

University of Groningen

Predictive value of serum sST2 in preschool wheezers for development of asthma with high FeNO

Ketelaar, Maria E; van de Kant, Kim D G; Dijk, F Nicole; Klaassen, Ester M M; Grotenboer, Néomie; Nawijn, Martijn C; Dompeling, Edward; Koppelman, Gerard H

Published in:
Allergy

DOI:
[10.1111/all.13193](https://doi.org/10.1111/all.13193)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Final author's version (accepted by publisher, after peer review)

Publication date:
2017

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Ketelaar, M. E., van de Kant, K. D. G., Dijk, F. N., Klaassen, E. M. M., Grotenboer, N., Nawijn, M. C., Dompeling, E., & Koppelman, G. H. (2017). Predictive value of serum sST2 in preschool wheezers for development of asthma with high FeNO. *Allergy*, 72(11), 1811-1815. <https://doi.org/10.1111/all.13193>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Title: (Word count 15, Characters 95)

Predictive value of serum sST2 in preschool wheezers for development of asthma with high FeNO.

Short title: (Word count 7, Characters 46)

Predictive value of sST2 in preschool wheezers

Author List:

Shared 1st: Maria E. Ketelaar, MSc^{1,2,*}; Kim D. G. van de Kant, PhD³
Shared 2nd: F. Nicole Dijk, MD¹; Ester M.M. Klaassen, MD PhD⁴
5th: Néomi Grotenboer, MSc^{1,2}
6th: Martijn C Nawijn, PhD²
Shared last: Edward Dompeling, MD PhD³; Gerard H. Koppelman, MD PhD¹

Affiliations:

1. University Medical Center Groningen, University of Groningen, Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, Groningen Research Institute for Asthma and COPD (GRIAC), Groningen, The Netherlands.
2. University Medical Center Groningen, University of Groningen, Department of Pathology and Medical Biology, Laboratory of Experimental Pulmonology and Inflammation Research (EXPIRE), Groningen Research Institute for Asthma and COPD, Groningen, The Netherlands.
3. Department of Pediatric Pulmonology, School for Public Health and Primary Care (CAPHRI), Maastricht University Medical Center (MUMC+), Maastricht, The Netherlands.
4. Department of General Practice, School for Public Health and Primary Care (CAPHRI), Maastricht University Medical Center (MUMC+), Maastricht, The Netherlands.

***Corresponding author:**

M.E. Ketelaar, MSc
Dept. Pathology & Medical Biology, University Medical Center Groningen
Laboratory of Experimental Pulmonology and Inflammation Research (EXPIRE),
T2.212, EA52
Hanzeplein 1, 9713GZ, Groningen, The Netherlands
T: +31 50 36 10262 E: m.e.ketelaar@umcg.nl

Abstract: (Word count 149)

Wheezing is common in childhood. However, current prediction models of paediatric asthma have only modest accuracy. Novel biomarkers and definition of subphenotypes may improve asthma prediction. *Interleukin-1-receptor-Like-1* is a well-replicated asthma-gene and associates with eosinophilia. We investigated whether serum sST2 predicts asthma and asthma with elevated exhaled NO (FeNO), compared to the commonly used Asthma Prediction Index (API). Using logistic regression modeling, we found that serum sST2 levels in 2-3y old wheezers do not predict doctors' diagnosed asthma at age 6y. Instead sST2 predicts a subphenotype of asthma characterized by increased levels of FeNO, a marker for eosinophilic airway inflammation. Herein, sST2 improved the predictive value of the API (AUC=0.70, 95CI 0.56-0.84), but had also significant predictive value on its own (AUC=0.65, 95CI 0.52-0.79). Our study indicates that sST2 in preschool wheezers has predictive value for the development of eosinophilic airway inflammation in asthmatic children at school age.

Keywords: childhood asthma, fraction of exhaled NO, prediction, preschool wheezers, serum sST2

List of Abbreviations: ADEM study: Asthma DEtection and Monitoring study; API: Asthma Prediction Index; FeNO: Fraction of exhaled Nitric Oxide; IL-1RL1: Interleukin-1 Receptor Like 1; PIAMA study: Prevalence and Incidence of Asthma and Mite Allergy study; SNP: Single Nucleotide Polymorphism; y=years.

Author contributions:

M. Ketelaar/F. Dijk/M. Nawijn/G. Koppelman/E. Dompeling/K. van de Kant/E. Klaassen contributed to the design of the study. M. Ketelaar/K. van de Kant/E. Klaassen performed the statistical analyses. E. Dompeling/K. Van de Kant/E. Klaassen collected clinical samples and patient information. M. Ketelaar/ N. Grotenboer performed experimental analyses of clinical samples. All authors contributed to the design, interpretation of data, and revision of the article. All authors had access to the data and read and approved the final manuscript.

