Relationships among aeroallergen sensitization, peripheral blood eosinophils, and periostin in pediatric asthma development

Halie M. Anderson, MD,[a] Robert F. Lemanske, Jr, MD,[a] Joseph R. Arron, MD, PhD,[b] Cecile T. J. Holweg, PhD,[b] Victoria Rajamanickam, MS,[c] Ronald E. Gangnon, PhD,[c] James E. Gern, MD,[a] and Daniel J. Jackson, MD[a] *Madison, Wis, and South San Francisco, Calif*

Background: Biomarkers, preferably noninvasive, that predict asthma inception in children are lacking.
Objective: Little is known about biomarkers of type 2 inflammation in early life in relation to asthma inception. We evaluated aeroallergen sensitization, peripheral blood eosinophils, and serum periostin as potential biomarkers of asthma in children.
Methods: Children enrolled in the Childhood Origins of ASThma study were followed prospectively from birth. Blood samples were collected at ages 2, 4, 6, and 11 years, and serum-specific IgE levels, blood eosinophil counts, and periostin levels were measured in 244 children. Relationships among these biomarkers, age, and asthma were assessed.
Results: Serum periostin levels were approximately 2- to 3-fold higher in children than previously observed adult levels. Levels were highest at 2 years (145 ng/mL), and did not change significantly between 4 and 11 years (128 and 130 ng/mL). Age 2 year periostin level of 150 ng/mL or more predicted asthma at age 6 years (odds ratio [OR], 2.3; 95% CI, 1.3-4.4). Eosinophil count of 300 cells/μL or more and aeroallergen sensitization at age 2 years were each associated with increased risk of asthma at age 6 years (OR, 3.1; 95% CI, 1.7-6.0 and OR, 3.3; 95% CI, 1.7-6.3). Children with any 2 of the biomarkers had a significantly increased risk of developing asthma by school age (≥2 biomarkers vs none: OR, 6.6; 95% CI, 2.7-16.0).
Conclusions: Serum periostin levels are significantly higher in children than in adults, likely due to bone turnover, which impairs clinical utility in children. Early life aeroallergen sensitization and elevated blood eosinophils are robust predictors of asthma development. Children with evidence of activation of multiple pathways of type 2 inflammation in early life are at greatest risk for asthma development. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Biomarkers, children, asthma development, periostin, aeroallergen sensitization, peripheral blood eosinophils*

Asthma is one of the most common chronic diseases in children. Recurrent wheezing during the preschool years is frequently the presenting sign of asthma; however, many children who wheeze during early life do not go on to develop childhood asthma.[1] Predicting which children will develop asthma remains challenging. Furthermore, once asthma is established, there is significant heterogeneity in response to therapy. The emergence of biomarkers in asthma and allergic airway disease is being used for the paradigm of "personalized" medicine and is an area of active research, although this has been primarily in adult patient populations to date. Biomarkers that predict asthma inception and/or response to therapy in children are currently lacking.

Type 2 inflammation is characterized by the production of a unique profile of cytokines by a host of cells including epithelial cells, mast cells, innate lymphoid cells, and T_H2 cells. These proinflammatory cytokines include, but are not limited to, IL-4, IL-5, IL-13, IL-25, IL-33, and thymic stromal lymphopoetin, and are thought to play a central role in the pathophysiology of allergic asthma.[2] A small number of biomarkers of type 2 inflammation have emerged as potentially useful and relevant for asthma.

Personal atopic history in early life is one of the most important factors determining an individual's risk of persistent asthma.[3] In fact, a National Institutes of Health expert panel on biomarkers in asthma concluded that of all the biomarkers, only multiallergen screening to define atopy is recommended as a core asthma outcome for asthma clinical trials.[4] Many studies have demonstrated a significant relationship between inhalant aeroallergen

From the [a]Division of Allergy, Immunology and Rheumatology, Department of Pediatrics, University of Wisconsin School of Medicine & Public Health, Madison; [b]Genentech, Inc, South San Francisco; and [c]the Department of Biostatistics and Medical Informatics, University of Wisconsin School of Medicine & Public Health, Madison.

