Prediction of asthma by common risk factors: a follow-up study in Cuban schoolchildren

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Abstract

Objective: To determine which common risk factors, including environmental factors, are predictors for the development of asthma in Cuban schoolchildren.

Methods: A longitudinal study was conducted in 1042 schoolchildren without asthma at baseline in two Cuban municipalities. Asthma status in 2007, diagnosed using the International Study of Asthma and Allergies in Childhood questionnaire, was related to a set of common risk factors assessed in 2003/2004 in a multivariable logistic regression model. Multiple imputation was used for missing values. The final prediction model was obtained by backward selection (P<0.15). The model's prognostic accuracy (R^2) and discriminative ability (Area Under the receiver operating characteristic Curve [AUC]) were assessed and internal validation by bootstrapping was performed.

Results: Family history of atopic diseases (OR 2.19; 95%CI 1.19-4.04), allergic sensitization (OR 1.83; 95%CI 0.94-3.55), municipality (OR 0.34; 95%CI 0.15-0.74), and use of antibiotics in the child's first year of life (OR 1.66; 95%CI 0.89-3.11) were predictors for asthma development. The model had an R^2 of 8.0% and a moderate discriminative ability (AUC 0.69; 95%CI 0.60-0.78). Internal validation hardly influenced the model's performance.

Conclusions: Antibiotics use, genetic predisposition and allergic sensitization were predictors of asthma in Cuban schoolchildren. Although known as common risk factors they could only partly predict asthma development. Poverty-related factors, such as low income and education, and parasitic infections, did not have an effect. Other or additional environmental predictors need to be identified, as these are potential targets for prevention and control of childhood asthma in affluent as well as non-affluent countries.

Introduction

Childhood asthma is an important public health problem worldwide (1) and its prevalence has increased in the past decades (2). Risk factors include genetic, environmental and host factors. Common risk factors are gender, socioeconomic status, (prenatal) exposure to tobacco smoke (3), exposure to air pollution (4), diet (3, 5), climate (4, 6), infection (3, 7), obesity (8), antibiotic use, allergic sensitization, lung function, breastfeeding, family structure, exposure to (furry) animals (3), a family history of atopic diseases (3, 9), low birth weight (10), premature birth (11), and day-care attendance (12).

So far, most research has focused on the association of asthma with single risk factors. Prediction models, based on a set of risk factors, are scarce and have so far only been performed in affluent countries (13, 14). In developing countries other or additional factors, such as socioeconomic status (15) and parasitic infections (16), may be influential.

A family history of atopic diseases or genetic predisposition is known to be an important factor in the aetiology of asthma (3). However, genetics alone cannot explain the global rise in asthma prevalence in the last decades. Environmental factors, alone and by interacting with genes, are assumed to be responsible (3, 17). Unlike genetic and host factors, environmental factors can be modified, thus making them potential targets for intervention and prevention strategies. Using prediction model, we aimed to identify which environmental and other common risk factors contribute to the development of asthma in Cuban schoolchildren.

Methods

Study population and design

A longitudinal study was performed in primary schoolchildren in San Juan y Martínez (SJM) and Fomento, two municipalities in Cuba. Using Survey select, SAS version 8.0 (SAS Institute Inc., Cary, NC, USA), primary schools were selected randomly after double stratification for municipality (SJM or Fomento) and area of residence (urban or rural), and all children from each school were included. The inclusion period was December 2003 to May 2004. In SJM, there were 398 children: 207 (52%) from 2 urban and 191 children (48%) from 3 rural primary schools. In Fomento, there were 923 children: 483 children (52%) from 2 urban and 440 children (48%) from 12 rural primary schools. The children were followed up between February and May 2007. Further details have

been described elsewhere (18, 19). Of these 1321 children only those who did not have asthma at the start of the study (*N*=1042) were used for the development of the prediction model.

Informed written consent was obtained from the parents or guardians of each participating child. The study was performed within the framework of an institutional collaboration between the Institute of Tropical Medicine (ITM) in Antwerp, Belgium, the National Institute for Hygiene, Epidemiology and Microbiology (INHEM) and the Pedro Kuri Institute (IPK) of Tropical Medicine in Havana, Cuba. Approval was obtained from the Ethical Committees of these institutes.

