Original Article

Systemic Corticosteroid Responses in Children with Severe Asthma: Phenotypic and Endotypic Features

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What is already known about this topic? Children with severe asthma are heterogeneous and have variable responses to systemic corticosteroids. Assessment of these responses is challenging due to a lack of a criterion standard and challenges with systemic corticosteroid delivery.

What does this article add to our knowledge? Clinical phenotypic predictors were of limited utility in discriminating triamcinolone response. Systemic mRNA expression of inflammatory mediators related to IL-2, IL-10, and TNF pathways strongly differentiated children who failed to achieve control after triamcinolone administration.

How does this study impact current management guidelines? Current definitions of severe asthma in children are associated with varied responses to systemic corticosteroids. Molecular endotypic as well as clinical phenotypic features should be considered in the evaluation of systemic corticosteroid responses.

BACKGROUND: Severe asthma in children is a heterogeneous disorder associated with variable responses to corticosteroid treatment. Criterion standards for corticosteroid responsiveness assessment in children are lacking.

OBJECTIVE: This study sought to characterize systemic corticosteroid responses in children with severe asthma after treatment with intramuscular triamcinolone and to identify phenotypic and molecular predictors of an intramuscular triamcinolone response.

METHODS: Asthma-related quality of life, exhaled nitric oxide, blood eosinophils, lung function, and inflammatory cytokine and chemokine mRNA gene expression in peripheral blood mononuclear cells were assessed in 56 children with severe asthma at baseline and 14 days after intramuscular triamcinolone injection. The Asthma Control Questionnaire was used to classify children with severe asthma into corticosteroid response groups.

RESULTS: Three groups of children with severe asthma were identified: controlled severe asthma, children who achieved control after triamcinolone, and children who did not achieve control. At baseline, these groups were phenotypically similar. After triamcinolone, discordance between symptoms, lung function, exhaled nitric oxide, and blood eosinophils was noted. Clinical phenotypic predictors were of limited utility in predicting the triamcinolone response, whereas systemic mRNA expression of inflammatory cytokines and chemokines related to IL-2, IL-10, and TNF signaling pathways, namely, *AIMP1*, *CCR2*, *IL10RB*, and *IL5*, strongly differentiated children who failed to achieve control with triamcinolone administration.

CONCLUSIONS: Systemic corticosteroid responsiveness in children with severe asthma is heterogeneous. Alternative prediction models that include molecular endotypic as well as clinical phenotypic features are needed to identify which children derive the most clinical benefit from systemic corticosteroid step-up therapy given the potential side effects. © 2016 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2017;5:410-9)

Key words: *Childhood asthma; Phenotype; Refractory asthma; Severe asthma; Gene expression; Corticosteroid*

Severe refractory asthma in children is a complicated disorder that is often difficult to evaluate in the clinical setting. A recent Task Force Report[1] defined "severe asthma" as the requirement for high doses of inhaled corticosteroids (ICSs) plus additional controller medications to maintain asthma control and/or prevent future exacerbations, implying that corticosteroid insensitivity is a fundamental feature underlying the disorder. Indeed, *ex vivo* studies in populations with severe asthma have noted

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Abbreviations used
ACQ- Asthma Control Questionnaire
AQLQ- Asthma Quality of Life Questionnaire
ICS- inhaled corticosteroid
PBMC- peripheral blood mononuclear cell
SABA- short-acting beta agonist

improvements in cellular function and inflammation with inhibition of signaling pathways that are normally suppressed by glucocorticoid receptor activation.[2,3] However, in clinical practice, variable responses to corticosteroid treatment have been observed,[4,5] highlighting the phenotypic and potentially endotypic heterogeneity among children with severe asthma.

Because the biological mechanisms associated with corticosteroid sensitivity in children with severe asthma are likely numerous and complex,[6-9] clinical assessment of corticosteroid responses are difficult and can further be confounded by poor medication adherence and delivery. Moreover, the lack of clinically applicable definitions of corticosteroid "responsiveness" has limited research in this field. Although studies in adults have relied on changes in lung function as an indicator of corticosteroid responsiveness,[10,11] children with severe asthma often have less airflow limitation than do adults[12,13] and do not always have concordance between lung function measures and symptoms.[14] Furthermore, lung function is only one component of asthma control and is best assessed in combination with current symptoms.[15]

Given the lack of a criterion standard for the assessment of corticosteroid responsiveness in children, the purpose of this study was to (1) characterize systemic corticosteroid responses in children with severe asthma after treatment with intramuscular triamcinolone and (2) identify phenotypic and molecular predictors of a response to intramuscular triamcinolone administration. Using a clinically available and validated questionnaire of asthma control, we identified 3 groups of children with severe asthma with similar baseline phenotypic features but differing systemic mRNA expression of inflammatory cytokines and chemokines related to IL-2, IL-10, and TNF signaling pathways. The findings highlight the heterogeneity of severe asthma in children as defined by current guidelines and further demonstrate the complicated nature of corticosteroid responsiveness assessment in these children.