Disclosure of potential conflict of interest: H. M. Anderson receives research support from the National Heart, Lung, and Blood Institute (NHLBI) and travel support from the AAAAI FIT Travel Scholarship. R. F. Lemanske, Jr is an employee of the University of Wisconsin, receives research support from NHLBI and Pharmaxis; serves as a consultant for Merck, Sepracor, SA Boney and Associates, GlaxoSmithKline, American Institute of Research, Genetech, Double Helix Development, Health Star Communications, and Boerhinger Ingelheim; receives speaker fees from Michigan Public Health, Allegheny General Hospital, AAP, West Allegheny Health, California Chapter 4, Colorado Allergy Society, Pennsylvania Allergy Society, Howard Pilgrim Health, California Society of Allergy, NYC Allergy Society, World Allergy Organization, APAPARI, Western Society of Allergy, Asthma and Immunology, Kuwait Allergy Society, Lurie Childrens Hospital, Boston Children's Hospital, LA Children's Hospital, Northwestern University, Asthma and Allergy Foundation of America, Alaska Chapter, and Egyptian Allergy Society; payment for manuscript preparation from the AAAAI; and Royalties from Elsevier and UpToDate. J. R. Arron is an empoyee of Genentech, Inc; has a patent with Genentech, Inc; and holds stock with the Roche Group. C. T. J. Holweg is an employee of Genetech Inc. V. Rajamanickam receives research funding from the University of Wisconsin, Madison. R. E. Gangnon receives research support from the NHLBI. J. E. Gern receives research support from the National Institutes of Health and GlaxoSmithKline; serves as a consultant for Genetech, Amgen, Novartis, PREP Biopharm, Inc, Janssen, Regeneron, and GlaxoSmithKline; receives payment for the development of educational presentations from Boehringer Ingelheim; and receives travel support from Boehringer Ingelheim. D. J. Jackson receives grant funding from the National Institutes of Health and serves as a consultant for Vectura.

Received for publication February 25, 2016; revised April 30, 2016; accepted for publication May 16, 2016.

Corresponding author: Halie M. Anderson, MD, 600 Highland Ave, CSC K4/936, Madison, WI 53792. E-mail: Handerson@pediatrics.wisc.edu.

0091-6749/$36.00

© 2016 American Academy of Allergy, Asthma & Immunology

http://dx.doi.org/10.1016/j.jaci.2016.05.033

Abbreviations used
COAST: Childhood Origins of ASThma
GM: Geometric mean
OR: Odds ratio

sensitization and asthma development.[5-9] Specific patterns of aeroallergen sensitization have identified differential impact on asthma risk, and sensitization to multiple allergens in early life has been strongly associated with an increased asthma risk.[10]

Peripheral blood eosinophils have been identified as a surrogate marker of type 2 inflammation, and eosinophils are considered a principle effector cell in the pathophysiology of asthma.[11] High levels of peripheral blood eosinophils are recognized as an important biomarker for the eosinophilic asthma phenotype and has been identified as a readily available biomarker that correlates with disease severity and may predict response to asthma therapy, specifically as it relates to immunologic-based interventions.[12,13] However, the role of peripheral blood eosinophilia and risk of asthma development is less clear. The pediatric asthma predictive index[14,15] identifies peripheral blood eosinophil percentage (≥4% of total white blood cells) as a minor risk factor; however, existing predictive models for asthma development in children have inadequate accuracy and are, generally, better at excluding asthma than at predicting it.[16] Small prospective cohorts of infants hospitalized with wheezing illness have demonstrated that elevated eosinophils at convalescence predicted increased asthma risk later in life.[17,18] Notably, there is a clear association between peripheral blood eosinophilia and atopy in children, which potentially complicates the utility of peripheral blood eosinophils as an isolated biomarker in children.[11,19]

Periostin is a matricellular protein whose expression can be induced by type 2 inflammatory cytokines IL-4 and IL-13, as well as by other stimuli such as TGF-β. Specifically, IL-13 induces the secretion of periostin from bronchial epithelial cells.[20] Periostin is secreted basally from airway epithelial cells where it has pleotropic effects on epithelial cell function and on the development of airway fibroblasts, which is thought to promote airway remodeling in patients with asthma, even from a young age.[21] Airway epithelial cells from children with allergic asthma produce greater amounts of periostin than do airway epithelial cells from healthy children.[21] In addition to bronchial epithelial cells, there are other tissue sources of periostin including skin, tendon, and bone.[22] Periostin levels in peripheral blood have been identified as an easily obtained systemic biomarker of type 2 airway inflammation in adults and may also predict responsiveness to therapy.[20,23] Although the literature is quickly evolving, there is a paucity of published studies of periostin in childhood, with conflicting results. One study suggests that elevated serum periostin level correlates with airway hyperresponsiveness, whereas another found that high periostin level was not a predictor of asthma morbidity.[24,25] There are no published studies of early life serum periostin levels in children, specifically as it relates to asthma inception.

Biomarkers, preferably noninvasive, that predict asthma inception and/or response to therapy in children are desirable, but currently lacking. In this study, we used samples from the Childhood Origins of ASThma (COAST) study to assess these biomarkers and their relationship to asthma inception.

METHODS

Study population

Two hundred eighty-nine newborns at high risk for the development of asthma and allergic disease were recruited from November 1998 through May 2000 into the COAST study. Details of the study population and design have been described previously.[26] Briefly, to qualify for the study, at least 1 parent was required to have *respiratory allergies* (defined as ≥1 positive aeroallergen skin test results) and/or a history of physician-diagnosed asthma. Of these children, 244 had serum samples available from age 2 years and were included in this study. Informed consent was obtained from the parents, and the Human Subjects Committee at the University of Wisconsin-Madison approved the study (institutional review board approval no. H-2007-0044).[10]

