Can serum periostin, YKL-40, and osteopontin levels in pre-school children with recurrent wheezing predict later development of asthma?

Guvenir H¹, Buyuktiryaki B^{1,2}, Kulhas Celik I¹, Civelek E¹, Kilic Suloglu A³, Karaaslan C⁴, Genc S⁵, Dibek Misirlioglu E¹, Toyran M¹, Ginis T¹, Kocabas CN⁶

¹MD, Department of Pediatric Allergy and Immunology, University of Health Sciences, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital, Ankara, Turkey

²MD, Department of Pediatric Allergy, Koc University, Faculty of Medicine, Istanbul, Turkey

³PhD, Department of Biology, Zoology Section, Hacettepe University, Faculty of Science, Ankara, Turkey

⁴PhD, Department of Biology, Molecular Biology Section, Hacettepe University Faculty of Science, Ankara, Turkey

⁵Bsc, Department of Biology, Molecular Biology Section, Hacettepe University Faculty of Science, Ankara, Turkey

⁶MD, Department of Pediatric Allergy and Immunology, Mugla Sitki Kocman University Faculty of Medicine, Mugla, Turkey

ABSTRACT

Background: Currently, there are no reliable clinical tools available for predicting asthma in preschool-aged children with recurrent wheezing. The aim of this study is to evaluate the usefulness of serum periostin, YKL-40 and osteopontin biomarkers in wheezy preschool-aged children for predicting the development of asthma in school-ages.

Methods: The study was prospectively conducted between 2011 and 2017. The clinical features of the preschool-aged children with recurrent wheezing and the levels of serum periostin, YKL-40, osteopontin were measured. The same participants were re-evaluated in school-age period and participants with asthma were identified. Relative risk (RR) for the development of asthma were analyzed.

Results: Of the 197 preschool-aged children with recurrent wheezing who were re-evaluated in school age years, 32% of them had asthma. Serum periostin, YKL-40, osteopontin levels at admission could not predict participants who would have asthma symptoms in school age years. The RR for continuing of asthma symptoms were higher in participants who had their first wheezing episode before 1 year of age, preterm birth, cesarean section delivery, prenatal smoking exposure, multi-trigger wheezing, parental asthma, modified asthma predictive index

positivity, prophylactic vitamin D intake $\leq$ 12 months, breastfeeding time $\leq$ 12 month, and aeroallergen sensitivity [RR (95% CI) and P value: 2.813(1.299-6.091), 0.002; 1.972(1.274-3.052), 0.009; 1.929(1.195-3.114), 0.004; 2.232(1.463-3.406), <0.001 ; 3.152(1.949-5.097), <0.001 ; 1.730(1.144-2.615), 0.016; 2.427(1.559-3.777), <0.001 ; 2.955(1.558-5.604), <0.001 ; 1.767(1.084-2.881), 0.016; 0.765(0.556-1.053), 0.016; respectively].

Conclusion: Results has shown that clinical features were more valuable than biomarkers in predicting having asthma in school-age years in participants who had recurrent wheezing in preschool-age period.

Keywords: asthma, biomarker, children, wheezing, periostin, osteopontin, YKL-40

Abbreviations:

API-Asthma Predictive Index

mAPI-Modified Asthma Predictive Index

CI: Confidence interval

GINA-Global Initiative for Asthma

IQR: Interquartile range

OR: Odds ratio

PIAMA-Prevention and Incidence of Asthma and Mite Allergy

SD-Standard deviations

INTRODUCTION

Wheezing is one of the common respiratory symptoms in childhood.¹ Studies have demonstrated that almost one in two children experience at least one wheezing episode during the preschool period. In total, 60% of wheezing children recover from wheezing before the age of 6 years. However, wheezing persists in some of them, and such children are subsequently diagnosed with asthma during school period or at adult age.²

It is difficult to predict which children with recurrent wheezing in preschool period will develop asthma. This is because respiratory symptoms suggesting asthma in this group may co-occur with other pediatric diseases such as respiratory tract infections and congenital airway anomalies.¹ Currently, there is no tool to reliably predicting asthma in wheezing children. Some clinical indexes [such as Asthma Predictive Index (API)³, modified API (mAPI)⁴, Isle of Wright score⁵, and Prevention and Incidence of Asthma and Mite Allergy (PIAMA) risk score⁶] have been developed to help diagnose asthma in the early period. Among these, modified API (mAPI) is the most frequently used index that is also recommended by the guidelines.⁷ However, clinical indexes usually have low sensitivity and positive predictive values and are of limited use in predicting which wheezing child will develop asthma.⁸

