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Predictors of severe asthma attack re-attendance in Ecuadorian children: a cohort study

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Take home message: Among children in a low low-resource setting in Latin America, younger age, an established asthma diagnosis and history of severe asthma attacks in the previous year were associated with recurrence of severe asthma attacks, irrespective of biomarkers.

Word count manuscript: 3040

ABSTRACT:

Asthma is a common cause of emergency care attendance in low and middle-income countries (LMICs). While few prospective studies of predictors for emergency care attendance have been undertaken in high-income countries, none have been done in a LMIC.

We followed a cohort of 5-15 year olds treated for asthma attacks in Emergency Rooms (ERs) of public health facilities in Esmeraldas City, Ecuador. We collected blood and nasal wash samples, and did spirometry and Fractional exhaled Nitric Oxide measurements. We explored potential predictors for recurrence of severe asthma attacks requiring emergency care over 6 months follow-up.

We recruited 283 children of whom 264 (93%) were followed up for at least 6 months or until their next asthma attack. Almost half (46%) had a subsequent severe asthma attack requiring emergency care. Predictors of recurrence in adjusted analyses were (adj. OR; 95% CI) younger age (0.87 per year; 0.79-0.96), previous asthma diagnosis (2.2; 1.2-3.9), number of parenteral corticosteroid courses in previous year (1.3; 1.1-1.5), food triggers (2.0; 1.1-3.6), and eczema diagnosis (4.2; 1.02-17.6). A parsimonious Cox regression model included the first three predictors plus urban residence as a protective factor (adj. HR: 0.69; 0.50-0.95). Laboratory and lung function tests did not predict recurrence.

Factors independently associated with recurrent emergency attendance for asthma attacks were identified in a low-resource LMIC setting. This study suggests a simple risk-assessment tool could potentialy be created for ERs in similar settings to identify higher risk children on whom limited resources might be better focussed.

Words: 245/250

INTRODUCTION

Severe asthma attacks requiring emergency care, hospital admission or systemic corticosteroids[1] are a common source of preventable morbidity in children. Asthma attacks are associated with impaired lung function [2], anxiety in patients and families [3], and elevated healthcare and family costs [4]. Many asthma attacks are preventable, either by avoiding triggers or by appropriate preventive treatment. Inhaled corticosteroids (ICS) reduce the probability of asthma attacks by around 40% [5] and can attenuate the decline in lung function associated with asthma attacks [2]. Because ICS have associated side-effects[6-8] and costs, use should be targeted at those at greater risk of attacks or persistent symptoms, especially when resources are limited. Identifying children with a greater risk of asthma attacks is crucial for optimization of asthma treatment, particularly in low resource settings where underdiagnosis and lack of capacity for long-term management are major problems.

In most Latin American countries where health resources and specialist follow-up care are limited [9,10], children with asthma are mainly seen in emergency rooms (ER) during acute attacks [11,12]. The ER represents an opportunity to identify children at higher risk of future attacks. Factors that have been previously associated with recurrent asthma attacks requiring emergency care in paediatric cohort studies include history of previous ER attendance for attacks, younger age, black ethnicity and low socioeconomic status [13-16]. However, these studies have been almost exclusively done in North America and did not evaluate potential usefulness of biomarkers commonly available in higher income settings [16]. No prospective studies investigating emergency room reattendance for asthma from lower income settings were identified in a recent systematic review [16].

We undertook a prospective cohort study to identify clinical factors and biomarkers associated with recurrent severe asthma attacks in children presenting with an asthma attack to regional ERs in a limited resource setting in Latin America.

METHODS

Study population and design

This prospective cohort study was done in the city of Esmeraldas (population 150,000 of mainly Afro-Ecuadorian ethnicity) in the north-western coastal province of Esmeraldas. Patients were recruited from the city's public hospital, Delfina Torres de Concha Hospital (DTCH) that offers free attention and treatment, the Instituto Ecuatoriano de Seguro Social

Esmeralda's Hospital (IESS) which offers free treatment to those paying national insurance, and the three largest public health centres in the city with 24-hour emergency care.

Children aged 5-15 years treated at emergency departments for asthma attacks (defined as bronchodilator-responsive wheeze) were recruited between May 2014 and September 2015. Children with other chronic conditions were excluded. Written consent was obtained from the child's caregiver and minor assent from children over 7 years. A total of 283 children were recruited. The protocol was approved by the Bioethics Committees of the Liverpool School of Tropical Medicine and the Universidad San Francisco de Quito, Quito, Ecuador.