Measurements

Peripheral blood samples were obtained at annual visits, at ages 2, 4, 6, and 11 years. Serum was stored at −80°C before measurement of periostin level. The serum samples were analyzed using the clinical trial version of the Elecsys Periostin assay (Roche Diagnostics, Penzberg, Germany) intended for use on the Cobas e601. The Elecsys Periostin assay is an automated electrochemiluminescence immunoassay, based on the sandwich principal.[27]

Peripheral blood eosinophil numbers were measured by standard methods. Total and allergen-specific IgE levels were measured by fluoroenzyme immunoassay (UniCAP 100, Pharmacia Diagnostics AB, Uppsala, Sweden) as previously described.[10]

Serum concentrations of specific IgE antibodies for 5 common inhalant allergens (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat, dog, and *Alternaria alternata*) were determined at ages 2, 6, and 11 years. In addition, allergen-specific IgE levels to ragweed, silver birch, timothy grass, and cockroach were measured at ages 6 and 11 years. The detection limit of the assay with regard to specific IgE level was 0.35 kU_A/L, and *aeroallergen sensitization* was defined as at least 1 value of 0.35 kU_A/L or more.

Clinical definitions

Asthma was diagnosed clinically at ages 6 and 11 years as previously described.[28] Briefly, current asthma was defined on the basis of the documented presence of 1 or more of the following during the previous year: (1) physician diagnosis of asthma, (2) use of albuterol for coughing or wheezing episodes (prescribed by physician), (3) use of a daily controller medication, (4) use of a step-up plan including the use of albuterol or short-term use of inhaled corticosteroids during illness, and (5) use of systemic corticosteroids for asthma exacerbation.

Statistical analysis

Periostin measurements over the years were assessed using a longitudinal mixed effect model with fixed effect for sex and years. Periostin levels (ages 2, 4, 6, and 11 years) and eosinophil counts (ages 2, 6, and 11 years) were compared with asthma diagnosis at age 6 and 11 years using longitudinal mixed effect models with fixed effects for years, sex, and asthma status and a random effect for the subject to account for repeated measure. Both outcomes were log-transformed to attain normality. Least square means on the log scale were back-transformed to geometric means (GMs) and associated 95% CIs.

The dichotomous outcome of aeroallergen sensitization at years 2, 6, and 11 years was compared with asthma status at ages 6 and 11 years using generalized linear mixed models adjusted for sex. These relationships were summarized using odds ratios (ORs) with 95% CI.

For the purpose of this article, we determined cutoff points to identify a positive biomarker: peripheral blood eosinophil count, 300 or more (literature-based)[12,13]; any aeroallergen sensitization (at least 1 value ≥0.35 kU_A/L), and serum periostin level of 150 ng/mL or more (approximate median of periostin levels at age 2 years in our population). Logistic regressions were used to predict asthma diagnosis at age 6 and 11 years with both individual and combinations of these 3 markers. A 2-sided P value of less than .05 was

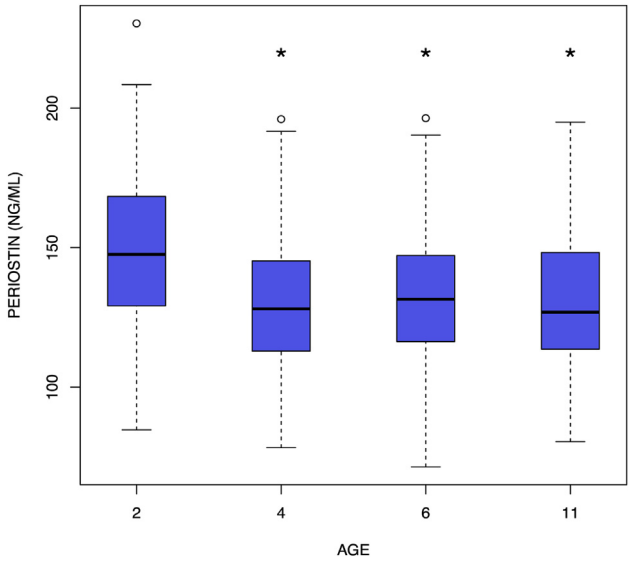


FIG 1. Serum periostin level decreases with age. The *shaded boxes* represent the interquartile range; the *black horizontal line* represents the median. The *whiskers* represent the minimum and maximum of all data, with the exception of outliers, which are denoted by a *small circle*. The * above age 4, 6, and 11 years denotes a statistically significant difference compared with age 2 years.

regarded as statistically significant. Analyses were performed in SAS statistical software, version 9.4 (SAS Institute, Inc, Cary, NC).

RESULTS

Demographic characteristics

Data were analyzed from 244 children at age 2 years with periostin, peripheral blood eosinophils, and/or aeroallergen sensitization assessed. There was no significant difference in the characteristics of the children who had the markers measured at all years compared with those who had missing data. Briefly, 43% of the children were female and 57% male, 29% had asthma at age 6 years and 31% had asthma at age 11 years, and 36% had asthma, either at age 6 years or at age 11 years. Of those who had asthma at age 6 years, 47% had mild intermittent asthma, 35% had mild persistent asthma, and 18% had moderate-severe persistent asthma. Allergic sensitization to aeroallergens for the entire cohort of patients occurred early (1-2 years) in 23% and later (3-11 years) in 33%, and 44% never developed allergic sensitization.