Declaration of Funding: Supported by unrestricted grants from the Dutch Lung Foundation (AF 3.2.09.081JU and AF 3.4.05.033) and the Stichting Astmabestrijding (SAB 2006/018), and the Maastricht University Medical Center (PF 294). MEK was the recipient of an ERS/EMBO Fellowship LTRF-2013_2060.

Conflicts of interest:

The authors declare to have no conflicts of interest.

Word count main text: 1649

To the editor:

Approximately 40% of all preschool children (aged <6 years [y]) encounter one or more episodes of respiratory symptoms, such as wheezing, coughing and dyspnoea. However, only some (20-40%) of these preschool children with respiratory problems will develop asthma at school age.¹ Current prediction models such as the asthma prediction index [API] are based on familiar predisposition, history of eczema, and presence of eosinophils or sensitization, and have only modest accuracy in predicting asthma.[1,2] To enable better prediction of asthma development at young age, novel biomarkers associated with asthma are required, which could include expression levels of well replicated asthma genes.[2]

One potential biomarker for asthma is soluble Interleukin-1-receptor-like1 [IL-1RL1-a or sST2], which is encoded by the *IL1RL1* gene (chr 2) and can be detected in serum. *IL1RL1* is an asthma susceptibility gene identified in genetic studies of paediatric and adult asthma patients.[3] *IL1RL1* has also been linked to blood eosinophilia, IgE (sensitization), eczema and hay fever.[3,4] ST2 is the receptor for interleukin-33 (IL-33), a cytokine thought to initiate and amplify a Th-type-2 response in inflammatory diseases such as asthma.[5] Soluble ST2 has been proposed to act as a decoy receptor, sequestering IL-33, thereby preventing its role in the induction of an immune response, and particularly its modulation of a Th-type-2 reaction.[5,6]

Previously, we found that *IL1RL1* SNPs associate with sST2 levels in childhood asthma in a Dutch birth cohort, the Prevalence and Incidence of Asthma and Mite Allergy [PIAMA] cohort. In this study, asthma-risk alleles were consistently associated with lower serum sST2 levels, indicating a putative protective effect of high sST2.[7] Moreover, *IL1RL1* SNPs were associated with intermediate onset and late onset wheezing phenotypes.[8] These children start to wheeze at age 2-3y, often have allergen sensitization at age 4y, and are at high risk of subsequent asthma development at school age. However, the expression levels of *IL1RL1* in preschool wheezers are unknown, as well as whether these levels could identify those children who will eventually develop asthma.

Therefore, we hypothesized that serum sST2 levels measured in wheezing preschool children contribute to the prediction of asthma at school age. Moreover, since *IL1RL1* was previously associated with blood eosinophilia, our second aim was to determine whether serum sST2 levels predict exhaled NO, as a marker of eosinophilic asthma at school age.

We investigated our hypotheses in children of the ADEM [Asthma DEtection and Monitoring] study (clinicaltrial.gov: NCT 00422747). The ADEM study is a unique longitudinal cohort designed to study the added value of biomarkers to clinical information (API) for an early asthma diagnosis. A detailed study protocol has been published previously.[9] This study included 202 wheezing children and 50 healthy controls who were enrolled from primary care practices in The Netherlands at age 2-3y and were followed up annually until age 6y. At age 6y, a final asthma diagnosis was made based on symptoms, use of asthma medication and lung function by experienced pediatricians in the field of respiratory medicine and by a computer algorithm. Corticosteroids were stopped 4 weeks before measurements when applicable. Wheezing at preschool age was defined as two or more wheezing episodes before inclusion, according to the questionnaire developed by the International Study of Asthma and Allergies in Childhood. sST2 serum levels at age 2-3y were quantified using a commercially available ELISA (R&D Systems Quantikine ELISA kit #DST200, Abingdon,

UK), which was selected after a series of validation steps comparing specificity, sensitivity, assay recovery and inter-assay variability (see also Supplemental Methods).

For the current study, in analogy to earlier analyses of the ADEM study [2], a logistic regression model was built to predict asthma diagnosis at age 6y comparing the API, sST2 and sST2 combined with API as predictors. Model performance was assessed by testing the contribution of a predictor to the model (F-test) and by quantifying discrimination (Area Under the Curve, AUC) using a receiver operating characteristics (ROC) curve. In the ADEM cohort, 40% of the preschool wheezers developed asthma at age 6y, while the remainder were transient wheezers. Predictive analyses were performed in the group with available sST2 levels at age 2-3y, which were 171 out of the 202 preschool wheezers. The subgroup of children with available sST2 serum levels did not significantly differ from the overall group in general characteristics. sST2 levels were square root transformed to meet normality criteria.