Measurements of exposures and outcomes

Data were collected in Spanish from the child's caregiver using a modified version of the International Study of Asthma and Allergies in Childhood (ISAAC) Phase II questionnaire [17] that has been extensively field-tested[18,19], together with additional questions regarding potential risk factors for recurring asthma attacks. A detailed description of variables studied is included in online supplementary table S1. The Childhood Asthma Control Test (C-ACT)[20] was completed by the child and guardian for children <12 years of age or the Asthma Control Test[21] for those ≥12. Weight and height were measured. A blood sample was drawn for haematocrit, blood count, and plasma assays. Total and specific IgE (sIgE) for *D. pteronyssinus* and *B. tropicalis* were measured using the CAP system (Pharmacia Diagnostics) in 86 random plasma samples, of which 60 were tested for German cockroach and food mix (egg white, milk, cod, wheat, peanut, soy) specific IgE. A positive assay for sIgE was defined as >0.70 kU/l. A nasal wash with saline was done to determine the relative proportion of granulocytes (eosinophil vs. neutrophils) using a protocol modified from a previous study [22] (see online supplementary file for detailed technique). Participants returned 2 weeks after recruitment (or up to 3 weeks if during the 2-week appointment they were still taking daily salbutamol or oral corticosteroids or had wheezing) for pre and post-bronchodilator spirometry (Microloop spirometer, Micro Direct, UK) and Fractional Exhaled Nitric Oxide (FeNO: NObreath, Bedfont Scientific, UK). At this time, the Pediatric Asthma Quality of Life Questionnaire (PAQLQ)[23] and Newcastle Asthma Knowledge Questionnaire (NAKQ)[24,25] were completed. Asthma severity was classified using the Ecuadorian Asthma Consensus 2011[26] which closely follows GINA 2008[27] and Spanish 2009 asthma guidelines (GEMA 2009)[28]. Participants were offered inhaled beta$_2$-agonists as relievers with or without corticosteroids for long-term treatment in accordance with these guidelines. They were also provided with a standardised

short education on asthma, recommendations for future asthma attacks, and written asthma action plans.

Each participant was followed up with twice-monthly telephone calls asking about asthma attacks (number, severity, duration and treatment received) and told to contact the study team immediately following an attack. A severe asthma attack was defined as an acute exacerbation of respiratory symptoms (difficulty breathing and wheezing that improved with bronchodilators) requiring either i) urgent care at an ER or ii) unscheduled medical visit with prescription of systemic corticosteroids (for at least 3 days if oral)[1]. Hospital and health centres' records were checked twice-weekly to record unscheduled visits for attacks.

Statistical analysis

The primary outcome was recurrence of an asthma attack requiring emergency care and occurring between 2 weeks and 6 months after the index attack. Only the first recurrence was considered in the analysis among those with more than one attack during follow-up. Only variables measured at baseline were studied as risk factors. Continuous variables were compared using Student's t or the Mann-Whitney U tests, as appropriate. Categorical variables were compared using Fisher's exact test. Multivariable logistic regression was used to evaluate the effects of multiple risk factors for emergency care re-attendance on risk of recurrence of asthma attacks. Only children followed-up until their first asthma attack recurrence or for a minimum of 6 months exacerbation-free were included in this analysis. A time-to-event analysis was done using a multivariable Cox proportional hazard model, including all children that completed at least 2 weeks follow-up. Collett's method[29] of variable selection was used with a P value threshold of 0.2, and confounding and interactions between variables were assessed. The parsimonious logistic and Cox models were selected based on explained variation (R^2). The proportional hazards assumption required by the Cox model was tested via the inclusion of time-dependent variables. The assumption was valid for all included variables.

The predictive ability of the models was evaluated by estimating the area under the Receiver Operating Characteristics curve (ROC)[30] and its 95% confidence interval, with 0.5 indicating a model with no discriminating power and 1.0 a perfectly discriminating model[31]. Internal validity was then assessed by bootstrapping using 200 random bootstrap samples with replacement, to evaluate potential bias (over- or under-fitting). Optimism in regression coefficients due to overfitting was estimated by measuring the difference between the

model's c-statistic (apparent c-statistic) and the c-statistic computed by nonparametric bootstrap resampling (internal bootstrap validation c-statistic)[32].

Statistical analyses were done using STATA 13.1 with significance set at $P < 0.05$. Details of sample size calculations and missing data strategy are provided in online supplementary methods.

RESULTS

Of the 283 children recruited, thirteen (4.6%) were lost to follow-up after the initial visit, and 264 (93%) were followed up until their next asthma attack or a minimum of 6 months exacerbation-free. Of these, 121 (46%) had at least one subsequent asthma attack requiring emergency care during the first 6 months of follow-up (figure 1). The median follow-up time was 408 days (IQR: 265-541 days, range 44-697 days) and the median time to a subsequent asthma exacerbation was 91 days (IQR: 39-178 days). Figure 2 shows time to first recurrence of a severe asthma attack.

(Figure 1 and 2 here)

Table 1 and 2 show characteristics for the 264 children completing 6 months follow-up or until first asthma attack recurrence, stratified by readmission status at 6-months follow-up. Available data for each variable are shown in the online supplementary table S1. Data for allergy and inflammatory markers and lung function are shown in figure 3. Thirty nine percent (107/272) of all children had blood eosinophilia (>500cells/µl) and 72% had nasal eosinophilia (>5%) [33]. Median FeNO for the whole sample was 33ppb (IQR: 3-79). Median predicted FEV_1 in 223 children who underwent spirometry was 97% (IQR: 86-107) of whom 30 (13%) had values below the lower limit of normal. Median Pre-FEV_1/FVC ratio was 91% (IQR: 85-97), median FEV_1 increase after inhaled bronchodilator was 6.4% (IQR: 2-14) and 109 (49%) had a positive bronchodilator response (increase in FEV_1 >12%). IgE was measured in a random sample of 86 participants: total IgE (median, 770 kU/l, IQR: 329-1376), and mite (*D. pteronyssinus* and *B. tropicalis*) IgE (81% positive). Of the 60 samples tested for specific IgE for cockroach and food allergens, 82% were positive for any allergen.