Developmental levels of periostin with age and sex

We first assessed the effects of age on serum periostin levels. Periostin was highest at age 2 years (GM, 145 ng/mL, $P < .0001$ for age 2 years vs ages 4, 6, and 11 years). Periostin levels did not change from age 4 to 11 years (GMs, 128, 130, and 130 ng/mL at ages 4, 6, and 11 years, respectively) (Fig 1).

We next examined the relationships of periostin and age stratified by sex. There were no significant differences in periostin levels for males versus females at ages 2, 4, and 6 years (Fig 2). However, levels of serum periostin were significantly higher in girls at age 11 years (GM, females 136 vs males 125 ng/mL; $P = .01$).

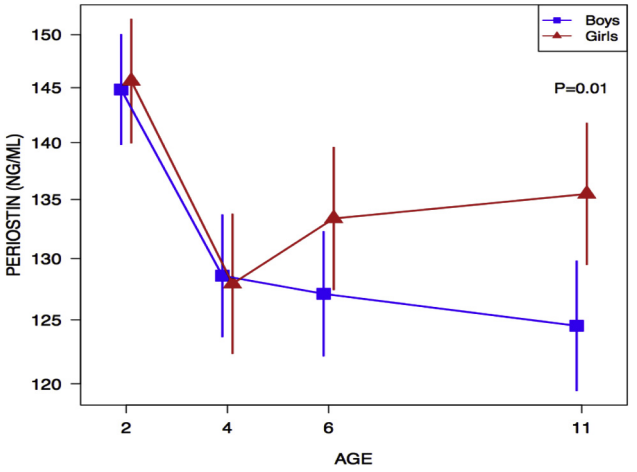


FIG 2. Serum periostin level is significantly higher in girls at age 11 years. GM with 95% CI. The *vertical lines* represent the 95% CI.

Developmental levels of eosinophils and aeroallergen sensitization with age and sex

We next assessed the developmental levels of peripheral blood eosinophils at ages 2 (GM, 167 cells/μL; 95% CI, 149-187), 6 (GM, 148; 95% CI, 131-167), and 11 (GM, 156; 95% CI, 137-179) years. Eosinophils levels did not change significantly between age 2 and 11 years (age 2 years vs 6 years, $P = .10$; age 2 years vs 11 years; $P = .42$; age 6 years vs 11 years; $P = .48$). Peripheral blood eosinophils did not differ between males and females at age 2 ($P = .30$), 6 ($P = .42$), or 11 ($P = .96$) years.

As anticipated, aeroallergen sensitization increased with age from age 2 years to age 11 years in our cohort (data not shown). There was no significant difference in rates of aeroallergen sensitization between males versus females at age 2 ($P = .24$), 6 ($P = .49$), or 11 ($P = .76$) years.

Cross-sectional evaluation of serum periostin and peripheral blood eosinophils and relationship to asthma development

Periostin and blood eosinophils were measured and evaluated cross-sectionally in relation to asthma at age 6 and 11 years to assess whether children with asthma at ages 6 and 11 years had higher levels of serum periostin and/or eosinophils compared with age-matched individuals without asthma (Table I). Children with asthma had higher periostin levels compared with children without asthma at age 6 years, although the difference was not statistically significant (GM, asthma 135 vs nonasthma 128 ng/mL; $P = .12$), and 11 years (GM, asthma 136 vs nonasthma 127 ng/mL; $P = .04$).

Children with asthma had significantly higher peripheral blood eosinophil counts than did age-matched children without asthma at both ages 6 years (GM, asthma 190 vs nonasthma 132 cells/μL; $P = .006$) and 11 years (GM, asthma 223 vs nonasthma 134 cells/mL; $P = .006$).

Predictive value of early life serum periostin and peripheral blood eosinophils and relationship to asthma development

We next assessed whether elevated early life periostin and peripheral blood eosinophil levels predict the development of asthma at age 6 and 11 years (Table I). Children who developed

TABLE I. Early life periostin level and peripheral blood eosinophils predict the development of asthma and cross-sectional assessment reveals higher periostin levels and peripheral blood eosinophils in children with asthma than in age-matched children without asthma

Periostin level at age 6 y					
	No asthma		Asthma		
Age (y)	N	GM (95% CI)	N	GM (95% CI)	*P* value
2	141	143 (139-148)	59	154 (146-161)	.02
4	100	125 (121-129)	45	139 (131-146)	.002
6	100	128 (124-133)	45	135 (128-143)	.12
Periostin level at age 11 y					
	No asthma		Asthma		
Age (y)	N	GM (95% CI)	N	GM (95% CI)	*P* value
2	119	143 (138-148)	54	151 (144-160)	.07
4	90	126 (122-131)	44	132 (125-139)	.23
6	90	127 (123-132)	44	134 (127-141)	.15
11	91	127 (122-132)	42	136 (129-144)	.04
Eosinophil count at age 6 y					
	No asthma		Asthma		
Age (y)	N	GM (95% CI)	N	GM (95% CI)	*P* value
2	161	148 (130-168)	69	226 (185-276)	.001
6	149	132 (115-151)	56	190 (152-238)	.006
Eosinophil count at age 11 y					
	No asthma		Asthma		
Age (y)	N	GM (95% CI)	N	GM (95% CI)	*P* value
2	138	142 (123-164)	63	239 (193-295)	<.0001
6	124	128 (110-149)	60	188 (151-234)	.005
11	111	134 (115-158)	51	223 (176-282)	.001