A negative association was found between *IL1RL1* genotype (using rs1420101, representing a major LD block in *IL1RL1* [3]) and sST2 protein expression in serum (ANOVA $P < 0.001$). Carriers of the asthma-risk allele (A) had lower sST2 levels, which is in the same direction as previously reported in the PIAMA cohort.[7]

However, serum levels of sST2 measured at age 2-3y could not distinguish which of the preschool wheezing children eventually developed asthma at school age (AUC=0.50 [95CI 0.41-0.59, $P=0.98$], $B=-0.002$ [OR=0.998, $P=0.89$]). No difference in serum sST2 levels at age 2-3y was found between children with transient wheeze, true asthmatics or healthy controls at age 6y ($P=0.881$, ANOVA). Consequently, serum sST2 levels at 2-3y did not significantly add to the prediction of an asthma diagnosis of the commonly used API (*API alone*: AUC= 0.60 [95CI 0.52-0.68, $P=0.02$]; *API+IL1-RL1-a*: AUC= 0.57 [95CI 0.49-0.66, $P=0.12$]). These results show that, although *IL1RL1* SNPs may affect *IL1RL1* expression levels, serum sST2 levels in wheezing children at 2-3y do not have added value in the prediction of doctors' diagnosed asthma as general phenotype at school age.

Possible reasons for this finding is the heterogeneity of the asthma phenotype in childhood, or the fact that our cohort of children was derived from a primary care setting, likely leading to an *a priori lower* asthma risk compared to a hospital setting. Since sST2 serum levels had previously been associated with blood eosinophil numbers in childhood asthma [10], we hypothesized that sST2 levels at 2-3y may predict measures of eosinophilic asthma rather than a general diagnosis of asthma at school age. In the ADEM cohort, levels of nitric oxide in exhaled breath (FeNO), considered a surrogate marker of eosinophilic airway inflammation in asthma patients [11], were measured at 6y (NIOX®; Aerocrine, Solna, Sweden).

Interestingly, we found that serum levels of sST2 measured at age 2-3y, although modestly, were negatively correlated with FeNO levels at 6y in children who had developed asthma (Pearson's $R=-0.24$, $P=0.046$, $N=59$), while no significant correlation was observed in transient wheezers (Pearson's $R=0.08$, $P=0.47$, $N=89$), see figure 1. This suggests that serum levels of sST2 at preschool age predict increased FeNO levels as a marker of eosinophilic airway inflammation in those children who will develop asthma. To investigate whether sST2 levels indeed could be a predictive biomarker for asthma with elevated FeNO levels, we next divided our population of asthmatic children at age 6y into a group with likely eosinophilic airway inflammation (FeNO \geq 20ppb, $n=15$) and asthmatics unlikely to have eosinophilic airway inflammation (FeNO $<$ 20ppb, $n=60$), based on the ATS guideline of FeNO.[11] We then performed logistic predictive modeling of asthma with elevated FeNO (Y/N) in wheezing children. Indeed, sST2 serum levels negatively predicted asthma with high

FeNO in preschool wheezers (OR=0.96, P=0.04, figure 2A/B), having a predicted AUC of 0.65 (95CI 0.52-0.79). When sST2 serum levels were combined with the API, the predictive model slightly, and significantly improved to distinguish preschool wheezers who developed asthma with elevated FeNO at school age (predicted AUC of 0.70, 95CI 0.56-0.84). We acknowledge that the sample size of the group with likely eosinophilic airway inflammation at age 6y (FeNO \geq 20ppb) of this analysis is limited, and propose that our findings should be replicated in future studies with larger sample size.

FeNO levels in 2-3y old wheezers did not have predictive value for FeNO at school age in our cohort (data not shown), nor for asthma development.[12] Moreover, although FeNO levels have previously been found useful in prediction of management of established asthma [13,14], FeNO could not predict treatment response in preschool wheezers.[15] .