Because clinical parameters have been insufficient in predicting asthma, several studies have been performed on the biomarkers involved in the pathophysiology of asthma.⁹ Periostin, osteopontin and YKL-40 (also known as Chitinase-3-Like Protein-1) are among these biomarkers studied in children with asthma.¹⁰ Studies in children mostly focused on determining the relationship between biomarkers and asthma severity and phenotype.^{11, 12} However, the roles of these biomarkers in predicting development of asthma in wheezing children are not known yet.¹⁰

In the present study, we aimed to evaluate the use of serum periostin, YKL-40, and osteopontin measurements in predicting asthma in children with recurrent wheezing in the preschool period.

PATIENTS and METHODS

The study was prospectively conducted between 2011 and 2017 in the Pediatric Immunology and Allergy outpatient clinic of Ankara Pediatric Hematology Oncology Training and Research Hospital. The study was approved by the ethics committee of our hospital, and consent was obtained from the parents of all the participants before the study.

Between 2011 and 2012, all participants who were evaluated at the pediatric immunology and allergy outpatient clinic due to recurrent wheezing (>3 wheezing episodes) at preschool age were included in the study. Participants with other chronic diseases (cystic fibrosis, bronchopulmonary dysplasia, and bronchiolitis obliterans) and congenital heart diseases were

not included in the study. Nine participants did not want to continue working and were therefore excluded from the study. All the remaining participants (including children whose wheezing improved during follow-up) were prospectively followed up yearly. The final evaluation was made in 2017 when participants were at school age. Flowchart of the study is shown in Figure 1.

Collection of participant' data

Participants' age, sex, birth time, mode of delivery, duration of breastfeeding, duration of prophylactic vitamin D use, prenatal smoking exposure, age at the first wheezing episode, wheezing phenotype, concomitant allergic diseases, family history of allergic disease, eosinophil count, serum IgE level, and aeroallergen sensitivity were recorded.

Wheezing phenotype: Participants who had wheezing, which is generally associated with viral respiratory tract infections, but no wheezing between episodes were evaluated as those with *episodic wheezing*; and participants with wheezing that was observed both during the episode and within the period between episodes, which worsens with triggers such as cigarette smoke, exercise, and allergens, were evaluated as those with the *multi-trigger wheezing* phenotype.¹³

mAPI: mAPI was evaluated as positive if one major (history of asthma in the parents, atopic dermatitis, or aeroallergen sensitivity diagnosed by a physician) criterion or two minors (food allergy, wheezing without cold, and $\geq 4\%$ eosinophilia) criteria is /are present along with >3 wheezing episodes.⁴

Assessment at school age

Clinical evaluation: All participants were reevaluated by a pediatric allergy specialist in 2017 (approximately six years later) and participants with asthma were identified. Global Initiative for Asthma (GINA) guidelines were used to identify participants with asthma at school age.¹⁴

Measurement of serum periostin, YKL-40, and osteopontin levels: Serum samples were collected from participants and stored at -80°C until use. Periostin, YKL-40, and osteopontin protein levels were measured by DY1433, DY2599, and DY3548B ELISA kits respectively (R&D Systems Minneapolis, MN, USA) according to manufacturer's protocol. ELISA dilution factor was determined according to the preliminary experiment for each biomarker

separately. Fifteen samples were randomly selected to measure the protein levels. The dilutions were chosen in the limitations of the manufacturer's guide. After three repeats of the analyses, the dilution was selected 1:1 for periostin, 1:2 for osteopontin, and 1:3 for YKL-40. The final concentration of the protein was calculated by using the dilution factor. The detection limit of the assay is 62.5 pg/ml for OPN, 31.2 pg/ml for CHI3L1, and 62.5 pg/ml for OSF-2. All data were in the detection limitations. The average of three repeated measurements for each biomarker was used for analysis. Coefficient of variation for periostin, YKL-40 and Osteopontin 14.1%, 8.1%, and 6.6% respectively.