Early life periostin level is significantly higher at age 2 and 4 years in individuals who develop asthma by age 6 years. Similarly, periostin level is higher at ages 2, 4, and 6 years in individuals who develop asthma by age 11 years, although not statistically significant. Peripheral blood eosinophil count of ≥300 is significantly higher at age 2 years in children who develop asthma by age 6 y and is also significantly higher at ages 2 and 6 years in children who develop asthma by age 11 years. Cross-sectional assessment of serum periostin level and peripheral blood eosinophils demonstrates that children with asthma at ages 6 and 11 years have higher levels of periostin and eosinophils when compared with aged-matched individuals. Least square means (Lsmean) are summarized by GMs and its 95% CI. The data were adjusted for sex. Statistical significance is a *P* value of <.05.

asthma by age 6 years had increased serum periostin at age 2 years, when compared with children who did not develop asthma by age 6 years (GM, asthma 154 vs nonasthma 143 ng/mL; $P = .02$). Similarly, children who developed asthma by age 6 and 11 years had higher eosinophil counts at 2 years, when compared with children who did not develop asthma by age 6 or 11 years (GM age 6 years, asthma 226 vs nonasthma 148 cells/μL; $P = .001$; GM age 11 years, asthma 239 vs nonasthma 142 cells/μL; $P < .001$).

Aeroallergen sensitization and asthma development

The relationship of aeroallergen sensitization to asthma development was assessed (Table II). If a child was sensitized to inhalant aeroallergen at age 2 years, the risk of asthma at age 6 years was 51% (OR, 3.3; 95% CI, 1.7-6.3; $P = .0005$) and at age 11 years was 53% (OR, 3.2; 95% CI, 1.6-6.6; $P = .001$). If a child was not sensitized to aeroallergen at age 2 years, the risk of asthma development at age 6 years was 24% and at age 11 years was 26%. Cross-sectional evaluation reveals that aeroallergen sensitization at age 6 years is associated with a 40% risk of asthma (OR, 2.6; 95% CI, 1.4, −4.9; $P = .003$) compared with an 18% risk for those children who are not aeroallergen sensitized at age 6 years. Similarly, cross-sectional evaluation at age 11 years demonstrates that sensitization at age 11 years confers an asthma risk of 41% (OR, 3.0; 95% CI, 1.5-6.3; $P = .003$) compared with an 18% risk for asthma in those children who do not have aeroallergen sensitization at age 11 years.

TABLE II. Early life aeroallergen sensitization increases asthma risk and cross-sectional assessment reveals increased asthma risk for children with aeroallergen sensitization

Asthma at age 6 y					
Age (y)	Aeroallergen sensitization	N	Asthma at 6 y, n (%)	OR (95% CI)	*P* value
2	Yes	47	24 (51)	3.3 (1.7-6.3)	.0005
	No	196	47 (24)		
6	Yes	91	36 (40)	2.6 (1.4-4.9)	.003
	No	111	20 (18)		
Asthma at age 11 y					
Age (y)	Aeroallergen sensitization	N	Asthma at 11 y, n (%)	OR (95% CI)	*P* value
2	Yes	40	21 (53)	3.2 (1.6-6.6)	.001
	No	168	43 (26)		
6	Yes	82	38 (46)	3.2 (1.7-6.1)	.0004
	No	100	20 (20)		
11	Yes	103	42 (41)	3.0 (1.5-6.3)	.003
	No	63	11 (18)		

The presence of aeroallergen sensitization at age 2 years predicts greater asthma risk at age 6 and 11 years compared with age-matched individuals without aeroallergen sensitization. Aeroallergen sensitization at age 6 years is associated with increased asthma risk at age 6 years and the same is demonstrated at age 11 years. ORs are reported with 95% CI. The model was adjusted for sex. Statistical significance is a *P* value of <.05.