Given the timespan (three to four years) between the measurement of sST2 and FeNO, and the negative correlation, it is tempting to speculate that sST2 levels could have a protective effect on the development of eosinophilic airway inflammation in asthmatic children. However, no data on eosinophil counts were available in the current cohort to further study this relationship. Nevertheless, in a previous study an inverse relationship between sST2 levels and blood eosinophil counts has been reported during exacerbations of childhood asthma.[10] Further evidence indicating a potential protective effect of sST2 in (eosinophilic) asthma are more experimental (murine) model studies of asthma, wherein delivering sST2 (respectively intraperitoneally/intranasally) significantly decreased inflammatory airway disease, including reduced eosinophil counts in BAL and methacholine induced airway hyperresponsiveness.[16,17] That a protective effect of sST2 might be rather disease specific is indicated by findings that sST2 levels *positively* predict other conditions, including mortality in cardiovascular disease [18,19] and disease activity in autoimmune diseases such as juvenile arthritis.[20]

In summary, we show that sST2 serum levels in preschool wheezers do not add to the prediction of doctors' diagnosed asthma as general phenotype at school age. However, sST2 serum levels at age 2-3y inversely correlate with FeNO levels in asthmatic children at 6y. Likewise, IL1-RL1-a serum levels in preschool wheezers contributed to the prediction of a subtype of asthma with elevated FeNO at age 6y, showing a negative direction of effect. Therefore, our study indicates that sST2 might play a protective role in the development of eosinophilic airway inflammation in children who experience asthma at school age. Our findings suggest that sST2 has potential to be further explored as a biomarker in wheezing children to predict the development of asthma with predominant eosinophilic inflammation. A combination of several markers is likely necessary to accurately predict asthma on an individual level. So far exhaled volatile organic compounds demonstrated great potential for the prediction of asthma at age 6y.[2] Furthermore, in future biomarker studies investigating the role of IL-1RL1-a, other measures likely relevant in the development of eosinophilic inflammation should be considered, including sputum and blood eosinophil counts.

Maria E. Ketelaar, MSc^{1,2}

Kim D. G. van de Kant, PhD³

Nicole Dijk, MD¹

Ester M.M. Klaassen, MD PhD³

Néomi Grotenboer, MSc^{1,2}

Martijn C Nawijn, PhD²

Prof. Edward Dompeling, MD PhD³

Prof. Gerard H. Koppelman, MD PhD¹

1. University Medical Center Groningen, University of Groningen, Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, Groningen Research Institute for Asthma and COPD (GRIAC), Groningen, The Netherlands.

2. University Medical Center Groningen, University of Groningen, Department of Pathology and Medical Biology, Laboratory of Experimental Pulmonology and Inflammation Research (EXPIRE), Groningen, The Netherlands.

3. Department of Pediatric Pulmonology, School for Public Health and Primary Care (CAPHRI), Maastricht University Medical Center (MUMC+), Maastricht, The Netherlands.

4. Department of General Practice, School for Public Health and Primary Care (CAPHRI), Maastricht University Medical Center (MUMC+), Maastricht, The Netherlands.

Acknowledgements:

The authors like to thank all the parents and children who participated in this study. This work was supported by unrestricted grants from the Dutch Lung Foundation (AF 3.2.09.081JU and AF 3.4.05.033) and the Stichting Astmabestrijding (SAB 2006/018), and the Maastricht University Medical Center (PF 294). MEK was the recipient of an ERS/EMBO Fellowship LTRF-2013_2060.

References:

1. Savenije OE, Kerkhof M, Koppelman GH, Postma DS. Predicting who will have asthma at school age among preschool children. *J Allergy Clin Immunol.* 2012;**130**(2):325-331. doi:10.1016/j.jaci.2012.05.007.
2. Klaassen EM, van de Kant KD, Jöbsis Q, van Schayck OC, Smolinska A, Dallinga JW et al. Exhaled biomarkers and gene expression at preschool age improve asthma prediction at 6 years of age. *Am J Respir Crit Care Med.* 2015; **191**(2):201-207. doi:10.1164/rccm.201408-1537OC.
3. Grotenboer NS, Ketelaar ME, Koppelman GH, Nawijn MC. Decoding asthma: translating genetic variation in IL33 and IL1RL1 into disease pathophysiology. *J Allergy Clin Immunol.* 2013;**131**(3):856-865. doi: 10.1016/j.jaci.2012.11.028.
4. Nygaard U, Hvid M, Johansen C, Buchner M, Fölster-Holst R, Deleuran M et al. TSLP, IL-31, IL-33 and sST2 are new biomarkers in endophenotypic profiling of adult and childhood atopic dermatitis. *J Eur Acad Dermatol Venereol.* 2016; **30**(11):1930-1938. doi:10.1111/jdv.13679.
5. Cayrol C, Girard JP. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. *Curr Opin Immunol.* 2014;**31**:31-37. doi:10.1016/j.coi.2014.09.004.
6. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol.* 2010;**10**(2):103-110. doi: 10.1038/nri2692.
7. Savenije OE, Kerkhof M, Reijmerink NE, Brunekreef B, de Jongste JC, Smit HA et al. Interleukin-1 receptor-like 1 polymorphisms are associated with serum IL1RL1-a, eosinophils, and asthma in childhood. *J Allergy Clin Immunol.* 2011;**127**(3):750-756.e1-5. doi: 10.1016/j.jaci.2010.12.014.
8. Savenije OE, Mahachie John JM, Granell R, Kerkhof M, Dijk FN, de Jongste JC et al. Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. *J Allergy Clin Immunol.* 2014;**134**(1):170-177. doi: 10.1016/j.jaci.2013.12.1080.
9. van de Kant KD, Klaassen EM, Jöbsis Q, Nijhuis AJ, van Schayck OC, Dompeling E et al. Early diagnosis of asthma in young children by using non-invasive biomarkers of airway