Statistical Analysis

The statistical analysis was performed using the SPSS program version 23.0 (IBM Corporation, Armonk, New York, USA). We expressed categorical variables as percentages. The distribution of quantitative data was determined according to Shapiro-Wilk test and visual inspection of histograms and normal Q-Q plots. The values are presented as means and standard deviations (SD) for data that demonstrated a normal distribution and as medians and interquartile ranges for data that did not demonstrate a normal distribution. Chi-square test was performed to compare the categorical variables, Mann-Whitney test was used to compare the non-normally distributed continuous variables, and the Student's T-test was used for normally distributed continuous data to compare participants with transient wheezing and asthma. The correlation status between eosinophil count with serum biomarker levels and the relationship of biomarkers with each other were evaluated with Spearman's correlation test. Relative risk (RR) analysis was performed to determine the risk factors for the continuation of asthma. The results of the risk analysis were expressed as relative risk (RR) and 95% confidence intervals (95% CIs). A p value of <0.05 was considered statistically significant.

RESULTS:

A total of 197 participants aged between 2.3 and 3.9 years diagnosed with recurrent wheezing were evaluated in the study. The first wheezing episode developed at <1 year of age in 77% (n = 152) of them. Overall, 54.8% (n = 108) of the participants had the multi-trigger wheezing phenotype. Modified API was negative in 54.8% and positive in 45.2% of the participants. The characteristics of participants are presented in Table 1.

When the participants were reevaluated at 5.91 ± 0.57 years after presentation, their mean $\pm$ SD age was 8.4 ± 0.9 years, and 32% (n = 63) had asthma. It was observed that the first

wheezing episode occurred earlier, frequency of wheezing within the first 1 year of age, C/S delivery, and preterm birth were higher, the duration of breastfeeding and prophylactic vitamin D use was shorter, and multi-trigger wheezing phenotype was more common in participants with asthma. In addition, the rate of mAPI positivity was higher for children with asthma than for children with transient wheezing (Table 1). According to the current study, the sensitivity of mAPI was 66.6%, specificity was 64.9%, positive predictive value was 47.1%, and negative predictive value was 80.5%.

In total, 23.8% (n = 15) of the participants had mild persistent and 76.2% (n = 48) had moderate persistent asthma, and there were no participants with severe asthma. In total, 134 participants in the transient wheezing group did not have asthma symptoms for median (IQR): 3.5 (2–4) years.

Evaluation of the association between serum periostin, YL-40, osteopontin levels and asthma:

When participants with asthma were compared with participants with transient wheezing, there was no statistical difference between the levels of periostin, osteopontin, and YKL-40 in serum samples collected at the time of admission (preschool-aged) (p value 0.371, 0.889, and 0.614; respectively) (Table 1, Figure 2).

In addition, there was no statistical difference between the periostin, osteopontin, and YKL-40 levels in the last (school-aged) serum samples of participants with asthma and of participants with transient wheezing (p value 0.126, 0.054, and 0.389; respectively) (Table 1, Figure 2).

There was no correlation between serum biomarker levels of preschool children with recurrent wheezing and sex, mode and time of delivery, wheezing phenotype, prenatal smoking exposure, aeroallergen sensitivity, concomitant allergic disease, family history of allergic disease, and eosinophilia ($\geq 4\%$) (Table E1). It was observed that eosinophil count had a positive correlation with periostin and YKL-40, and no correlation with osteopontin ($r = 0.422$, $p < 0.001$; $r = 0.150$, $p = 0.035$ and $r = -0.043$, $p = 0.552$, respectively).

There was a positive correlation between the level of periostin and YKL-40 in both recurrent wheezing and asthma at pre-school age ($r=0.491$, $p=0.001$; $r=0.418$, $p=0.003$; respectively). However, there was no correlation between periostin, YKL-40, and osteopontin in school-age (Table E2).

Determination of risk factors for asthma:

The risks for continuing of asthma symptoms were higher in participants who had their first wheezing episode before 1 year of age, preterm birth, cesarean section delivery, prenatal smoking exposure, multi-trigger wheeze, parental asthma, modified asthma predictive index positivity, prophylactic vitamin D intake ≤ 12 months, breastfeeding time ≤ 12 month, and aeroallergen sensitivity [RR (95% CI) and P value: 2.813(1.299-6.091), 0.002; 1.972(1.274-3.052), 0.009; 1.929(1.195-3.114), 0.004; 2.232(1.463-3.406), <0.001 ; 3.152(1.949-5.097), <0.001 ; 1.730(1.144-2.615), 0.016; 2.427(1.559-3.777), <0.001 ; 2.955(1.558-5.604), <0.001 ; 1.767(1.084-2.881), 0.016; 0.765(0.556-1.053), 0.016; respectively] (Figure 3).