Table 1: Sociodemographic characteristics, personal and family history of 264 participants followed-up for 6 months or until first asthma attack recurrence, stratified by recurrence status at 6 months

		Total Cohort (N=264)	Second asthma attack within 6 months		
			Yes (N=121)	No (N=143)	P
Sociodemographic Characteristics					
Age (years) (median, IQR)		8 (6-11)	7 (5-10)	9 (7-11)	**<0.001**
Sex male (%)		59	60	59	0.900
Ethnicity (%)	Afro-Ecuadorian	54	52	55	0.963
	Mestizo	43	45	42	
	White	2	2	1	
	Other	2	2	2	
BMI (kg/m^2) (median, IQR)		16.6 (15.1-19.4)	16.4 (15.0-19.2)	16.9 (15.1-19.7)	0.265
At least one sibling (%)		90	89	91	0.547
No. of years study by mother + No. of years by father (median, IQR)		24 (18-28)	24 (18-28)	24 (19-29)	0.304
Pets at home (%)		43	45	42	0.708
Second hand smoke exposure (%)		18	17	21	0.474
Humid household (%)		47	4	51	0.137
Urban setting (%)		66	60	70	0.094
Intense traffic near house (%)		30	30	30	1.000
Monthly household income (USD) (median, IQR)		400 (255-700)	400 (260-700)	350 (250-700)	0.981
Personal and Family History					
Early life respiratory illness (%)		49	59	40	**0.013**
Allergic rhinitis ever (%)		72	74	70	0.490
Allergic rhinitis diagnosis (%)		21	20	21	0.878
Eczema ever (%)		13	14	12	0.713
Eczema diagnosis (%)		4	7	2	0.119
Paternal/maternal asthma (%)		46	53	41	0.175
Paternal/maternal asthma/rhinitis/eczema (%)		68	70	66	0.788

IQR: Interquartile range; BMI: Body Mass Index; USD: US dollars.
Bold: statistically significant differences (p<0.05)

Table 2: Asthma characteristics* of 264 participants followed for 6 months or until first asthma attack recurrence, stratified by readmission status at 6-months.

		Total Cohort (N=264)	Second asthma attack within 6 months		
			Yes (N=121)	No (N=143)	P
Wheezing ever (%)		94	98	90	**0.021**
Wheezing last 12m (%)		87	93	81	**0.003**
No. attacks last 12m (median, IQR)		3 (2-6)	5 (2-6)	3 (1-5)	**<0.001**
Days since last attack (median, IQR)		60 (30-120)	60 (30-120)	60 (30-150)	0.120
Wheezing at night last 12m (%)	Never	19	16	21	0.424
	<1 night per week	1	2	0.7	
	≥1 night per week	14	17	11	
	Only during attacks	66	66	67	
Severe attack last 12m (%)		36	44	30	**0.014**
Wheezing with exercise (%)	No	36	30	41	0.188
	Yes	40	44	37	
	Only during attacks	24	27	23	
Previous asthma diagnosis (%)		64	77	54	**<0.001**
Number of triggers (median, IQR)		4 (2-6)	4 (3-6)	4 (2-5)	0.117
Food as trigger (%)		37	46	29	**0.006**
Doctor visit for acute asthma last 12m (%)	None	15	8	21	**<0.001**
	1-3	49	46	53	
	4-12	27	32	23	
	>12	8	14	3	
Doctor visit for asthma control last 12m (%)	None	68	65	71	0.592
	1-3	24	26	23	
	4-12	7	8	7	
	>12	0.4	0.8	0	
ICS treatment (%)		2	0.9	3	0.374
ER visit last 12m for asthma (%)		76	79	74	0.388
No. ER asthma visits last 12m (median, IQR)		2 (1-4)	3 (1-6)	2 (0-3)	**0.006**
No. IV/IM CS courses last 12m for asthma (median, IQR)		0 (0-1)	1 (0-3)	0 (0-1)	**<0.001**
Ever admitted for asthma (%)		25	34	18	**0.004**
Admitted for asthma last 12m (%)		8	12	4	**0.034**
Ever admitted to ICU for asthma (%)		10	13	7	0.102

IQR: Interquartile range; ICS: Inhaled corticosteroids; ER: emergency room; IV/IM: intravenous or intramuscular; CS: corticosteroids; ICU: Intensive Care Unit; 12m: 12 months. * All the variables were measured at baseline, at the moment of the index asthma attack.
Bold: statistically significant differences (p<0.05)

(*Figure 3 here*)

In univariable analyses (tables 1 and 2), risk factors for recurrence were younger age, early life severe respiratory illness, food triggers, previous asthma diagnosis, number and severity of asthma attacks over the previous year, and lower haemoglobin levels (recurrence, mean (SD) 12.9 (0.87) vs. no recurrence, 13.1 (0.84), p=0.032). There were no differences in laboratory or lung function parameters (figure 3). Positivity for IgE specific to any allergen did not vary by recurrence status (recurrence, 33/42 [79%] vs non-recurrence, 37/44 [84%]). The parents of children with recurrence had less specific asthma knowledge (NAKQ score) (OR: 0.91 per 1 point, 95% CI: 0.85-0.98) and children had lower PAQLQ emotional domain (OR: 0.72 per 1 point, 95% CI: 0.55-0.94) scores (online supplementary table S2). No differences were seen between the two groups for asthma control (ACT and C-ACT scores) or PAQLQ total, symptoms or activity scores (online supplementary table S2). Further results concerning food triggers for asthma attacks are included in the Supplementary files.

The multivariable logistic regression model for risk of severe asthma attack recurrence showed independent risk factors to be younger age, a previous asthma diagnosis, food triggers, number of parenteral corticosteroid courses for acute asthma in the previous 12 months, and previous eczema diagnosis (table 3). The area under curve (AUC) of the model was 0.73 (95% CI: 0.67-0.79) (c-statistic) and the model explained 13% of variation. After internal validation (bootstrapping), AUC reduced to 0.72 and R^2 to 14.1%, indicating little overfitting of regression coefficients.