Predicting asthma development using biomarkers of type 2 inflammation

For the purpose of this article, we determined cutoff points to identify a positive biomarker: peripheral blood eosinophils, 300 cells/μL or more (literature-based)[12,13]; any aeroallergen sensitization (at least 1 value ≥0.35 kU_A/L), and serum periostin level of 150 ng/mL or more (approximate median of periostin levels at age 2 years in our population). Using the aforementioned cutoff points, at age 2 years, we assessed the risk of asthma at age 6 and 11 years (Fig 3). Eosinophil count of 300 cells/μL or more and aeroallergen sensitivity at age 2 years were each associated with a similar risk of asthma at age 6 years (OR, 3.1; 95% CI, 1.7-6.0 and OR, 3.3; 95% CI, 1.7-6.3, respectively). Similar findings were observed for eosinophil count of 300 cells/μL or more and any aeroallergen sensitization at age 2 years for asthma at age 11 years (OR, 3.1; 95% CI, 1.6-6.1 and OR, 3.2; 95% CI, 1.6-6.5, respectively). Although a periostin level of 150 ng/mL or more at age 2 years predicted asthma at age 6 years (OR, 2.3; 95% CI, 1.3-4.4), the effect was not sustained for predicting asthma at age 11 years (OR, 1.0; 95% CI, 0.5-1.9).

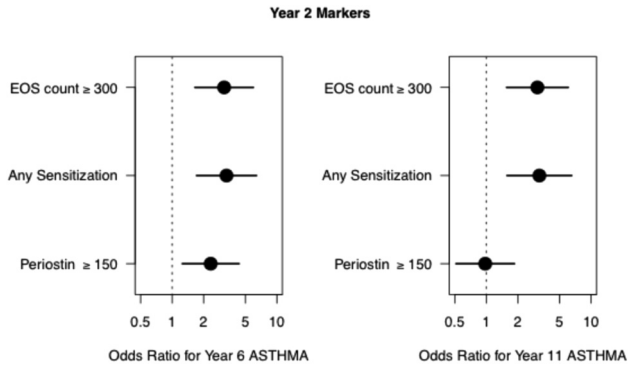


FIG 3. Positive biomarkers at age 2 years have increased ORs for asthma development at ages 6 and 11 years. Using cutoff points to identify positive biomarkers, we assessed the risk for asthma. The presence of a positive biomarker does not necessarily mean that other biomarkers are not also positive. ORs are depicted with *black circle*, with the *horizontal bars* representing the 95% CI. *EOS*, Eosinophil.

We next aimed to assess whether combining biomarkers could enhance predictive value (Table III). For the 196 children who had data for all biomarkers for asthma development at age 6 years, we found that having *only* 1 positive biomarker was not associated with an increased risk of asthma (OR, 1.1; 95% CI, 0.5-2.4) for any 1 compared with none. However, if any 2 (or all 3) of the biomarkers were present, children had a significantly increased risk of developing asthma by school age (any 2 or more vs none: OR, 6.6; 95% CI, 2.7-16.0; any 2 or more vs *only* 1: OR, 6.0; 95% CI, 2.63-13.7). Similarly, for the 170 children who had data for all biomarkers for asthma development at age 11 years, a similar pattern was identified (any 2 or more vs none: OR, 3.2; 95% CI, 1.3-7.9; any 2 or more vs *only* 1: OR, 3.3; 95% CI, 1.4-7.6).

DISCUSSION

Asthma is a common chronic illness in children, and we continue to seek ways to better understand, identify, and predict the development of asthma in the pediatric population. One area of active interest is that of biomarker identification. Using our prospective COAST cohort of high-risk children, we were able to evaluate current biomarkers of interest (aeroallergen sensitization, peripheral blood eosinophil count, and serum periostin level) and their relationship to pediatric asthma development in this population.

Periostin has been identified as a promising biomarker in adults. To our knowledge, this is the first study to evaluate and report on early life peripheral blood periostin levels in children, specifically as it relates to asthma development. Our findings demonstrated significant effects of age on periostin levels. Serum periostin levels were several-fold higher in children than those previously observed in adults. Using the same periostin assay, the median serum periostin level in healthy adults is about 44 ng/mL with an interquartile range of 36 to 54 ng/mL, whereas in adult patients with moderate to severe asthma the median serum periostin level is about 50 ng/mL with an interquartile range of 44 to 59 ng/mL.[29] Children enrolled in our study had serum periostin levels ranging from 120 to 150 ng/mL. Serum periostin levels were highest at age 2 years, regardless of the subsequent development of asthma. Serum periostin levels at ages 4, 6, and 11 years were significantly lower than at age 2 years and there was no significant change in the distribution of serum periostin levels between ages 4 and 11 years. This developmental change observed in serum periostin levels may be related to bone turnover and growth, as periostin is a component of the extracellular matrix and regulates type I collagen formation, which is important for skin, tendon, and bone development.[22] Most healthy infants and children grow in a predictable fashion.[30] Height velocity varies with age, with the most rapid period of growth occurring during the first year of life, followed by a gradual slowing of height attainment and a relative plateau of height velocity from age 4 years until puberty. There is an expected deceleration in height velocity before the pubertal growth spurt. Thus, developmental changes in serum periostin in this study appear to parallel changes in growth velocity.

