

# Exhaled volatile biomarkers for differentiating noisy breathing infants: a pilot study

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## Abstract

**Background** Early differentiation of rattling infants, frequently misdiagnosed as wheezing, is important to prevent under- and overtreatment. Exhaled breath biomarkers reflect metabolic processes and can potentially aid differential diagnosis. This study investigated the potential of exhaled biomarkers in differentiating rattling infants. **Methods** Exhaled breath collected from infants (2-18months) with an adjusted breath sampler was analysed using gas chromatography mass spectrometry (GC-MS) and selected ion flow tube mass spectrometry (SIFT-MS). Linear discriminant analysis was used to classify recovered, mild, moderate and severe rattling infants in a one-vs-all approach. The potential of parent reported outcome about symptoms and burden to improve the discriminant models was also investigated. **Results** Classifying the diagnostic groups (recovered, mild, moderate, severe rattling) based on exhaled breath showed potential with accuracies between 69.12-75.0% for GC-MS and 59.21-69.74% for SIFT-MS. Highest accuracy and specificity was achieved for severe rattling vs all other diagnostic groups. Adding parent reported symptoms in past the three days to the discriminant model increased accuracies (69.12-86.76% GC-MS; 65.79-88.16% SIFT-MS), particularly for moderate and severe rattling infants. The differentiating VOCs were of the type alkane, acids, amine, imine, triazine and ketone. **Conclusion** Exhaled breath analysis has potential to differentiate infants with different rattling severities and recovered infants. Additionally, combining parent reported symptoms in the past three days with exhaled breath biomarkers improved the performance of the diagnostic models.

## Exhaled volatile biomarkers for differentiating noisy breathing infants: a pilot study

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## Running title (max 40 char incl spaces)

Exhaled biomarkers for rattling infants

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The authors have no conflict of interest to declare.

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### **Abstract**

#### Background

Early differentiation of rattling infants, frequently misdiagnosed as wheezing, is important to prevent under- and overtreatment. Exhaled breath biomarkers reflect metabolic processes and can potentially aid differential diagnosis. This study investigated the potential of exhaled biomarkers in differentiating rattling infants.

#### Methods

Exhaled breath collected from infants (2-18months) with an adjusted breath sampler was analysed using gas chromatography mass spectrometry (GC-MS) and selected ion flow tube mass spectrometry (SIFT-MS). Linear discriminant analysis was used to classify recovered, mild, moderate and severe rattling infants in a one-vs-all approach. The potential of parent reported outcome about symptoms and burden to improve the discriminant models was also investigated.

#### Results

Classifying the diagnostic groups (recovered, mild, moderate, severe rattling) based on exhaled breath showed potential with accuracies between 69.12-75.0% for GC-MS and 59.21-69.74% for SIFT-MS. Highest accuracy and specificity was achieved for severe rattling vs all other diagnostic groups. Adding parent reported symptoms in past the three days to the discriminant model increased accuracies (69.12-86.76% GC-MS; 65.79-88.16% SIFT-MS), particularly for moderate and severe rattling infants. The differentiating VOCs were of the type alkane, acids, amine, imine, triazine and ketone.

#### Conclusion

Exhaled breath analysis has potential to differentiate infants with different rattling severities and recovered infants. Additionally, combining parent reported symptoms in the past three days with exhaled breath biomarkers improved the performance of the diagnostic models.

### **Keywords**

Noisy breathing infant, wheezing, rattling, breathomics, volatile organic compounds, exhaled breath biomarkers

### **Introduction**

The nature and management of the noisy breathing infant and preschool child is always a challenge for the paediatrician and general physician. In clinical practice the evaluation of respiratory sounds is based on parental history, describing noises heard at a distance and on doctors' physical qualification of auscultatory findings. Although different lung sounds are well defined<sup>1</sup>, the evaluation by parents and even by physicians

can be problematic. Parents and caregivers use a variety of rather descriptive terms to report respiratory sounds that could often be misleading. They use a variety of terms for the same sound and the same terms for different sounds.<sup>2-4</sup> Parental misinterpreting is well known especially in infants and young children with overuse of the term “wheeze”, describing many different respiratory sounds.<sup>3,5,6</sup> An observational study by Elphick et al. demonstrated that “rattle” in infants younger than 18 months is often labelled by parents as “wheeze”.<sup>6</sup>