Asthma outcome

At baseline and in 2007 a parent or guardian of each child was interviewed using the standard Spanish version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (20). Asthma was defined as an affirmative answer to the second ISAAC core asthma question on current wheeze ("Has your child had wheezing or whistling in the chest in the last 12 months?") (21).

Potential predictors

Potential predictors of asthma, based on previous literature, were assessed in 2003-2004. Antibiotic use during the child's first life year, furry pet ownership inside the house during the child's first life year, smoking by the mother during the child's early life (pregnancy and/or first year of life), current smoking inside the child's home, preschool day-care attendance, low birth weight (<2500 gram), premature birth (<37 weeks of gestational age), crowding (>2 persons/bedroom), sibship (≥1 siblings), area of residence (rural vs. urban), municipality (SJM vs. Fomento), education level of the mother (< high school vs. ≥ high school), monthly household income (≤250 pesos (≈ 7 euro)/month vs. >250 pesos/month), any breastfeeding for at least six months, infection with soil-transmitted helminths (STHs, i.e. *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm), and infection with protozoa were considered as potential environmental predictors. Potential genetic and host factors, i.e. age (continuous), sex, allergic sensitization (atopy) and a family history of atopic diseases (in mother, father and/or sibling) as proxy for genetic predisposition, were also taken into account. Apart from infection and allergic sensitization, information on the predictors was collected by means of a structured parental questionnaire.

One fresh stool sample was collected from each child and used for one direct smear and two 25 mg Kato-Katz examinations (22), with recording of all parasites detected. Infection with STHs was defined as the presence of STH eggs detected by either of the methods. Protozoa infection was defined as the presence of any protozoa cysts and/or trophozoites by direct smear.

Skin prick testing for allergic sensitization was performed using extracts of seven allergens (*Dermatophagoides pteronyssinus*, *D. farinae*, cat dander, mixed tree, mixed grass, *Alternaria alternata*, and cockroach) produced by ALK (Nieuwegein, The Netherlands). Histamine (10 mg/mL) was used as a positive and allergen diluent as a negative control. The extracts and controls were placed on the volar side of the left forearm using separate ALK lancets. Skin response was measured after 15 minutes; a wheal of 3 mm or larger in the absence of significant reactivity to the diluent control and a positive reaction to histamine was considered a positive reaction. Allergic sensitization was defined as a positive reaction to at least one of the seven allergens applied.

Statistical analysis

For the development of the prediction model we followed the guidelines of Harrell (23), Royston *et al.* (24), and Altman *et al.* (25), i.e. backward regression, with assessment of model performance and validation. Performance and validation are important to determine to which extent a model reflects reality and how it will perform in other populations.

To avoid bias due to missing values that could result from a complete case analysis, multiple imputation was applied to account for the missing values (26). This resulted in five multiple imputed data sets. Univariate logistic regression analyses were conducted to assess the relationship of each potential predictor with the outcome measure separately ($P<0.05$). Subsequently, all potential predictors were entered into a multivariable logistic regression model. To obtain the final model the prognostic variables were selected taking into account all five imputed data sets, i.e. by applying Rubin's Rules (27) in combination with backward regression analysis. For the selection of variables a *P*-value of less than 0.15 was used. We chose this less strict *P*-value as using a value of 0.05 can lead to selection bias and optimism as a result of overfitting, meaning that the model is too closely adapted to the data (24).

The prognostic accuracy of the models was estimated by their model-fit (calibration) using Hosmer–Lemeshow (H-L) test statistic and the explained variation (R^2). The discriminative ability of the model, i.e. the probability to distinguish between asthma-

positive and -negative children, was estimated by assessing the Area Under the receiver operating characteristic Curve (AUC) with 0.5 indicating a model with no discriminating power and 1.0 a perfectly discriminating model. The final model was internally validated by bootstrapping techniques. We used 200 bootstrap samples. Optimism in regression coefficients due to overfitting was estimated by the calibration slope of the observed proportions plotted against the predicted probabilities, with a slope of 1.0 indicating no optimism by overfitting. Accuracy measures were estimated in each imputed dataset and subsequently averaged (except for the H-L test statistic). SPSS Statistics 17.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all analyses except for the model validation, which was performed with R version 2.10 (R Foundation for Statistical Computing, Vienna, Austria) (28).

