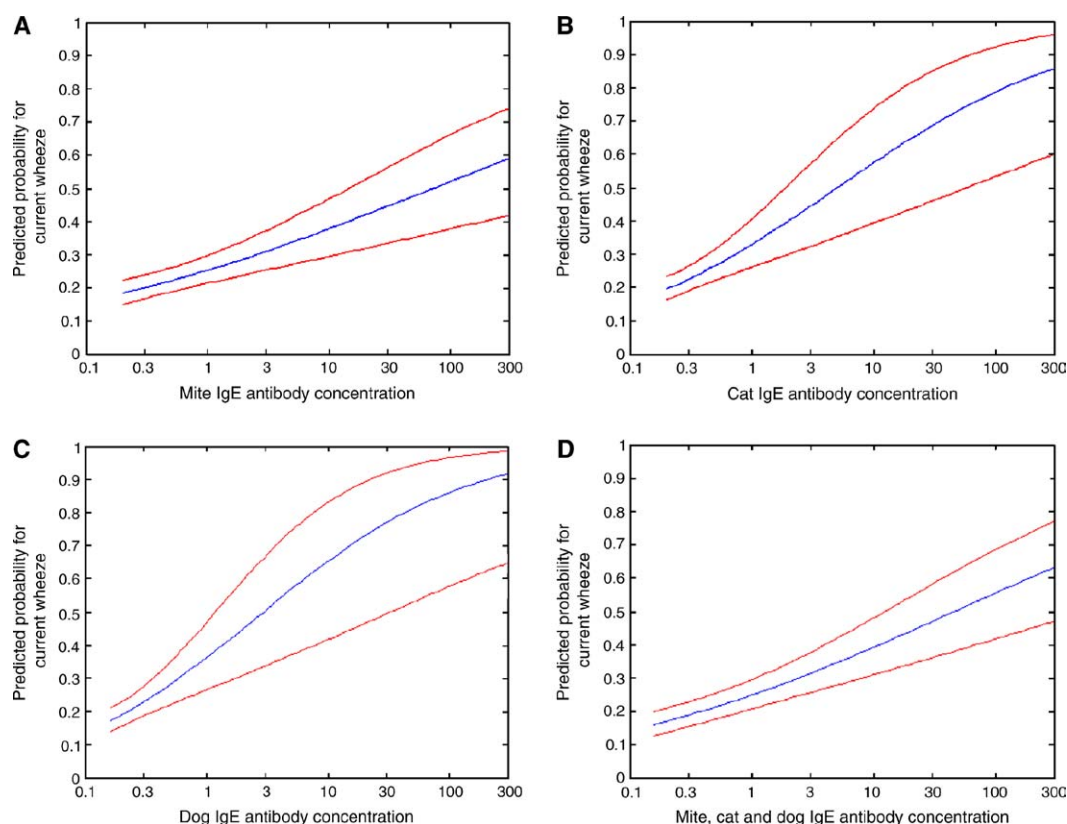


FIG 1. Fitted predicted probability curves (and 95% CIs) for current wheeze at a given IgE value for mite (A), cat (B), dog (C), and the sum of mite, cat, and dog (D) derived from the logistic regression analysis.

(excluded from this analysis), and 957 children were followed in the observational cohort. Of the children in the observational cohort, 840 (87.8%) attended the 5-year follow-up. All of these children had questionnaire data, and 521 provided a blood sample for IgE measurement (62%). Of the 319 children who did not have IgE measurements, 280 (87.8%) had skin tests. Children excluded did not differ from those included in terms of family history, parental smoking, maternal age, socioeconomic status, gestational age, birth weight, history of wheeze, and skin test results.

Of the 521 children, 184 had a raised specific IgE to 1 or more allergens (99 mite, 48 cat, 54 dog, 95 grass, 49 egg, 63 milk, 36 peanut); 69 children were sensitized to 3 or more allergens. Current wheeze at age 5 years was reported by the parents of 114 children (21.9%); 257 children never wheezed, 107 had transient early wheezing, 26 had late-onset wheezing, and 70 had persistent wheezing (61 children could not be classified into 1 of the 4 wheeze phenotypes). Sensitization (IgE) was present in 51.8% of children with current wheeze and 29.6% of never-wheezers.

