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Predictors And Potential Mechanisms Of Improvement In Asthma Control In Children Following Adenotonsillectomy

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Predictors and Potential Mechanisms of Improvement in Asthma Control in Children Following Adenotonsillectomy

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PREDICTORS AND POTENTIAL MECHANISMS OF IMPROVEMENT IN ASTHMA CONTROL IN CHILDREN FOLLOWING ADENOTONSILLECTOMY.

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Recent small observational studies suggest that asthmatic children receive clinical benefit in asthma control following adenotonsillectomy (TA), but little is known about which clinical and biological characteristics impact improvement. We enrolled 213 children undergoing TA, including 136 children with asthma and 78 controls, in a longitudinal observational cohort study (YCAAD). An asthma questionnaire, Asthma Control Test (ACT) scores, and serum asthma biomarkers levels were obtained at baseline and at six-months. Interim analysis compared patient characteristics to a historical cohort (CT-Kids) of 49 children with asthma who underwent TA. Urgent care visits ($P < 0.001$), oral steroid courses ($P < 0.001$), and ACT scores ($P < 0.001$) all improved in children with asthma following TA. Serum Th2 inflammatory markers, including IL-4 ($P = 0.022$) and IL-5 ($P = 0.002$), decreased following TA. Decreased IL-5 levels following surgery correlated with improvement in urgent care visits ($P = 0.021$), decreases in oral steroids ($P = 0.02$), and overall improvement in asthma control ($P = 0.008$). Children who were low or healthy weight, younger, female, had a history of sinusitis, and/or had a history of persistent asthma were more likely have improvement in their asthma following surgery. Elevations of serum IL-2, IL-4, IL-5, IL-13, IFN-γ, TNF-α, and GM-CSF levels were found in children whose asthma improved after TA. These clinical characteristics and biomarkers may help predict which children will receive maximum benefit in asthma control following TA.

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INTRODUCTION

Asthma is a common inflammatory disease of the lower airways with significant morbidity and mortality worldwide. There is a growing understanding that the pathophysiologic processes that contribute to asthmatic inflammation exist beyond the lower airway. Recent work has demonstrated that upper airway disease, including adenotonsillar disease in children, may worsen asthma control. [1, 2] Despite the prevalence of adenotonsillar disease in childhood, there has only been limited investigation on the impact of adenotonsillectomy (TA) on asthma control.

The Economic Burden of Asthma is Substantial

Asthma is one of the most common chronic morbidities in children in Western countries. In the United States approximately 14% of children are diagnosed with asthma during their lifetime and 9.6% of children carry a current diagnosis of asthma. [3] Over 300 billion people worldwide of all ages carry a diagnosis of asthma [4] with a significant of proportion of disease in the pediatric population. There is increasing recognition that asthma is a global disease, with a significant increase in disease prevalence in Latin America, Northern Europe, Eastern Europe, and Africa. Over a five to ten year period in the International Study of Asthma and Allergies in Childhood (ISAAC)—a large multicountry cross sectional-survey of over 50 countries—there was increasing global prevalence of asthma of 0.13% per year worldwide. [5] Although high-income countries have higher prevalence rates of asthma in childhood, severe disease is more common in less affluent countries. [6] With the increasing prevalance of asthma worldwide and increasing recognition of impact disease in the less affleunt countries, the economic burden of disease will continue to expand unless better disease interventions are found.

The current economic burden of asthma is substantial. The treatment of asthma alone accounts for eight billion dollars of US health care expenditure annually [7], with $3,856 spent per asthmatic per year. [8] In 2004 in the United States it was estimated that pediatric asthma was accountable for 12.8 million missed school days, 750,000 emergency room visits, 198,000 hospitalizations, and 186 childhood deaths. [6] As the prevalence of asthma only continues to increase, having interventions that improve and modulate asthma control are necessary to reduce disease burden.

What is asthma?

Asthma is an inflammatory disease of the lower airways that results in intermittent airway obstruction as evidenced by increased airway hyperreactivity; symptoms of dyspnea, wheezing, shortness of breath; and/or nocturnal coughing. [9, 10] A major in challenge both the diagnosis and treatment of asthma is that is a heterogeneous disease; the natural history of disease varies largely between individuals and depends on factors such as age of first symptoms onset, overall disease severity, and the patient's sex. [1, 11]

Although descriptions of asthma exacerbations date back to the second century A.D.[12], the exact etiology of asthma remains unclear. It is hypothesized that asthma occurs as a combination of both early environmental exposures and underlying genetic and biological susceptibility. [9] Over one hundred gene loci are associated with asthma including genes candidates that are part of inflammatory pathways (e.g. IL-4 cluster on chromosome 5), remodeling (e.g. ADAM33), and medication response (beta-adrenergic receptor mutations). But genetic susceptibility alone fails to fully explain the clinical spectrum of disease. Early infantile and prenatal exposures, such as early childhood viral

bronchiolitis, allergen exposure, secondhand smoke exposure, and pollutants exposure, also contribute to childhood risk of asthma. As such, the exact nature of the interaction between the biologic susceptibility and environmental exposures is poorly understood and currently an active area of research. For example, although it has been described that early childhood bronchiolitis with wheezing in infants is a risk factor for development of asthma, the majority of children with early respiratory virus associated wheezing do not develop asthma [13]. Recent work with the COAST birth cohort demonstrated that children with a homozygous gene mutation in the 17q21 locus who had an episode of rhinovirus-associated wheezing as an infant had an increased risk (OR=26.1) of developing asthma compared to children without this genetic susceptibility. [14] Connecting underlying genetic susceptibility with environmental triggers may be essential in helping to understand the pathophysiologic processes that drive development in asthma and to help with management of disease.

Inflammation and asthma

Although the exact etiology of asthma remains to be elucidated, there is strong evidence that inflammation of the lower airways drives the airway hyperresponsiveness that is responsible for the intermittent wheezing and shortness of breath that characterizes asthma. [15] In allergic asthma—the most common form of asthmatic inflammation found in children (See *Clinical Manifestations of Asthma in the Pediatric Population*)—the walls of the lower airways are infiltrated with mononuclear (primarily CD4+ T-helper cells) and eosinophils, although other proinflammatory cells such as mast cells, macrophages and neutrophils are also in abundance. T-helper cells Type II (Th2) are thought to both initiate and perpetuate asthmatic inflammation by priming the allergic

airway and increasing antigen-induced allergic inflammation. [16] After initial sensitization of the airway to aeroallergen, Th2 cells upregulate inflammation through the expression of cytokines including IL-4, IL-5, and IL-13 that promote inflammatory cell recruitment and perpetuate of the allergic response. This inflammation is further worsened through increased expression of other proinflammatory molecules such as TGF-beta, GM-CSF, and MMP may also trigger airway remodeling, with increase in respiratory smooth muscle mass that is also associated with airway hyperresponsiveness. [17]

One of the challenges with characterizing the exact immune response in pediatric asthma specifically is that it is a clinically heterogeneous disease that affects children differently depending on age of onset and disease severity. Therefore, it is reasonable that the underlying biochemical and physiologic basis of disease could differ between different phenotypes of asthma. In asthmatic mouse models there are a significant pathologic differences found sensitized infantile mice compared with juvenile and adult mice. [18] Hypersensitized infantile mice developed goblet cell hypertrophy compared to juvenile mice who were more likely to develop airway smooth muscle hypertrophy and have increased IL-5 expression, suggesting a fundamental pathological difference between asthma development based on age of symptom onset. Therefore, the exact pathophysiology of asthmatic inflammation may vary based on asthmatic phenotype.

Clinical Manifestations of Asthma in the Pediatric Population

The heterogeneity of asthma makes it a challenging disease to both diagnose and treat. There is a growing interest to classify asthmatic phenotypes to better predict natural history of disease, to connect clinical outcomes with underlying pathophysiologic

differences, and to individualize treatment regimens. The heterogeneity of clinical course of asthma and early childhood wheezing is evident even in the pediatric population making both diagnosis and management of the disease challenging. A major goal in pediatric asthma care is to improve in early identification of asthma subtypes that might improvement long-term management of disease.

Pediatric asthma is frequently classified into three major phenotypes: 1) early onset allergic, 2) exercise-induced, and 3) obesity-related disease. [19] Early onset allergic onset asthma covers a full range of severity and is often associated with allergy, atopy and rhinitis. On a molecular level this phenotype is connect with increased serum IgE, elevation of Th2 cytokines, and potentially related to 17q12 gene mutations. In contrast, exercised induced asthma in children tends to be mild, intermittent, and airway hyperreactivity related to mast-cell activation, intermittent Th2 cytokine spikes, and cysteinyl leukotrienes. A third major clinical asthma phenotype in children, primarily found in adolescent females, is an obesity-associated phenotype. Obesity-associated asthma may be a distinct pathophysiologic entity from general childhood allergic asthma characterized by increased Th1 inflammation and a non-atopic phenotype. [20-23] Although these clinical phenotypes have been described, often clinical characteristics are not easily evident in early childhood, making it challenging to predict long-term pulmonary outcomes.

Another major challenge in the diagnosis of asthma in childhood is that wheezing is common in children under the age of three; up to thirty-three percent of all children have wheezing prior to this age but only 40% of that population goes on to develop persistence of disease. [13] *Martinez et al.* in a birth cohort of 1,246 infants in Tucson,

AZ identified three major phenotypes of childhood asthma: 1) early onset intermittent wheezing, 2) early onset persistent wheezing, and 3) late onset wheezing (where age of onset is demarcated by the being less or earlier than age three). [13] These wheezing phenotypes correlated with long-term response to treatment and lung function. Using patient characteristics associated with wheezing the Tucson group developed an "Asthma Prediction Index" where if an infant with greater than or equal to four wheezing episodes neither met one major criteria (parental history of asthma or physician diagnosis of atopic dermatitis) or two minor criteria (physician diagnosed allergic rhinitis, wheezing unrelated with colds, or blood eosinophilia) they had a NPV of 91.6% for developing asthma by 6 years of age. [24] Despite the importance of clinical prediction of disease, the Tucson classification does not explain on a molecular basis the variation in asthma phenotypes nor aide in clinical decision-making.

Interestingly, despite the specific characteristics underlying the aforementioned classification strategies, there has been less success in connecting these clinical characteristics with specific inflammatory markers that predict long-term outcomes and explain biological differences between phenotypes. Because of concern for bias in the phenotypic classification of asthma, there has been increasing interest in using unbiased approaches in classification schemes. Especially as the majority children have relatively normal lung function during symptom free periods, even with clinically severe asthma, there is a need to identify high-risk individuals to better target interventions. In the Severe Asthma Research Program (SARP) in school-aged children, four phenotypes of pediatric asthma were identified using an unbiased cluster analysis: 1) late onset symptomatic asthma with normal lung function 2) early onset atopic asthma with normal

lung function 3) early onset atopic asthma with mild airflow limitation 4) early onset atopic asthma with advanced airflow limitation. [25] These phenotypes correspond directly with asthma duration, number of asthma medication, baseline FEV1 and potentially with the biomarker eNO. This understanding of these phenotypes may eventually help practitioners to better target disease and to be able to educate parents on a child's course of disease.

Alternatively, Woodruff *et al* used a genome-wide profiling of airway epithelial cells of moderate and found two major phenotypes of asthma: Th2 high (associated with the regulatory genes for IL-4, IL-13, and IL-5 expression) and Th2 low. [26] Interestingly, the Th2 high group airway hyperesponsiveness had a much-improved response to corticosteroids compared to the Th2 low phenotype [27], suggesting that a better understanding molecular characteristics of disease may help clinicians guide treatment in a more directed fashion.

Approach to the management of asthma in children

The main goal of asthma care is to control symptoms with minimal medical intervention. Asthma control is defined by the degree to which symptoms, functional impairment, risk of adverse events is minimized by treatment. [9] Asthma control in children is monitored by patient description of symptoms of breathlessness, interference with normal activity, and nighttime/early daytime awakenings. Validated questionnaires of asthma control, such as the Asthma Control Test (ACT), the Childhood Asthma Control Test (c-ACT) and Test for Respiratory and Asthma Control (TRACK) are particularly useful in both clinical practice and in research as they both evaluate a

patient's control over a defined period of time and have well-defined clinical thresholds for changing medical management. [28-30]

A major challenge in asthma care is the limitation in available objective data that guides diagnosis and management of disease. Pulmonary function tests are the cornerstone of objective testing in both diagnosis and treatment in asthma in both pediatric and adult patients. Although FEV1 in the presence or absence of bronchodilator response helps clinicians to diagnose and manage of disease, most children under the age of six cannot reliably perform the full exhalation needed for testing. As 80% of children have symptom onset by one year of age [9, 12], there is a need for more reliable testing in children of younger age groups. Because of the limitations of PFTs in younger children, there has been growing interest in the role of biomarkers to diagnose, monitor control, and evaluate treatment response of asthma in young children (see *Asthma Biomarkers Section*).