DISCUSSION

In the present study evaluating 197 children with recurrent wheezing in preschool period, there was no difference in the measured levels of serum periostin, osteopontin, and YKL-40 between children with transient wheezing and children with asthma in both preschool and school periods. Therefore, the biomarkers measured in children with recurrent wheezing may not be useful in predicting the development of asthma.

There is only one study with a design similar to that of the current study. Klaassen et al¹⁵ followed 202 participants with recurrent wheezing at preschool age and identified those who developed asthma at the age of 6 years. In addition, exhaled breath condensate biomarkers (IL1 alpha, IL2, IL4, IL5, IL10, IFN gamma, Eotaxin, and ICAM1), volatile organic compounds, and expression of inflammation genes has been analyzed. They demonstrated that when exhaled volatile organic compounds, expression of inflammation genes, and API were evaluated together could correctly predict asthma in around 90% of recurrent wheezing with preschool age. However, exhaled breath condensate biomarkers in preschool age were not found useful in predicting the diagnosis of asthma at six years of age.¹⁵

There are no studies on serum periostin, osteopontin, and YKL-40 levels in predicting asthma in preschool children with recurrent wheezing in the literature. Periostin is the extracellular matrix protein secreted by airway epithelial cells in response to interleukin-13.^{16,17} There was higher periostin expression in airway epithelial cells and in serum collected from children with asthma than in those from healthy children.¹⁸⁻²⁰ Therefore, it is considered that determining the level of serum periostin in children aged ≥ 6 years may be helpful in the diagnosis of asthma. Conversely, Habernau Mena et al.²¹ have shown that children with severe uncontrolled asthma have lower periostin levels than children with severe controlled

asthma. Therefore, it is unclear whether periostin measurements in developing children with asthma are useful.

YKL-40, a chitinase-like protein, is a biomarker that can be detected in the serum and respiratory tract of adults with asthma; it is associated with disease severity indices, including subepithelial basement membrane thickness and airflow limitation.¹⁶ In a study conducted by the Konradsen et al,²² it has been demonstrated that in children with severe and treatment-resistant asthma, YKL-40 levels increase more than in children with controlled asthma and that YKL-40 can be used as an indicator of asthma severity. Contrary to this, Santos et al.²³ found that the serum YKL-40 levels in children with severe persistent asthma were lower than those in children with mild and moderate persistent asthma and adults with persistent asthma.

Osteopontin is an extracellular matrix protein that plays a role in airway remodeling and is expressed in various cells, such as bronchial epithelial cells, eosinophils and T cells.¹⁶ In a study conducted by Akelma et al,²⁴ serum osteopontin levels were found to be higher in children with asthma than in controls and higher in those with allergic rhinitis than those without. In another study conducted by Nacaroglu et al,²⁵ no difference was found between children with asthma and the control group in terms of osteopontin levels. According to a recently published meta-analysis including these two studies, osteopontin expression in participants with asthma was found to be higher than that in the healthy controls. However, no association with the type and severity of asthma was found.²⁶

As is observed, studies conducted on periostin, YKL-40, and osteopontin levels in children with asthma have not clarified their usability in the diagnosis of asthma and in determining its severity. In our study, it was demonstrated that these biomarkers were not successful in predicting asthma in preschool children with recurrent wheezing. Besides, serum levels at school age were not found to be different between children with asthma and those with transient wheezing.

Modified API is the most commonly used index in predicting asthma in children with recurrent wheezing.^{4,7} In our current study, mAPI was positive in 66.7% of children with persistent asthma and in 35.1% of those with transient wheezing. Negative predictive value of mAPI was found to be 80.5% in the present study. Therefore, although it is poor in predicting which participants will develop asthma, it has been found to be successful in identifying children who will most likely not develop asthma.

Considering the studies evaluating the risk factors involved in the development of asthma in children with recurrent wheezing in the preschool period, male sex,^{27,28} prenatal exposure to smoking,²⁷ early sensitivity to food or aeroallergens,^{27,28} atopic dermatitis,^{27,28} food allergy,^{27,28} allergic rhinitis,^{27,28} family history of asthma,²⁷⁻²⁹ and onset of wheezing after 1 year of age²⁷ are found to be the risk factors for asthma. There are also studies showing that there is a correlation between obesity and psychological/social factors and non-atopic asthma.²⁹ In the present study showed that having the first wheezing episode before 1 year of age, preterm birth, C/S delivery, prenatal smoking exposure, multi-trigger wheezing, parental asthma, modified asthma predictive index positivity, prophylactic vitamin D intake ≤ 12 months, breastfeeding time ≤ 12 month, and aeroallergen sensitivity are the risk factors for the persistence of asthma. Our study data are mostly consistent with the literature. The only exception is that the onset of wheezing episodes before 1 year of age was found to be a risk factor for asthma in the present study unlike some other studies, in which the onset of wheezing episodes after 1 year of age was shown as a risk factor.