Specific IgE, skin prick tests, and current wheeze

When the IgE antibody measurement was used to define subjects as sensitized or not (ie, dichotomous variable), there was a clear association between current wheeze and

sensitization to inhalant allergens, with less consistent associations for the food allergens (Table I). Similar results were seen when the analysis was repeated by using persistent wheeze as the outcome measure (see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

Using a range of cutoff points to define sensitization did not materially alter the results (see Tables E2 and E3 in the Online Repository in the online version of this article at www.jacionline.org). A similar effect was seen for skin tests results, with the effect strongest for sensitization to cat and to dog, whereas no association was observed for the food allergens.

We then explored whether the absolute level of specific IgE or the size of the wheal diameter of the skin test was useful in predicting the probability of respiratory symptoms. Using specific IgE as a continuous variable, the risk of current wheeze increased significantly with increasing specific IgE to mite, cat, and dog (all P values < .0001; Table I; Fig 1, A-C). When the levels of specific IgE to mite, cat, and dog were summed, the probability of current wheeze increased 1.33-fold per logarithmic unit increase (95% CI, 1.21-1.47; P < .0001; Fig 1, D), corresponding to an OR of 3.10 at 10 kU_A/L and 4.25 at 30 kU_A/L (see Table E4 in the Online Repository in the online version of this article at www.jacionline.org). When all 7 specific IgE results were summed, the probability of current wheeze increased 1.27-fold (95% CI, 1.16-1.38; P < .0001).

TABLE II. Quantification of IgE antibodies and SPTs and current wheeze at age 5 years among sensitized children

	Quantitative IgE (n = 184)			Size of skin test (n = 141)		
	OR	95% CI	P value	OR	95% CI	P value
Dust mite	1.20	1.06-1.36	.004	0.99	0.88-1.11	.82
Cat	1.37	1.11-1.69	.004	1.12	0.99-1.26	.07
Dog	1.45	1.12-1.88	.005	1.13	0.98-1.29	.08
Grass	1.02	0.88-1.18	.83	0.95	0.84-1.07	.37
Milk	1.08	0.71-1.62	.73	0.99	0.65-1.52	.97
Egg	1.09	0.63-1.89	.75	1.0	0.78-1.27	.98
Peanut	1.14	0.85-1.51	.38	NA		
Mite + cat + dog	1.28	1.13-1.46	.0002	1.14	0.92-1.41	.24
Sum of all	1.27	1.10-1.46	.001	1.24	0.75-2.04	.40

NA, Not applicable because children were not skin tested to peanut at age 5 years.

We found a weaker association for total IgE (OR, 1.23; 95% CI, 1.08-1.40; $P = .002$). When total and specific IgE results were included in the multivariate analysis, total IgE was not associated with outcomes (OR = 0.96), whereas OR for the sum of IgEs remained unchanged.

Increasing size of the individual mean wheal diameter of the skin test to mite, cat, dog, and grass pollen was significantly associated with current wheeze in the whole group (Table I).

Levels of specific IgE antibodies, skin test size, and risk of wheeze in sensitized children

To investigate further the relationship between quantitative IgE antibodies and current wheeze, we repeated the analysis among children considered to be sensitized using our definition ($n = 184$). Even in this group, increasing levels of specific IgE to mite, cat, and dog were associated with a significantly increased risk of current wheeze (Table II). There was no association between current wheeze and increasing level of food or grass pollen-specific IgE. The sum of mite-specific, cat-specific, and dog-specific IgEs was most strongly associated with current wheeze (OR, 1.28; 95% CI, 1.13-1.46; $P < .001$). Neither total IgE nor the size of the mean wheal diameter to any allergen was associated with current wheeze in sensitized children.

Specific IgE antibodies and lung function

In the linear regression analysis, increasing level of the sum of specific serum IgE antibodies to mite, cat, and dog was significantly associated with increasing level of sR_{aw} (ie, reduced lung function; $P = .004$; Fig 2). We found no association between the skin test size and lung function.

Levels of specific IgE antibodies and prediction of persistence of wheeze

Blood sample for IgE measurement at age 3 years was available in a subsample of 186 children (55 had wheezed within the first 3 years of life). To test the hypothesis that sensitization at age 3 years predicts the persistence of wheeze by age 5 years, we compared children with transient early wheezing ($n = 30$) with those with persistent wheeze ($n = 25$). By using specific IgE as a dichotomous variable, we confirmed that sensitization to mite, cat, and dog was associated with an increase in the risk of per-