Respiratory noises can be considered as clinical features of certain conditions with underlying aetiologies consisting of pathologically distinct processes and different treatment options. Therefore, confusion in terminology must be avoided, and accurate and correct description of breath sounds is important. While early wheeze has become a predictor of subsequent persistent asthma<sup>7-9</sup>, other lung sounds could be a marker for different disease entities, not related to asthma. While the “wheezy infant” is seen as a distinct clinical entity, the “rattling infant” is not, perhaps wrongly. Infants who mainly “rattle”, were less likely to wheeze at older age and more likely to outgrow their noisy breathing.<sup>5,10</sup>

Rattles are believed to be caused by excessive airway secretions, often in combination with a viral infection, which move during normal airflow within the central airways. On occasion rattling disappears after coughing and clearing of airway secretions.<sup>11</sup> Clinical guidelines on the treatment of rattling infants and young children are lacking and only few studies focus on this topic. The need for treatment should depend on the presence of subjective discomfort to avoid needless medication. Wheezes on the other hand, are most commonly associated with airway obstruction due to various mechanisms, e.g., bronchoconstriction, airway wall oedema, intraluminal obstruction (e.g., foreign body or mass), external compression, or dynamic airway collapse.<sup>11</sup>

Differentiating the heterogeneous group of noisy breathing infants is important to further delineate the most appropriate approach, given that they have different aetiologies, natural histories and different responses to therapy.<sup>5</sup> An early differential diagnosis by the use of non-invasive or minimally invasive techniques is of high importance for a “precision medicine” approach preventing under- and overtreatment. Exhaled breath analysis has potential to improve noisy breathing diagnosis. Volatile organic compounds (VOCs) in exhaled breath can be valuable biomarkers that reflect metabolic processes. This study aims to investigate the clinical potential of exhaled volatile biomarkers in the differentiation of noisy breathing infants. The current manuscript examined whether the severity of rattling could be estimated from the exhaled breath profile.

## Methods

### *Study population*

Infants aged between 2 and 18 months diagnosed with noisy breathing, either clear wheezing or clear rattling, were recruited at a paediatric clinical practice in Hasselt, Belgium after parents provided informed consent. Exclusion criteria were preterm birth (gestational age < 37 weeks), congenital or genetic disorders, chronic respiratory diseases. All participants were treated according to standard clinical practice given that treatment did not depend on the study and vice versa. Two follow-up visits were planned three and six weeks after the first consult. At all three visits the paediatrician scored the intensity and duration of rattling and/or wheezing heard during auscultation. Based on the paediatrician’s rattle and wheeze scores, patients were classified into mild, moderate or severe rattling or recovered. Additionally, exhaled breath was collected at every visit. The study has been approved by the ethical review committee of the Jessa Hospital in Hasselt and with the advice of the medical ethics committee of the University of Hasselt (nr. B243201837634).

### *Questionnaires*

Parents filled out an initial questionnaire about medical history, living environment, and quality of life. In the period between the first and final visit ( $\pm$  six weeks), parents kept a questionnaire based journal about their child’s symptoms. The most clinically relevant parameters extracted from these questionnaires were age at onset of noisy breathing, age at first cold, number of colds, parent reported outcome about burden in the past three days (PRO<sub>b</sub>) and parent reported outcome about symptoms in the past three days (PRO<sub>s</sub>).