This is the first study to investigate the usability of periostin, YKL-40, and osteopontin in predicting the development of asthma in children with recurrent wheezing in the preschool period. The participants were followed up for a long period, and participants who developed asthma were identified. Therefore, we believe that the data from the current study is inspiring for similar studies to be conducted in the future. A limitation of the present study is that currently, we only know the asthma status of the participants during school period. Children with asthma in school period can go into remission in adolescence or adult age, or the opposite may also occur. Therefore, the follow-up of the participants is ongoing.

In conclusion, serum periostin, osteopontin, and YKL-40 measurements in children with recurrent wheezing in the preschool period were not found to be of use in predicting asthma. However, there is a need for further and more comprehensive studies on this subject involving different biomarkers. Finding methods based on objective measurement in predicting asthma can help identify children at risk of developing asthma early and with high accuracy or rule out an unnecessary asthma diagnosis.

Impact statement

This is the first study to investigate the usability of periostin, YKL-40, and osteopontin in predicting the development of asthma in children with recurrent wheezing in the preschool period. In preschool-aged with recurrent wheezing, the clinical features were more valuable

than biomarkers in predicting having asthma. Serum periostin, YKL-40, and osteopontin measured in children with recurrent wheezing cannot be used as a tool to predict the development of asthma in the future.

REFERENCES:

1. Ducharme FM, Tse SM, Chauhan B. Diagnosis, management, and prognosis of preschool wheeze. *Lancet* 2014; 383: 1593-604.
2. 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(3): 133–138.
3. 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.
4. Guilbert TW, Morgan WJ, Krawiec M, Lemanske RF. The Prevention of Early Asthma in Kids study: design, rationale and methods for the Childhood Asthma Research and Education network. *Control Clin Trials.* 2004; 25:286–310.
5. Kurukulaaratchy RJ, Matthews S, Holgate ST, Arshad SH. Predicting persistent disease among children who wheeze during early life. *Eur Respir J.* 2003; 22: 767-71.
6. Caudri D, Wijga A, Schipper CMA, Hoekstra M, Postma DS, Koppelman GH, et al. Predicting the long-term prognosis of children with symptoms suggestive of asthma at preschool age. *J Allergy Clin Immunol.* 2009; 124: 903-10.
7. National Asthma Education and Prevention Program Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. *J Allergy Clin Immunol* 2007; 120(Suppl): S94-138.
8. Castro-Rodriguez JA1, Cifuentes L, Martinez FD. Predicting Asthma Using Clinical Indexes. *Front Pediatr.* 2019; 7: 320.
9. Mogensen I, James A, Andrei Malinovski A. Systemic and Breath Biomarkers for Asthma: An Update. *Curr Opin Allergy Clin Immunol.* 2020; 20: 71-79.
10. Zissler UM, Esser-von Bieren J, Jakwerth CA, Chaker AM, Schmidt-Weber CB. Current and Future Biomarkers in Allergic Asthma. *Allergy.* 2016; 71: 475-94.

305 11. Licari A, Castagnoli R, Brambilla I, Marseglia A, Tosca MA, Marseglia GL, et al. Asthma
306 Endotyping and Biomarkers in Childhood Asthma. *Pediatr Allergy Immunol Pulmonol*. 2018;
307 31: 44-55.

308 12. Lee YJ, Fujisawa T, Kim CK. Biomarkers for Recurrent Wheezing and Asthma in
309 Preschool Children. *Allergy Asthma Immunol Res*. 2019; 11: 16-28.

310 13. Brand PL, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, et al.
311 Definition, assessment and treatment of wheezing disorders in preschool children: an
312 evidence-based approach. *Eur Respir J* 2008; 32: 1096–110.

313 14. Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention
314 (updated 2019). [https://ginasthma.org/wp-content/uploads/2019/06/GINA-2019-main-report-](https://ginasthma.org/wp-content/uploads/2019/06/GINA-2019-main-report-June-2019-wms.pdf)
315 [June-2019-wms.pdf](https://ginasthma.org/wp-content/uploads/2019/06/GINA-2019-main-report-June-2019-wms.pdf)

316 15. Klaassen EM, van de Kant KD, Jöbsis Q, van Schayck OC, Smolinska A, Dallinga JW, et
317 al. Exhaled biomarkers and gene expression at preschool age improve asthma prediction at 6
318 years of age. *Am J Respir Crit Care Med*. 2015; 191: 201-7.