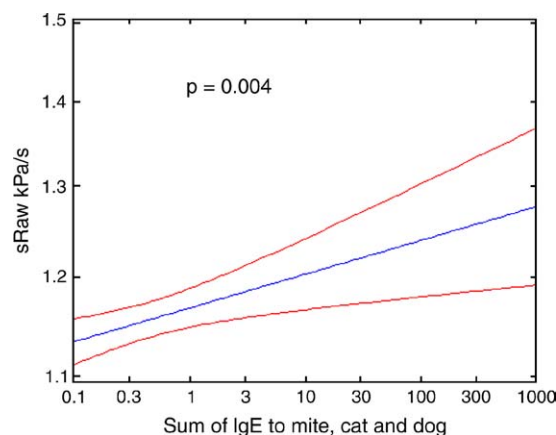


FIG 2. Association between lung function at age 5 years and a sum of mite, cat, and dog allergen-specific IgE antibodies (regression line with 95% CIs).

sistent wheeze (Table III). We conducted further analysis to investigate whether the level of the specific IgE gave additional information regarding the likely persistence of wheeze to age 5 years. For mite, cat, and dog, there was a trend toward increase in the risk of persistent wheeze with increasing specific IgE levels that failed to reach statistical significance. However, when the sum of the specific IgEs to mite, cat, and dog was considered, the risk of persistence of wheeze significantly increased with increasing sum of IgE (>2-fold per logarithmic unit increase in the specific IgEs; Table III; Fig 3). There was no significant association between total serum IgE and persistent wheeze (OR, 1.08; 95% CI, 0.88-1.31; $P = .47$).

For skin tests at age 3 years, using the results as a dichotomous variable, only sensitization to dog was associated with an increase in risk of persistence of wheeze. Using the size of the wheal diameter, the association was significant only for dog and added little to using skin tests as a dichotomous variable (Table III).

DISCUSSION

Our observations confirm that sensitization to inhalant allergens is a risk factor for wheezing. However, we have extended this observation in demonstrating that the

TABLE III. IgE antibodies and SPTs at age 3 years in 55 children who have wheezed previously and persistence of wheeze by age 5 years

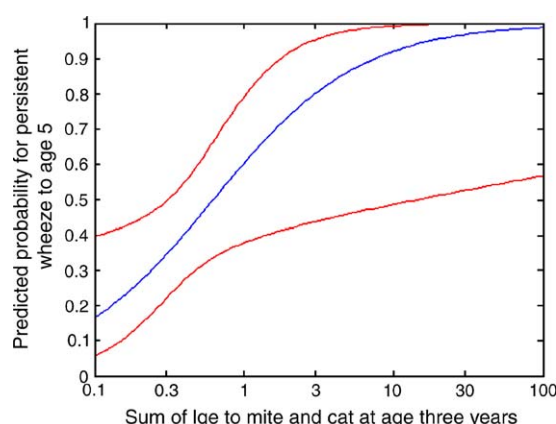
	Quantitative IgE			Dichotomous IgE*			Size of skin test			Dichotomous skin test†		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Dust mite	3.32	0.84-13.02	.09	5.4	1.02-29.19	.05	1.14	0.91-1.44	.26	2.05	0.51-8.30	.31
Cat	2.02	0.87-4.67	.10	9.15	1.02-82.13	.05	1.15	0.91-1.47	.24	3.50	0.62-19.87	.16
Dog	2.03	0.93-4.46	.08	5.44	1.02-29.19	.05	1.34	1.01-1.78	.04	5.44	1.02-29.19	.05
Grass	NA			NA			‡			‡		
Milk	2.54	0.55-11.77	.23	2.67	0.45-15.96	.28	‡			‡		
Egg	‡			‡			1.20	0.88-1.63	.24	3.96	0.39-40.64	.24
Mite + cat + dog	2.15	1.13-4.10	.02	NA			1.16	0.97-1.39	.10	NA		
Sum of all	2.25	1.16-4.35	.02	NA			1.13	0.96-1.36	.15	NA		

NA, Not applicable; IgE to grass pollen was not measured at age 3 years.

*Sensitization defined as specific IgE values >0.2 kU_A/L.

†Sensitization defined as a mean wheal diameter 3 mm greater than negative control.

‡Too few sensitized to analyze.