Table 3. Multivariable logistic regression for risk of ER re-attendance for severe asthma attacks during 6 months follow-up. (N=264)

	Crude OR	**95% CI**	**P value**	**Adjusted OR***	**95% CI**	**P value**
Age	0.87	0.79-0.95	0.001	0.87	0.79-0.96	0.006
Previous asthma diagnosis	2.78	1.62-4.76	<0.001	2.17	1.19-3.94	0.011
No. IV/IM CS courses during last 12m	1.36	1.15-1.61	<0.001	1.28	1.08-1.53	0.006
Food as trigger	2.11	1.25-3.55	0.005	1.99	1.11-3.55	0.020
Eczema diagnosis	3.26	0.84-12.6	0.086	4.22	1.02-17.54	0.048

*: Adjusted Odds Ratios were adjusted for all other variables in the model. OR: odds ratio, CI: confidence interval; IV: intravenous; IM: intramuscular; CS: corticosteroids.

Table 4 shows the results of multivariable analysis for time to asthma attack recurrence. All children with at least 2 weeks of follow-up were included in the analysis (N= 270). Children of younger age, with an existing asthma diagnosis, greater number of parenteral corticosteroid courses for acute asthma in the previous 12 months and not living in an urban setting, showed a higher rate of recurrent asthma attacks requiring emergency care. The AUC of the model was 0.65 (95% CI: 0.60-0.70) (c-statistic) and overfitting of the model was estimated as <1% after bootstrapping.

Table 4. Multivariable Cox regression model for ER re-attendance for severe asthma attacks. (N = 270)

	Crude HR	95% CI	P value	Adjusted HR*	95% CI	P value
Age	0.92	0.87-0.97	0.002	0.93	0.88-0.98	0.009
Previous asthma diagnosis	1.78	1.26-2.53	0.001	1.66	1.15-2.39	0.007
No. IV/IM CS courses during last 12m	1.17	1.10-1.24	<0.001	1.13	1.06-1.20	<0.001
Urban residence	0.65	0.47-0.89	0.007	0.69	0.50-0.95	0.023

*: Adjusted Hazard Ratios were adjusted for all other variables in the model. HR: hazard ratio, CI: confidence interval; IV: intravenous; IM: intramuscular; CS: corticosteroids.

DISCUSSION

We have identified independent predictors for emergency care re-attendance for severe asthma attacks among asthmatic children in a low resource setting in a tropical region of coastal Ecuador, the first such prospective study done to our knowledge in an LMIC setting [16]. Having a previous severe asthma attack was the most reliable predictor of future risk: each acute parenteral corticosteroid course received during the previous year for acute asthma increased the odds of a subsequent severe attack by a factor of 1.28. Other factors associated with risk of attack or time to next attack were similar including younger age (both), an existing asthma (both) or eczema (risk of recurrence) diagnosis, food triggers (risk of recurrence), and rural residency (time to recurrence). We were unable to identify a biomarker that could usefully predict future risk of severe attacks.

Comparison with previous studies and implications

The recurrence rate of asthma attacks over 6 months observed (46%) was higher than reported in similar studies from UK (37-38%) [34], or US (12-17%) [35], although consistent with our previous findings in this setting [11]. This and previous studies have identified a history of severe asthma attacks during the preceding year as a predictor for future asthma attacks [16]. Other predictors identified in univariate analysis were previous ER attendances, number of parenteral corticosteroid courses, and unscheduled contacts for acute asthma. However, the number of parenteral corticosteroid courses was the only predictor among these associated with asthma attack recurrence in the multivariable model, probably because of the specific characteristics of our study setting (low rates of oral corticosteroid prescriptions and variable degree of severity of attacks).

Among sociodemographic characteristics, younger children had a greater risk of subsequent asthma attacks, as observed previously in studies done in high-income countries [13,14]. One possible explanation is that younger children suffer more from upper respiratory viral infections, such as rhinovirus, an important trigger for asthma attacks [36]. Children living in the City of Esmeraldas had a lower risk of subsequent asthma attacks compared to those from the surrounding rural area, a finding which has not been described previously. Distance to the emergency care facilities (located in the city) could have biased the results with only children with more severe asthma traveling from rural areas to receive urgent medical attention. The association remained after controlling for variables such as educational level of parents and socioeconomic factors that have been associated with the risk of asthma attacks [14]. Two relevant characteristics that have been previously identified as risk factors for repeated asthma attacks did not predict future events in our study: low socioeconomic status (SES) and African ethnicity [14,15]. The population in which we worked in Esmeraldas was from poor neighbourhoods with a probable high degree of African genetic admixture resulting in a relatively homogeneous cohort and perhaps reducing power to identify such factors as predictors. A recent study showed that the association between black ethnicity and paediatric asthma readmissions was strongly confounded by socioeconomic factors [37], possibly explaining why Afro-Ecuadorian ethnicity was not a predictor in our study. Further studies in similar LMICs are necessary to clarify the respective roles of SES and ethnicity as determinants of asthma attack risk.

