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Differences between patients who achieved asthma control and those who remain uncontrolled after standardized severe asthma care strategy

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ABSTRACT

Objective: To assess clinical, functional, and inflammatory patterns of children and adolescents with severe uncontrolled asthma, and investigate the differences between patients who achieved asthma control and those who remain uncontrolled after standardized asthma care strategy.

Methods: Screening all children and adolescents with asthma from the Pediatric Pulmonology Outpatient Clinic of Unicamp, Brazil, and included those with severe uncontrolled asthma according to GINA guidelines criteria. Patients were assessed at baseline and after by demographic and medication data, questionnaires (Asthma Control Test and Pediatric Asthma Quality of Life Questionnaire), Six-Minute Walk Test, skin prick test, spirometry, induced sputum, and blood collection (total immunoglobulin E and eosinophil count). Cytokine dosage was analyzed in sputum supernatant and serum by Cytometric Bead Array.

Results: Thirty-three patients with severe uncontrolled asthma were included (median age 10.9 [7.00–17.60] years). All patients presented satisfactory adherence to treatment and 50% of them achieved good asthma control after six-month follow-up ($p < 0.001$). Patients who achieved asthma control reported higher intervals since their last exacerbation episode ($p = 0.008$) and higher quality of life scores ($p < 0.001$) as compared to patients who remained uncontrolled. We found no changes in lung function markers, inflammatory biomarkers, or cytokine levels between patients with uncontrolled and controlled asthma.

Conclusion: Participation of six months in a structured outpatient clinic for children with severe asthma had a notable improvement in control and quality of life of patients. This demonstrates the importance of a global assessment, focused on peculiarities presented by patients with severe uncontrolled asthma.

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Introduction

Asthma, a heterogeneous disease, affects approximately 1–18% of the world population, varying by country, and is responsible for 5 deaths/day and more than 120 000 asthma-related hospitalizations per year in Brazil (1,2). Patients with severe persistent asthma represent 5–10% of total population with this condition (3). Despite the lower prevalence, severe uncontrolled asthma can result in higher risk of severe exacerbations recurrence, with visits to the emergency room and hospitalizations, chronic morbidity, and death (4).

Thus, standardized treatment strategies have been developed to assist in the management of severe asthma (1,4,5). Among these strategies, the ones that stand out are, ensuring that the diagnosis of asthma is really correct, exploring the associated comorbidities, verifying the inhalation technique and treatment adherence, and identifying the environmental triggers (1,4,5). According to Global Initiative for Asthma (GINA), the treatment of these children consists of using high doses of inhaled corticosteroids and additional controllers (long-acting inhaled beta 2 agonists, montelukast, and/or theophylline), however, individualized and targeted therapies should be considered (1).

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Application of management protocols and identification of asthma phenotypes with biomarkers could enhance severe asthma evaluation and treatment, by assessing airway inflammation, response to therapies and to offer a personalized, targeted, and appropriate treatment for these patients (5–8). In addition, these children should be routinely monitored by a multidisciplinary team, even those who have achieved asthma control, as they represent a heterogeneous group of patients with highly varied clinical characteristics and asthma-related outcomes, patient safety, treatment burden, and quality of life (1,5–7).

It is of great relevance to carry out studies with pediatric population with uncontrolled severe asthma, by using World Health Organization (WHO) and GINA protocols and assessment of biomarkers (1,4,5). Therefore, the study aims to assess clinical, functional, and inflammatory patterns of children and adolescents with severe uncontrolled asthma and to investigate the differences between patients who achieve asthma control and those who remain uncontrolled after standardized asthma care strategy.

Methods

Participants and study protocol

This was a prospective and analytical clinical study performed with children and adolescents aged seven to 17 years old with diagnosis of severe uncontrolled asthma. They attended the Pediatric Pulmonology Outpatient Clinic of the State University of Campinas, Brazil, which is an academic outpatient clinic for asthma.