- inflammation and early lung function measurements: study protocol of a case-control study. *BMC Public Health* 2009;**29**: 9:210. doi: 10.1186/1471-2458-9-210.
10. Ali M, Zhang G, Thomas WR, McLean CJ, Bizzintino JA, Laing IA et al. Investigations into the role of ST2 in acute asthma in children. *Tissue Antigens*. 2009;**73**(3):206-212. doi: 10.1111/j.1399-0039.2008.01185.x.
11. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO et al. Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Application: an official ATS Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2011;**184**(5): 602-615. doi:10.1164/rccm.9120-11ST.
12. Klaassen EM, van de Kant KD, Jöbsis Q, Høvig ST, van Schayck CP, Rijkers GT et al. Symptoms, but not a biomarker response to inhaled corticosteroids, predict asthma in preschool children with recurrent wheeze. *Mediators Inflamm* 2012. doi:10.1155/2012/162571.
13. Petsky HL, Kew KM, Chang AB. Exhaled nitric oxide levels to guide treatment for children with asthma. *Cochrane Database Syst Rev*. 2016;**9**(11). doi:10.1002/14651858.CD011439.pub2
14. Malerba M, Radaeli A, Olivini A, Ragnoli B, Ricciardolo F, Montuschi P. The Combined Impact of Exhaled Nitric Oxide and Sputum Eosinophils Monitoring in Asthma Treatment: A Prospective Cohort Study. *Curr Pharm Des*. 2015;**21**(32):4752-4762.
15. van de Kant KD, Koers K, Rijkers GT, Lima Passos V, Klaassen EM, Mommers M et al. Can exhaled inflammatory markers predict a steroid response in wheezing preschool children? *Clin Exp Allergy* 2011;**41**(8):1076-1083. doi: 10.1111/j.1365-2222.2011.03774.x.
16. Lee HY, Rhee CK, Kang JY, Byun JH, Choi JY, Kim SJ et al. Blockade of IL-33/ST2 ameliorates airway inflammation in a murine model of allergic asthma. *Exp Lung Res*. 2014;**40**(2):66-76. doi: 10.3109/01902148.2013.870261.
17. Yin H, Li XY, Liu T, Yuan BH, Zhang BB, Hu SL et al. Adenovirus-mediated delivery of soluble ST2 attenuates ovalbumin-induced allergic asthma in mice. *Clin Exp Immunol*. 2012;**170**(1):1-9. doi: 10.1111/j.1365-2249.2012.04629.x.
18. Marino R, Magrini L, Orsini F, Russo V, Cardelli P, Salerno G. Comparison Between Soluble ST2 and High-Sensitivity Troponin I in Predicting Short-Term Mortality for Patients Presenting to the Emergency Department With Chest Pain. *Ann Lab Med*. 2017;**37**(2):137-146. doi: 10.3343/alm.2017.37.2.137.
19. Aimo A, Vergaro G, Passino C, Ripoli A, Ky B, Miller WL et al. Prognostic Value of Soluble Suppression of Tumorigenicity-2 in Chronic Heart Failure: A Meta-Analysis. *JACC Heart Fail*. 2016;**2**: S2213-1779; 30496-6. doi: 10.1016/j.jchf.2016.09.010. [Epub ahead of print]
20. Ishikawa S, Shimizu M, Ueno K, Sugimoto N, Yachie A. Soluble ST2 as a marker of disease activity in systemic juvenile idiopathic arthritis. *Cytokine*. 2013;**62**(2):272-277. doi: 10.1016/j.cyto.2013.03.007.