Biomarkers in Asthma Care

NIH criteria defines a biomarker as a measurable substance that characterizes a biologic, physiologic or pathologic response to either a therapeutic treatment or disease management [15]. In asthma care there has been limited success in finding readily available biomarkers of that correlate with clinical disease outcomes. Exhaled nitric oxide (eNO) is considered to be a marker of allergic inflammation in the lower airways and may be a useful biomarker for allergic asthma in younger children, as it testing for it is non-invasive, inexpensive, and can be reliably done in young children. Although elevations of eNO correlate with asthma diagnosis and have a dose dependent decrease after treatment with ICS, its clinical utility remains uncertain, especially as the marker is

also elevated in atopic children without asthma. [9] Th2 associated cytokines, such as IL-4, IL-5, and IL-13 have been to found to be elevated in the serum, bronchoalveolar lavage fluid (BALF) and the sputum of children with asthma. But as cytokines have a large variable range across the population, they have limited utility as a strict predictive biomarker of disease activity. Despite this limitation, changes in cytokine levels following treatment strategy may connect a biological response to a clinical intervention. [15, 31]

Another potential family of asthma biomarkers is the chitinase and chitinase-like proteins. Chitinases are evolutionarily conserved enzymes that degrade chitin, a complex polysaccharide found in insect and fungal debris that triggers host inflammation. In children, elevated chitotriosidase—the primary active chitinase of the lung—has been associated with clinical asthma severity. [32] The chitinase-like protein YKL-40, which binds and sequesters chitin, has been found to be elevated in the serum of asthmatics and correlates with disease severity. [33, 34] In children elevation of YKL-40 has been correlated with treatment resistant disease and asthma severity. [35] Therefore, chitinase and chitinase-like proteins may be important surrogate biomarkers that aid in the management of pediatric asthma.

Benefits and Limitations of Pharmacologic Management of Asthma Symptoms

Pharmacologic treatment of asthma focuses on both alleviating acute respiratory symptoms and improving chronic airway hyperresponsiveness. The long-term goal of management is to relieve both short-term impairment (e.g. shortness of breath, nocturnal cough) and to minimize risk of adverse events (e.g. intubation, death). Therefore, the

treatment strategy is two-fold: short term agents that help in acute exacerbation of airway responsiveness and long-term anti-inflammatory agents.

For short-term airway hyperresponsiveness, the cornerstone of management is the use of short acting beta-agonists such as albuterol. The goal of short acting beta-agonist use is to aid in improvement symptoms of airway hyperreactivity and prevent anticipated bronchospasm prior to exercise (specifically for patients with exercise sensitivity). For acute exacerbations, systemic corticosteroids may be required and are rarely required for long-term control of severe asthma in children. Although excellent at reducing inflammation and acute symptoms, the long-term consequences of systemic corticosteroid use are profound—including metabolic effects, growth suppression, osteoporosis, and cataract formation[9]. Therefore, a major goal in pediatric asthma care is to limit a child's exposure to oral corticosteroids by decreasing acute exacerbations.

Adequate treatment of persistent asthma with preventative asthma medication has shown to both decrease asthma morbidity and to improve asthma control. [36] The general approach taken to asthma pharmacotherapy in persistent asthmatics is a "step up, step down management plan" in which patients are placed on a medication regimen that completely controls symptoms and with a decrease in symptoms medications are tapered off. For persistent asthma, inhaled corticosteroids (ICS) are the preferred first-line therapy as they both improve lung function and asthma control. Although they aid in improving asthma, ICS do not alter the natural history of disease [9] and have significant adverse side effects including oral candidiasis and vocal dysphonia. In children, concern has also been raised that long-term use of ICS may result in growth suppression and osteoporosis; in the CAMP cohort—a large cohort of 1041 children being treated with

either with daily ICS or daily inhaled non-ICS—there was a 1.7 cm decrease in height of girls being treated with chronic ICS. [9, 37] Alternative agents to aid in improving asthma control include leukotriene receptor antagonists, cromones, and theophylline, although generally these medications are added as additional therapy in patients who do not respond to ICS compared to primary therapy. There is increasing interest in the use of long-acting beta-agonists in the support of moderate persistent asthma in children who suffer from nocturnal symptoms [38], although concern has been raised for worsening of status asthmaticus in children [9]. Although general incidence of preventative asthma medication use has doubled since 1988 [36], there is still a need for improved treatment adherence and optimization of management of disease beyond conventional treatment.

Management of Asthma Beyond Targeting the Lower Airway

Because of the impact that environmental exposures have on asthma, there is interest in expanding the treatment of asthma beyond targeting the lower airways. Although home environmental interventions (such as avoidance of allergens and second-hand smoke) have been a mainstay in pediatric asthma education, there is growing evidence that avoidance of allergens in early life may alter the natural course of disease. As allergy and asthma are frequently co-morbid, prevention of early life exposure to allergens associated with worse asthma control in high-risk children may impact development of disease. In Canada, in a high-risk cohort of 308 asthmatic children, in children who underwent early preventative measures to avoid allergen exposure and were breast fed there was a decreased prevalence (12.9%) of asthma compared to children without these measure in place (prevalence 25%) at seven years of age. [39] Similar studies in the Netherlands[40] and in the Isle of Wight[41] have shown consistent results

of decreasing asthma symptom development by decreasing early childhood allergen exposure. But greater sample size, follow-up time, and stratification of patient characterizes are needed to asses if the intervention does impact natural history of disease.[42]

There is also a growing understanding there is an interaction between chronic upper airway inflammation and worsening of asthma control, which is often termed the "One Airway Hypothesis." For example, rhinitis is a common co-morbidity with asthma, especially in the pediatric population. Approximately 90% of children with asthma have rhinitis[2] and up to 60-78% of children with asthma have allergic rhinitis. [43] Chronic rhinitis may worsen asthma by limiting the humidification and warming that nasal berathing normally provides and by filtering of allergens from environment.[2] Improvement in allergic rhinitis improves inflammation in the lower airways, asthma symptoms, and decreases asthma medication use. [9] Treatment of allergic rhinitis, including nasal steroids and anti-histamines is now considered a peripheral treatment for asthma. [2, 9, 44]

Adenotonsillar disease is a common form of upper airway disease in children with as many as 2-3% of children in the general population having sufficient adenotonsillar hypertrophy to cause polysomnographic evidence of obstructive sleep apnea.[45] In severe and moderate asthmatic children, there is a higher prevalence of obstructive sleep apnea compared to a general pediatric population. [46] Ross et al described in a cohort of 108 asthmatic children that individuals with sleep disordered breathing had a 5.02 OR of developing severe asthma over a one year period. [46] Despite the prevalence of adenotonsillar disease in childhood [47], there has been limited investigation of how

surgical management of adenotonsillar disease may impact asthma control (*See Asthma and Adenotonsillectomy*).

Adenotonsillar Disease in Children

The adenoids and tonsils are the largest accumulation of lymphoid tissues in Weldeyer's ring, the collection lymphoid tissue along the upper edge of pharynx. Dominated by B-cells, they are of thought to play a role in primary antigen surveillance of the upper airway and immunoglobulin production. [48, 49] The immune role of the adenoids and tonsils is most profund in children, as they involute in both size and activity during puberty.

Adentonsillectomy (TA) is one of the oldest described surgeries, with the first operation being performed in 10 A.D. by the Greek Physician Celsus. [49] In 2006, 530,000 adenotonsillectomies (TAs) were performed in the US alone and accounted for 16% of all ambulatory surgery in children. [48, 50] The two most common indications for tonsillectomy are recurrent infection (i.e. recurrent tonsillitis, recurrent adenoiditis, peritonsillar abscess) and for sleep disordered breathing. [47, 48] Sleep disordered breathing (SDB) represents a spectrum of upper airway disease that ranges from primary snoring to obstructive sleep apnea (OSA). Obstructive sleep apnea in children is defined at least one obstructive apneic episode or evidence of obstructive hypoventilation during nocturnal polysomnography. [51] Unlike in adults, adenotonsillar hypertrophy is a major contributor to dynamic airway obstruction in children, especially as the adenoid to airway ratio is largest in early childhood. [52] Airway narrowing in children with OSA occurs along the upper two-thirds of the airway and the maximal obstruction is where the tonsils overlap the adenoid tissue. [53] Concern has been raised about long-term complications

arising from chronic OSA in children—including worse neurocognitive outcomes, cardiovascular strain, and poor behavior—and now SDB is the most common indication for TA.

Although there still remains debate on the absolute indications for TA and timing of surgery [48, 54, 55], many children receive significant benefit from surgery in decreasing upper airway obstruction, decreasing incidence of infection, improving overall health and quality of life. [55] The current recommendation for TA in children with recurrent tonsillitis (RT) follows the Paradise Criteria: removal is indicated if a child has seven episodes of RT in one year, five in two consecutive years, or three in three consecutive years. For children with SDB, if SBD is accompanied by growth retardation, poor school performance, behavioral problems, and enuresis, TA should be considered. [55] For children with less clear symptoms of SDB but with a nocturnal PSG consistent with OSA, TA is indicated.

Asthma and Adenotonsillectomy

Despite the prevalence of adenotonsillar disease in childhood, there has been limited investigation of how surgical management of adenotonsillar disease may impact asthma control. In several small retrospective cohort studies, researchers observed that patient-reported asthma control improved following TA, with a decrease in use of asthma-related medications, a decrease healthcare utilization and an increase in Asthma Control Test (ACT) scores. [56-59] In the Childhood Adenotonsillectomy Trial (CHAT), a large cohort of 454 children undergoing either immediate adenotonsillectomy or tonsillectomy delayed for three months, it was noted that there were only three asthma

exacerbations for children receiving early adenotonsillectomy compared with 18 exacerbations in the watchful waiting arm, including three serious adverse events. [54]

Kheirandish-Gozal *et al.* found in cohort of 35 children with asthma and OSA after undergoing TA they had improvement in number of oral steroid courses, decrease in asthma symptom scores, and decrease in beta-agonist use. [58] Previous work in our lab, demonstrated in a cohort of sixty children with asthma undergoing TA [1, 60] that children with asthma had robust post-operative improvement in asthma control and decreased healthcare utilization after surgery. In addition to improvement in clinical asthma control, it was also found that chitinase activity—which reflects serum chitotriosidase activity—decreased following surgery selectively in asthmatic children and was elevated prior to surgery in children whose asthma improved following surgery suggesting that baseline chitinase may be useful in predicting which children's asthma will improve following surgery. [60] In addition to changes in chitinase expression, we found that in a limited sample of whole blood mRNA microarrays that the plasmin activation inhibitor SERPIN B2, a genomic signature that has previously been associated with asthma and related to IL-13 expression [26], may also decrease in asthmatic children with improvement in symptoms following surgery [1]. These findings suggest that mediators of asthmatic inflammation may decrease in children undergoing adenotonsillectomy and may play a role in explaining post-operative improvement in asthma.

HYPOTHESES AND AIMS

Despite a growing understanding of the impact that TA has on improving asthma control, there still has been only limited investigation on specific clinical and biological characteristics that influence which children have improvement in their asthma following surgery. We hypothesized that we could identify both patient characteristics and molecular signatures that correlate with post-operative improvement in asthma control. We postulate that molecular signatures related to asthmatic inflammation will change following TA and that these changes in molecular signature will correlate to improvement in asthma.

Specific Aims of the Study

I. To expand the established cohort of pediatric patients, both children with and without asthma who undergo TA for reasons other than asthma control.
II. Determine clinical asthma response following TA with a focus on change in ACT score and patient reported outcomes.
III. To investigate biochemical alterations following TA using proteomic analysis in order to better understand the changes in systemic inflammation that drive improvement in asthma symptomatology post-operatively.
IV. To identify clinical and biological characteristics that correlate with improvement after surgery.

METHODS

YCAAD cohort[a]

Over a 25-month enrollment period (December 2011-February 2013), as part of currently ongoing trial, children ages 2-18 who were undergoing TA for standard indications were enrolled at Yale New Haven Children's Hospital or at the North Haven Surgical Center. This study was approved by the Yale IRB; parental consent and child assent (if older than age 7) were obtained prior to enrollment in study.