This study was approved by the Research Ethics Committee of the Unicamp School of Medical Sciences (Protocol no. 438.481/2013). The participant's legal guardians signed an informed consent form (ICF) and adolescents signed an additional assent form.

Selection and assessment of severe uncontrolled asthma patients

Step 1: We screened all school-aged children and adolescents with asthma who attended our Outpatient Clinic. Patients with diagnosis of cardiac comorbidities, chronic lung disease other than asthma, severe immunodeficiency disease, anaphylaxis history due to the prick test and cognitive or motor limitations were excluded.

Step 2: Selection of patients with severe asthma according to the GINA criteria: patients who

remained uncontrolled despite being on steps treatment 4 or 5 (1). Asthma control was assessed by the asthma control test (ACT), a questionnaire with scores ranging from 5 to 25 points, wherein a cutoff point ≤ 19 determines uncontrolled asthma (1,9).

Step 3: Patients with severe uncontrolled asthma were assessed by physicians, physiotherapists, and study investigators in two moments (Visit 1 and Visit 2).

In Visit 1, patients underwent a clinical evaluation consisting of a structured questionnaire with information on the disease history, personal and family history, medications and environmental factors, quality of life assessment and classification of asthma severity and control. In addition, Six-minute walk test (6MWT), skin prick test (SPT), spirometry pre- and postbronchodilator, induced sputum, and blood collection were performed.

In Visit 2, six months after the first evaluation, all tests and exams were repeated, except for SPT. If patients presented asthma exacerbation on the day of test, they were properly treated by the medical staff and the assessment was rescheduled.

In compliance with standardized strategies for severe asthma, once the diagnosis of asthma was confirmed and the associated comorbidities have been explored, we evaluated environmental control, inhalation technique, and adherence to treatment according to the report of participants and their guardians (1,4,5). Where necessary, patients must be re-educated and retrained regarding the inhalation technique. Satisfactory adherence was considered when patients used at least 80% of prescribed doses in the week before, according to their own report (1,10). For all evaluation, treatment was also re-checked and re-adjusted if necessary.

Quality of life was assessed by Pediatric Asthma Quality of Life Questionnaire (PAQLQ), which consists of 23 questions specific to children and adolescents with asthma (11). The questionnaire was divided into three domains of activity limitation, symptoms, and emotional function. Answers were scored on a 7-point scale, on which 1 indicates the most severe impairment and 7 indicates no impairment at all (11). At the end, four arithmetic means were calculated, being three referring to each domain and one calculated of all 23 responses referring to overall score (11).

The 6MWT was performed according to the guidelines of the American Thoracic Society (ATS) (12), in which participants were instructed to walk as fast as possible in six minutes, as observers measured heart

rate, respiratory rate, systemic blood pressure, oxygen saturation, and Borg scale for dyspnea and overall fatigue at baseline and immediately after the test (12). Interruption criteria included severe chest pain, intolerable dyspnea, leg cramps, staggering, diaphoresis, and pale or ashen appearance (12). We calculated the percent of predicted distance in meters using reference formulas for the Brazilian population (13).

The SPT was applied to determine allergen sensitization according to European Standards (14). Allergen tests were considered positive when papule size was 3 mm larger than negative control (14). If patients presented at least one allergen positive, we considered the SPT positive.

Participants performed spirometry to assess lung function or the CPFS/D model (Medical Graphics Corporation, St. Paul, MN, USA), according to guidelines and recommendations of the European Respiratory Society (ERS) and the American Thoracic Society (15). Forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC ratio, and forced expiratory flow between 25% and 75% of FVC (FEF25–75%) were evaluated in all patients and the parameters were expressed as z-score (16).

Participants also performed sputum induction based on the method described by Pizzichini et al. (17–18) after 5 min of inhalation of hypertonic saline solution (HSS) 4.5%. For a better success of the technique, patients received mechanical vibration in posterior pulmonary region and executed the Forced Expiration Technique and the increased expiratory flow with hand compression made by the physiotherapist for five times (19).