METHODS

Children aged 6 to 17 years with physician-diagnosed asthma treated with high-dose ICS and a second controller medication were recruited from an outpatient severe asthma clinic in Atlanta, Georgia. All children met published criteria for severe asthma[1] including adherence to ICS evidenced by 10 or more monthly electronic prescription refills over the previous 12 months. Each participant had a history of either 12% or more reversibility in FEV_1 after bronchodilator administration or airway hyperresponsiveness to methacholine, evidenced by a provocative concentration of methacholine of 16 mg/mL or less. Exclusion criteria included premature birth before 35 weeks' gestation, aspiration disorders, vocal cord dysfunction, avascular necrosis, diabetes mellitus, historical or current bronchopulmonary aspergillosis, treatment with nonsteroidal anti-inflammatory drugs or omalizumab, chronic bone disorders, or bone fractures within the previous 6 months. All participants were stable at the time of baseline characterization with no signs of acute respiratory illnesses. If a recent exacerbation was reported, the first visit was conducted 4 weeks after completion of an oral or injectable systemic corticosteroid burst. Permission to proceed with this study was granted by the Emory University Institutional Review Board. Informed written consent was obtained from the legally authorized representatives of eligible children and assent was also obtained from participants aged 6 years and older.

Study design and group classification

Certified study personnel conducted the study under a standardized protocol and manual of procedures. After consent was obtained, participants completed 2 research characterization visits separated by 14 days. Intramuscular triamcinolone (1 mg/kg, 60 mg maximum dose) was administered in the gluteal muscle at the completion of the first visit to all participating children. Children were telephoned 24 to 48 hours after the injection to assess for adverse events. Daily short-acting beta agonist (SABA) use for asthma symptoms (excluding pretreatment before exercise) and corresponding symptoms were recorded in paper diaries between visits.

At the baseline visit, children were considered "controlled" if their Asthma Control Questionnaire (ACQ) score[16] was less than 0.75, which corresponds to "well-controlled asthma" with a positive predictive value of 0.73 and a negative predictive value of 0.85.[17] Children with uncontrolled asthma were classified as not achieving asthma control if their ACQ score was 1.5 or more (which corresponds to a positive predictive value of 0.88[17]) at the second visit after triamcinolone receipt.

Characterization procedures

Allergy skin prick testing with 12 extracts was performed at the first visit after a 3-day antihistamine withhold: tree mix (*Quercus alba*, *Ulmus americana*, *Platanus acerifolia*, *Salix caprea*, *Populus deltoides*), grass mix (*Cynodon dactylon*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Sorghum halepense*, *Paspalum notatum*), weed mix (*Artemisia vulgaris*, *Chrysanthemum leucanthemum*, *Taraxacum vulgare*, *Solidago virgaurea*), common ragweed (*Ambrosia artemisiifolia*), *Alternaria alternata*, *Aspergillus fumagatis*, *Cladosporidium herbarum*, dog dander, cat dander, German cockroach (*Blatella germanica*), *Dermatophagoides farinae*, and *Dermatophagoides pteronyssinus* (Greer Laboratories, Lenoir, NC). Histamine and saline served as positive and negative controls, respectively. Tests were considered positive if a wheal of 3 mm diameter or greater and flare 10 mm or more was present 15 minutes after application. Up to 10 mL of blood was obtained by venipuncture for total serum IgE (Children's Healthcare of Atlanta, Atlanta, Ga) and peripheral blood mononuclear cell (PBMC) isolation from whole blood through a density gradient.

Clinical outcomes

Clinical outcomes of interest included asthma quality of life as assessed by the Asthma Quality of Life Questionnaire (AQLQ), blood eosinophils, exhaled nitric oxide concentrations, and FEV_1 values. The AQLQ was completed with technical assistance as previously recommended.[18,19] Blood eosinophils were quantified by a local laboratory (Children's Healthcare of Atlanta, Atlanta, Ga) and exhaled nitric oxide concentrations were determined using online methods (NIOX MINO, Aerocrine, Morrisville, NC).[20] Spirometry (KoKo PDS, Ferraris, Louisville, Colo) was performed at baseline and the best of 3 vital capacity maneuvers was interpreted.[21] Participants were asked to withhold bronchodilator medication before