Results

The 1042 children from the baseline study population were aged 4 to 14 years (mean 8.6 years), consisted of 531 boys (51.0%) and 511 girls (49.0%); 492 (47.2%) children lived in a rural area, and 550 (52.8%) in an urban area. The monthly family income was 250 pesos or less in 558 children (54.0%) and for 551 children (53.2%) the mother had an educational grade less than high school. Of the 1036 children who provided stool samples, 101 (9.7%) were positive for *T. trichiura,* 60 (5.8%) for *A. lumbricoides*, 100 (9.7%) for hookworm, and 462 (44.6%) for protozoa infection.

In 2007, 890 children (85.4%) of the selected cohort were traced. The main reason for loss to follow-up was that they had moved outside the study area. This population was then aged 7 to 17 years (mean 11.4 years), and consisted of 456 boys (51.2%) and 434 girls (48.8%). The response rate to the questionnaires was 90.0% (*N*=801). Asthma was reported in 31 children (3.9%). Table 1 shows the characteristics of the study population according to asthma outcome. In children with asthma, low birth weight, antibiotics use in their first year of life, a family history of atopic diseases, and allergic sensitization were more prevalent than in those without.

From the 1042 children at baseline, complete data on asthma status in 2007 were available for 801 children (76.9%). Missing data in individual predictors from 2003/2004 ranged from 0% to 1.4%. Children with missing data on asthma status significantly differed from those with complete data regarding age, municipality, area of residence, and infection status. For this reason, multiple imputation was applied to account for missing values. Thus our results pertain to the imputed dataset, including all 1042 children.

Table 1. Characteristics of the study population with and without asthma.

	Asthma (*N*=31)	**No asthma (*N*=770)**
Age (median (IQR)), y	9 (2)	8 (4)
Female sex	19 (61.3)	378 (49.1)
Municipality (Fomento)	20 (64.5)	627 (81.4)
Area of residence (urban)	20 (64.5)	429 (55.7)
Education mother (≥ high school)	15 (48.4)	366 (47.9)
Income (>250 peso/month)	15 (48.4)	362 (47.4)
Trichuris trichiura infection	3 (9.7)	67 (8.7)
Ascaris lumbricoides infection	3 (9.7)	32 (4.2)
Hookworm infection	2 (6.5)	76 (9.9)
Any protozoa infection	13 (41.9)	353 (46.0)
Low birth weight	7 (22.6)	71 (9.3)
Premature birth	3 (9.7)	47 (6.1)
Breastfeeding (≥6 months)	10 (32.3)	383 (49.9)
Antibiotics use during child's first year of life	20 (64.5)	329 (43.2)
Family history of atopic diseases	20 (64.5)	303 (39.5)
Mother smoked during child's early life	6 (19.4)	99 (12.9)
Current smoking inside child's house	19 (61.3)	364 (47.3)
Furry pet ownership inside the house during child's first year of life	14 (45.2)	417 (54.3)
Preschool day-care attendance	6 (19.4)	133 (17.3)
Crowding (>2 persons/bedroom)	8 (25.8)	221 (28.9)
Sibship (≥1 siblings)	26 (83.9)	643 (82.3)
Allergic sensitization	11 (35.5)	141 (18.3)

Values are extracted from the complete, non-imputed cases (*N*=801) and are expressed as number (%) of patients, unless otherwise specified; asthma was assessed in 2007 and the characteristics were in 2003/2004.

Univariate and multivariable analysis

Antibiotic use in the child's first year of life, a family history of atopic diseases, and municipality were univariate predictors of the development of asthma (Table 2).

Table 2. Univariate associations of possible predictors with asthma.