**FIG 3.** Fitted predicted probability curve for persistent wheezing by age 5 years in relation to the sum of mite, cat, and dog allergens derived from the logistic regression analysis.

absolute specific IgE antibody levels offer more information than just the presence of specific IgE. We found total IgE to be a poorer predictor of wheeze than the sum of specific IgEs. In addition, increasing specific IgE antibody levels were associated with reduced lung function in preschool children. Our data also suggest that among children who have wheezed within the first 3 years of life, the sum of IgE antibodies to inhalant allergens at age 3 years strongly predicts the persistence of symptoms to age 5 years. These findings indicate that within the context of respiratory illness, IgE-mediated sensitization should not be considered a simple dichotomous variable (ie, an all or nothing phenomenon) because the probability of wheeze and reduced lung function increases with increasing specific IgE antibody levels. In contrast with the findings with inhalant allergens, food allergens (with exception of peanut) did not have a major influence on the presence or persistence of wheeze in this age group. This was further accentuated when their inclusion in the model as the sum of all allergens resulted in a weaker association with wheeze than the sum of only the indoor inhalant allergens. Adding peanut IgE to the sum of indoor inhalants did not improve the prediction model. We also found that increasing skin prick test (SPT)

wheel size to inhalant allergens predicted an increase in the risk of current wheeze (we emphasize that more than 95% of all of the skin tests were performed by the same investigator with the same batch of extracts, ensuring reproducibility).

Although retention in this population-based observational birth cohort is excellent, we analyzed data from approximately half of the subjects (mostly because the child refused venepuncture). We emphasize that there was no difference between children excluded or included in the analysis in any relevant parameter. Furthermore, the prevalence of allergic sensitization among the parents of the children is similar to that of young adults in the United Kingdom,²¹ suggesting the subjects are representative of the general population.

Various cutoff values have been used to define sensitization in epidemiological studies and clinical practice (eg, even within 1 study, investigators from different centers used different IgE levels or skin test size).²²⁻²⁴ However, our data suggest that labeling subjects as sensitized or not on the basis of an arbitrary cutoff is an oversimplification of a trait that is not dichotomous in its relationship to wheeze.

To use quantitative information in a reliable way, it is essential to have a repeatable and reproducible measure. Our findings regarding skin testing are unlikely to be generalizable to the clinical setting, where skin tests are performed by different operators using a range of extracts, often containing variable quantities of allergens. Our ability to measure allergen-specific IgE antibodies progressed from qualitative assays in the late 1970s to the recently developed quantitative assays that allow reporting the results in mass units or in absolute amounts (1 kU_A/L equivalent to 2.24 ng specific IgE²⁵). During this time, there have been continued technical improvements, including the use of a well-defined calibration linked to the World Health Organization-determined IgE standard, well-standardized allergen extracts to bind all antibodies without competition between classes, and absolute linearity of the test at different concentrations of IgE antibodies.²⁵ This has led to newer tests that can measure the IgE antibodies with very high precision in quantitative terms.^{26,27}

With respect to the assessment of sensitization status, the important point practicing physicians need to consider is how much sensitization contributes to the symptoms. Within any population, a proportion of individuals is sensitized but has no symptoms of allergic disease. Conversely, it is almost certain that in some sensitized individuals with symptoms suggestive of allergic disease (eg, wheeze), the sensitization is just a chance finding not contributing to the presence or the severity of their symptoms. Our data demonstrate that the probability of wheeze increases with increasing IgE levels to inhalant allergens. Importantly, this is also true for the sensitized subgroup—that is, even among children who are assigned as allergic by commonly used cutoff points to define the presence of sensitization, the probability of wheeze increases significantly with the increasing sum of specific IgE antibodies to the 3 dominant inhalant allergens in this area (mite, cat, and dog). However, in this subgroup, there was no association between current wheeze and the size of the wheal diameter on skin testing.

Our finding of an association between IgE antibody levels and lung function is consistent with a recent study demonstrating that quantification of IgE antibodies may be of use not only to diagnose allergic diseases in young children but also to serve as a marker of severity of asthma.^{28,29}

The other clinically important question is how to predict persistence of symptoms in children with early-life wheeze. It is well recognized that most such children who wheeze in the first 3 years of life do not go on to develop asthma. Our results suggest that within this subgroup of children, the sum of specific IgEs to mite, cat, and dog predicted persistence of wheeze, with a 2-fold increase in risk with each logarithmic unit increase in IgE.

We emphasize that although there was a highly significant relationship between quantitative IgE antibodies to inhalant allergens at age 5 years and current and persistent wheeze within this large birth cohort study, the predictive value of such testing for individual children is likely to be lower than in the case of food allergy because of an overlap between the various wheezing phenotypes. However, IgE antibody quantification may be a clinically useful tool by increasing confidence that sensitization has a role in the expression of wheeze and discriminating transient from persistent wheeze in comparison with simple information on the presence or absence of IgE antibody above a certain arbitrary cutoff point.

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