### *Exhaled breath collection and analysis*

Exhaled breath was collected using an adjusted ReCIVA<sup>®</sup> breath sampler (Owlstone Medical, Cambridge, UK) combined with the CASPER<sup>®</sup> clean air supply pump (Owlstone Medical, Cambridge, UK) which provided filtered inspiratory air to minimize background contamination from ambient air. Details about the adjustments of the breath sampler can be found in supplement section 1). The influence of the adjustments to the breath sampler was assessed separately and the VOC concentrations measured were similar using both the adjusted and the original sampler (see supplement section 2). Exhaled breath samples were analysed using thermal desorption (TD) gas chromatography mass spectrometry (GC-MS) and TD selected ion flow tube mass spectrometry (SIFT-MS). Details about the GC-MS and SIFT-MS analysis of the exhaled breath samples are provide in supplement section 3.

### *Statistical analysis*

Statistical analysis was performed in IBM SPSS Statistics for Windows version 28.0.1.0 (IBM Corp., NY, USA) for both GC-MS and SIFT-MS data. Kruskal-Wallis tests with pairwise comparisons were performed to determine significant differences between recovered, mild rattling, moderate rattling and severe rattling infants for each of the 32 VOCs detected by GC-MS. For SIFT-MS data, an additional dimension reduction was performed on all 167 detected features by means of principal component analysis. Again Kruskal-Wallis tests with pairwise comparisons were performed to determine significant differences between recovered, mild rattling, moderate rattling and severe rattling infants, for each of the principal components (PC). Discriminant analysis with leave-one-out cross-validation was performed with the significantly different VOCs for the GC-MS data and with the significantly different PCs for the SIFT-MS data. The quality of the discrimination models (DMs) was evaluated with receiver operating characteristics (ROC) curve analysis.

To investigate whether PRO parameters could improve the DM based on the VOCs for GC-MS data or the PCs for SIFT-MS data, Pearson correlation coefficients were used to assess the correlation between the paediatrician’s rattle score and the most clinically relevant parameters extracted from the questionnaires (age at onset of noisy breathing, age at first cold, number of colds, PRO<sub>b</sub> and PRO<sub>s</sub>). Furthermore, Kruskal-Wallis tests were used to assess if those clinically relevant parameters were significantly different between the four groups (recovered, mild rattling, moderate rattling and severe rattling infants). In case a PRO parameter correlated with the paediatrician’s rattle score or was significantly different between the four groups, is was added to the DMs. For comparison, a third DM including only the PRO parameter(s) was used.

## **Results**

### *Study population*

Twenty-nine patients participated in the study. Only two participants dropped out before the third visit and did not complete the questionnaires. In total 85 breath samples were collected. The study included two wheezing infants and two infant with a mixed (rattle-wheezing) phenotype. Table 1 shows the patient characteristics of the participants that were diagnosed with rattling and were included in the statistical analysis.

Fifty-eight of the 85 breath samples were collected easily while the child remained calm without any discomfort. Three samplings were unsuccessful because the child was restless and did not tolerate the mask of the breath sampler. Some samples were collected with more difficulty (6/86), while the child was crying (12/85) or while the child had a pacifier in his/her mouth in order to keep him/her calm (6/85).

### *Clinical parameters*

Only PRO<sub>s</sub> showed moderate Pearson correlations with the paediatrician’s rattle score (GC-MS dataset:  $r=0.553$ ,  $p<0.001$ ; SIFT-MS dataset:  $r=0.561$ ,  $p<0.001$ ). This clinical parameter differed significantly between recovered, mild rattling, moderate rattling and severe rattling infants (GC-MS dataset:  $p<0.001$ ; SIFT-MS dataset:  $p<0.001$ ).

### *GC-MS & SIFT-MS results*

Seven VOCs differed significantly between recovered, mild rattling, moderate rattling and severe rattling infants (table 2). No VOCs differed significantly between ‘recovered’ and ‘mild rattling’ infants. 2D scatterplot based on ethylenimine and methenamine showed overlap between recovered and mild rattling infants (figure 1). Additional analysis of the recovered and mild rattling group versus the moderate and severe rattling group showed that eight VOCs were significantly different (table 2).