	OR (95% CI)	*P*-value
Age (median (IQR)), y	1.01 (0.87-1.18)	0.877
Female sex	1.14 (0.59-2.19)	0.686
Municipality (Fomento)	0.38 (0.18-0.81)	0.016
Area of residence (urban)	1.16 (0.60-2.26)	0.650
Education mother (≥ high school)	0.95 (0.46-1.95)	0.886
Income (>250 peso/month)	0.98 (0.47-2.03)	0.954
Trichuris trichiura infection	1.13 (0.37-3.46)	0.819
Ascaris lumbricoides infection	1.83 (0.68-4.95)	0.227
Hookworm infection	0.74 (0.24-2.31)	0.602
Any protozoa infection	0.81 (0.45-1.44)	0.465
Low birth weight	1.67 (0.66-4.25)	0.271
Premature birth	1.04 (0.35-3.13)	0.940
Breastfeeding (≥6 months)	0.62 (0.26-1.47)	0.256
Antibiotics use during child's first year of life	1.92 (1.03-3.56)	0.041
Family history of atopic diseases	2.12 (1.13-3.99)	0.021
Mother smoked during child's early life	1.40 (0.56-3.45)	0.455
Current smoking inside child's house	1.27 (0.58-2.76)	0.531
Furry pet ownership inside the house during child's first year of life	0.80 (0.37-1.71)	0.541
Preschool day-care attendance	0.89 (0.39-2.00)	0.769
Crowding (>2 persons/bedroom)	1.07 (0.53-2.16)	0.851
Sibship (≥1 siblings)	1.16 (0.55-2.46)	0.700
Allergic sensitization	1.40 (0.74-2.67)	0.300

Values are extracted from the imputed cases (*N*=1042); asthma was assessed in 2007 and the characteristics in 2003/2004.

In the multivariable logistic regression analysis, antibiotic use in the child's first year of life, a family history of atopic diseases, allergic sensitization, and municipality were predictors of the development of asthma (Table 3). The H-L test statistic was not significant in any of the imputed datasets, indicating that the overall model fit was good. The model explained 8.0% of the variation in the outcome, and the AUC of the model was 0.69 (95%CI 0.60-0.78). After internal validation the R^2 reduced to 6.3% and the AUC to 0.67. The calibration slope was 0.92 indicating little optimism or overfitting of the regression coefficients.

Table 3. Multivariable prediction model for asthma

	OR (95% CI)	*P*-value
Antibiotics use during child's first year of life	1.66 (0.89-3.11)	0.108
Allergic sensitization	1.83 (0.94-3.55)	0.075
Family history of atopic diseases	2.19 (1.19-4.04)	0.013
Municipality (Fomento)	0.34 (0.15-0.74)	0.009

Values are extracted from the imputed cases (*N*=1042); asthma was assessed in 2007 and the characteristics in 2003/2004.

Discussion

So far, few studies have considered multiple risk factors in prediction models and those that have, have been performed in populations from affluent countries. We studied the contribution of a set of environmental and other common risk factors to asthma development in a population of Cuban schoolchildren in a three-year follow-up study. We found that antibiotic use during the child's first year of life, municipality, a family history of atopic diseases, and allergic sensitization were the most important predictors for the development of childhood asthma in Cuba. Poverty-related factors, such as low income and education, and parasitic infections, did not have any effect. The performance of the prognostic model was moderate.

Some limitations should be remarked on. The ISAAC questionnaire is a well-established, widely used and validated questionnaire for the measurement of asthma in childhood populations (20). Nevertheless, some information and recall bias may have occurred using this or other questionnaires in this study. Although a low incidence of asthma is normal in school-aged children (29, 30), this limited the power of our study. Therefore, we cannot rule out that we missed some weaker predictors. We are aware that the use of retrospective data to assess most predictors weakens our findings to some extent. Prospective longitudinal data recorded in real time and before the outcome of interest would be preferable, but they were not available. Nevertheless, our study provided a unique opportunity to explore, for an important childhood disease, many common predictors simultaneously in a developing country using a prediction model. We were not able to validate the model in an external dataset, which would have been the best approach. However, with our internal validation, we were able to obtain a realistic indication about how the model would perform in other populations. The rule of thumb in logistic models to consider at least 10 events per predictor variable (EPV) was not met in our model. However, Vittinghoff and McCulloch (31) elegantly showed in a large simulation study that this rule can be relaxed and that with less than 10 EPV only little bias in coefficient estimates can be expected. Furthermore, our model validation by

bootstrapping, a recommended method in models with less that 10 EPV (31), showed little bias of our estimates. Finally, although we used multiple imputation to account for missing values, we cannot totally exclude bias due to loss to follow-up.