319 16. Fitzpatrick AM. Biomarkers of asthma and allergic airway diseases. *Ann Allergy Asthma*
320 *Immunol*. 2015; 115: 335-40.

321 17. James A, Janson C, Malinovschi A, Holweg C, Alving K, Ono J, Ohta S, Ek A,
322 Middelveld R, Dahlen B, et al. Serum periostin relates to type-2 inflammation and lung
323 function in asthma: Data from the large population-based cohort Swedish GA(2)LEN.
324 *Allergy*. 2017; 72: 1753-1760.

325 18. Song JS, You JS, Jeong SI, Yang S, Hwang IT, Im YG, et al. Serum periostin levels
326 correlate with airway hyper-responsiveness to methacholine and mannitol in children with
327 asthma. *Allergy*. 2015;

328 19. Inoue T, Akashi K, Watanabe M, Ikeda Y, Ashizuka S, Motoki T, et al. Periostin as a
329 biomarker for the diagnosis of pediatric asthma. *Pediatr Allergy Immunol*. 2016; 27: 521-6.

330 20. El Basha NR, Osman HM, Abdelaal AA, Saed SM, Shaaban HH. Increased expression of
331 serum periostin and YKL40 in children with severe asthma and asthma exacerbation. *J*
332 *Investig Med*. 2018; 66: 1102-1108.

333 21. Habernau Mena A, Del Pozo Abejón V, Rodríguez Vidigal FF, Bobadilla González P.
334 Role of Periostin in Uncontrolled Asthma in Children (DADO study). *J Investig Allergol Clin*
335 *Immunol*. 2017; 27: 291-298.

336 22. Konradsen JR, Skantz E, Nordlund B, Lidegran M, James A, Ono J, et al. Predicting
337 asthma morbidity in children using proposed markers of Th2-type inflammation. *Pediatr*
338 *Allergy Immunol*. 2015; 26: 772-9.

23. Santos CB, Davidson J, Covar RA, Spahn JD. The chitinase-like protein YKL-40 is not a useful biomarker for severe persistent asthma in children. *Ann Allergy Asthma Immunol.* 2014; 113: 263-6.
24. Akelma AZ, Cizmeci MN, Kanburoglu MK, Bozkaya D, Catal F, Mete E, et al. Elevated level of serum osteopontin in school-age children with asthma. *Allergol Immunopathol (Madr).* 2014; 42: 275-81.
25. Nacaroglu HT, Gayret OB, Erol M, Buke O, Zengi O, Tasdemir M, et al. Biomarkers of airway and systemic inflammation in obese asthmatic paediatric patients. *Allergol Immunopathol (Madr).* 2017; 45: 534-540.
26. Xu H, Lou W, Fu F. Association between osteopontin expression and asthma: a meta-analysis. *J Int Med Res.* 2019; 47: 3513-3521.
27. Rodriguez-Martinez CE, Sossa-Briceno MP, Castro-Rodriguez JA. Factors predicting persistence of early wheezing through childhood and adolescence: a systematic review of the literature. *J Asthma Allergy.* 2017; 10: 83–98.
28. Bao Y, Chen Z, Liu E, Xiang L, Zhao D, Hong J. Risk Factors in Preschool Children for Predicting Asthma During the Preschool Age and the Early School Age: a Systematic Review and Meta-Analysis. *Curr Allergy Asthma Rep.* 2017; 17: 85.
29. Strina A, Barreto ML, Cooper PJ, Rodrigues LC. Risk factors for non-atopic asthma/wheeze in children and adolescents: a systematic review. *Emerg Themes Epidemiol.* 2014; 11:5.

Table 1. Characteristics of participants and comparison of participants with transient wheezing and asthma