Interestingly, there were no sex-related effects on periostin until age 11 years, at which point girls had significantly higher serum periostin than did boys. This observation also fits with the concept that periostin level is higher during times of growth, specifically linear growth. On average, females enter puberty at age 9 years and reach peak growth about 2.5 years into puberty.[31] On the contrary, males tend to enter puberty slightly later at around age 11 years, and peak growth occurs about 3 years later. Thus, if serum periostin levels reflect rates of bone growth, it is likely that sex effects on serum periostin may change during adolescence. It is unclear when the periostin levels decrease to expected, and much lower, adult levels, but we suspect this occurs sometime after puberty and once adult height has been attained.

We demonstrated that periostin level is elevated in children with asthma, cross-sectionally, and that it is also modestly predictive of asthma development from measurement as early as age 2 years. However, the ability to predict asthma development decreases with increasing age, which is somewhat counter to what one might expect given that periostin has been shown to have predictive capacity in adults, at least with regard to response to immunologic-based therapy.[6,13] Our study did not assess whether serum periostin levels in children with asthma predict response to treatment, which would be an important topic for future research. However, because of the varying levels by age and sex, it will be challenging to establish a universal cutoff for the pediatric population.

Many studies, including previously reported COAST cohort data, have demonstrated a significant relationship between aeroallergen sensitization and asthma development.[10] Similarly, peripheral blood eosinophils are also a key biomarker for allergic asthma, at least in regard to response to therapy in adult patients. Our data analysis confirms these relationships with asthma development. In early life, the presence of aeroallergen sensitization and/or peripheral eosinophil count of 300 cells/μL or more signifies an increased risk for developing asthma. Interestingly, the data from this study suggest that peripheral eosinophilia may be as robust of a predictor as aeroallergen sensitization for asthma development. Historically, the role of peripheral blood eosinophilia and risk of asthma development have not been clear. As alluded to previously, the asthma predictive index identifies peripheral eosinophil percentage greater than or equal to 4% as a minor risk factor, but our data suggest that absolute eosinophil counts of 300 cells/μL or more should be considered a worthy biomarker for asthma inception risk in children. Although periostin (≥150 ng/mL) has modest predictive value for asthma development at age 6 years, its predictive effect wanes for asthma by age 11 years.

TABLE III. Combinations, of ≥2 positive biomarkers, predict higher risk for asthma development at age 6 and 11 years

Asthma at age 6 y					
Positive biomarker	N (%)	Asthma at 6 y	N (%)	OR (95% CI) vs None	OR (95% CI) vs *Only* 1
None	68 (35)	Yes	14 (21)	Reference	
		No	54 (79)		
Only 1	90 (46)	Yes	20 (22)	1.1 (0.5-2.4)	Reference
		No	70 (78)		
Any 2 or more	38 (19)	Yes	24 (63)	6.6 (2.7-16.0)	6.0 (2.6-13.7)
		No	14 (37)		
Asthma at age 11 y					
Positive biomarker	N (%)	Asthma at 11 y	N (%)	OR (95% CI) vs None	OR (95% CI) vs *Only* 1
None	58 (34)	Yes	15 (26)	Reference	
		No	43 (74)		
Only 1	78 (46)	Yes	20 (26)	1.0 (0.5-2.2)	Reference
		No	58 (74)		
Any 2 or more	34 (19)	Yes	18 (53)	3.2 (1.3-7.9)	3.3 (1.4-7.6)
		No	16 (47)		

Using the data from the children in the cohort who had data on all biomarkers, we assessed the utility of combinations of biomarkers. ORs are reported with associated 95% CI comparing none, *only* 1, and any 2 as it relates to development at age 6 and 11 years.

These data analyses do suggest that combinations of positive biomarkers (any 2 or more) at age 2 years can provide additional predictive information for asthma inception. The presence of only 1 positive biomarker did not add significant predictive value, but any combination of 2 or more was associated with an increased risk, which could have clinical utility. This suggests that children with evidence for activation of multiple pathways of type 2 inflammation are at greatest risk of asthma development. Furthermore, it is important to recognize that the predictive value of the aforementioned biomarkers is irrespective of personal wheezing history.

The strengths of this study are the prospective design and longitudinal follow-up with high retention rates. In addition, the children are carefully characterized with respect to allergy and asthma phenotypes. One limitation to this study is that an enrollment criterion included only patients at higher risk for the development of asthma based on parental history of atopy or asthma. In addition, the COAST cohort is composed primarily of white children, so little information regarding potential effects of race on biomarkers in children, including serum periostin levels, is available from this study. Also, periostin assays are currently clinically unavailable for the pediatric population, further limiting its clinical utility in practice at this time.

In conclusion, our study found that early life aeroallergen sensitization and peripheral blood eosinophilia are both strong predictors of asthma inception. Serum periostin levels are significantly higher in children compared with published adult values and change developmentally, which may be due to bone turnover. Although serum periostin may have modest predictive value for asthma development at age 6 years, the confounding by growth limits its clinical utility in children. Notably, children with evidence of activation of multiple pathways of type 2 inflammation in early life are at greatest risk for the subsequent development of asthma.

Clinical implications: Children with evidence of activation of multiple pathways of type 2 inflammation in early life are at greatest risk for the subsequent development of asthma.