YCAAD Enrollment and Follow-Up

As previously described [60] parents completed an asthma questionnaire and either age-appropriate ACT (ACT or c-ACT) or TRACK score on the day of surgery. A child was classified as asthmatic if a primary care provider, allergist, or pulmonologist had diagnosed the child with asthma prior to enrollment in the study. The questionnaire included an extensive pulmonary history (including age of onset of asthma symptoms, asthma triggers, current asthma medication use, urgent care visits, and oral steroid courses), presence of asthma-associated comorbidities (including sinus disease, allergy, atopy, and GERD) and demographic data. Race and Ethnicity data were self-reported according to the guidelines of the US Census. TRACK and c-ACT scores were rescaled to a 25 scale to be compared with the teen ACT score as described previously. [60] Intraoperatively, with the help of the anesthesia team, serum serum blood samples were obtained.

Six months following surgery, patients were contacted for follow-up visit either by telephone or email. A modified questionnaire was completed either over the phone or

[a] This thesis is a continuation of the work started by Jonathan Levin, MD. Subjects were enrolled by Lisa Gagnon, APRN, Jonathan Levin, MD, or this author. Chitinase assays were either performed by Jonathan Levin or this author.

at a clinical site visit. Venous sampling was additionally offered to children at the follow-up visit, and children who agreed to participate received compensation. Incidence of clinical events, were rate-adjusted to a standardized twelve-month period as described previously[60].

CT Kids Cohort[b]

Asthmatic children ages 2-18 who were undergoing TA for standard indications were enrolled over a 24-month period from 2008-2010. All children included in the study had previous clinician diagnosis of asthma. This study was independently approved by the Yale IRB; parental consent and child assent (if older than age 7) were obtained prior to enrollment in study.

Parents completed an asthma questionnaire prior to the day of surgery that included healthcare utilization over the last year (number of emergency room visits and pediatrician visits), socioeconomic burden of disease (missed school days and missed work days), asthma medication use, asthma phenotype, and other co-morbidities (sinusitis, GERD, atopy). One-year following surgery parents were contacted for follow-up, at which time the baseline asthma questionnaire was repeated.

Weight Data (Both CT Kids and YCAAD)

Pre-operative height and weight were obtained either by nursing staff on the day of surgery or from communication with the parents about their child's measured height and weight within six months of the surgical date. Each child's body mass index (BMI) and age appropriate BMI percentile was calculated using the Center for Disease Control and Prevention BMI calculator. [61] Children were classified as overweight if their BMI

[b] CT-Kids Cohort data was collected by Lisa Gagnon, APRN. All statistics run on this patient population were performed by the primary author of this study.

was greater than the 85th percentile or low/healthy weight (LHW) if their BMI was less than the 85th percentile. For the YCAAD Cohort, parents were asked on the day of surgery about their perception of child's weight as Low Weight, Normal, Overweight, or Obese. For children with available BMI data, the BMI data was compared with parent's reported weight perception. If the child BMI did not correlate with parent's description, they were re-categorized based on BMI. Children were then clustered into LHW or Overweight groups to form the composite variable of parent-adjusted weight (PAW).

Serum Sample Collection and Processing (YCAAD Only)

Serum was isolated by centrifugation of whole blood 405 xg for 10 minutes to separate RBC from serum immediately after collection. The samples were stored at either -20°C or -80°C until biological assays were performed.

Chitinase activity

Chitinase activity was determined using a standard fluorometric assay described previously. [60, 62] Briefly, 20 μL of serum was added to 180μL of 22 μMol solution of 4-methylumbelliferyl-β-D-N,N',N''-triacetylchiotrioside in McIlvain Buffer. The reaction was incubated at 37°C for 30 minutes after which enzymatic reaction was terminated with the addition of 2 mL of 0.3 M glycine-NaOH, pH 10.6. Samples were excited at 365 nm and emission was measured at 445 nM using a Sequoia Turner fluorometer. Enzymatic activity was calculated in nM/mL*h using a standard curve of 4-methylumbelliferone.

YKL-40 ELISA Assay

Serum YKL-40 levels were determined by ELISA per manufacturer's protocol (Quidel, San Diego, CA). In brief, 20 μL of serum or standards were added to

streptavidin ELISA coated-strips. Samples were incubated with 100 µL of capture solution (mouse monoclonal anti-YKL-40 fAb conjugated to biotin) for one hour at room temperature. All incubation steps were performed covered (out of direct light) to prevent photobleaching. Reaction wells were emptied and washed with 300 µL of wash buffer solution three times. To the reaction wells, 100 µL of enzyme conjugate solution (polyclonal rabbit polyclonal anti-YKL-40 antibody conjugated to alkaline phosphate) was added and allowed to incubate at room temperature for one hour. Reaction wells were emptied and washed three times as described previously. One hundred µL of substrate solution (diethanolamine and magnesium chloride solution) was then added to well, and allowed to incubate for another hour at room temperature. To the final reaction solution, 100 µL of Stop solution (0.5 M NaOH) was added. Optical density of ELISA was read at 405 nm within 15 minutes of addition of stop solution. All samples were run in duplicate and concentrations were averaged. Any sample with confidence interval (CI) of greater than 25% between wells was rerun until the CI was less than 25%.

Bio-Plex Assay

Serum cytokine levels were determined using magnetic bead Luminex platform (Bio-Plex Pro Human Th1/Th2 Cytokine Panel, Biorad, Hercules, CA) per the manufacturer's protocol. Briefly, the provided lyophilized standard was reconstituted in 500 µL standard diluent for 30 minutes on ice and diluted as recommended. Samples were then diluted 1:4 in sample diluent. Magnetic beads were diluted to 1X concentration in assay buffer from 10X stock and 50 µL aliquots were distributed to each well of the fluorescent plate. Beads were washed two times with 100 µL of wash buffer. Once samples and standards were brought to room temperature, 50 µL of samples and

standards were distributed to each well. All samples and standards were run in duplicate. Reaction was incubated covered for one hour at room temperature with shaking at 600 rpm. The plate was washed three times with 100 μL of wash buffer then 25 μL of 1X detection antibody solution was added to each well. Reaction was incubated covered for thirty minutes at room temperature with shaking at 600 rpm. The sample was again washed three times with 100 μL of wash buffer then 50 μL of 1X streptavidin was added to each well. The reaction was incubated covered for ten minutes at room temperature with shaking at 600 rpm. The plate was washed three times as described previously and magnetic beads were resuspended in 150 μL of assay buffer. The plate was read on a Bio-plex (Bio-plex 200 System, Biorad) and data was acquired (Bio-plex Manager Software 5.0, Biorad). Outliers on standard curve were removed after visual inspection. Concentrations for cytokines were calculated based on the average readings between duplicate cells.

Statistical Analysis

Clinical data from the YCAAD cohort was uploaded onto the YCAAD computer online database. Statistical analysis was performed using SPSS software version 20 (IBM, Armonk, NY). Baseline values were only compared in children who also had available follow-up data. Nominal data was compared using the Fisher's exact and Pearson's Chi-Squared tests. All scaled data was confirmed to be of non-normal distribution using Shapiro-Wilk's test, therefore, non-parametric tests, including Wilcoxon Signed-Rank, Mann-Whitney, and Sign test were used.

Definition of improvement in Asthma Control

Improvement in asthma following surgery was defined by four distinct variables: ACT/TRACK reached MCID, any improvement in urgent care visits, any improvement in steroid courses, and composite improvement. The minimal clinically indicated difference (MCID) is a previously defined term that indicates the minimal difference in ACT or TRACK score that is associated with significant clinical outcomes. [63, 64] We used the previously described MCID scores as an improvement in ACT score greater than or equal to three or an improvement in TRACK score greater than or equal to 10. Although the MCID has not been formally described for the c-ACT questionnaire, we used the same cut-off that has been described for the teen/adult ACT score. We also used a composite score for improvement (composite improvement) in asthma control as defined previously [60] as any improvement in albuterol use frequency, decreased rate of steroid courses, decreased rate of urgent care visits, or a change in ACT /TRACK score that reached MCID.

RESULTS

Baseline demographics YCAAD Cohort

In an ongoing trial, over a 25-month period 136 children with asthma undergoing TA were enrolled with an average follow-up time of 7.2 months. Formal analysis was conducted when the first 100 children with asthma completed follow-up, with a follow-up rate of 74%. One child was excluded from analysis because there was no clinician diagnosis asthma despite symptoms and medication use consistent with disease. The cohort was generally young (mean age 6.5), predominately male (63%), and with a large percentage of Hispanic children (44%). Consistent with current trends in TA for all children [47], 74% of children had their tonsils removed for SDB and 22% for recurrent tonsillitis. About one-third of children had either GERD or sinusitis and almost three-quarters of children with asthma were atopic (Table 1).

In parallel, 78 children without a diagnosis of asthma were also enrolled as controls. Fifty-seven of those children completed follow-up for a follow-up rate of 73%. Three children were excluded from analysis as they were diagnosed with asthma during the course of the study. Compared with asthmatic peers, control subjects had less asthma related co-morbidities (i.e. sinusitis $P = 0.018$, GERD $P = 0.004$, and atopy $P = 0.003$, Mann-Whitney U), were less likely to be Hispanic, and were more likely to come from a higher socioeconomic background (Table 1).

Improvement in Asthma Control Following TA

Evidence of improvement in asthma symptoms after TA was demonstrated by comparing paired changes from baseline to follow-up in parent reported clinical outcomes. Urgent care visits (mean decrease from 2.37 to 0.78 visits per year, $P < 0.001$)

oral steroid courses (mean decrease from 1.74 to 0.5 visits per year, $P < 0.001$), and ACT/TRACK scores (median increase of adjusted ACT scores from 20 to 22, $P < 0.001$), all decreased in children following surgery (Figure 1). Children also had decreased use of controller medications (decrease from 52% to 37%, P = 0.004) but no significant change in albuterol use ($P = 0.27$).

Circulating Inflammatory Markers after TA

At baseline, children with asthma had higher levels of serum IL-5 (median 2.4 pg/mL versus 1.0 pg/mL, $P = 0.002$) and IL-13 (median 1.7 pg/mL versus 0.9 pg/mL, $P = 0.008$) compared to non-asthmatic peers (Figure 2). In contrast, children without asthma had higher baseline levels of chitinase activity compared at baseline compared to children without asthma (median 3.8 nMol /mL*hr versus 3.2 nMol/mL*hr vs $P = 0.014$) (Summarized in Table 2).

We found that neither chitinase activity ($P = 0.63$) nor YKL-40 levels ($P = 0.26$) changed following surgery (Table 2). The inflammatory cytokines IL-4 and IL-5 significantly decreased ($P = 0.022$ and $P = 0.002$ respectively*)* selectively in children with asthma following TA (Figure 3). IL-13 levels decreased in both children with and without asthma following surgery but were only significant for children without asthma ($P = 0.088$ and $P = 0.029$ respectively). All other cytokines did not significantly change following TA (Table 2).

Effect of weight on improvement in asthma control following TA

Because of the hypothesized impact of obesity on asthma control and the limited BMI data available in the YCAAD cohort, we compared the impact that a child's weight had

on asthma control in two cohorts: the YCAAD Cohort (interim analysis of 88 children) and CT-Kids Cohort.

Baseline demographics CT Kids Cohort

Over a one-year period, 88 children with asthma undergoing TA were enrolled. One-year follow-up was completed for 76 (86%) children. BMI data was available for 49 (66%) of the children who completed follow-up. LHW children made up of 61% of the cohort (n = 30) and overweight children 39% (n =19). There was no significant difference between LHW and overweight children in age, gender, race/ethnicity, indication for tonsillectomy, or asthma related co-morbidities (summarized in Table 3A).

Baseline demographics interim YCAAD Cohort

An interim analysis on the effect of weight on asthma control was carried on the first 62 (86%) children with asthma that completed follow-up in the YCAAD Cohort. Strict BMI data was available for 47 (76%). Sixty-two percent of the cohort was LHW (n = 29) while 38% was overweight (n = 18). There was no significant difference between LHW and overweight children in sex, race/ethnicity, or asthma-related co-morbidities (Table 3A). Children in the overweight group were more likely to be older (range 2-17) compared with LHW peers (range 2-12, $P = 0.01$) and have tonsillectomy for sleep disordered breathing ($P = 0.05$).