Asthma in the presence of atopy is associated with other allergic diseases including eczema and rhinoconjunctivitis. Children with concomitant allergic diseases may have a higher risk of

future asthma attacks[38], as observed here for children with a previous eczema diagnosis. Eczema has been associated with poor asthma control among children in urban Brazil [38]. Individuals with allergic asthma appear to be particularly susceptible to virus-induced exacerbations [39], and suppression of allergic mediators reduces virally-mediated attacks [40]. However, in our study we did not observe associations between allergy markers (blood and nasal eosinophilia, and FeNO) or allergic sensitisation (specific IgE) and risk of asthma attack recurrence. Further studies are necessary to better understand the nature of this association.

Similarly, there was a greater risk of repeated severe asthma attacks among children describing food as a trigger (food allergies, colorants or cold foods). A previous population-based study from the UK has shown an association between food-induced wheeze in children and more frequent attacks of wheeze and healthcare use [41]. However, the mechanisms involved in food-induced asthma are not well understood and are not solely immunological, making it difficult to diagnose [42]. This was observed in our cohort, where there was no association between reported food triggers and a positive IgE to food extracts. Similarly, most children reported cold drinks/foods (physical agents) as the triggers of their food-induced asthma.

Predictors of recurrent asthma attacks could be combined into a risk assessment tool to identify children with good symptom control but at high risk of attacks, a group representing half of those in our study. To be able to reduce the risk in these children, a complete asthma management package would need to be implemented: supply of free medications, individualised asthma action plan, training for inhaler technique, close monitoring of adherence, and control of co-morbidities and modifiable life-style and environmental risk factors.

Strengths and limitations

Our study had a number of limitations. First, because all children were treated in accordance with local guidelines, the fact that this cohort of asthmatic children were followed-up, educated about their asthma and provided a written asthma action plan, likely modified their outcomes through improved asthma knowledge, adherence to treatment, and management of attacks. Second, although we initially aimed to start children on ICS following the Ecuadorian guidelines, the large number of children recruited and limited human resources available meant it was not possible to monitor the children and their adherence as closely as we would have liked. Third, the study population has certain characteristics (including ethnicity,

socioeconomic status, asthma knowledge, low proportion of ICS use) that may differ from other Latin American settings and limit generalizability. Although attendance at ERs for treatment of attacks is part of the definition of a severe asthma attack according to ATS/ERS[1]. However, the objective of the study was to identify predictors in a real world setting to facilitate working towards a risk-assessment tool that can be applied in ERs for any patient presenting with bronchodilator-responsive wheeze. Strengths of this study were adequate power to identify potential predictors and a high follow-up rate. The comprehensive questionnaire, inflammatory markers and lung function measurements were designed to explore a broad range of potential risk factors for asthma attack recurrence. By including all bronchodilator-responsive wheeze irrespective of previous asthma diagnosis or lung function parameters, this relatively non-selective sample represented more closely patients with real-life asthma attending emergency rooms.

Conclusion

In conclusion, we did a prospective study of children presenting with asthma attacks to ERs in a resource-poor setting in Ecuador and identified predictors of reattendance with attacks and time to next attack. Approximately half of the recruited asthmatic children suffered a subsequent asthma attack within six months of follow-up. Several predictors were identified including a previous asthma diagnosis and an asthma attack during the previous year requiring systemic corticosteroids. A combination of these predictors could be used as a simple risk-assessment tool in ERs to identify asthmatic children at a higher risk of recurrent attacks. Such a tool would be extremely useful in LMICs where limited resources should be targeted towards those most in need of continuing support and treatment. Further studies are now required to validate our findings in different low-resource settings.

Funding Source: This research was funded by a Wellcome Trust Clinical PhD Fellowship granted to Cristina Ardura-Garcia (grant 099938/B/12/Z).

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Figure 1: Recruitment and follow-up of participants

