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

Chapter 8
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Abstract

Wheezing is common in childhood. However, current prediction models of pediatric asthma have only modest accuracy. Novel biomarkers and definition of subphenotypes may improve asthma prediction. *Interleukin-1-receptor-like-1* (*IL1RL1* or ST2) 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 2-3 years-old wheezers do not predict doctors' diagnosed asthma at age 6 years. 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, 95% CI 0.56-0.84), but had also significant predictive value on its own (AUC=0.65, 95% CI 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.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADEM study</td>
<td>Asthma DEtection and Monitoring study</td>
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<tr>
<td>API</td>
<td>Asthma Prediction Index</td>
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<tr>
<td>FeNO</td>
<td>Fraction of exhaled Nitric Oxide</td>
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<td>IL-1RL1</td>
<td>Interleukin-1 Receptor Like 1</td>
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<tr>
<td>PIAMA study</td>
<td>Prevalence and Incidence of Asthma and Mite Allergy study</td>
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<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
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<td>y</td>
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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. 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. 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.

One potential biomarker for asthma is soluble interleukin-1-receptor-like 1 (IL-1RL1-a or sST2), which is encoded by the IL-1RL1 gene (chr 2) and can be detected in serum. IL-1RL1 is an asthma susceptibility gene identified in genetic studies of pediatric and adult asthma patients. IL-1RL1 has also been linked to blood eosinophilia, IgE (sensitization), eczema, and hay fever. 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. 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.

Previously, we found that IL-1RL1 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. Moreover, IL-1RL1 SNPs were associated with intermediate-onset and late-onset wheezing phenotypes. These children start to wheeze at age 2-3 years, often have allergen sensitization at age 4 years, and are at high risk of subsequent asthma development at school age. However, the expression levels of IL-1RL1 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, as IL-1RL1 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 (clinicaltrials.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. This study included 202 wheezing children and 50 healthy controls who were enrolled from primary care practices in The Netherlands at age 2-3 years and were followed up annually until age 6 years. At age 6 years, 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 2-3 years 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 interassay variability (Further details are provided in the Supplemental Material).

For the current study, in analogy to earlier analyses of the ADEM study, a logistic regression model was built to predict asthma diagnosis at age 6 years 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 6 years, while the remainder were transient wheezers. Predictive analyses were performed in the group with available sST2 levels at age 2-3 years, which were 171 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 \( \text{IL-1RL1} \) genotype (using rs1420101, representing a major LD block in \( \text{IL-1RL1} \)) and sST2 protein expression in serum (ANOVA \( P<.001 \)). Carriers of the asthma-risk allele (A) had lower sST2 levels, which is in the same direction as previously reported in the PIAMA cohort. However, serum levels of sST2 measured at age 2-3 years could not distinguish which of the preschool wheezing children eventually developed asthma at school age (AUC=0.50 [95CI 0.41-0.59, \( P=.98 \]), \( B=0.002 \) [OR=0.998, \( P=.89 \)]. No difference in serum sST2 levels at age 2-3 years was found between children with transient wheeze, true asthmatics, or healthy controls at age 6 years (\( P=.881 \), ANOVA). Consequently, serum sST2 levels at 2-3 years did not significantly add to the prediction of an asthma diagnosis of the commonly used API (API alone: AUC= 0.60 [95% CI 0.52-0.68, \( P=.02 \]); API+IL1-RL1-a: AUC= 0.57 [95CI 0.49-0.66, \( P=.12 \)]. These results show that, although \( \text{IL-1RL1} \) SNPs may affect \( \text{IL-1RL1} \) expression levels, serum sST2 levels in wheezing children at 2-3 years 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. As sST2 serum levels had previously been associated with blood eosinophil numbers in childhood asthma, we hypothesized that sST2 levels at 2-3 years 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, were measured at 6 years (NIOX, Aerocrine, Solna, Sweden). Interestingly, we found that serum levels of sST2 measured at age 2-3 years, although modestly, were negatively correlated with FeNO levels at 6 years in children who had developed asthma (Pearson’s \( R=-0.24, P=.046, N=59 \)), while no significant correlation was observed in transient wheezers (Pearson’s \( R=0.08, P=.47, N=89 \)), 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.