Baseline Asthma Control by Weight Category

Baseline asthma control, as assessed by medication use, emergency room or urgent care visits, and missed school/work days was comparable between LHW children and overweight children in both cohorts (Table 3B). Additionally, in the YCAAD cohort, ACT scores were similar between LHW children and overweight children ($P = 0.30$).

Baseline oral steroid use was significantly lower for overweight compared to LHW children in the YCAAD cohort ($P = 0.013$) but was equivalent in the CT Kids cohort ($P = 0.57$).

Post-operative Asthma Medication Use by Weight Category

At the follow-up visit—either at six months (YCAAD) or at one-year (CT-Kids)—LHW children had decreased inhaled corticosteroid (ICS) use post-operatively (YCAAD, $P = 0.008$, CT Kids $P = 0.016$), while overweight children had no significant change in use (Table 3C, Table 3D). In the YCAAD cohort, frequency of albuterol use decreased in LHW children (median initial use of one time a week versus follow-up of no weekly albuterol use, $P = 0.002$), but did not change for overweight children. Leukotriene receptor antagonists (LTRA) were used less frequently post-operatively only for LHW children in the CT Kids cohort ($P = 0.016$). There was no significant change in long-acting β_2-agonists (LABA) in either cohort for both LHW and overweight children. The number of oral steroid courses decreased post-operatively for both cohorts of LHW children ($P < 0.05$, $P < 0.02$) but did not decrease significantly in overweight children in either cohort.

Asthma Control and Health Care Utilization by Weight Category

In the YCAAD cohort, ACT scores were significantly improved in LHW children ($P = 0.001$) but not for overweight children ($P = 0.48$) following surgery (Table 3D). In the CT Kids Cohort, both LWH and overweight children had post-operative improvement in the number of emergency visits ($P < 0.01$, $P < 0.05$, respectively) and pediatrician visits ($P = 0.001$, $P < 0.001$) (Table 3C). Urgent care visits so decreased in both LWH and

overweight children in the YCAAD cohort but this change was not significant (Table 3D).

Clinical and Biomarker Characteristics that Impact Improvement in Asthma Control following TA

For the first one hundred children in the YCAAD cohort with asthma who achieved follow-up, we identified baseline clinical and molecular signatures that correlated with improvement in asthma after TA.

Minimally Clinical Indicated Difference in ACT and TRACK Scores

Paired ACT, c-ACT, or TRACK scores were available for 74% of children. Improvement in asthma as defined by a MCID cutoff of a change of greater than three for the rescaled ACT score (see methods) and greater than 10 for the TRACK score. Improved asthma control occurred for 46% (n = 34) of children after TA. Children whose asthma improved were younger (median age 5.5 versus 7, $P = 0.027$), female (53% versus 27%, $P = 0.03$), on controller medications at baseline (68% versus 32% $P < 0.001$), and children who had never been symptom free for one year (6% versus 23%, $P = 0.055$) were more likely to have improvement in ACT/TRACK score (Table 4). Indication for tonsillectomy, demographics, asthma triggers, and previous hospitalization history for asthma did not impact improvement in ACT/TRACK score following surgery (Table 4).

For the children with ACT/TRACK score data, 80% (n = 60) had baseline cytokine data available and 95% (n = 70) had baseline chitinase activity/YKL-40 levels. Children whose asthma improved by MCID level following surgery had higher baseline

levels of IL-2 (P = 0.015), IFN-γ (P = 0.039), GM-CSF (P = 0.02) and TNF-α (P = 0.04) (Table 4).

Improvement in Urgent Care Visits

Urgent care visits decreased for 41% (n = 41) of children with asthma following TA. Children who had improvement in ER visits were more likely to have exercise (P = 0.023) and "other" (P = 0.003) listed as asthma triggers, to be on controller medications (P < 0.001), to have moderate or severe asthma (P = 0.002), children who had never been symptom free for one year (P = 0.007), and to have had a higher number of lifetime hospitalizations (P = 0.04). There was also a higher frequency of children with sinusitis (P = 0.02) in the population who had a decrease in ER visits following TA (See Table 5).

Cytokine data was available for 83% (n = 83) of children and chitinase and YKL-40 levels were available for 94% (n = 94). Children who had improvement in ER visits had elevated IL-4 (P = 0.014), IL-4 (P = 0.023), and IL-13 (P = 0.013) (Table 5).

Improvement in Oral Steroid Courses

Steroid courses decreased for 39% (n = 39) of asthmatic children after TA. Children who had improvement in steroid courses were more likely to have "other" (P = 0.002) as an asthma trigger, have asthma that limited normal activities of daily life (P = 0.043), to be on controller medications (P < 0.001), to have moderate or severe asthma (P < 0.001), to have had a higher number of lifetime hospitalizations (P = 0.018), to have been hospitalized in the year prior to surgery (P = 0.002). There was also a higher frequency of children with sinusitis (P = 0.007) in the population who had a decrease in steroid visits following TA (See Table 6).

Cytokine data was available for 83% (n = 82) of children and chitinase and YKL-40 levels were available for 95% (n = 94). No baseline biomarker levels correlated with improvement in steroid courses following surgery (Table 6).

Composite variable improvement

Asthma improved for 70% (n = 70) of children with asthma following TA using the composite variable that has been previously described. [60] Children whose asthma improved more likely to be younger ($P = 0.012$), have "other" ($P = 0.002$) as an asthma trigger, to be on controller medications ($P < 0.001$), to have moderate or severe asthma ($P = 0.001$), and to never have been symptom free for one year ($P = 0.001$) (Table 6).

Cytokine data was available for 83% (n = 83) of children and chitinase and YKL-40 levels were available for 94% (n = 94). No baseline biomarker levels correlated with improvement in steroid courses following surgery (Table 6). Children whose asthma improved had elevated IL-4 ($P = 0.042$), IL-4 ($P = 0.025$), and IL-13 ($P = 0.029$) and TNF-α ($P = 0.041$) levels at baseline compared to peers whose asthma did not improve (Table 6).

Decreases in IL-5 Correspond to Improvement in Asthma Control

To observe if changes in inflammatory cytokines impacted improvement in asthma control after surgery, we compared median changes in cytokines levels in children whose asthma improved versus did not improve. Interleukin-5 levels dropped in children who had improvement in urgent care visits (-1.3 pg/mL versus -0.2 pg/mL, $P = 0.021$), number of steroid courses (-1.25 pg/mL versus -0.15 pg/mL, $P = 0.022$), and overall composite control of asthma (-1.2 pg/mL versus -0.05 pg/mL, $P = 0.008$) after surgery compared to peers who did not improve. Neither changes of IL-4 (composite

improvement, $P = 0.09$; urgent care improved $P = 0.13$; steroids improved, $P = 0.95$) nor IL-13 (composite improvement, $P = 0.51$; urgent care improved $P = 0.11$; steroids improved, $P = 0.70$) correlated with improvement in asthma.

DISCUSSION

Though retrospective cohort studies have examined the role of TA on asthma control and healthcare utilization, this is the first study that identifies inflammatory markers and specific patient characteristics that may predict improvement in asthma after TA. In addition, this is the first study that demonstrates that TA modulates Th2 inflammation in asthmatic children, which may play an important role in explaining symptoms resolution after surgery.

Baseline Cohort Demographics

In general, our cohort is similar to other pediatric asthma cohorts. The male predominance, large Hispanic population, and higher rates of asthma co-morbidities compared with the general population, are consistent with other pediatric asthma cohorts. One notable difference is that the mean age is significantly younger in our cohort. The younger age may be explained by the general trend in pediatric asthma research to delay enrollment of children until age six. Because wheezing is a common occurrence in the pre-school aged child [13], frequently birth cohorts delay official diagnosis of asthma until a child can undergo pulmonary function testing with bronchodilator response at age six. [9, 14, 37] Therefore, because our study does not rely on PFTs for diagnosis we may capture a few children with recurrent wheezing of childhood rather than asthma. On the other hand, this study may capture an earlier life intervention that could impact natural history of disease.

Another notable difference between our asthmatic population and control subjects was the difference in maternal education level. Control subjects were more likely to have parents with an advanced educational background compared to asthmatic subjects.

Although there is not an evident explanation for this distinction, it is possible this effect may be partially explained by a reluctance of parents with a less education to enroll their children in clinical research if the results do not directly apply to their child.

Asthma Improves Following TA

There was robust clinical improvement in asthma control—including improvement in ACT/TRACK scores, urgent care visits, and steroid courses—following TA. This clinical improvement is likely a multifactorial resulting from improvement in overall airway resistance, decreasing potential for microaspiration, and decreasing systemic inflammation.

There has been increasing interest in the role for microaspiration in the worsening of asthma control. Bacterial toxins and bacterial colonizers of the nasal passage, adenoids, and tonsils may be silently aspirated in the lungs, thereby triggering worsening inflammation of the lower airways. [65] In children with adenoid hypertrophy there may be increased stasis of nasal secretions and increased bacterial colonization of tissue leading to increased susceptibility for antigen triggering of the lower airways, thereby worsening asthma control. Therefore, by removing the nidus of inflammation, there may decreased triggering of the lower airways and improved asthma control.

Alternatively TA may improve asthma symptoms by decreasing systemic inflammation. In children with adenotonsillar disease there may be low-level systemic inflammation as a consequence of misregulation of normal adenoid and tonsillar immune function[49] or from systemic inflammation associated with long-standing OSA/SDB. Sleep disordered breathing in children is associated with higher levels of pro-inflammatory cytokines associated with asthma including hs-CRP, leptin, and adiponectin.[46] Therefore, if

surgery decreases low-level circulating pro-inflammatory agents, it may contribute to improving airway inflammation leading to overall improvement in asthma control (See below*)*.

Biomarkers of Th2 associated asthmatic inflammation improve following TA

Although improvement in asthma control following TA has been described previously, less is known about its impact on asthmatic inflammation. Inflammatory cytokines are an appealing target as a post-tonsillectomy biomarker as Th2 inflammatory cytokines are expressed by CD4+extrafollicular T-cells in pediatric tonsillar tissue. [66] Additionally, it was previously found in our lab that Serpin B2—a gene that is regulated by IL-4 and IL-13 in vitro [60, 67]—expression levels decrease in children whose asthma improves following surgery [1], thereby suggesting a connection between Th2 cytokine pathways and TA.

There was a significant decrease in IL-4 and IL-5 levels in the serum of asthmatic children following TA. Interleukin-4 and IL-5 are two of the classically described cytokines involved with Th2 allergic inflammation in asthmatic children. Therefore, if TA modulates expression of inflammatory cytokines involved with asthma pathogenesis, it may explain the improvement in asthma symptoms following surgery.

Interleukin-4 has a broad range of clinical activities but in asthma it may initiate allergic sensitization and prime the Th2 immune response leading to recruitment of eosinophils into the airway, increasing bronchial hyperreactivity, and elevating IgE levels. [68, 69] Expression of IL-4 is increased at both the proteomic and genomic level in the bronchoalveolar lavage fluid (BALF) of asthmatics. A decrease in IL-4 levels

following surgery may modulate Th2 response and decrease allergic sensitization, thereby improving asthma symptoms.

Despite the decrease in IL-4 levels in children following TA, this decrease did not correlate with clinical improvement of asthma. The absence of improvement is consistent with efforts to target IL-4 therapeutically in adult asthmatics; Phase II trials that targeted IL-4 expression using either soluble IL-4R or anti-IL-4 antibodies did not improve asthma symptoms or improve FEV1. [70, 71] The failure of anti-IL-4 agents suggests that IL-4 may be more important in the initial allergic sensitization phase compared to long-standing remodeling and disease persistence. [72, 73] Additionally, IL-4 levels were difficult to detect in the serum, therefore differences in expression were subtle and the entire impact of the effect of surgery on this biomarker may not be fully captured by our assay. Therefore, modulation of expression of IL-4 in the serum of asthmatic children, may have less clinical relevance in describing improvement in asthma following TA.

Interleukin-5 is another important Th2 cytokine in allergic asthmatic inflammation that contributed to response to TA in our cohort. Interleukin-5 promotes the maturation, activation, and mobilization of eosinophils within the lung in atopic asthmatic individuals. Exogenous exposure to IL-5 increases sputum eosinophilia and airway hyperresponsiveness. [74] Interleukin-5 is overexpressed in the serum and in the BALF in asthmatic individuals and mRNA expression has been shown to correlate with asthma severity. [75, 76] Similarly, we found that children with asthma had higher levels of circulating IL-5 compared with non-asthmatic peers. As atopic asthmatic inflammation—the most commonly described type of asthmatic inflammation in children—is characterized by eosinophillic infiltration and bronchial

hyperresponsiveness, a decrease in IL-5 levels could decrease asthmatic inflammation following surgery.