The induced sputum samples were analyzed based on recommendations of the European Respiratory Society (ERS) (5,10,17,18), and were considered satisfactory if they contained $\leq 20\%$ squamous cells, and $\geq 50\%$ viable cells, and had ≥ 400 inflammatory cells. Sputum inflammatory cell profile was classified in: (1) eosinophilic if eosinophils $> 2.5\%$, (2) neutrophilic if neutrophils $> 54\%$, (3) mixed granulocytic if eosinophils $> 2.5\%$ and neutrophils $> 54\%$, and (4) paucigranulocytic if eosinophils $\leq 2.5\%$ and neutrophils $\leq 54\%$ (5,10,17,18).

We collected venous blood samples for assessment of total immunoglobulin E (IgE) and cytokines in serum and eosinophil count in a hemogram. After performing all data collection, blood and sputum samples for cytokine dosage were processed in specific laboratories and subsequently stored at -80°C .

Cytokine concentration was determined by Cytometric Bead Array (CBA, BD Biosciences, USA)

according to manufacturer's protocol in a FACSCanto flow cytometer (BD Biosciences, USA), and analyzed with FCAP Array Software (version 3.0.1., BD Biosciences, USA). For statistical purposes, the limits of detection (pg/ml) were 9.4, 0.3, 3.9, 2.2, 1.2, 2.4, 1.8, 1.9, and 1.3, respectively, for IL-17, IL-13, IL12/IL23p40, IL-10, IL-6, IL-4, IL-2, IFN- γ , and TNF. For the cytokine analysis, only patients with satisfactory sample of sputum and blood in both evaluations were studied in comparison to Visit 1 and Visit 2.

Statistical analysis

Data were processed with the Statistical Package for Social Sciences for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA).

For characterization of patients with severe uncontrolled asthma, the qualitative variables were presented in absolute and relative frequency and quantitative variables were described in median, minimum, and maximum. The Kolmogorov–Smirnov test and the Shapiro-Wilk test were applied in order to verify the normality of the quantitative variables, and in both, variables with values $p > 0.05$ were considered to have normal distribution.

In order to compare participants who achieved asthma control with those who remained classified as uncontrolled in Visit 2, we used Chi-square, Fisher test, or Fisher-Freeman-Halton test, with bilateral probability estimated by the Monte Carlo method for categorical variables. The Mann–Whitney test was used to compare variables with non-normal distribution between groups. In all cases, the level of significance was set at 5%.

Results

We evaluated all children and adolescents with asthma in our outpatient clinic and found that 61 patients out of 251 were affected by severe asthma (24%) and of these, 33 were classified as uncontrolled and included in this study. The selection of all patients, as well as all tests applied, are shown in Figure 1.

Baseline demographic, anthropometric, clinical, and laboratorial characteristics of the patients are shown in Table 1.

Table 2 shows the comparisons of clinical characteristics, step of treatment, spirometric parameters, 6MWT, and quality of life scores between the patients who remained with uncontrolled asthma and those who improved to controlled asthma in Visit 2.