	All patients (n= 197)	Transient wheezing (n=134)	Asthma (n=63)	P value*
Age of first wheezing episode (month), mean±SD	7.97±5.25	8.67±5.82	5.92±3.04	0.007
First wheezing episode age <1 year, n (%)	152 (77.2)	95 (70.9)	57 (90.5)	0.002
Gender, n (%)				
Male/Female	133 (67.5)/64 (32.5)	86 (64.2)/48 (35.8)	47 (74.6)/16 (25.4)	0.145
Birth time, n (%)				
Preterm/Term	21 (10.7)/176 (89.3)	9 (6.7)/125 (93.3)	12 (19)/ 51 (81)	0.009
Mode of delivery, n (%)				
Cesarian section/Normal delivery	115 (58.4)/82 (41.6)	69 (51.5)/65 (48.5)	46 (73)/17 (27)	0.004
Breastfeeding time (month) mean±SD	13.06±6.6	14.02±6.97	11.0±5.21	0.007
Breastfeeding time, n (%)				
≤12 months	123 (62.4)	76 (56.7)	47 (74.6)	0.016
>12 months	74 (37.6)	58 (43.3)	16 (25.4)	
Prophylactic vitamin D taking time (month) mean±SD	13.6±4.43	14.53±4.34	11.65±4.01	<0.001
Prophylactic vitamin D taking time, n (%)				
≤12 months	132 (67)	78 (58.2)	54 (85.7)	<0.001
>12 months	65 (33)	56 (41.8)	9 (14.3)	
Phenotype of wheezing, n (%)				
Multi-trigger wheezing	91 (46.2)	45 (33.6)	46 (73)	<0.001
Concomitant allergic disease, n (%)	37 (18.8)	24 (17.9)	13 (20.6)	0.648
Prenatal smoking exposure, n (%)	83 (42.1)	39 (29.1)	44 (69.8)	<0.001
Maternal smoking	32 (16.2)	16 (11.9)	16 (25.4)	0.001
Maternal+paternal smoking	51 (25.9)	23 (17.2)	28 (44.4)	0.002
Allergic diseases in family, n (%)	68 (34.5)	42 (31.3)	26 (41.3)	0.172
mAPI, n (%)				
Positive	89 (45.2)	47 (35.1)	42 (66.7)	<0.001
mAPI major criteria, n (%)				
Parental asthma	37 (18.8)	19 (14.2)	18 (28.6)	0.016
Atopic dermatitis	16 (8.1)	9 (6.7)	7 (11.1)	0.292
Aeroallergen sensitivity	37 (18.8)	19 (14.2)	18 (28.6)	0.016
mAPI minor criteria, n (%)				
Wheezing without colds	91 (46.2)	45 (33.6)	46 (73)	<0.001
Food allergy	3 (1.5)	2 (1.5)	1 (1.6)	0.688
Eosinophilia (≥4%)	60 (30.5)	36 (26.9)	24 (38.1)	0.110
Total IgE (kU/L), median (IQR)	20.7 (10.1-71.4)	18.2 (7.6-67.8)	29.7 (16.4-115.5)	0.111
Preschool age				
Periostin (pg/ml), median (IQR)	704.7 (324.6-1466.3)	695.4 (405.1-1712.1)	749.61 (265.1-1422.6)	0.371
YKL-40 (pg/ml), mean±SD	3145.7±780.9	3136.9±702.1	3155.1±863.3	0.899
Osteopontin (pg/ml), median (IQR)	552.8 (324.2-1008.5)	574.4 (318.5-1031.6)	525.4 (341.5-1001.06)	0.614
School age				
Periostin (pg/ml), median (IQR)	525.5 (442.8-660.3)	552.6 (444.07-740.8)	504.6 (439.64-592.4)	0.126
YKL-40 (pg/ml), mean±SD	3779.3±711.3	3658.3±704.4	3908.7±701.7	0.054
Osteopontin (pg/ml), median (IQR)	1450.07 (1116.1-2008.4)	1390.8 (1078.8-1894.08)	1465.03 (1132.5-2028.8)	0.389
IQR: Interquartile range, SD: Standard deviation, mAPI: Modified asthma predictive index min: minimum, max: maximum				
*P values of statistical difference between groups using either Student's T-test, Mann-Whitney U test or Pearson's chi- square test depending on characteristics of the data.				

376 **Table E1. Evaluation of the association between biomarkers and clinical features**