Interleukin-5 levels decreased in our cohort following TA and a decrease in circulating levels correlated with improved urgent care visits and overall control. There may be elevation of serum IL-5 in children with acute asthma exacerbation and levels decreases once asthma is stabilized [77], suggesting a decrease in IL-5 levels may correlate with improved asthma control. Unlike attempts to target IL-4, therapeutic agents that target IL-5 have had modest success in improving asthma symptoms in patient cohorts with significant sputum eosinophilia with a history of frequent exacerbations. [75] The best-described anti-IL-5 agent is the humanized monoclonal antibody Mepolizumab (SB240563, GlaxoSmithKline, Research Triangle Park, NC), which in phase II trials in patients with significant sputum eosinophilia on chronic ICS has shown to decrease asthma exacerbations. [78] In adults with significant response to Mepolizumab decreasing peripheral eosinophil counts, rather than measuring serum IL-5, are the standard of response to therapy. Although serum eosinophil counts were unavailable for our cohort, future correlation in circulating eosinophils would enrich our understanding of response to surgery., In children who have a change in IL-5 expression following surgery there may be improvement in symptoms secondary to decreasing airway eosinophilia.

Interleukin-13 levels were also elevated in the serum of asthmatic children compared to controls. Interleukin-13 is another Th2 cytokine also present in the asthmatic airway. Although similar in structure and function to IL-4, it is thought perpetuate of allergic airway response rather than to initiate response.[79] Unlike IL-4 and IL-5 levels,

IL-13 did not significantly decrease in asthmatic children following surgery. It is possible that IL-13 levels did not fall following surgery as in general they were expressed at lower levels in the serum compared to IL-4 and IL-5 and levels may be suppressed by oral steroid use, therefore biological proteomic assays may not be able to detect levels are readily as other cytokines. In contrast, IL-13 did decrease significantly in children without a clinician diagnosis of asthma. This change suggests that even in children without asthma, TA may impact systemic inflammation.

Previously in the YCAAD cohort we found that asthmatic children undergoing TA, in addition to having post-operative improvement in asthma control, had a decrease in chitinase activity.[60] With enlargement of the cohort, this effect was eliminated; there was no significant decrease in chitinase activity in children with or without asthma following surgery. Additionally, control subjects had higher baseline chitinase activity compared with asthmatic peers. This contradicts previous findings that serum chitotriosidase is elevated in the serum of asthmatic individuals compared with controls. [32] As chitotriosidase has been found to be overexpressed in the hypertrophied adenoids of children with chronic rhinosinusitis, otitis media with effusion, and allergic rhinitis [80], in children with adenotonsillar disease chitinase activity may reflect degree of adenotonsillar inflammation rather than asthmatic inflammation, therefore decreasing its utility as an asthma biomarker in the setting of adenotonsillar disease. Alternatively, this study did not address the role of genetic mutations of chitotriosidase and difference in expression may be a result of differing genetic background between asthmatic and non-asthmatic children in our cohort.

Clinical Characteristics that May Predict Improvement in Asthma Following Surgery

Though previous studies have examined the role of TA on asthma control and healthcare utilization, this is the first study that identifies specific clinical characteristics—such as being LHW, being younger, being female, having a history of sinusitis, and having persistent asthma—correlated with improvement in asthma following surgery.

Children who are overweight receive less benefit from TA for asthma control compared to LHW peers

We found in two independent cohorts that LHW children are more likely to have clinical improvement in asthma control and medication usage following TA compared to their overweight peers. LHW children used less asthma medications post-TA and had a more robust increase in ACT scores while overweight peers had no significant change in medication use or ACT scores. The strong decline in oral steroid courses post-operatively for LHW children in both cohorts suggests a decline in frequency of asthma exacerbations following surgery that was more robust in LHW children compared to overweight peers.

The absence of change in ICS use for overweight children may reflect a general increased resistance to inhaled corticosteroids in obese asthmatic children. Forno *et al* found that overweight asthmatic children were less likely to have improvement in lung function following budesonide administration compared with non-overweight weight peers. [81] Obesity-associated inflammation may contribute to persistence of clinical resistance to ICS post-operatively, especially as pro-inflammatory cytokines have been

implicated in the regulation of the expression of the β-isoform of the glucocorticoid receptor associated with resistance to ICS in asthmatic adults. [82, 83]

Overweight children may receive less benefit in asthma control following TA because of persistent mechanical obstruction of the upper airway secondary to redundant neck soft-tissue and adiposity. Even at baseline in the YCAAD cohort, overweight children were more likely to have TA for obstructive airway disease compared to recurrent tonsillitis. Chu *et al* observed that in children undergoing TA for OSA, 59% of obese children have persistent polysomnographic evidence of OSA post-operatively compared with less than 22% of non-obese children. [84] Persistence of upper airway obstruction after TA may contribute to lack of improvement in asthma control.

Alternatively, obesity-associated asthma may be associated with Th1 inflammation compared with the Th2 atopic phenotype found in classical pediatric asthma. [20, 85] If removal of the adenoids and tonsils predominately impacts Th2 inflammatory pathways, the impact on asthmatic inflammation may be less dramatic for obese asthmatic children. Persistence of upper airway obstruction in obese children post-operatively may contribute to continued activation of inflammatory pathways that influence asthma control, especially as both obstructive sleep apnea and sleep disordered breathing are associated with increased expression of pro-inflammatory markers such as IL-6 and hs-CRP. [46, 86, 87]

Interestingly, with enlargement of the cohort to 100 children in the YCAAD cohort, neither BMI percentile nor parent adjusted weight correlated with improvement in asthma. The effect of being overweight may have been diminished in larger sample size as the robust improvement following TA may modulate the effect of obesity on

improvement. A more detailed understanding of the obesity sub-phenotypes, including measurements such as waist-circumference and percent body fat, may further elucidate the importance of obesity on post TA improvement in asthma control.

Asthma control in younger children is more likely to improve following TA

Younger children were more likely to have improvement in their asthma control after TA as measured by the ACT, c-ACT, or TRACK scores and by the composite variable for improvement. Younger children with asthma are at higher risk for hospitalization and have significantly longer hospitalizations compared with older peers. [88] As younger children have anatomically narrower lower airways compared to older peers, they are at increased risk for obstruction with inflammation, often in the setting viral infections. Therefore, TA has the potential to reduce disease burden in this at risk population.

Age is also an important factor both anatomically and physiologically in adenotonsillar disease. The adenoids to airway ratio size is largest in children ages two to eight [52], therefore, in younger children there is an increased likelihood that adenoid hypertrophy may cause obstruction of airflow. As nasal airflow plays an important role in humidification, warming, and filtering inspired antigens, the increased airway obstruction in younger children may play a significant role in exacerbating asthma control. [2, 89] Additionally, adenotonsillar disease is considered to be the primary risk factor for OSA and SDB in younger children. Magnetic resonance imaging shows a direction correlation between increasing adenoid and tonsillar volume in relationship to airway size with an increasing apnea-hypopnea index. [53] Bhattacharjee *et al* found in a cohort of 578 children that following adenotonsillectomy, children had improvement in apnea-

hypopnea index and total sleep time, suggesting improvement in OSA following surgery.[58] Interestingly, this effect was diminished in subjects older than age seven, suggesting TA may have greater benefit in improving OSA symptoms for younger children. Sleep disordered breathing and OSA may worsen asthma control through by increasing systemic circulatory inflammation [46] increasing neurocirculatory bronchoconstriction, or worsening of GERD. [2] Therefore, if there is greater improvement OSA and SDB following TA in younger children, they may receive superior improvement in asthma control.

Beyond anatomical considerations, the tonsils and the adenoids are most immunologically active in younger children. Although the exact immune function of the tonsils remains to be elucidated, they are thought to play a significant role in antigen surveillance, immunoglobulin production, and the development of adaptive immune response. [49, 90] In children with OSA, there is increased proliferation of T-cells and expression of the pro-inflammatory cytokines TNF-alpha, IL-1 alpha, and IL-6 within tonsillar tissue, suggesting that adenotonsillar hypertrophy may contribute to increasing overall systemic inflammation. [90, 91] Especially as lymphocytes activated in the tonsillar tissue can selectively migrate to the lung [92], increased tonsillar inflammation may lead to lower airway inflammation in children with asthma. Therefore, in younger children who have more immunologically robust tonsillar tissue, there may be and increased role in adenotonsillar disease and contribution to lower airways inflammation.

One of the major challenges with assessing asthma in younger children is that clinician diagnosis of disease frequently occurs without objective pulmonary function tests. Considering that wheezing is common in early childhood, it is possible that we are

capturing improvement in children transient wheezing of childhood rather than with a true diagnosis of asthma. Nevertheless, the improvement in clinical outcomes suggests that TA may be particularly beneficial in a young pediatric cohort.

Asthma control is more likely to be improved in girls than boys following adenotonsillectomy

After TA we found that females were more likely to have a clinically significant improvement ACT or TRACK scores compared to male peers. There is well-described gender imbalance in pediatric asthma, where boys are twice as likely to wheeze under the age of ten compared to girls. [93, 94] This trend reverses during puberty with an increasing incidence in asthma in females during adolescence. It has been hypothesized that male children are at increased risk for asthma in early childhood because of smaller airway diameter [95] and increased allergen sensitization compared to female peers. Males have increased level of IgE compared to females. [96, 97] and are more likely to have positive allergy skin testing compared to female peers. [96, 98] This gender inequality also translates into clinical disease severity as younger boys were more likely to have increased length of hospitalization in childhood compared to female peers. [88] Therefore, if male children have underlying anatomic and inflammatory susceptibility that puts them at increased risk for worse control, they may not receive the same benefit from surgery. The fact that gender only significantly impacted the asthma control scores and not changes in urgent care visits, steroid courses, or composite improvement, it likely plays a lesser role in predicting improvement after TA.

Children with baseline sinusitis have decreased urgent care visits and steroid courses following TA

A history of baseline sinusitis correlated with improvement in urgent care visits and steroid courses following TA. Sinus disease has a higher prevalence in both children and adults with asthma than in the general population. [2, 99] Chronic rhinosinusitis may trigger asthmatic inflammation through post-nasal drainage of inflammatory mediators, shared mucosal inflammatory mediators between the nasal passages and the lower airways, and pharyngobronchial reflux. [65] Previously, it has been shown that both medical treatment (nasal steroids, antimicrobial agents, and oral decongestants)[100] and surgical management (functional endoscopic sinus surgery) [101, 102] decreased requirements for glucocorticoids, decreased asthma-related hospitalizations, missed school days, and improved lung function. [65]

The high of baseline history of sinus disease in our cohort suggests that sinusitis worsens baseline asthma control in the setting of adenotonsillar disease. Adenoid hypertrophy aggravates sinus disease by acting as a local reservoir for the local spread of bacterial flora to the sinuses and by mechanical outflow obstruction of nasal secretions. [103, 104] Adenoidectomy is an alternative treatment for refractory sinusitis in children. [105] Vandeburg *et al* found that 50-79% of children with rhinosinusitis refractory to medical management had improvement in symptoms following adenoidectomy.[106] Consistent with our findings, for asthmatic children with chronic rhinosinusitis TA may be particularly beneficial in improving asthma symptoms following surgery.

Children with Persistent Asthma have improvement in asthma control following TA

The impact of persistent asthma on lung function is profound. In the Tucson cohort children who have persistent wheezing by age six are more likely to have long-term decrease in lung function. [13] Persistent asthma is especially challenging to manage clinically in children. The mainstay of treatment for persistent asthma is inhaled corticosteroids, but in younger children adequate medication delivery through inhalers is less reliable and there are concerns for long-term metabolic consequences. Although there is recent evidence that oral LTRA may be an alternative therapy to improve asthma symptoms in young children [107], there is still a need for improvement in management of persistent disease.

The decrease in controller medication use following surgery suggests that TA may play a role in altering inflammatory pathways that lead to persistence of disease. Not only did controller use decrease as a result of TA, but also children on controller medications were also more likely to have improvement in asthma following surgery in all improvement variables. Additionally, children who had a one-year symptom free period were less likely to have improvement in asthma control and health care utilization for asthma. At baseline, children with persistent disease might have a higher likelihood to improve because they had worse baseline metrics (higher number of emergency room visits, lower baseline ACT/TRACK scores, and higher hospitalization rate) compared to children not on controllers. But beyond baseline metrics, children on controller medications were more likely to have chronic allergies and rhinosinusitis, suggesting a more atopic phenotype with higher baseline levels of IL-4 and IL-5. Therefore, a baseline inflammatory Th2 profile may contribute to why children improve following surgery.