Three DMs were developed for each of the four diagnostic groups and compared in a one-vs-all approach. DM 1 included the significant VOCs, DM 2 also included PRO<sub>s</sub> and DM 3 only included PRO<sub>s</sub>. The same DMs were developed for the differentiation between recovered/mild rattlers and moderate/severe rattlers. ROC-AUC, accuracy, sensitivity and specificity are shown in table 3 and table 4. Results of the SIFT-MS were very similar to those of the GC-MS (supplement section 4). Accuracies of the 3 DMs based on exhaled breath ranged between 59.21-69.74%, while for the DMs based on exhaled breath and PRO<sub>s</sub> they ranged between 65.79-88.16%.

## Discussion

This prospective study in real-life paediatric secondary care setting investigated the potential of exhaled volatile biomarkers in the differentiation of noisy breathing infants presenting with wheezing or rattling. Due to the low number of wheezing infants included in the study, the statistical analysis focused on the differentiation of rattling infants with different severity from recovered infants. The severity was a classification based on the paediatrician’s diagnosis. Collecting exhaled breath samples from infants using an in-house adjusted commercial breath sampler was feasible and acceptable. In order to avoid difficulties during breath sampling, placing the child on the parent’s lap, distracting it with toys or movies and assistance from the parent were very important.

Offline GC-MS and SIFT-MS analysis showed similar performance in discriminating the different patient groups (recovered, mild rattling, moderate rattling and severe rattling). The differentiation of recovered/mild rattlers and moderate/severe rattlers showed slightly better performance than the four group discrimination. Both the four group and the two group discrimination improved by combining exhaled breath data with PRO<sub>s</sub>. For comparison, we also ran a discriminant model using only PRO<sub>s</sub>. This resulted in much lower performance, particularly for the discrimination of mild, moderate and severe rattling.

We used both GC-MS and SIFT-MS analytical techniques to explore their diagnostic capacity. GC-MS allows the detection and identification of compounds, and is the current golden standard for breath VOC analysis. However, it cannot be used for real-time exhaled breath measurements<sup>12</sup>, which limits its potential as a point-of-care application. The instrumental compacity and simplicity of use make SIFT-MS a candidate as point-of-care breath screening system.<sup>13</sup> However, SIFT-MS could not be used for identification of VOCs. Multiple studies have reported the potential of breath VOCs to diagnose respiratory illnesses in adults and children.<sup>14-19</sup> Exhaled VOCs may reflect reactions in the target organ and therefore, reflect a person’s health status. The fact that VOC metabolites are directly measured in the exhaled air, makes them interesting as a potential easy to use point-of-care tool.

Some of the differentiating VOCs identified by GC-MS in the current study, have been previously reported to be associated with asthma, wheezing or viral infections. 2-butanone has been associated with asthma.<sup>20,21</sup> Pentanoic acid was reported as one of the discriminating VOCs for the differentiation of preschool children (1.9-4.5 years) with recurrent wheeze from children without wheeze.<sup>22</sup> Exhaled 2,8-dimethylundecane in adults was shown to be associated with oxidative stress induced by viral infections.<sup>23</sup> A very similar compound, 3,9-dimethylundecane has been described to be altered in wheezing or asthmatic children compared to controls.<sup>19</sup>

The comparison of the different discriminating models in the current study demonstrated that exhaled breath markers can be of value for the discrimination of rattling infants from recovered infants and the differentiation based on severity. PRO<sub>s</sub> also had its value in combination with the exhaled breath markers, but on this own it was less valuable for discrimination of the different groups. However, PRO<sub>s</sub> is subjective and depends on the knowledge of the parents. In this pilot study all parents were specifically informed by a paediatrician

specialized in pulmonology who used sound recordings of wheeze and rattle in infants to demonstrate the specific traits of the sounds, and the difference. Furthermore, the paediatrician explained carefully what the indications of burden to the child were (not eating, not sleeping, less playful). That information may have influenced the added value of the parent reported parameter. The quality of parent's reporting might be lower in a non-informed population. This would be especially the case in primary care where general physicians (GP) are not always aware of the difference between wheezing, rattling, and its severity.