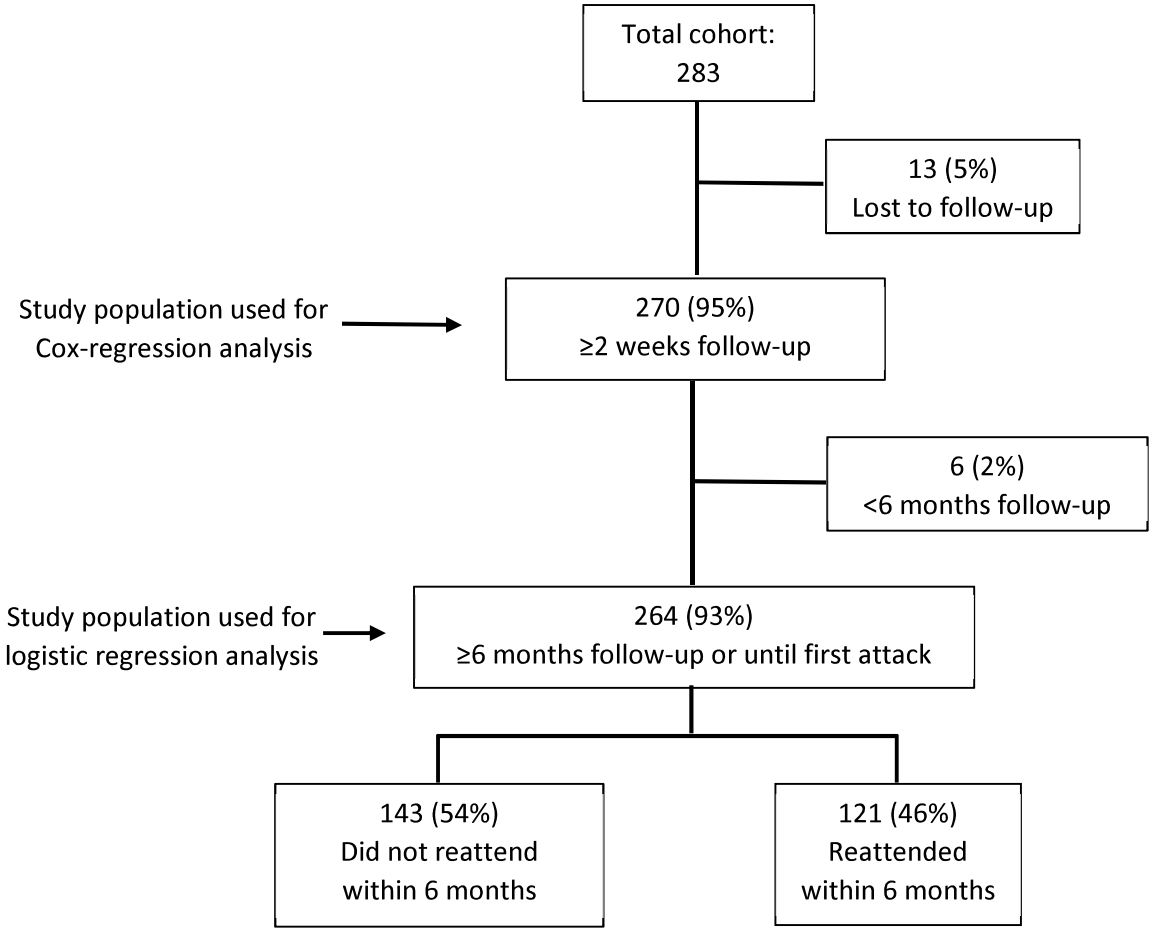


Figure 2: Kaplan-Meier curve of time to first recurrence of severe asthma attacks.

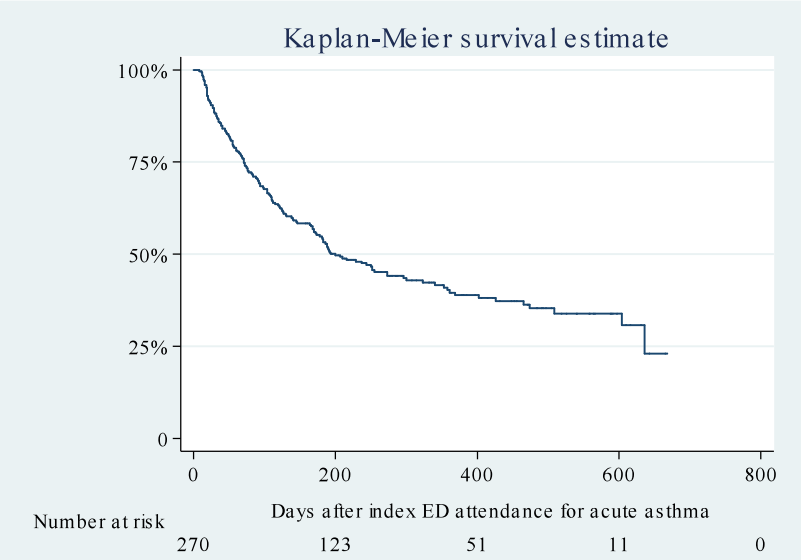
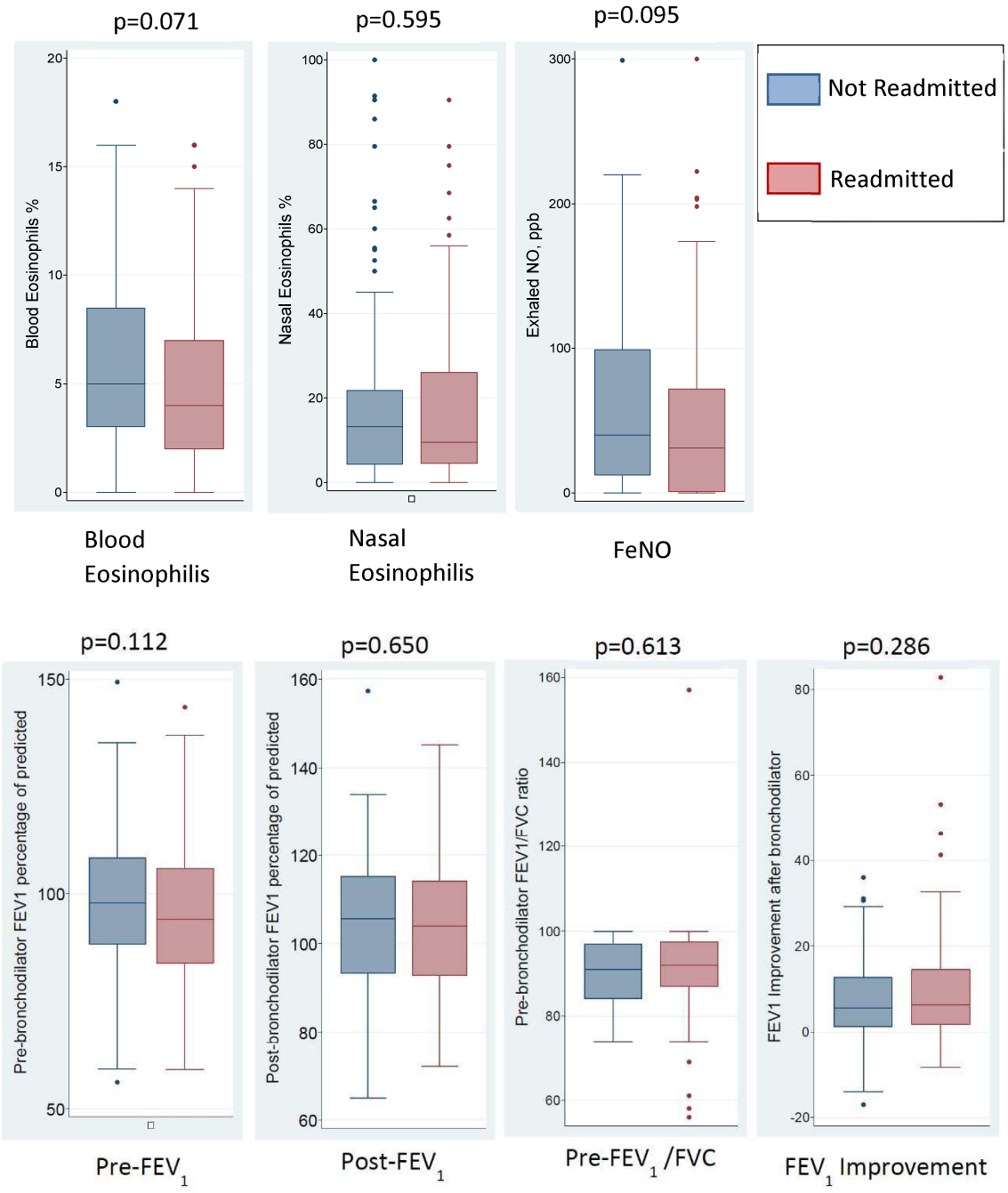