Not only did persistence of asthma symptoms influence improvement, but severity of asthma impacted which children received benefit from surgery; moderate to severe asthmatics had a greater improvement in asthma control compared to mild asthmatics. This difference may be explained partially by inequality of baseline metrics, but also may reflect the relationship between upper airway obstruction and worsening asthma control in severe asthma. Severe and moderate asthmatics are more likely to have OSA compared to mild-asthmatics. [46] Therefore, in children—where adenotonsillar disease is the major contributor to OSA—removal of the tonsils and adenoids may have a more profound impact on children with moderate to severe disease rather than to children with milder disease.

Inflammatory asthma biomarkers are elevated in children's whose asthma improves following TA

Children whose asthma improves following TA have elevation of Th2 Cytokines prior to surgery

Serum levels of IL-4, IL-5, and IL-13 were elevated in children who had improvement in overall asthma control and in urgent care visits following TA. Although the specific actions of these cytokines in asthmatic inflammation have been discussed previously (See *Biomarkers of Asthmatic Inflammation Improve Following TA*), it is worth noting the importance of these markers in the classical Th2 inflammatory response associated with allergic asthma. Because an increase in Th2 immune response is thought to be a driving force for inflammatory infiltration and development of bronchial hyperreactivity in asthma in early-onset atopic asthma, elevation of these cytokines at baseline suggests that the individuals who receive the most benefit from surgery are children who have an

asthma phenotype associated with early-onset Th2 inflammation.[108] Additionally, expression of IL-5, and IL-13 also correspond with asthma severity and poorer control. [109, 110] As more severe asthmatics are more likely to have improvement following surgery, elevation of cytokine levels may reflect baseline severity in addition to atopic phenotype associated with improvement.

Non-Th2 inflammatory cytokines are elevated in asthmatics whose asthma improve after surgery

In our cohort we found that TNF- α, GM-CSF, IL-2, and IFN-gamma were elevated in children who had clinically significant improvement in ACT/TRACK scores after surgery. Tumor necrosis factor-α is an inflammatory cytokine that is essential for leukocyte recruitment, expression of vascular endothelial growth factors, and stimulation of fibroblasts and smooth muscles cells. Inhalation of exogenous TNF-α leads to airway hyper-responsiveness and increased sputum neutrophils counts, similar to asthmatic inflammation. [16] Tumor necrosis factor-α may be essential in that pathophysiology of acute exacerbations as acute exacerbations are related increased sputum neutrophilia. [111] The baseline elevation of TNF-α in our cohort may be related to poorer baseline control and recent asthma exacerbation as children with lower baseline scores (thus suggesting increased likelihood of recent exacerbation) as lower baseline scores correlate significantly with change in ACT/TRACK score after TA. As the ACT evaluates asthma control over relatively short time period of four weeks, if a child has had worsening asthma control and associated changes in circulating TNF-α during that period it would be captured in a lower baseline score.

Children whose ACT score reached MCID following adenotonsillectomy also were more likely to have increased baseline expression of GM-CSF compared to those who did not improve. Overexpression of GM-CSF in the lungs in animal shows severe lung eosinophilia, alveolar macrophage expansion and fibrosis consistent with asthmatic inflammation. [112] In humans, genetic polymorphisms of GM-CSF are related to asthma. [113] Although physiologic expression GM-CSF may play an essential role in priming Th1 immunity, with its overexpression there is an imbalance of cytokine and chemokine response as overexpression leads to transient increased expression of IL-4 and IL-5. [114] Therefore, in children with elevated GM-CSF at baseline it is possible that they have misregulation of Th2 cytokine expression. As improvement in asthma following surgery correlates with IL-5 regulation, misregulation of GM-CSF may contribute to a baseline inflammatory profile that identifies children whose asthma improves following surgery.

We found that baseline elevation of IL-2 and IFN-γ was associated with clinically significant improvement in ACT/TRACK scores following surgery. Interferon-γ and IL-2 are cytokines most frequently associated with Th1 response, a response that is generally suppressed in asthmatics. [16, 115] Interferon-γ specifically manipulates the balance of Th1 and Th2 inflammation. But studies looking at BALF of asthma found that in severe asthmatics there is elevation of IFN-γ and IL-2. [16, 116] Additionally, there may be overproduction of IFN-γ by CD8+ T-cells in asthmatic individuals, but levels are not significant enough to exceed the exaggerated Th2 response. [115] Therefore, the elevation of IFN-γ and IL-2 in children whose asthma improves following surgery suggests a phenotype associated with generalized elevation systemic inflammation. As

increased total systemic inflammation is associated with asthma persistence and severity, baseline elevation of IFN-γ and IL-2 may help identify persistent asthmatics who may improve post-operatively. (See *Children with Persistent Asthma have improvement in asthma control following TA).*

Study Strengths and Limitations

A major strength of this study is that we correlated both clinical and biological data to improvement in asthma after surgery. To our knowledge this is the largest cohort of pediatric asthma patients undergoing adenotonsillectomy and the only cohort that has both clinical and biological data.

Continued follow-up of this cohort would demonstrate if the effect of TA has a long-standing effect on the natural history asthma or a more transient effect in the relatively short follow-up time. Further comparison of the biological and clinical response to TA with asthmatic children undergoing surgery other than TA would aide in understanding the direct relationship of clinical and biologic response after TA. Comparison with an asthma surgical control group would also help to account for the placebo effect of surgery on improving asthma control.

Additionally, the majority of therapeutic research in the pediatric population occurs in school-aged children in order to minimize the risk of treatment in the very young child. Therefore the largest pediatric asthma cohorts have an average age of about nine [37], thereby making interpretation of clinical outcomes for our younger cohort compared to other interventions more difficult. Furthermore, characterization of OSA pre-operatively would also help characterize the degree of upper airway obstruction in children prior to surgery. Nocturnal polysomnography (PSG) is increasingly used in

pediatric population for the diagnosis and management of OSA. As children with recurrent tonsillitis and OSA can have tonsillar hypertrophy and SDB, further characterization with nocturnal PSG may aide in helping to determine the role of tonsillar hypertrophy and functional obstruction on asthma control.

CONCLUSIONS AND FUTURE DIRECTIONS

This is the first study that specifically connects specific patient characteristics—both clinical and biological—to improvement in asthma after TA. By characterizing a phenotype of child whose asthma responds to surgery, we eventually may be able to predict which children with asthma will have a response to TA. By predicting which children will have a good response to surgery, it is possible that asthmatic children without standard indications for TA could be identified that may receive benefit from TA selectively for asthma control. Additionally, to our knowledge, this is the first time anyone has demonstrated that TA modulates the allergic response found in asthmatic children and that modulation of this response corresponds to improvement in asthma. Further characterization of this allergic response is needed to better understand the complex pathways that drive the marked clinical improvement and to determine if this modulation has long-lasting impact on asthma control.

TABLES

Variable	Asthma n = 100	Controls n=54	*P*-value
Age Mean [Range]	6.5 [2-17]	5.7 [2-18]	0.09°
Male Gender (%)	63	50	0.13*
Race (%)			0.086*
White/Caucasian	77	72	
African American	17	26	
Asian	0	2	
Other	6	0	
Latino Ethnicity (%)	44	21	**0.02≠**
BMI Percentile Mean [Range]	65 [0-100]	68 [0.2-99]	0.92°
Gross Income (%)			0.09≠
< 19,999	26	19	
20,000-39,999	24	17	
40,000-59,999	14	7	
60,000-99,999	18	24	
>100,000	16	33	
Declined	2	0	
Maternal Education (%)			**0.009≠**
< 8th Grade	0	4	
Some HS	10	4	
HS Graduate	43	30	
Trade School	17	13	
College Graduate	23	24	
Graduate School	6	24	
Declined	1	1	
Location (%)			0.51≠
Rural	12	15	
Suburban	51	59	
Urban	36	26	
Declined	1	0	
Second Hand Smoke	31	29	0.84*
Indication TA			0.31≠
SDB/OSA	74	80	
RT	22	20	
Other	4	0	
Asthma Comorbidities (%)			
Sinusitis	39	20	**0.018***
Atopy	78	53	**0.003***
GERD	36	13	**0.004***

Table 1. Baseline Demographics of YCAAD Cohort. *P*-values represent comparisons between asthma and control populations. *Fisher's exact, ≠Pearson's χ^2, °Mann-Whitney U Test.

Biomarker	Asthma			Controls			Baseline
	Pre	Post	∞*P*-value	Pre	Post	∞*P*-value	°*P*-value
Chitinase Activity	3.2 [0.2-35]	3.6 [0.2-22]	0.63	3.8 [1.0-15]	4.0 [0-11.5]	0.26	**0.014**
YKL-40	32 [13-194]	34 [8-171]	0.58	32 [9-234]	31 [7-85]	084	0.51
IL-2	2.4 [0-1062]	3.3 [0-628]	0.23	2.5 [0-100]	0 [0-74.14]	0.39	0.39
IL-4	**0.5 [0-6.6]**	**0.3 [0-2.7]**	**0.022**	0.5 [0-13]	0.3 [0-8.15]	0.21	0.82
IL-5	**2.4 [0-31.6]**	**1.2 [0-7.2]**	**0.002**	1.0 [0-35]	0.9 [0-14]	0.15	**0.002**
IL-10	7.5 [0-1918]	6.7 [0-787]	0.66	9.6 [0-66]	5.1 [0-76]	0.41	0.80
IL-12	7.5 [0-6787]	2.6 [0-2729]	0.79	0 [0-368]	0 [0-340]	0.44	0.06
IL-13	1.7 [0-64]	0.9 [0-20]	0.088	**0.7 [0-37]**	**0.6 [0-6.1]**	**0.029**	**0.008**
IFN-γ	30 [0-1014]	24 [0-494]	0.062	41 [0-680]	29 [0-905]	0.329	0.57
TNF-α	6.9 [0-500]	5.1 [0-971]	0.95	4.0 [0-186]	2.5 [0-65]	0.40	0.064
GM-CSF	12 [0-1136]	11 [0-158]	0.45	19 [0-162]	9.9 [0-192]	0.11	0.67

Table 2. Asthma Biomarkers in response to TA. *P*-values represent ∞Wilcoxon Rank Sum and °Mann-Whitney U. Baseline represents comparison of biomarker data between children with asthma and controls on the day of surgery. Values represent median and range is demarcated by [ ]. Cytokine levels are in pg/mL, YKL-40 in ng/mL and Chitinase Activity in nMol/mL*hr.

	YCAAD (n=47)			CT Kids (n = 49)		
	LHW (n=29)	Overweight (n=18)	P-value	LHW (n=30)	Overweight (n= 19)	P-value
Age	5.8 [2-10]	8.7 [2-17]	**0.023***	6 [2-16]	7.5 [2-15]	0.24*
Gender			0.55§			0.77§
Male	18 (62)	9 (50)		19 (63)	11 (58)	
Female	11 (38)	9 (50)		11 (37)	8 (42)	
Race			0.06≠			0.93≠
White	26 (90)	12 (67)		27 (90)	12 (90)	
African-American	2 (7)	6 (33)		2 (7)	1 (5)	
Other	1 (3)	0		1 (3)	1 (5)	
Hispanic	12 (41)	11 (61)	0.24§	3 (10)	2 (11)	1.00§
Indication			**0.05≠**			0.11≠
OSA	16 (55)	16 (89)		21 (72)	10 (59)	
Tonsillitis	11 (38)	5 (11)		1 (3.4)	4 (23.5)	
Other	2 (7)	0		7(24)	3 (18)	
Co-morbidities						
Atopy	18 (64)	15 (88)	0.20§	23 (77)	12 (63)	0.35§
Sinusitis	9 (31)	8 (44)	0.37§	4 (13)	2 (10.5)	1.00§
GERD	14 (48)	4 (22)	0.12§	4 (13)	4 (21)	0.69§

Table 3A. Baseline demographics interim YCAAD Cohort and CT-Kids Cohort. Age expressed as mean, all other values represent n. () represent percentage, [ ] represent range, * Mann-Whitney, ≠Pearson's χ^2 test, and §Fisher's Exact Test.