The main limitation of this study was its small sample size which may result in overfitting of the statistical models used for classification. To avoid this, validation of the discriminant model is mandatory i.e. ideally, the sample size should be large enough to split the dataset into a training set (used to train the classification method) and validation set (used to test the trained classification model).<sup>24</sup> Instead of splitting the data, we applied leave-one-out cross validation which is usually used when the number of available samples per class is low (around 20 per class).<sup>24</sup> Another limitation, was the identification of VOCs measured by GC-MS based on the National Institute of Standards and Technology (NIST) library. Although the NIST standard reference database is one of the most popular mass spectral databases for metabolite identification, results show a high rate of false identifications of metabolites.<sup>6</sup> Therefore, identification of VOCs is not 100% certain and standards should be used in follow up studies to check retention time and mass spectrum in order to confirm identification.

Confounders known to effect breath VOC composition are food intake<sup>25</sup>, age and gender.<sup>26</sup> Especially the timing of food intake is difficult to control in the infant population. These confounders may also have played a role in our population. The children were between 5 and 16 months old at the time of inclusion. This age difference may cause differences in exhaled metabolites due to differences in metabolism and food pattern. Medication is also a known confounder<sup>27,28</sup>, however parents were instructed not to give their child medication on the day of the paediatrician visit. Blanchet et al. demonstrated that although the VOC breath profile of males and females are different, the separation between the two groups is not very marked and the difference is not sufficient to discriminate males and females.<sup>26</sup> Additionally, not accounting for confounders resulted in the recruitment of a study population that represents the infant population presenting to the real clinical practice as close as possible.

## Conclusion

In conclusion, exhaled breath VOCs of the type alkanes, acids, amine, imine, triazine and ketone, showed to differentiate between different rattling severities and recovered infants, and especially between recovered/mild and moderate/severe rattlers. Accuracy of the discriminant model based on exhaled breath was higher than the model based only on parent reported outcome, particularly for moderate and severe rattling infants. Additionally, combining this relevant clinical parameters reported by parents with assessment of breath markers showed the potential to improve the diagnosis.

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## Key Message

Wheezing and rattling are two distinct types of noisy breathing with different underlying pathological mechanisms, different treatment options and different long-term scenarios. However, rattling infants are often mislabelled as wheezing leading to overtreatment with bronchodilators and/or inhaled corticosteroids (ICS). ICS will not benefit the rattling infant, but can have adverse effects on linear growth potentially causing significant growth reduction. Currently there are no objective diagnostic tools to diagnose noisy breathing infants and the clinician can solely rely on auscultation, parent reports and physical examination. There is a need for non-invasive and objective techniques to achieve early differential diagnosis which is of high importance for a "precision medicine" approach to prevent under- and overtreatment. In the Non-invasive Noisy breathing Infant study showed that the analysis of exhaled volatile biomarkers could have potential

as a clinical tool for detection and classification of rattling in infants. Future research should investigate the potential of exhaled breath to differentiate wheezing and rattling infants.