	Periostin median (IQR)	YKL-40 mean±SD	Osteopontin median (IQR)
Gender			
Male	850.33 (336.01-1592.64)	3240.99±785.67	611.37 (344.33-1038.71)
Female	873.06 (430.58-1709.73)	3163.78±788.61	431.37 (273.94-890.44)
<i>P</i>	0.667	0.520	0.052
Mode of delivery			
Vaginal delivery	906.17 (484.16-1557.23)	3266.77±776.88	543.28 (276.26-1026.1)
Caesarean section delivery	807.35 (310.68-1717.2)	3179.63±792.88	543.0 (330.25-990.4)
<i>P</i>	0.437	0.444	0.901
Birth time			
Preterm	695.05 (156.56-1958.04)	3002.09±739.52	461.02 (318.13-758.7)
Term	906.17 (426.9-1625.4)	3241.42±788.87	566.24 (301.49-1013.27)
<i>P</i>	0.434	0.188	0.686
Phenotype of wheezing			
Episodic	756.99 (438.09-1592.64)	3165.16±770.67	511.95 (301.92-850.05)
Multi-trigger	904.77 (323.37-1661-80)	3256.87±798.36	611.37 (318.26-1064.87)
<i>P</i>	0.624	0.417	0.233
Asthma predictive index			
Positive	903.38 (329.99-1735-22)	3234.94±841.18	543.0 (292.87-1055.2)
Negative	795.16 (419.91-1529.77)	3200.22±739.99	543.28 (320.56-933.82)
<i>P</i>	0.879	0.758	0.747
Prenatal smoke exposure			
Yes	870.41 (314.7-1723.54)	3306.78±831.1	516.04 (314.01-990.4)
No	850.33 (405.18-1615.88)	3149.74±747.23	571.44 (304.65-1010.54)
<i>P</i>	0.648	0.166	0.518
Aeroallergen sensitivity			
Yes	908.68 (493.41-1739.04)	3113.27±884.44	478.17 (280.62-875.54)
No	811.02 (350.63-1606.37)	3238.08±763.59	572.36 (318.5-1023.97)
<i>P</i>	0.485	0.395	0.511
Concomitant allergic diseases			
Yes	906.17 (422.75-1331.71)	3030.65±742.68	562.79 (288.08-1112.52)
No	811.02 (365.22-1648.07)	3258.74±791.06	541.79 (320.56-989.47)
<i>P</i>	0.835	0.112	0.703
Eosinophilia (≥4%)			
Yes	941.24 (412.26-1716.81)	3248.82±875.85	577.08 (358.51-971.32)
No	809.18 (361.57-1615.88)	3201.49±745.4	514.3 (303.28-1011.91)
<i>P</i>	0.625	0.698	0.569
Allergic diseases in family			
Yes	795.16 (296.2-1439.26)	3154.65±779.26	567.99 (348.13-1146.12)
No	914.32 (473.01-1701.48)	3248.19±789.76	534.87 (288.08-874.93)
<i>P</i>	0.157	0.428	0.197
Parental asthma			
Yes	742.0 (279.85-1396.43)	3193.03±772.09	634.89 (344.33-1265.19)
No	909.62 (431.05-1648.07)	3221.19±790.8	526.99 (301.92-891.66)
<i>P</i>	0.432	0.845	0.186

IQR: Interquartile range, SD: Standard deviation

377

378

379

380

381

382 **Table E2. Evaluation of the correlation between periostin, YKL-40, and osteopontin**

			Pre-school age								
			All patients			Recurrent wheezing			Asthma		
			Periostin	YKL-40	Osteopontin	Periostin	YKL-40	Osteopontin	Periostin	YKL-40	Osteopontin
Pre-school age	Periostin	r	1	0.447	0.044	1	0.491	0.157	1	0.418	-0.092
		P		<0.001	0.670		0.001	0.299		0.003	0.527
	YKL-40	r	0.447	1	0.184	0.491	1	0.212	0.418	1	0.170
		P	<0.001		0.044	0.001		0.099	0.003		0.203
	Osteopontin	r	0.044	0.184	1	0.157	0.212	1	-0.092	0.170	1
		P	0.670	0.044		0.299	0.099		0.527	0.203	
			School age								
			All patients			Recurrent wheezing			Asthma		
			Periostin	YKL-40	Osteopontin	Periostin	YKL-40	Osteopontin	Periostin	YKL-40	Osteopontin
School age	Periostin	r	1	0.160	-0.045	1	0.244	-0.115	1	0.136	0.108
		P		0.080	0.623		0.056	0.375		0.310	0.420
	YKL-40	r	0.160	1	0.111	0.244	1	-0.004	0.136	1	0.215
		P	0.080		0.229	0.056		0.978	0.310		0.104
	Osteopontin	r	-0.045	0.111	1	-0.115	-0.004	1	0.108	0.215	1
		P	0.623	0.229		0.375	0.978		0.420	0.104

Figure 1. Flowchart of the study

Pre-school age

School age

Figure 2. Comparison of pre-school and school-aged serum biomarker levels in participants with recurrent wheezing and asthma.

Figure 3. Determination of risk factors for asthma