	YCAAD (n=47)			CT Kids(n = 49)		
	LHW (n =29)	Overweight (n=18)	P-value	LHW (n=30)	Overweight (n=19)	P-value
Medication Use						
ICS	19(65)	10 (55)	0.55§	26 (87)	12 (63)	0.08§
LTRA	7 (24)	4 (22)	1.00§	16 (53)	8 (42)	0.56§
LABA	5 (17)	1 (6)	0.35§	2 (7)	1 (5.3)	1.00§
Albuterol Use			0.34≠	30 (100)	19 (100)	1.00§
≥3 times per day	4 (14)	1 (6)				
1-2 times per day	3 (11)	2 (12)				
2-3 times per week	3 (11)	1 (6)				
Once a week or less	5 (19)	8 (47)				
Never	12 (44)	5 (29)				
Oral Steroid Courses	1.45 [0-8]	0.33 [0-4]	**0.013***	0.97 [0-4]	0.95 [0-4]	0.57*
Urgent Care Visits	2.86 [0-50]	0.72 [0-6]	0.15*	0.67 [0-5]	0.53 [0-3]	0.43*
Pediatrician Visits	N/A	N/A		6.48 [0-30]	4.94 [0-17]	0.65*
Baseline ACT™	18 [7-27]	22 [17-27]	0.30*	N/A	N/A	N/A

Table 3B. Baseline Asthma Control and Healthcare Utilization of interim YCAAD Cohort and CT-Kids Cohort. Medication use and Albuterol expressed as n, all other values represent mean. () represents percentage, [ ] represent range, *Mann-Whitney, ≠Pearson's χ^2 test, and §Fisher's Exact Test.

	LHW			Overweight		
	Baseline	Follow-Up	P-value	Baseline	Follow-Up	P-value
Medication Use						
ICS	26 (86.7)	18 (60)	**0.008°**	12 (63.2)	9 (47.4)	0.38°
LTRA	16 (53.3)	9 (30)	**0.016°**	8 (42.1)	4 (21.1)	0.22°
LABA	2 (6.7)	3 (10)	1.00°	1 (5.3)	1 (5.3)	1.0°
Albuterol	30 (100)	27 (90)	0.25°	19 (100)	18 (95)	1.0°
Oral Steroid Courses	0.97 [0-4]	0.47 [0-3]	**0.048∞**	0.95 [0-4]	0.21 [0-2]	0.056∞
ER Visits	0.67 [0-5]	0.07 [0-5]	**0.003∞**	0.53 [0-3]	0	**0.04∞**
Pediatrician Visits	6.48 [0-30]	2.57 [0-15]	**0.001∞**	4.94 [0-17]	0.84 [0-8]	**<0.001∞**

Table 3C. Post-operative change medication usage and healthcare utilization in the CT Kids Cohort. Medication use and Albuterol expressed as n, all other values represent mean. () represents percentage, [ ] represent range. °Sign test, ∞Wilcoxon-Signed Rank Test.

	LHW			Overweight		
	Baseline	Follow-Up	P-value	Baseline	Follow-Up	P-value
Medication Use						
ICS	19(65)	12 (41)	**0.016°**	10 (55)	7 (39)	0.25°
LTRA	7 (24)	9 (31)	0.50°	4 (22)	3 (17)	1.00°
LABA	5 (17)	3(10)	0.50°	1 (6)	1 (6)	1.00°
Albuterol Use			**0.002°**			0.453°
≥3 times per day	4 (14)	2 (8)		1 (6)	0	
1-2 times per day	3 (11)	2 (8)		2 (12)	2 (11)	
2-3 times per week	3 (11)	1 (4)		1 (6)	2 (11)	
Once a week or less	5 (19)	3 (11)		8 (47)	5 (22)	
Never	12 (44)	18 (69)		5 (29)	10 (56)	
Oral Steroid Courses	1.45 [0-8]	0.41 [0-5]	**0.014∞**	0.33 [0-4]	0	0.18
Urgent Care Visits	2.86 [0-50]	1.45 [0-8]	0.12∞	0.72 [0-6]	0.0	0.18∞
ACT Score	18 [7-27]	23 [7-27]	**0.001∞**	22 [17-27]	23 [17-27]	0.48∞

Table 3D. Post-operative change medication usage and healthcare utilization in the YCAAD Cohort. Medication use and Albuterol expressed as n, all other values represent mean. () represents percentage, [ ] represent range. °Sign test, ∞Wilcoxon-Signed Rank Test.

Variable	+ MCID n = 34	- MCID n = 40	*P*-value
Age median [range]	5.5 [2-17]	7 [2-14]	**0.023**°
Male %(n)	47(16)	73(29)	**0.033***
Latino Ethnicity %(n)	44(15)	45(18)	0.1*
Race %(n)			0.073≠
Caucasian	77(26)	77(31)	
African American	14(5)	23 (9)	
Other	9(3)		
BMI Percentile median [range]	78 [2-100]	79 [0-100]	0.72°
Overweight (PAW) %(n)	44(15)	38(15)	0.64*
Inner City %(n)	33(11)	43(17)	0.47*
Second Hand Smoke %(n)	21(7)	40(15)	0.13*
Indication TA %(n)			0.77*
SDB/OSA	76(26)	78(31)	
RT	24(8)	17(7)	
Asthma Co-Morbidities %(n)			
Chronic Allergies	68(23)	68(26)	1.00*
Atopy	76(25)	82(31)	0.57*
Sinusitis	47(14)	33(13)	0.46*
GERD	33(11)	39(15)	0.81*
Age Asthma Diagnosis median [range]	2.0 [1-16]	2.0 [1-12]	0.77°
Age Lung Sx Onset median [range]	1.0 [1-14]	1.0 [1-8]	0.94*
Asthma Triggers %(n)			
Respiratory Infection	88(30)	85(33)	0.74*
Seasonal	62(21)	62(24)	1.00*
Environment	41(14)	36(14)	0.81*
Home	32(11)	18(7)	0.18*
Exercise	56(19)	59(23)	0.82*
Other	3(1)	10(4)	0.36*
Asthma Limits Activity %(n)	12(4)	13(13)	0.32*
One year symptom free %(n)	6(2)	23(9)	0.055*
Controller %(n)	68(23)	32(12)	**0.004***
Mild vs. Mod/Severe %(n)	35(12)	17.5(7)	0.11*
History ICU for asthma %(n)	3 (1)	5 (2)	1.00*
Lifetime Hospitalizations median [range]	0 [0-8]	0 [0-20]	0.46°
Hospitalized last year %(n)	6(2)	5(2)	1.00°
Chitinase Activity	3.2 [0.2-35]	3.3 [0.2-21]	0.70°
YKL-40	34 [13-172]	29 [13-194]	0.37°
IL-2	8.9 [0-1062]	1.6 [0-253]	**0.015**°
IL-4	0.6 [0-6.6]	0.4 [0-2.6]	0.064°
IL-5	2.4 [0.4-7.8]	1.8 [0-18]	0.24°
IL-10	12 [0-1918]	5.9 [0-113]	0.33°
IL-12	16 [0-6787]	7.3 [0-3221]	0.12°
IL-13	2.4 [0-13]	1.3 [0-23]	0.14°
IFN-γ	38 [3.3-1014]	25 [0-295]	**0.039**°
TNF-α	13 [0-310]	5.3 [0-500]	**0.04**°
GM-CSF	29 [0-279]	5.6 [0-91]	**0.02**°

Table 4. Baseline characteristics that influence if child has clinically significant improvement in ACT/TRACK score. All biological data is represented as median [range]. Values represent percentage (number). *Fisher's exact, ≠Pearson's χ^2, °Mann-Whitney U Test. Cytokine levels are in pg/mL, YKL-40 in ng/mL and Chitinase Activity in nMol/mL*hr.

Variable	Improved n = 41	Not Improved n = 59	*P*-value
Age median [range]	5 [2-17]	6 [2-14]	0.28°
Male %(n)	61(25)	64(38)	0.83*
Latino Ethnicity %(n)	44(19)	43(25)	0.42*
Race %(n)			0.26≠
Caucasian	83(34)	73(43)	
African American	10(4)	22(13)	
Other	7(3)	9 (3)	
BMI Percentile median [range]	73 [2-100]	82 [0-100]	0.91°
Overweight (PAW) %(n)	42(17)	41(24)	0.97*
Inner City %(n)	37(15)	26(15)	1.00*
Second Hand Smoke %(n)	39(15)	26(15)	0.26*
Indication TA %(n)			0.15*
SDB/OSA	81(33)	70(41)	
RT	65(6)	27(16)	
Other	8(2)	3(2)	
Asthma Co-Morbidities %(n)			
Chronic Allergies	77(30)	60(35)	0.12*
Atopy	82(31)	75(43)	0.62*
Sinusitis	54(22)	29(17)	**0.02***
GERD	46(18)	31(18)	0.14*
Age Asthma Diagnosis median [range]	2.0 [1-16]	2.0 [1-9]	0.08°
Age Lung Sx Onset° median [range]	1.0 [1-14]	1.0 [1-8]	0.70°
Asthma Triggers %(n)			
Respiratory Infection	83(34)	86(50)	0.78*
Seasonal	59(24)	55(32)	0.84*
Environment	39(16)	41(24)	0.84*
Home	15(6)	28(16)	0.15*
Exercise	71(29)	47(16)	**0.023***
Other	20(8)	2(1)	**0.003***
Asthma Limits Activity %(n)	24(10)	17(10)	0.40*
One year symptom free %(n)	3(1)	23(13)	**0.007***
Controller %(n)	82(32)	34(20)	**< 0.001***
Mild vs. Mod/Severe %(n)	46(19)	17(10)	**0.002***
History ICU for asthma %(n)	7.3(3)	3.4(2)	0.40*
Lifetime Hospitalizations median [range]	0 [0-35]	0 [0-10]	**0.04°**
Hospitalized last year %(n)	20(8)	2(1)	**0.003***
Chitinase Activity	2.6 [0.2-35]	3.4 [0.2-21]	0.09°
YKL-40	35 [13-172]	30 [13-194]	0.61°
IL-2	1.2 [0-1062]	2.6 [0-253]	0.73°
IL-4	0.8 [0-6.6]	0.4 [0-2.7]	**0.014°**
IL-5	3.6 [0.4-18]	1.8 [0-32]	**0.023°**
IL-10	7.4 [0-1918]	7.7 [0-187]	0.85°
IL-12	7.3 [0-6787]	9.8 [0-495]	0.80°
IL-13	3.2 [0-23]	1.4 [0-64]	**0.021°**
IFN-γ	36 [0-1014]	28 [2.7-503]	0.43°
TNF-α	11 [0-310]	6.3 [0-500]	0.33°
GM-CSF	6 [0-279]	18 [0-1136]	0.17°

Table 5. Baseline characteristics that influence if child has improvement in Urgent Care Visits following surgery. All biological data is represented as median [range]. Values represent percentage (number). *Fisher's exact, ≠Pearson's χ^2, °Mann-Whitney U Test. Cytokine levels are in pg/mL, YKL-40 in ng/mL and Chitinase Activity in nMol/mL*hr.