## References

1. Cugell DW. Lung sounds nomenclature. *Am J Respir Dis* . 1987;136(4):1016. doi:https://doi.org/10.1164/ajrccm/136.4.1016
2. Elphick HE, Ritson S, Rodgers H, Everard M. When a wheeze is not a wheeze Acoustic analysis of breath sounds in infants. *Eur Respir J* . 2000;16:593-597. doi:10.1034/j.1399-3003.2000.16d04.x
3. Saglani S, Malmström K, Pelkonen A, et al. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. *Am J Respir Crit Care Med* . 2005;171(7):6. doi:10.1164/rccm.200410-1404OC
4. Michel G, Silverman M, Strippoli MP, et al. Parental understanding of wheeze and its impact on asthma prevalence estimates. *Eur Respir J* . Dec 2006;28(6):1124-30. doi:10.1183/09031936.06.00008406
5. Elphick HE, Sherlock P, Foxall G, et al. Survey of respiratory sounds in infants. *Arch Dis Child* . 2001;84:35-39. doi:10.1136/ad.84.1.35
6. Aggio RB, Mayor A, Reade S, Probert CS, Ruggiero K. Identifying and quantifying metabolites by scoring peaks of GC-MS data. *BMC Bioinformatics* . 2014-12-01 2014;15(1)doi:10.1186/s12859-014-0374-2
7. Ducharme FM, Tse SM, Chauhan B. Diagnosis, management, and prognosis of preschool wheeze. *The Lancet* . 2014;383(9928):1593-1604. doi:10.1016/s0140-6736(14)60615-2
8. Morgan WJ, Stern DA, Sherrill DL, et al. Outcome of Asthma and Wheezing in the First 6 Years of Life: Follow-up through Adolescence. *Am J Respir Crit Care Med* . 2005;172(10):1253-58. doi:10.1164/rccm.200504-525OC
9. Turner SW, Palmer LJ, Rye PJ, et al. The relationship between infant airway function, childhood airway responsiveness, and asthma. *Am J Respir Crit Care Med* . Apr 15 2004;169(8):921-7. doi:10.1164/rccm.200307-891OC
10. Turner C, Parekh B, Walton C, Spanel P, Smith D. An exploratory comparative study of volatile compounds in exhaled breath and emitted by skin using selected ion flow tube mass spectrometry. *Rapid Commun Mass Sp* . 2008;22(4):526-532. doi:10.1002/rcm.3402
11. Fouzas S, Anthracopoulos MB, Bohadana A. Clinical Usefulness of Breath Sounds. In: Priftis KN, Hadjileontiadis LJ, Everard ML, eds. *Breath Sounds: From Basic Science to Clinical Practice* . Springer International Publishing AG; 2018:33-52:chap 3.
12. Horvath I, Barnes PJ, Loukides S, et al. A European Respiratory Society technical standard: exhaled biomarkers in lung disease. *Eur Respir J* . Apr 2017;49(4)doi:10.1183/13993003.00965-2016
13. Stefanuto PH, Zanella D, Vercammen J, et al. Multimodal combination of GC x GC-HRTOFMS and SIFT-MS for asthma phenotyping using exhaled breath. *Sci Rep* . Sep 30 2020;10(1):16159. doi:10.1038/s41598-020-73408-2
14. Smolinska A, Klaassen EMM, Dallinga JW, et al. Profiling of Volatile Organic Compounds in Exhaled Breath As a Strategy to Find Early Predictive Signatures of Asthma in Children. *PLoS ONE* . 2014;9(4):e95668. doi:10.1371/journal.pone.0095668
15. van der Schee MP, Hashimoto S, Schuurman AC, et al. Altered exhaled biomarker profiles in children during and after rhinovirus-induced wheeze. *European Respiratory Journal* . 2015-02-01 2015;45(2):440-448. doi:10.1183/09031936.00044414

16. Vijverberg S, Brinkman P, Raaijmakers J, et al. Asthmatic children that are uncontrolled despite inhaled corticosteroids have a distinct breathprint (the pacman2 study). *Clin Transl Allergy* . 2015-03-23 2015;5(S2):O6. doi:10.1186/2045-7022-5-s2-o6

17. Schleich FN, Zanella D, Stefanuto P-H, et al. Exhaled Volatile Organic Compounds Are Able to Discriminate between Neutrophilic and Eosinophilic Asthma. *Am J Respir Crit Care Med* . 2019-08-15 2019;200(4):444-453. doi:10.1164/rccm.201811-2210oc

18. Cavaleiro Rufo J, Madureira J, Fernandes EO, Moreira A. Volatile organic compounds in asthma diagnosis: a systematic review and meta-analysis. *Allergy* . Feb 2016;71(2):175-88. doi:10.1111/all.12793