![Figure 1. Correlation of sST2 serum levels (2-3 y) and FeNO (6 y). sST2 serum levels measured in wheezers at age 2-3 y negatively correlate with fraction of exhaled nitric oxide levels (FeNO) in children who have developed asthma at age 6 y. Boxplots show the quartiles and outliers of the distribution of sST2 (x-axis) and FeNO (y-axis). ppb, parts per billion.](image-url)
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 6 years into a group with likely eosinophilic airway inflammation (FeNO≥20ppb, n=15) and asthmatics unlikely to have eosinophilic airway inflammation (FeNO<20ppb, n=60), based on the ATS guideline of FeNO. 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=.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, 95% CI 0.56-0.84). We acknowledge that the sample size of the group with likely eosinophilic airway inflammation at age 6 years (FeNO ≥20 ppb) of this analysis is limited and propose that our findings should be replicated in future studies with larger sample size.

FeNO levels in 2- to 3-y-old wheezers did not have predictive value for FeNO at school age in our cohort (data not shown), nor for asthma development. Moreover, although FeNO levels have previously been found useful in prediction of management of established asthma, FeNO could not predict treatment response in preschool wheezers. Given the time span (3-4 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. Further evidence indicating a potential protective effect of sST2 in (eosinophilic) asthma is 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 hyper-responsiveness. 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 and disease activity in autoimmune diseases such as juvenile arthritis.
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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≥20ppb to the Reference Line (representing the 0-hypothesis of a predicted area under the curve (AUC)= 0.5). (B) Calculations of the AUC and parameters of the logistic regression model of the prediction of asthma with FeNO≥20 ppb. sST2 serum levels in 2- to 3-y-old wheezers (n=171) significantly predict this eosinophilic subtype of asthma at age 6 y (n=15, P=.04) with an average AUC of 0.65 (95% CI 0.52-0.79, P=.059). The combined logistic model of API+sST2 serum levels has an average AUC of 0.70 (95% CI 0.56-0.84, P=.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's 95% CI; 95% confidence interval; B, regression coefficient; NPV, negative predictive value; OR, Odds ratio; PPV, positive predictive value; Sens, sensitivity; Spec, specificity.

P-values of the AUC are compared to the Reference Line (0-hypothesis AUC=0.5).
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 2-3 years inversely correlate with FeNO levels in asthmatic children at 6 years. Likewise, sST2 serum levels in preschool wheezers contributed to the prediction of a subtype of asthma with elevated FeNO at age 6 years, 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 6 years. Furthermore, in future biomarker studies investigating the role of sST2, other measures likely relevant in the development of eosinophilic inflammation should be considered, including sputum and blood eosinophil counts.

**Acknowledgments**

The authors like to thank all the parents and children who participated in this study.

**Clinical implications**

Asthma associated genetic variants cannot be used to predict childhood asthma.
References


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Supplemental Material
Supplemental Methods

Study descriptives
The Asthma DEtection and Monitoring (ADEM) study (clinicaltrial.gov: NCT 00422747) 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). 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. 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).

Asthma diagnosis
At the age of 6y, a diagnosis of ‘healthy’ (=never-wheezing), ‘transient wheezer’ (=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. 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. 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.

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. 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.

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), and seen for IL-33 ourselves, 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.
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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 study2, 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 References


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F. Nicole Dijk, Charlotte Folkersma, Olena Gruzieva, Ashish Kumar, Alet H. Wijga, Ulrike Gehring, Inger Kull, Dirkje S. Postma, Judith M. Vonk, Erik Melén, and Gerard H. Koppelman