Variable	Improved n = 39	Not Improved n = 60	*P* - value
Age median [range]	5 [2-17]	6 [2-17]	0.16°
Male %(n)	64 (25)	62 (37)	0.84*
Latino Ethnicity %(n)	47(17)	45(27)	0.84*
Race %(n)			0.07≠
Caucasian	82(32)	73(44)	
African American	8(3)	23(14)	
Other	10(4)	4(2)	
BMI Percentile median [range]	71 [2-100]	86 [0-100]	0.40°
Overweight (PAW) %(n)	39(15)	43(26)	0.68*
Inner City %(n)	28(11)	42 (25)	0.20*
Second Hand Smoke %(n)	37(13)	28(17)	0.49*
Indication TA %(n)			0.47*
SDB/OSA	77(30)	72(43)	
RT	18(7)	25(15)	
Other	5(2)	3(2)	
Asthma Co-Morbidities %(n)			
Chronic Allergies	77(28)	63(37)	0.26*
Atopy	81(29)	76(44)	0.80*
Sinusitis	56(22)	28(17)	**0.007***
GERD	42(16)	32(19)	0.39*
Age Asthma Diagnosis median [range]	2 [1-12]	2 [1-12]	0.84°
Age Lung Sx Onset° median [range]	1 [1-5]	1[1-5]	0.60°
Asthma Triggers %(n)			
Respiratory Infection	85(33)	85(51)	1.00*
Seasonal	51(20)	60(36)	0.41*
Environment	41(16)	40(24)	1.00*
Home	23(9)	22(13)	1.00*
Exercise	64(25)	52(42)	0.07*
Other	21(8)	2(1)	**0.002***
Asthma Limits Activity %(n)	31(12)	13(8)	**0.043***
One year symptom free %(n)	8(3)	19(11)	0.24*
Controller %(n)	84(32)	34(20)	**< 0.001***
Mild vs. Mod/Severe %(n)	62(24)	8(5)	**<0.001***
History ICU for asthma %(n)	10.3(4)	2(1)	0.07*
Lifetime Hospitalizations median [range]	0 [0-35]	0 [0-20]	**0.018°**
Hospitalized last year %(n)	21(8)	2(1)	**0.002***
Chitinase Activity	2.9 [0.2-35]	3.3 [0.2-21]	0.58°
YKL-40	36 [13-172]	31 [13-194]	0.30°
IL-2	2.0 [0-171]	2.6 [0-1062]	0.97°
IL-4	0.6 [0-6.6]	0.4 [0-6.6]	0.36°
IL-5	3.0 [0.4-9]	2.2 [0-32]	0.17°
IL-10	7.6 [0-108]	7.6 [0-1918]	0.54°
IL-12	7.0 [0-267]	10 [0-6787]	0.51°
IL-13	2.3 [0-23]	1.4 [0-64]	0.37°
IFN-γ	34 [0-704]	30 [0-1014]	0.68°
TNF-α	11 [0-310]	5.8 [0-500]	0.20°
GM-CSF	9.3 [0-193]	18 [0-1136]	0.57°

Table 6. Baseline characteristics that influence if child has clinically significant improvement in steroid courses. All biological data is represented as median [range]. Values represent percentage (number). *Fisher's exact, ≠Pearson's χ^2, °Mann-Whitney U Test. Cytokine levels are in pg/mL, YKL-40 in ng/mL and Chitinase Activity in nMol/mL*hr.

Variable	Improved n = 70	Not Improved n = 30	*P* - value
Age median [range]	5 [2-17]	8 [2-14]	**0.012**°
Male %(n)	59(41)	73(22)	0.18*
Latino Ethnicity %(n)	45(30)	47(14)	1.00*
Race %(n)			0.082≠
Caucasian	79(55)	73 (22)	
African American	13 (9)	27 (8)	
Other	9 (6)		
BMI Percentile median [range]	78.9 [2-100]	63 [0-100]	0.25*
Overweight (PAW) %(n)	43(30)	37(11)	0.66*
Inner City %(n)	36(25)	37(11)	1.00*
Second Hand Smoke %(n)	33(22)	27(8)	0.64*
Indication TA %(n)			0.43*
SDB/OSA	77(54)	67(28)	
RT	20(14)	27(8)	
Other	3(2)	7(2)	
Asthma Co-Morbidities %(n)			
Chronic Allergies	74(52)	55(16)	0.16*
Atopy	79(52)	76(22)	0.79*
Sinusitis	44(31)	27(8)	0.12*
GERD	40(27)	30(9)	0.50*
Age Asthma Diagnosis median [range]	2 [1-12]	2 [1-16]	0.69°
Age Lung Sx Onset° median [range]	1 [1-5]	1 [1-14]	0.98°
Asthma Triggers %(n)			
Respiratory Infection	86(60)	83(24)	0.76*
Seasonal	57(40)	55(16)	1.00*
Environment	40(28)	41(12)	1.00*
Home	21(15)	24(7)	0.79*
Exercise	60(42)	48(14)	0.37*
Other	13(16)	0(0)	**0.043***
Asthma Limits Activity %(n)	23(16)	14(4)	0.18*
One year symptom free %(n)	6(4)	35(10)	**0.001***
Controller %(n)	68(46)	20(6)	**< 0.001***
Mild vs. Mod/Severe %(n)	39(27)	7(2)	**0.001***
History ICU for asthma %(n)	7(5)	0(0)	0.32*
Lifetime Hospitalizations median [range]	0 [0-35]	0 [0-10]	0.36°
Hospitalized last year %(n)	13(9)	0(0)	0.055*
Chitinase Activity	3.2 [0.2-35]	3.1 [0.2-7.9]	0.25°
YKL-40	34 [13-172]	30 [13-194]	0.70°
IL-2	4.1 [0-1062]	1.8 [0-253]	0.17°
IL-4	0.6 [0-6.6]	0.4 [0-2.6]	**0.042**°
IL-5	3.0 [0.4-18]	1.5 [0-32]	**0.025**°
IL-10	8.3 [0-1918]	4.5 [0-187]	0.17°
IL-12	7.4 [0-6787]	8.5 [0-495]	0.50°
IL-13	2.4 [0-23]	1.0 [0-64]	**0.029**°
IFN-γ	36 [0-1014]	25 [2.7-503]	0.16°
TNF-α	11 [0-310]	4.9 [0-500]	**0.041**°
GM-CSF	12 [0-279]	12 [0-1136]	0.88°

Table 7. Baseline characteristics that influence if child has clinically significant improvement in Composite Improvement. All biological data is represented as median [range]. Values represent percentage (number). *Fisher's exact, ≠Pearson's χ^2, °Mann-Whitney U Test. Cytokine levels are in pg/mL, YKL-40 in ng/mL and Chitinase Activity in nMol/mL*hr.

FIGURES

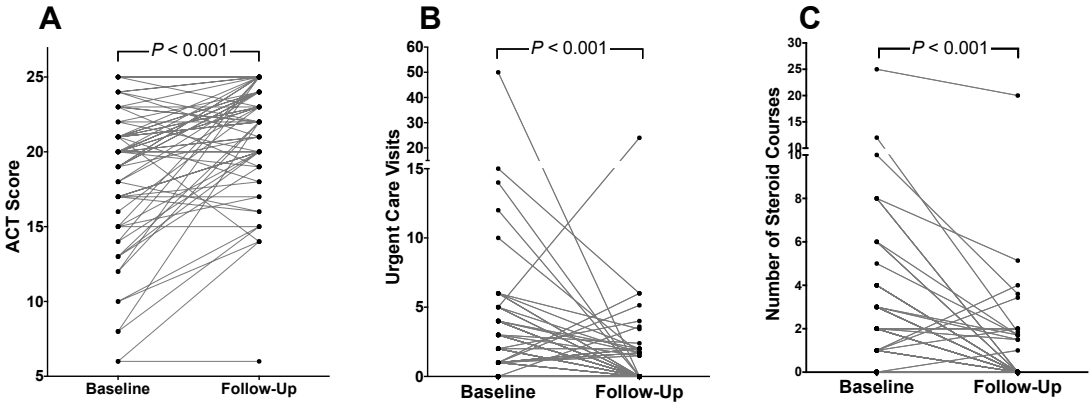

Figure 1. Children's asthma improves following TA. *P*-values represent Wilcoxon Signed-Rank test A) ACT and TRACK scores rescaled to 25-point scale. B) Number of urgent care visits (per year). C) Number of steroid courses (per year).

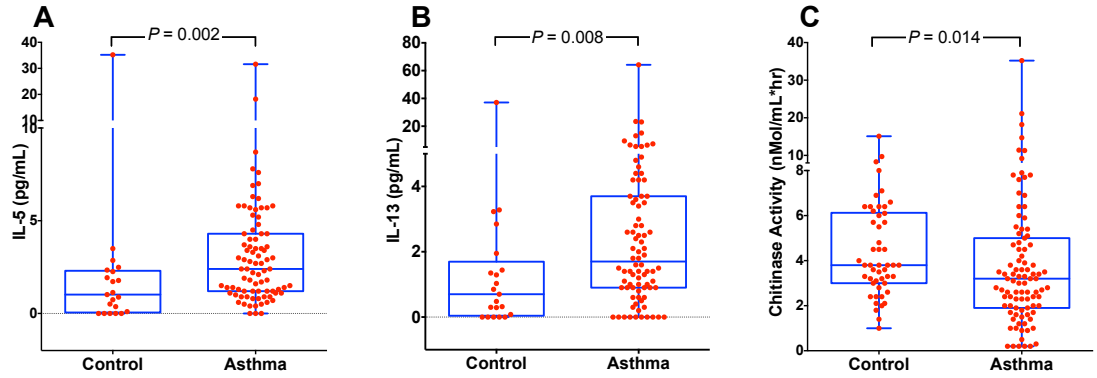

Figure 2. Serum biomarker levels differ in children with and without asthma prior to TA. *P*-values represent Mann-Whitney-U Test. Median bar is displayed with interquartile ranges. Error bars represent minimum and maximum values. A) IL-5. B) IL-13. C) Chitinase Activity.

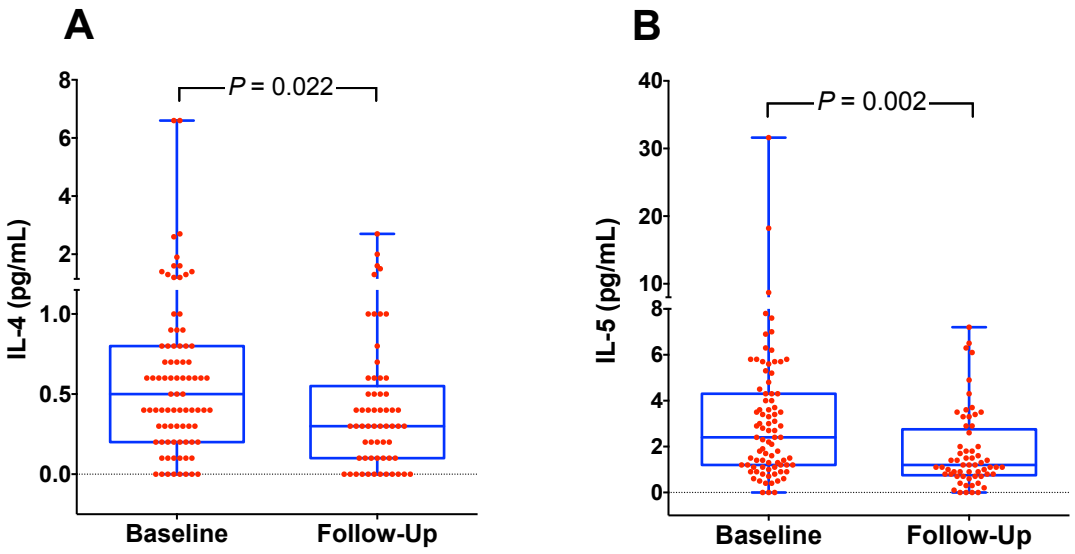

Figure 3. Th2 cytokines decrease in children with asthma following TA. *P*-values represent Wilcoxon Signed-Rank Test. Median bar is displayed with interquartile ranges. Error bars represent minimum and maximum values. A) IL-4. B) IL-5.

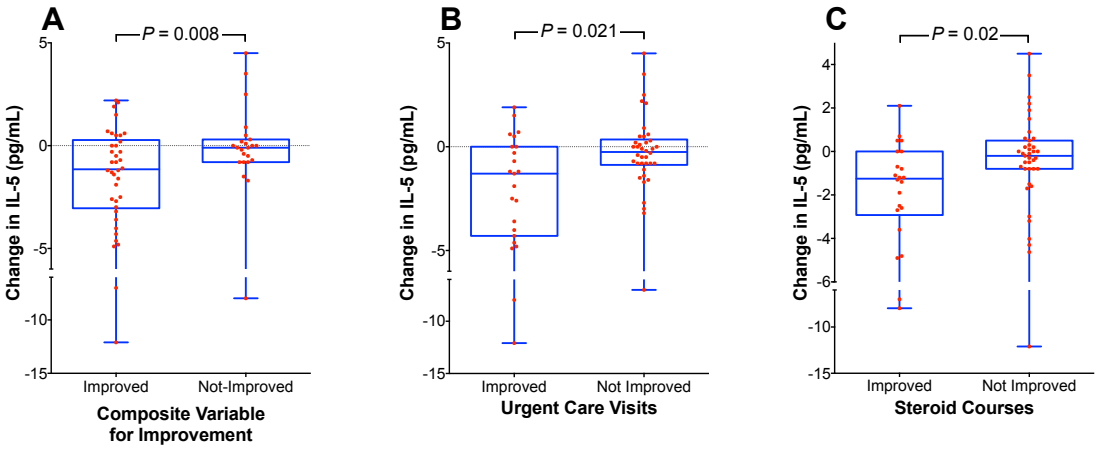

Figure 4. Improvement in Asthma Correlates with Decreases in IL-5. Asthma improves measured using the composite variable for improvement. *P*-value represents Mann-Whitney-U.

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