REFERENCES

1. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995;332:133-8.
2. Klein Wolterink RG, Hendriks RW. Type 2 innate lymphocytes in allergic airway inflammation. Curr Allergy Asthma Rep 2013;13:271-80.
3. Sly PD, Boner AL, Bjorksten B, Bush A, Custovic A, Eigenmann PA, et al. Early identification of atopy in the prediction of persistent asthma in children. Lancet 2008;372:1100-6.
4. Szefler SJ, Wenzel S, Brown R, Erzurum SC, Fahy JV, Hamilton RG, et al. Asthma outcomes: biomarkers. J Allergy Clin Immunol 2012;129:S9-23.
5. Brockow I, Zutavern A, Hoffmann U, Grubl A, von Berg A, Koletzko S, et al. Early allergic sensitizations and their relevance to atopic diseases in children aged 6 years: results of the GINI study. J Investig Allergol Clin Immunol 2009; 19:180-7.
6. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood: a prospective study. N Engl J Med 1990;323:502-7.
7. Gaffin JM, Phipatanakul W. The role of indoor allergens in the development of asthma. Curr Opin Allergy Clin Immunol 2009;9:128-35.
8. Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet 2006;368:763-70.
9. Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. J Allergy Clin Immunol 2007;119: 1105-10.
10. Stoltz DJ, Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Gern JE, et al. Specific patterns of allergic sensitization in early childhood and asthma & rhinitis risk. Clin Exp Allergy 2013;43:233-41.
11. Arbes SJ Jr, Calatroni A, Mitchell HE, Gergen PJ. Age-dependent interaction between atopy and eosinophils in asthma cases: results from NHANES 2005-2006. Clin Exp Allergy 2013;43:544-51.
12. Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, et al. Dupilumab in persistent asthma with elevated eosinophil levels. N Engl J Med 2013;368: 2455-66.
13. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 2012;380:651-9.
14. Castro-Rodriguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. Am J Respir Crit Care Med 2000;162:1403-6.
15. Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefler SJ, et al. Long-term inhaled corticosteroids in preschool children at high risk for asthma. N Engl J Med 2006;354:1985-97.
16. Luo G, Nkoy FL, Stone BL, Schmick D, Johnson MD. A systematic review of predictive models for asthma development in children. BMC Med Inform Decis Mak 2015;15:99.
17. Backman K, Nuolivirta K, Ollikainen H, Korppi M, Piippo-Savolainen E. Low eosinophils during bronchiolitis in infancy are associated with lower risk of adulthood asthma. Pediatr Allergy Immunol 2015;26:668-73.
18. Piippo-Savolainen E, Remes S, Korppi M. Does blood eosinophilia in wheezing infants predict later asthma? A prospective 18-20-year follow-up. Allergy Asthma Proc 2007;28:163-9.
19. Genuneit J. Interaction between atopy and blood eosinophils in the development of childhood wheeze. J Allergy Clin Immunol 2013;132:1237-9.e1235.
20. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med 2011;365: 1088-98.

21. Lopez-Guisa JM, Powers C, File D, Cochrane E, Jimenez N, Debley JS. Airway epithelial cells from asthmatic children differentially express proremodeling factors. J Allergy Clin Immunol 2012;129:990-7.e996.
22. Kudo A. Periostin in fibrillogenesis for tissue regeneration: periostin actions inside and outside the cell. Cell Mol Life Sci 2011;68:3201-7.
23. Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, et al. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. J Allergy Clin Immunol 2012;130:647-54.e610.
24. Song JS, You JS, Jeong SI, Yang S, Hwang IT, Im YG, et al. Serum periostin levels correlate with airway hyper-responsiveness to methacholine and mannitol in children with asthma. Allergy 2015;70:674-81.
25. Konradsen JR, Skantz E, Nordlund B, Lidegran M, James A, Ono J, et al. Predicting asthma morbidity in children using proposed markers of Th2-type inflammation. Pediatr Allergy Immunol 2015;26:772-9.
26. Lemanske RF. The childhood origins of asthma (COAST) study. Pediatr Allergy Immunol 2002;13:38-43.
27. Sherman J, Holweg C, Kincaid H, Sidobre S, Ma L, Maciuca R, et al. The Elecsys Periostin assay as a companion diagnostic for the novel asthma drug lebrikizumab. Clin Chem 2014;60:S25-6.
28. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL Pappas TE, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med 2008;178:667-72.
29. Arron JR, Choy DF, Scheerens H, Matthews JG. Noninvasive biomarkers that predict treatment benefit from biologic therapies in asthma. Ann Am Thorac Soc 2013;10:S206-13.
30. Nichols J. Normal growth patterns in infants and prepubertal children. [Internet; closed database.] UpToDate [Last updated, May, 2016]. Available at: https://www-uptodate-com/contents/normal-growth-patterns-in-infants-and-prepubertal-children?source=search_result&search=Normal+growth+patterns+in+infants+and+prepubertal+children.&selectedTitle=1~150.
31. Abbassi V. Growth and normal puberty. Pediatrics 1998;102:507-11.