19. Neerincx AH, Vijverberg SJH, Bos LDJ, et al. Breathomics from exhaled volatile organic compounds in pediatric asthma. *Pediatr Pulmonol* . 2017;52(12):1616-1627. doi:10.1002/ppul.23785

20. Ibrahim B, Basanta M, Cadden P, et al. Non-invasive phenotyping using exhaled volatile organic compounds in asthma. *Thorax* . 2011;66(9):804-809. doi:10.1136/thx.2010.156695

21. van Mastrigt E, Reyes-Reyes A, Brand K, et al. Exhaled breath profiling using broadband quantum cascade laser-based spectroscopy in healthy children and children with asthma and cystic fibrosis. *J Breath Res* . 2016;10(2)doi:https://doi.org/10.1088/1752-7155/10/2/026003

22. van de Kant KD, van Berkel JJ, Jobsis Q, et al. Exhaled breath profiling in diagnosing wheezy preschool children. *Eur Respir J* . Jan 2013;41(1):183-8. doi:10.1183/09031936.00122411

23. Philips M, Cataneo R, Chaturvedi A, et al. Effect of influenza vaccination on oxidative stress products in breath. *Journal of Breath Research* . 2010;4doi:https://doi.org/10.1088/1752-7155/4/2/026001

24. Smolinska A, Hauschild AC, Fijten RRR, Dallinga JW, Baumbach J, van Schooten FJ. Current breathomics — a review on data pre-processing techniques and machine learning in metabolomics breath analysis. *J Breath Res* . 2014;8(027105):20. doi:10.1088/1752-7155/8/2/027105

25. Smolinska A, Baranska A, Dallinga JW, et al. Comparing patterns of volatile organic compounds exhaled in breath after consumption of two infant formulae with a different lipid structure: a randomized trial. *Sci Rep* . 2019-12-01 2019;9(1)doi:10.1038/s41598-018-37210-5

26. Blanchet L, Smolinska A, Baranska A, et al. Factors that influence the volatile organic compound content in human breath. *J Breath Res* . 2017-02-22 2017;11(1):016013. doi:10.1088/1752-7163/aa5cc5

27. van der Schee MP, Palmay R, Cowan JO, Taylor DR. Predicting steroid responsiveness in patients with asthma using exhaled breath profiling. *Clin Exp Allergy* . 2013;43(11):1217-1225. doi:10.1111/cea.12147

28. Bannier MAGE, Kienhorst S, Jöbsis Q, et al. Exhaled Breath Analysis for Investigating the Use of Inhaled Corticosteroids and Corticosteroid Responsiveness in Wheezing Preschool Children. *J Clin Med* . 2022-08-31 2022;11(17):5160. doi:10.3390/jcm11175160

**Tables:**

**Table 1** Patient characteristics

N	N
First paediatrician visit (months, mean & range)	First paediatrician visit (months, mean & range)
Gender (M/F)	Gender (M/F)
First rattle episode (months, mean & range)	First rattle episode (months, mean & range)
Frequency of rattling (often/sometimes/rarely)	Frequency of rattling (often/sometimes/rarely)
Time between rattling episodes (<1week/2-3weeks/>1month)	Time between rattling episodes (<1week/2-3weeks/>1month)
Started with cold (yes/no)	Started with cold (yes/no)
Age at first cold (months, mean & range)	Age at first cold (months, mean & range)
Medication used	<b>Without effect</b>



N	N
Bronchodilators	19 (70.37%)
Inhaled corticosteroids	12 (44.44%)
Leukotriene receptor antagonist	1 (3.70%)

**Table 2** VOCs significantly different between the four patient groups: recovered, mild rattling, moderate rattling and severe rattling infants, based on Kruskal-Wallis test results