Figure 3: Allergy and inflammatory markers, and lung function of participants who had recurrent severe attacks (readmitted) or not (not readmitted) at 6 months follow-up. P values represent inter-group differences using Mann-Whitney U-tests.



FeNO: Fraction of exhaled Nitric Oxide; FEV1: Forced Expiratory Volume 1st second; FVC: Forced Vital Capacity; Pre: pre-bronchodilator; Post: post-bronchodilator

SUPPLEMENTARY FILES

Supplementary Methods

Nasal wash procedure

Obtaining nasal wash sample: We atomize 2ml of warm (37°C) phosphate-buffered saline (PBS) solution into the child's nostril using a MAD Nasal® atomizer during breath-holding. The child is sitting, leaning slightly forward with the head bent slightly forward while holding a 15 mL centrifuge tube into which is placed the neck of a glass funnel (10-15 ml diameter). The funnel is placed below the nose while we atomize slowly the PBS aerosol into the nostril, ensuring that the child holds their breath. Once the syringe is emptied and removed from the nostril, the child blows strongly through the nose into the funnel while the opposite nostril is occluded. This same procedure is repeated in the other nostril and then we wash the funnel with 2ml of PBS solution before removing the 15ml centrifuge tube.

Processing of nasal wash sample: After shaking the sample gently on a mixer for 15 minutes, we centrifuge at 6000 RPM for 10 minutes. We then store some samples at -80°C and discard the remaining supernatant fluid. We place 250µl of pellet into two Cytospin funnels (125µl each) and centrifuge at 700 RPM for 4 minutes (Rotofix cytocentrifuge). We dry and stain slides with Wrights for direct observation under the microscope.

Spirometry estimations

The percentage of predicted spirometry values for age and height were estimated using Global Lung Initiative standards [1] and the FEV1 post-bronchodilator improvement was calculated as: (Post FEV1 - Pre FEV1 / Pre FEV1) x 100%.

Indications for starting inhaled corticosteroids (ICS)

The most updated Ecuadorian guidelines that we applied are based on the GINA 2008[2] and Spanish GEMA 2009[3] guidelines. They recommend controller ICS in children with persistent asthma or frequent episodic asthma (not more than 1 exacerbation every 5-6 weeks, with a maximum of 6-8 exacerbations per year and without symptoms between the exacerbations).

Statistical analysis:

Sample size estimation

We calculated that a cohort of 250 children with a severe asthma attack would provide 80% power to detect factors that reduce the proportion of children re-attending the emergency room within 6 months from 50% to 31.4% (hazard ratio of ≥1.46), and 90% power to detect factors that reduce the proportion of children re-attending emergency room within 1 year from 50% to 33.9% (hazard ratio of ≥1.37). The expected proportion of children suffering a subsequent asthma attack requiring emergency care was estimated to be 50% over the following 6 months, based on our previous findings in this same setting[4].

Missing data strategy

To investigate the effect of missing values for variables with greater than 5% missing data, we performed a sensitivity analysis using the "ice"[5] procedure for multiple imputation in Stata 13.1. The "mim" procedure in Stata[6] was then used to average the estimates of results across the 20 imputed data sets created, according to Rubin's rules[7]. The imputation models included all variables selected, the outcome of interest, and the Nelson Aalen estimator of the cumulative baseline hazard [8]. There was no difference in the final logistic regression and Cox regression multivariable models obtained when using the multiple imputation dataset compared to the original dataset (data not shown).

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Supplementary Results

With respect to food triggers, of the 93 children who reported food-induced asthma attacks, 70 reported cold drink or foods as triggers, 28 reported specific foods (milk, nuts, etc.), 5 reported both, and 2 did not specify the food trigger. The most common specific food triggers reported were artificial drinks and food colorants (both reported in 7 children). Allergen specific IgE for food mix was measured in a random sample of 59 children, with a positive result in 25 (42%) of them. There was no association between a positive food mix IgE and recurrence (OR: 1.08; 95% CI: 0.39-3.04). There was also no association between having reported a food trigger for asthma attacks and having a positive food mix IgE result (OR: 0.77; 95% CI:0.26-2.31).