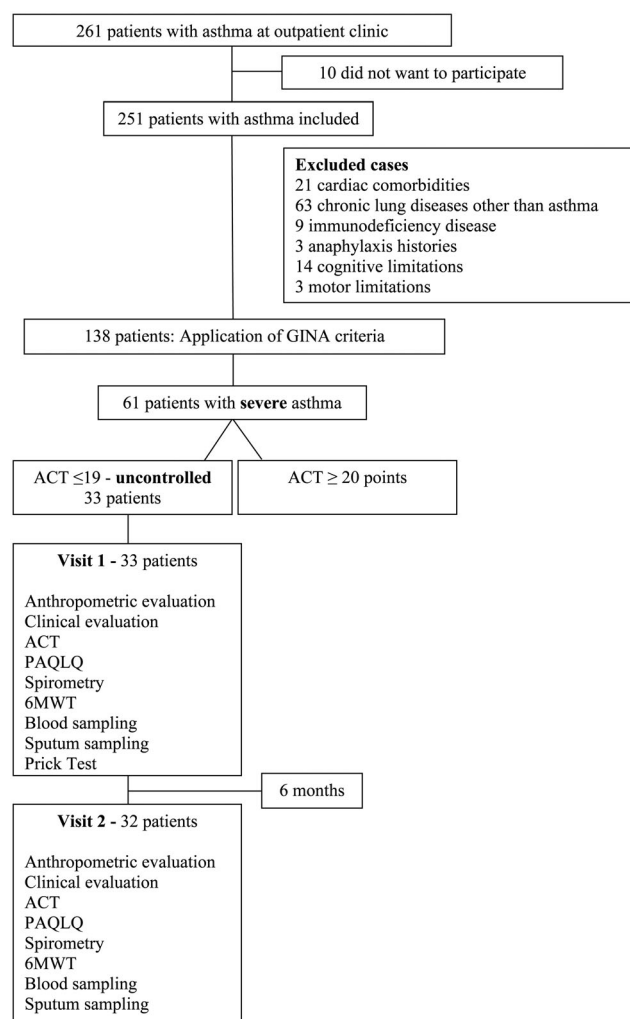


Figure 1. Flowchart of patient selection and tests applied in Visit 1 and Visit 2. ACT: Asthma Control Test; PAQLQ: Quality of life was assessed by Pediatric Asthma Quality of Life Questionnaire; 6MWT: Six-minute walk test.

After six-months, despite the improvement in adherence for all patients and use of medications in high doses (steps 4 and 5), 50% maintained the uncontrolled asthma condition, when compared to Visit 1 ($p < 0.001$), which highlights the portion of population with true severe asthma. Patients with controlled asthma reported higher intervals since their last exacerbation episode ($p = 0.008$) and had higher scores of quality of life in activity limitation domain ($p < 0.001$), symptoms domain ($p < 0.001$), emotional function domain ($p < 0.001$) and overall score ($p < 0.001$) than patients who remained classified as uncontrolled asthma. No statistically significant differences were observed in the other variables analyzed.

Table 3 shows the comparison of results in blood exams, sputum induced profile and cytokines in sputum, and serum of patients with uncontrolled and

controlled asthma, with no significant differences between them. In Visit 2, the levels of IL-2, IL-4, IL-10, IL-13, and TNF were undetectable or very low.

Discussion

In the present study, the management of children with severe asthma was standardized in accordance with WHO and GINA guidelines and we could observe that, as it is a referral center for severe asthma, our sample had a high percentage of children and adolescents with severe asthma, 61 of 251 patients (24%). Besides that, all children and adolescents with severe uncontrolled asthma were able to achieve satisfactory adherence to treatment, 50% of whom achieved asthma control (1,4,5). Those patients showed a decrease in the recurrence of exacerbations and improved quality of life scores when compared to the ones who did not.

WHO and GINA created protocols for management in patients with severe asthma, which aimed to reduce their high morbidity (1,4,5). They advise to review asthma control level, to re-check comorbidities associated with lack of asthma control, to remove potential risk factors, to verify inhalation technique and adherence to treatment, and to consider step-up treatment in order to provide adequate asthma control level (1,4,5,20). As in the present study, de Andrade et al. also applied the WHO protocol in children and adolescents with uncontrolled severe asthma and they observed an increased proportion of patients with correct inhaler technique and decreased asthma exacerbations after the intervention (10).

Adherence is defined as the ratio between the amount of medication prescribed by the physician and the amount of medication used by patient, and poor adherence is the main factor associated with lack of asthma control (1,10,21). Sometimes, adherence levels in children with severe asthma is not satisfactory, but it can be improved after adequate intervention, and provides better asthma control level, morbidity reduction, and less exacerbation and hospitalization, as shown in this study (21).