To our knowledge only two other prediction models for asthma development have been developed as suggested by Harrell (23), Royston *et al.* (24), and Altman *et al.* (25), namely those by Balemans *et al.* (13) and Caudri *et al.* (14). Both studies concerned Dutch populations and had a clinical focus, which influenced the predictors investigated. Nevertheless, like us, they also addressed some genetic, host, and environmental predictors.

The role of genetic predisposition and allergic sensitization in asthma development is well known (3) and was confirmed by our study and the Dutch studies (13, 14). Childhood asthma is more common in boys, reflected by the study of Caudri *et al.* (14), while in adolescence this trend reverses and asthma becomes more common in females (3), as shown by Balemans *et al.* (13). This time-dependent effect of sex on asthma may explain why sex was not a predictor in our study as our Cuban study population consisted of both children and adolescents.

Among the environmental predictors that were considered in all three studies, i.e. sibship, breastfeeding, infection, smoking, birth weight, day-care attendance, pet ownership, and antibiotic use, we only found the last factor to be of importance. Antibiotic use has been positively associated with asthma although the mechanism is not clear. It has been suggested that antibiotics could influence the immune system by changing the bowel flora and as such cause asthma (3). Furthermore, they could be a proxy for the number of early-life (viral) infections (3). Some of these infections, such as viral respiratory tract infections, are generally associated with increased asthma risk (3, 32). Respiratory tract infections increase asthma risk and were identified as predictors of asthma by Balemans *et al.* (13) and Caudri *et al* (14). Passive smoking exposure is in general associated with an increased risk of asthma (3), but this was only confirmed by Balemans *et al.* and not by Caudri *et al.* or by our study. Apart from differences in study populations, the inconsistencies in study results may also be due to differences in used asthma definitions, the age of assessing predictors and outcome, and the time between measurements.

Poverty-related factors, such as low income and education, and parasitic infections, did not have a significant effect on the development of asthma in Cuban children. So far the association between socioeconomic status and asthma prevalence has been inconclusive (3). Based on the hygiene hypothesis, an inverse association is generally assumed between STHs and asthma (33), although helminth-specific effects have been reported recently (34). However, our prediction model suggests that STH infections do

not play an important role in the development of asthma in Cuban schoolchildren. This may be due to the relatively low prevalence of STH infections in these children or to the timing of infection assessment, as infections occurring during infancy appear to be more important than those occurring later in childhood (35).

Studies assessing risk factors concurrently have been performed in developing countries (36-38), but not according to the approach of Harrell (23) and Royston *et al.* (24), and Altman *et al.* (25). In line with our study, these studies suggest that a family history of atopic diseases and allergic sensitization are predictors of asthma development (36, 37). Environmental factors found included smoking exposure (36), education (36, 38), breastfeeding (38), sibship (38), and day-care attendance (37). Typical poverty-related factors were not studied.

To date, all prediction models for asthma, including ours, have only shown moderate performance. While a large range of common risk factors has been assessed, they still appear to be insufficient to explain the development of a multifactorial disease like asthma. Other –environmental– factors would appear to be of importance. For example, concurrently with the rise of asthma in the last decades, diets have changed considerably, particularly in westernized countries (5). Air pollution has been positively associated with asthma, and people in urban areas, where air pollution is greater, tend to have more asthma than those in rural areas (4). Climate, by means of temperature and humidity for example, and subsequently climate change can influence pollution and pollen levels in the air and consequently induce asthma (4). Municipality, an independent predictor in our study, could be a proxy for these factors, but also other unidentified environmental factors.

In conclusion, the role of genetic predisposition and allergic sensitization in asthma development in a non-affluent country was confirmed by our study. Antibiotic use during early life was the only important environmental predictor for asthma development in Cuban schoolchildren. Poverty-related factors, such as low income and education, and parasitic infections, do not seem to have an effect. More prediction models should be developed, taking into account additional environmental factors, to identify important predictors that can be targeted for prevention and control of asthma in affluent as well as non-affluent countries.

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