Figure 1

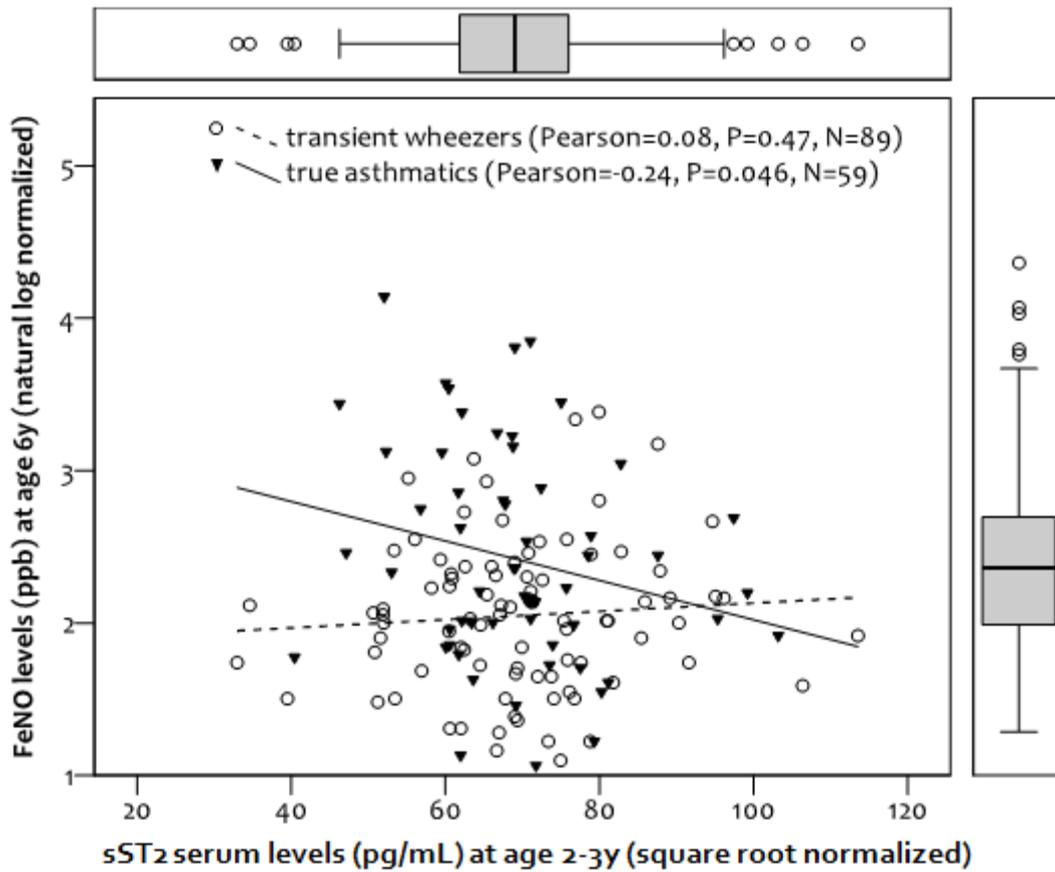


Figure 1- Correlation of sST2 serum levels (2-3y) and FeNO (6y). sST2 serum levels measured in wheezers at age 2-3y negatively correlate with fraction of exhaled nitric oxide levels (FeNO, online method) in children who have developed asthma at age 6y. Boxplots show the quartiles and outliers of the distribution of sST2 (x-axis) and FeNO (y-axis). *ppb*=parts per billion.