In severe asthma, potentiating health education about environment control, inhalation technique and adherence to therapy, addressing risk factors, and targeting social and psychological domains can improve quality of life by achieving better asthma control (22). We found a statistically significant increase in values of symptom domain, emotional function domain, and overall score after protocol, and higher scores of quality of life in all domains and overall score in patients

Table 1. Baseline characteristics from patients included in the study (Visit 1).

Variable	Severe Uncontrolled Asthma (n = 33)
Age (median[<i>min-max</i>])	10.9 [7.00 – 17.60]
BMI (median[<i>min-max</i>])	21.0 [14.2 – 31.1]
Male gender <i>n</i> (%)	21 (63.6)
Caucasian <i>n</i> (%)	18 (54.5)
Second hand smoking <i>n</i> (%)	10 (30.3)
Presence of rhinitis- <i>n</i> (%)	30 (90.9)
SPT positive <i>n</i> (%)	27 (81.8)
Satisfactory adherence <i>n</i> (%)	28 (84.8)
Spirometry parameters (median[<i>min-max</i>])	
FEV1 (%)	85 [45.0 – 116.0]
FEV1/FVC ratio	80 [55.0 – 107.0]
FEF25-75% (%)	65 [20.0 – 111.0]
6MWT (median[<i>min-max</i>])	
Percent of predicted distance	80 [7.4 – 106.1]
Blood eosinophil (%) (median[<i>min-max</i>])	7.5 [0.0 – 21.2]
Total IgE (IU/ml) (median[<i>min-max</i>])	805 [4.0 – 6560.0]

N: Number of cases; %n Percentage of cases; Min: Minimum; Max: Maximum; BMI: Body mass index; SPT: Skin prick test; FEV1: Forced expiratory volume in the first second; FVC: Forced vital capacity ; FEF25-75%: Forced expiratory flow between 25% and 75% of FVC; 6MWT: Six-minute walk test; IgE: Immunoglobulin; %: Percentage; IU/ml: International nits por mililiter.

Table 2. Clinical and functional characteristics evaluated in Visit 2.

Variable	Uncontrolled Asthma (n = 16)	Controlled Asthma (n = 16)	<i>p</i>
Rhinitis	15 (53.6)	13 (46.4)	0.600 ^a
Obesity	10 (62.5)	6 (37.5)	0.157 ^b
Satisfactory adherence	16 (100.0)	16 (100.0)	N/A
Last exacerbation (months)	1 [1 – 6]	1 [5 – 11]	0.008 ^c
Step treatment			
Step 4	13 (48.1)	14 (51.9)	1.000 ^a
Step 5	3 (60.0)	2 (40.0)	
Spirometry parameters			
FVC (z-score)	–0.39 [–2.33 to +2.60]	0.54 [–2.34 to +2.50]	0.478 ^c
FEV1 (z-score)	–0.75 [–3.45 to +2.86]	–0.70 [–4.58 to +2.41]	0.833 ^c
FEV1/FVC ratio (z-score)	–0.84 [–2.77 to +0.93]	–1.66 [–4.06 to +1.04]	0.357 ^c
FEF25-75% (z-score)	–1.21 [–3.56 to +1.48]	–1.52 [–4.98 to +1.40]	0.678 ^c
6MWT			
Percent of predicted distance	78.2 [27.1 – 104.6]	84.5 [70.7 – 105.9]	0.054 ^c
Quality of life			
Activity limitation	3.6 [1.8 – 5.6]	5.6 [4.0 – 7.0]	<0.001 ^c
Symptoms	4.6 [1.5 – 6.4]	6.6 [4.6 – 7.0]	<0.001 ^c
Emotional function	4.4 [1.2 – 6.9]	7.0 [4.6 – 7.0]	<0.001 ^c
Overall score	4.3 [1.5 – 6.2]	6.6 [4.5 – 7.0]	<0.001 ^c

Absolute frequency (% relative frequency); Median [minimum – maximum]; FEV1: Forced expiratory volume in the first second; FVC: Forced vital capacity (FVC); FEF25-75%: Forced expiratory flow between 25% and 75% of FVC; 6MWT: Six-minute walk test; N/A: Not applicable. ^aFisher Test; ^bChi-square test; ^cMann–Whitney Test.

who controlled asthma when compared to those who remained uncontrolled.