Table S1: Exposures studied as potential predictors of recurrent asthma attacks, and available data out of 270 children that completed at least 2 weeks of follow-up (included in Cox regression analysis).

Exposure / predictor	Available data (N)	Method
Demographics		
- Age	270	Questionnaire
- Sex	270	Questionnaire
- Ethnicity	266	Questionnaire
- Siblings	269	Questionnaire
- Household location	268	Questionnaire
- Overcrowding	269	Questionnaire
- Monthly household income	248	Questionnaire
- Parental level of education	266	Questionnaire
Personal history		
- Gestational age	261	Questionnaire
- Birthweight	126	Questionnaire
- Breastfeeding	266	Questionnaire
- Bronchiolitis/pneumonia as infant	261	Questionnaire
Asthma history		
- Wheezing ever	267	Questionnaire
- Wheezing last 12m	266	Questionnaire
- Doctor's asthma diagnosis	266	Questionnaire
- Date last asthma attack	228	Questionnaire
- Number of severe exacerbations last year	265	Questionnaire
- ER attendance last year	268	Questionnaire
- Number of ER attendance/s last year	267	Questionnaire
- Hospitalization/s last year / ever	268	Questionnaire
- Admitted to ICU ever	267	Questionnaire
Asthma treatment previous year		
- Inhaled corticosteroids	266	Questionnaire
- Leukotriene inhibitors	266	Questionnaire
- Inhaled/nebulised SABA	266	Questionnaire
- No. oral corticosteroids courses	264	Questionnaire
- No. parenteral corticosteroids courses	264	Questionnaire
Asthma symptoms		
- Asthma control	267	C-ACT; ACT
- Quality of life	219	PAQLQ
- Triggers (number and specific triggers)	165	Questionnaire
- Total time last year with symptoms	268	Questionnaire
- Nocturnal symptoms	267	Questionnaire
- Symptoms with exercise	260	Questionnaire
- Pulmonary function	227	Lung function
Asthma knowledge		
- Poor asthma knowledge	253	NAKQ score
- Asthma education sessions	269	Questionnaire
Family history		
- Maternal rhinitis/asthma/eczema	261	Questionnaire
- Paternal rhinitis/asthma/eczema	259	Questionnaire
Co-morbidities		
- Allergic rhinitis (Doctor's diagnosis)	267	Questionnaire
- Eczema (Doctor's diagnosis)	268	Questionnaire
- Obesity	249	BMI (weight and height)

Environmental exposures		
- Air pollution at home area	269	Questionnaire
- Tobacco exposure at home	268	Questionnaire
- Humid household	268	Questionnaire
- Mould in household	269	Questionnaire
- Carpets in household	269	Questionnaire
- Pets	268	Questionnaire
Inflammatory markers		
- Blood eosinophilia	261	Blood sample
- Nasal eosinophilia	157	Nasal wash
- Fraction of exhaled nitric oxide	229	FeNO measurement

BMI: Body Mass Index; C-ACT: Childhood Asthma Control Test; ACT: Asthma Control Test; NAKQ: Newcastle Asthma Knowledge Questionnaire; PAQLQ: Pediatric Asthma Quality of Life Questionnaire; FeNO: Fraction of exhaled nitric oxide.

Table S2: Asthma control, asthma knowledge and quality of life of 264 participants followed up for 6 months or until first asthma attack recurrence, stratified by readmission status at 6 months.

	Total Cohort	**Second asthma attack within 6 months**		
	(N=264)	**Yes (N=121)**	**No (N=143)**	**P**
C-ACT score (mean, SD)	16.0 (3.75)	15.4 (4.05)	16.5 (3.41)	0.077
ACT score (median, IQR)	16.0 (13-18)	16.0 (14-18)	15.5 (13-18)	0.936
NAKQ score (median, IQR)	**18 (16-20)**	**17 (15-20)**	**18 (16-21)**	**0.041**
PAQLQ total score (median, IQR)	3.5 (3.0-4.0)	3.5 (2.9-4.0)	3.5 (3.0-4.2)	0.275
PAQLQ symptom score (median, IQR)	3.5 (2.8-4.1)	3.6 (2.8-4.0)	3.5 (2.8-4.2)	0.479
PAQLQ activity score (median, IQR)	3.4 (2.6-4.0)	3.4 (2.6-4.0)	3.4 (2.6-4.2)	0.525
PAQLQ emotional score (median, IQR)	**3.7 (3.1-4.5)**	**3.5 (3.0-4.2)**	**3.9 (3.2-4.6)**	**0.030**

IQR: Interquartile range; C-ACT: Childhood Asthma Control Test; ACT: Asthma Control Test; NAKQ: Newcastle Asthma Knowledge Questionnaire; PAQLQ: Pediatric Asthma Quality of Life Questionnaire. Bold: statistically significant differences ($p<0.05$)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3, 4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4, 5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4, 5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4, 5
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	Sup. files
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	Sup. files
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	Sup. files
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6, Fig 1
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	Fig 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1-2, S2
		(b) Indicate number of participants with missing data for each variable of interest	6, table S1
		(c) Summarise follow-up time (eg, average and total amount)	7,

			Fig 2
Outcome data	15*	Report numbers of outcome events or summary measures over time	6, Fig 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-10, tables 1-4 and S2
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.
NA: Not applicable

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.