Figure 2A

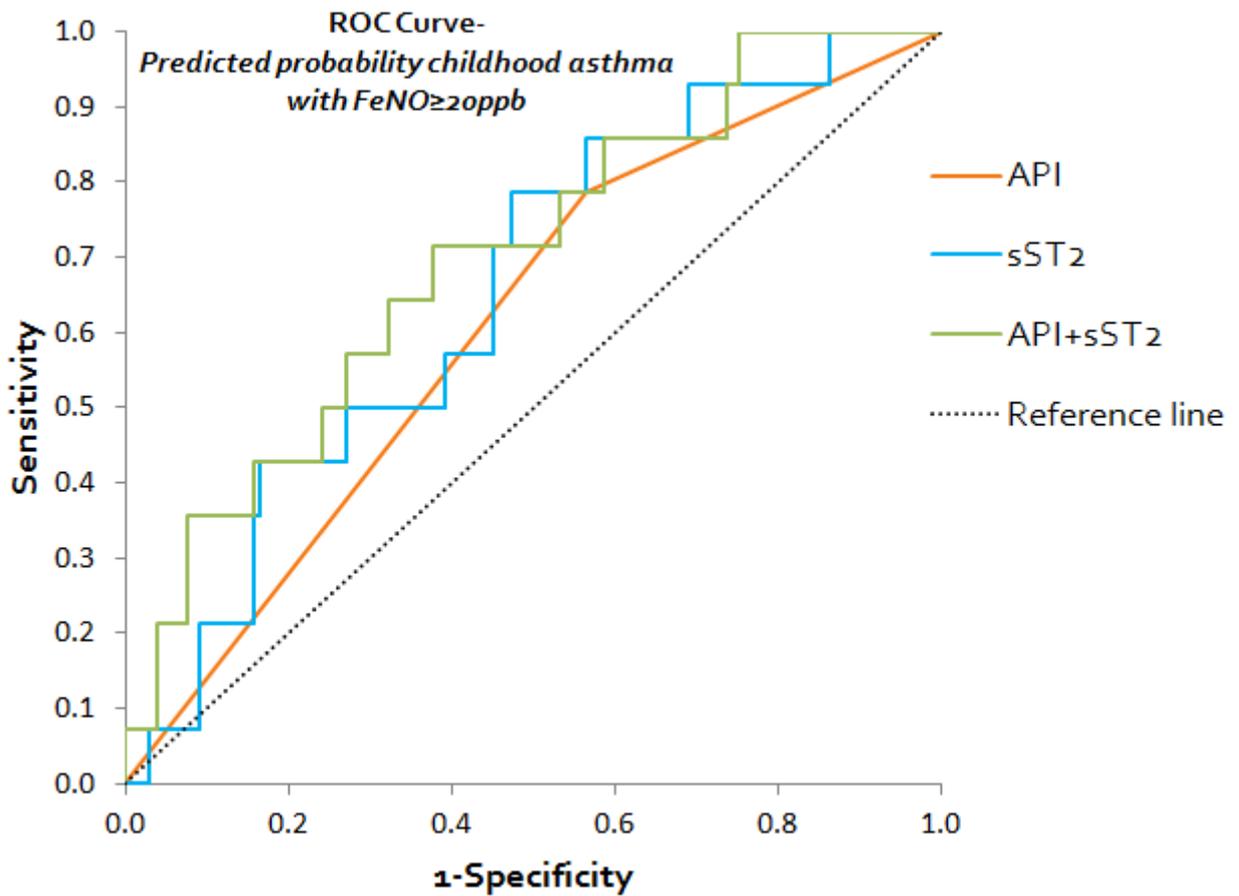


Figure 2- Prediction of asthma with elevated FeNO in childhood using sST2 serum levels and the API
A- Receiver Operating Characteristics (ROC) curve comparing the predictive value of serum sST2 levels, the Asthma Prediction Index (API), and their combined predictive value for development of childhood asthma with FeNO \geq 20ppb to the Reference Line (representing the o-hypothesis of a predicted area under the curve (AUC)= 0.5).

Figure 2B

	MODEL: sST2 only or API only				MODEL: sST2 and API combined							
	Pred AUC (95CI)	B	OR	P value (OR)	Pred AUC (95CI)	B	OR	P value (OR)	PPV	NPV	Sens	Spec
Constant	-	0.24	1.28	P=0.85	-	-0.07	0.93	P=0.89				
Serum sST2	0.65 (0.52-0.79) (P=0.059)	-0.04	0.96	P=0.04	0.65 (0.52-0.79) (P=0.059)	-0.05	0.96	P=0.03	0.11	0.96	0.86	0.33
API	0.62 (0.49-0.76) P=0.11	1.18	3.26	P=0.053	0.61 (0.47-0.76) (P=0.17)	1.23	3.43	P=0.049	0.12	0.96	0.80	0.45
API+ Serum sST2	-	-	-	-	0.70 (0.56-0.84) (P=0.01)	-	-	-	0.14	0.95	0.71	0.52

Figure 2- Prediction of asthma with elevated FeNO in childhood using sST2 serum levels and the API
B- Calculations of the AUC and parameters of the logistic regression model of the prediction of asthma with FeNO \geq 20ppb. sST2 serum levels at in 2-3y old wheezers (n=171) significantly predict this eosinophilic subtype of asthma at age 6y (n=15, P=0.04) with an average AUC of 0.65 (95CI 0.52-0.79, P=0.059). The combined logistic model of API+sST2 serum levels has an average AUC of 0.70 (95CI 0.56-0.84, P=0.01).
API: Asthma Prediction Index (based on parental asthma, eczema, allergic rhinitis, wheezing apart from cold, atopy as determined by Phadiatop). Pred AUC: predicted area under the curve. 95CI: 95% confidence interval. B: regression coefficient. NPV=negative predictive value. OR: Odds Ratio. PPV=positive predictive value. Sens=sensitivity. Spec=specificity. Note: P-values of the AUC are compared to the Reference Line (o-hypothesis AUC=0.5).

M.E. Ketelaar et al.