In a previous study, our group evaluated the quality of life in children and adolescents with asthma and concluded that better quality of life appears to be directly related to well-controlled asthma and lower severity (23). Other study showed worse quality of life associated with higher asthma exacerbation recurrence and severity, number of triggers at baseline, presence of dyspnea and chest tightness, symptoms of sleep disorder, and exercise-induced dyspnea (24).

We did not observe differences between groups with controlled and uncontrolled asthma in spirometry and 6MWT. Although FEV1 and FEV1/FVC are important parameters in assessment of bronchial obstruction in asthma, they did not present good correlation with asthma control in pediatric age group

(25,26). 6MWT is a simple and highly applicable test that can assess presence of dyspnea and desaturation during physical activity, aerobic capacity for practice exercises, and response to therapeutic or rehabilitation programs. Therefore, although there are no differences in this study, we highlight the importance of this tool in evaluation of pulmonary diseases (12,27).

Currently, besides clinical evaluation, studies cited many biomarkers from blood, induced sputum, bronchoalveolar lavage, respiratory epithelium, fractional exhaled nitric oxide, and exhaled breath condensate that could help to monitor and to identify asthma phenotypes in children and adolescents (7,8,28). In a review, James et al. emphasizes the importance of these biomarkers in phenotyping and monitoring asthma in children (28).

We found no differences in blood exams, induced sputum profile, and cytokines levels between patients

Table 3. Blood exams, sputum profile and levels of cytokines analyzed in sputum and serum by flow cytometry of patients, in Visit 2.

Variable	Uncontrolled asthma	Controlled asthma	<i>p</i>
Blood exams			
Eosinophils (%)	6.4 [1.0 – 17.7]	6.9 [0.9 – 12.4]	0.651 ^a
Total IgE (IU/ml)	678 [4 – 8310]	615 [98 – 4140]	0.976 ^a
Sputum profile			
Eosinophilic	1 (16.7)	5 (83.3)	0.091 ^b
Neutrophilic	0 (0.0)	0 (0.0)	
Mixed granulocytic	2 (100.0)	0 (0.0)	
<i>Paucigranulocytic</i>	12 (54.5)	10 (45.5)	
Cytokines (pg/ml)			
IL-17			
Sputum	9.4 [9.4 – 74.8]	9.4 [9.4 – 40.4]	0.538 ^a
Serum	9.4 [9.4 – 48.1]	9.4 [9.4 – 69.1]	0.810 ^a
IL-13			
Sputum	0.3 [0.3 – 1.3]	0.3 [0.3 – 0.5]	0.784 ^a
Serum	0.3 [0.3 – 1.6]	0.3 [0.3 – 0.9]	0.731 ^a
IL-12/IL23p40			
Sputum	3.9 [3.9 – 1375.6]	3.9 [3.9 – 46.4]	0.832 ^a
Serum	167.0 [3.9 – 960.5]	67.9 [3.9 – 194.5]	0.088 ^a
IL-10			
Sputum	2.2 [2.2 – 2.2]	2.2 [2.2 – 2.2]	1.000 ^a
Serum	2.2 [2.2 – 2.2]	2.2 [2.2 – 2.2]	1.000 ^a
IL-6			
Sputum	5.2 [1.2 – 306.2]	6.1 [1.2 – 605.5]	0.979 ^a
Serum	1.2 [1.2 – 6.2]	1.2 [1.2 – 6.3]	0.852 ^a
IL-4			
Sputum	2.4 [2.4 – 2.4]	2.4 [2.4 – 2.4]	1.000 ^a
Serum	2.4 [2.4 – 2.4]	2.4 [2.4 – 2.4]	1.000 ^a
IL-2			
Sputum	1.3 [1.3 – 1.3]	1.3 [1.3 – 1.3]	1.000 ^a
Serum	1.3 [1.3 – 6.6]	1.3 [1.3 – 2.8]	0.538 ^a
IFN- γ			
Sputum	12.4 [1.8 – 36.6]	1.8 [1.8 – 47.6]	0.077 ^a
Serum	17.0 [1.8 – 47.6]	21.4 [1.8 – 47.6]	0.437 ^a
TNF			
Sputum	1.9 [1.9 – 1.9]	1.9 [1.9 – 1.9]	1.000 ^a
Serum	1.9 [1.9 – 1.9]	1.9 [1.9 – 1.9]	1.000 ^a