Serum IL-1RL1-a levels predict exhaled NO levels in preschool wheezers who develop asthma

Supplemental Materials and Method:

Study descriptives

The Asthma DEtection and Monitoring [ADEM] study (clinicaltrial.gov: NCT 00422747)[9] is a longitudinal prospective study, aimed to develop an instrument to diagnose asthma at early age, by using biomarkers of airway inflammation. In total, 202 wheezing children and 50 non-wheezing children (healthy controls) were prospectively followed from the age of 2-3y until 6y. Wheezing was defined as two or more wheezing episodes before inclusion, according to the questionnaire developed by the International Study of Asthma and Allergies in Childhood [ISAAC].[9] Four wheezing children were lost during follow-up due to personal constraints of the parents. One child in the control group was diagnosed with asthma at age 6y. The study design of the ADEM cohort has been published before.[9] For the current study, serum levels of soluble Interleukin-1-receptor-like1 (IL-1RL1-a) were studied in the context of the commonly used asthma prediction index [API].[2,9] In total, 171 wheezing children and 39 controls had IL-1RL1 measures available and a diagnosis at age 6y. These groups did not significantly differ from the total population.

Asthma diagnosis

At the age of 6y, a diagnosis of 'healthy' (=never-wheezing), 'transient wheezing' (=wheezing before or at preschool age, but no asthma symptoms at age 6y), or 'true asthmatic' was made by an experienced pediatrician in the field of respiratory medicine. This was based on symptoms, lung function (reversibility to a beta-2-agonist and bronchial hyperresponsiveness) and medication use. In addition, this clinical diagnosis was assessed by a computer-calculated algorithm as described previously.[9] Non-invasive measures of asthma were determined at age 6y, including fraction of exhaled nitric oxide (FeNO). FeNO was measured using the online method (NIOX®; Aerocrine, Solna, Sweden) as published previously.[2,9]

Asthma with elevated FeNO was defined as asthma with FeNO > 20ppb (n=15), representing a group of asthmatic children with intermediate to high level of eosinophilic airway inflammation, following the ATS guide for interpretation of FeNO.[11] The group of asthmatic children with FeNO > 20ppb represented the upper quartile of FeNO in the current cohort.

Asthma prediction measures, determination of IL-1RL1-a protein and IL1RL1 genotype

Asthma prediction index (API) was assessed based on parental asthma, eczema, allergic rhinitis, wheezing apart from cold, and atopy. Besides atopy, variables were reported by questionnaires.[2,9] A positive score on atopy was determined by a specific IgE concentration against a mixture of inhalant and food allergies (Phadiatop infant test, Phadia, Uppsala, Sweden) of ≥ 0.35 kU/L determined at inclusion.[2]

IL-1RL1-a protein levels were determined in serum at inclusion (age 2-3y) using ELISA (IL-1RL1 human Quantikine kit R&D Systems®, Abingdon, United Kingdom). Considering the importance of validating this type of immuno-assays for use in serum/plasma samples, as also highlighted by Nygaard et al (2016)[4], and seen for IL-33 ourselves[S1], the assay was validated for specificity, sensitivity, assay recovery and inter-assay variability using a spiking approach with serum, serial dilutions, freeze/thaw experiments, and reproducibility testing on

separate plates/days. We have tested three kits (R&D DuoSet sST2 ELISA kit, # DY523B, R&D Quantikine ELISA kit #DST200 and MBL sST2 ELISA kit #7638), out of which the R&D Quantikine ELISA was selected for use in the current study, having the best overall performance.

Statistical analysis:

Analyses were done using SPSS Statistics 23 (IBM, Amsterdam, The Netherlands). ANOVA was used to compare serum IL-1RL1-a levels among groups, with Tukey's post-hoc testing in case of $P < 0.05$. In analogy to earlier analyses in the ADEM study [2], a logistic regression model was built to predict asthma diagnosis at age 6y using the API and/or serum protein levels of IL-1RL1-a at 2-3y as predictors. Pearson correlation was used to correlate IL-1RL1 serum levels at age 2-3y with FeNO levels at age 6y. IL-1RL1-a serum values were square root transformed and FeNO levels natural log transformed to meet normality criteria. F-test was used to assess additive value of a predictor to the model, with statistical significance at $P < 0.05$. The model performance was further investigated by quantifying discrimination (Area Under the Curve, AUC) using a receiver operating characteristics (ROC) curve, plotting sensitivity and 1-specificity. Herein, model performance was assessed compared to the Reference Line, representing a predicted AUC of 0.5.

Supplemental Specific References:

S1. Ketelaar ME, Nawijn MC, Shaw DE, Sayers I, Koppelman GH. The challenge of measuring IL-33 in serum using commercial ELISA: lessons from asthma. *Clin Exp Allergy*. 2016;**46**(6):884-887. doi: 10.1111/cea.12718.