Absolute frequency (% relative frequency); Median [minimum – maximum]; IL: Interleukin; IFN- γ : Interferon gama; TNF: Tumor Necrosis Factor; ^aMann–Whitney Test; ^bFisher–Fremman–Halton. Two patients did not present satisfactory sample of sputum and one of blood.

with uncontrolled asthma and those who obtained control. As in our study, some authors have shown that serum levels of eosinophils were not correlated with sputum eosinophils in children with severe asthma (28,29). Therefore, we highlight the use of more than one biomarker for tissue eosinophilia and inflammatory cytokines, regardless of the age group studied.

The analysis of sputum profile as eosinophilic, neutrophilic, mixed granulocytic, and paucigranulocytic can classify different asthma phenotypes (30). Studies observed high percentage of sputum neutrophils, IL-10, GMCSF, IFN- γ , and TNF- α in children and adolescents with severe therapy-resistant asthma and high concentrations of sputum eosinophil, IL-4, IL-5, IFN γ , IL-2, and IL-12p70 in atopic asthmatic children analyzed by flow cytometry (31,32), when compared to nonatopic asthma children and healthy children.

It is possible that undetectable cytokine levels measured in sputum and serum with CBA technique could have occurred due to the sensitivity of the available kit.

Thus, we underline the importance of evaluating biomarkers in children and adolescents with severe asthma in order to improve management of this disease, responsible for high infant mortality, and to perform an individualized drug therapy.

In spite of decreased number of asthma-related hospitalizations, in-hospital mortality rate increased in Brazil (2). We believe that the differential of our research was the follow-up of patients with uncontrolled severe asthma according to WHO and GINA protocol, the assessment of inflammatory cytokines in both sputum and serum samples, and great results obtained in improvement in asthma control level and quality of life scores, which allowed us to better understand and manage this disease (1,4,5).

The character of our study, real-life observational cohort study, made it difficult to preview power analysis and sample size of patients can be a limiting factor in determining biomarker for uncontrolled asthma as well as when we say that there is no difference between patient groups. In addition, data related to treatment adherence were obtained through reports from patients

and their guardians, and not through objective measures such as dose counters, canister weights, and electronic monitoring device, which may have overestimated our result. We also believe that the detection limit of the kit available for measuring cytokines measured in sputum and serum using the CBA technique may have influenced the results obtained.

The lacking of noninvasive inflammatory monitoring, such as fraction of exhaled nitric oxide (FeNO) might be seen as another limitation for this study. FeNO has been suggested as a useful tool to guide the treatment of asthma in childhood (33). Nevertheless, there are contradictory results and it is not yet standard recommended for all children with asthma (6,34). Besides that, due to the potential interferences in the FeNO reading, we chose to measure inflammatory markers directly in the blood and sputum.

Conclusion

We concluded that children and adolescents with severe uncontrolled asthma who reached control after six months of management, according to structured protocols, showed improvement in quality of life and number of exacerbations. However, we were unable to find changes in lung function markers, inflammatory biomarkers, or changes in cytokine levels in sputum and serum samples.

Therefore, our study reaffirmed the need to follow a standard assessment of patients with severe asthma, as they represent a heterogeneous group of patients with highly varied clinical characteristics and also that further research must be carried out in order to enhance treatment and improve patient outcomes, thereby saving financial resources and reducing the country's morbidity and mortality.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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