Recovered vs mild vs moderate vs severe rattlers	Recovered vs mild vs moderate vs severe rattlers	Recovered/mild rattl
VOC	<b>p-value</b>	<b>VOC</b>
ethylenimine	0.0004	ethylenimine
methenamine	0.0359	methenamine
2-butanone	0.0083	2-butanone
2,8-dimethylundecane	0.0350	2,8-dimethylundecan
1,3,5-Triazine	0.0010	methyl ester acetic a
pentanoic acid	0.0274	4-ethyl-2,2,6,6-tetran
3,3-dimehtylpentane	0.0486	2,6,6-trimethyl-octan
		Methyl ester tridecar

**Table 3** results of three discriminant models based on GC-MS data differentiating recovered, mild rattling, moderate rattling and severe rattling infants in a one-vs-all approach; PRO<sub>s</sub>: parent reported outcome about symptoms in the past three days

Discriminant model	Diagnostic class	AUC (95% CI)	Accuracy	Sensitivity	Specificity	PPV	NPV
7 VOCs	Recovered	0.833 (0.731-0.935)	69.12%	68.75%	69.23%	40.74%	87.80%
	Mild	0.899 (0.826-0.972)	70.59%	91.67%	66.07%	36.67%	97.37%
	Moderate	0.865 (0.770-0.961)	70.59%	68.75%	71.15%	42.31%	88.10%
	Severe	0.869 (0.772-0.967)	75.00%	66.67%	79.55%	64.00%	81.40%
7 VOCs + PRO <sub>s</sub>	Recovered	0.952 (0.903-1)	86.76%	81.25%	88.46%	68.42%	93.88%
	Mild	0.930 (0.871-0.990)	69.12%	83.33%	66.07%	34.48%	94.87%
	Moderate	0.901 (0.820-0.983)	70.59%	62.50%	73.08%	41.67%	86.36%
	Severe	0.893 (0.799-0.987)	83.82%	83.33%	84.09%	74.07%	90.24%
PRO <sub>s</sub>	Recovered	0.845 (0.713-0.977)	89.71%	75.00%	94.23%	80.00%	92.45%
	Mild	0.532(0.355-0.709)	33.82%	83.33%	23.21%	18.87%	86.67%
	Moderate	0.644 (0.508-0.781)	45.59%	100.00%	28.85%	30.19%	100.00%
	Severe	0.638 (0.507-0.769)	54.41%	95.83%	31.82%	43.40%	93.33%

Legend: PPV=positive predictive value, NPV= negative predictive value

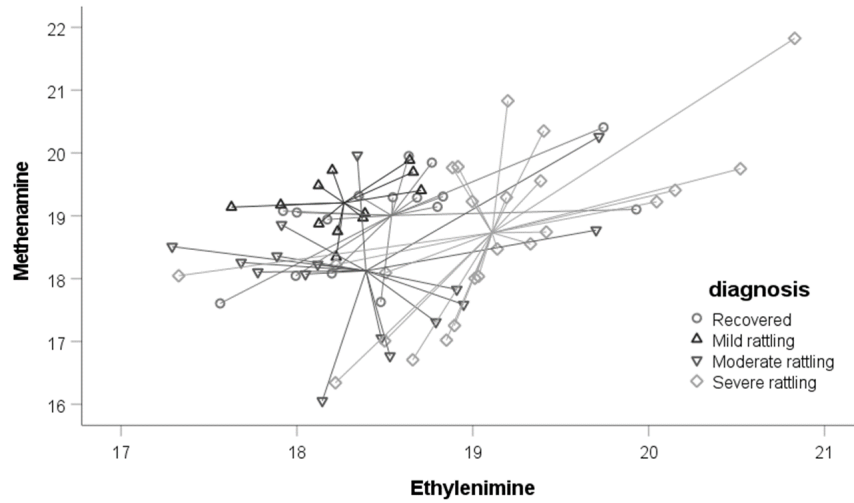
**Table 4** results of three discriminant models based on GC-MS data differentiating recovered/mild rattling and moderate/severe rattling infants; PRO<sub>s</sub>: parent reported outcome about symptoms in the past three days

Discriminant model	AUC (95% CI)	Accuracy	Sensitivity	Specificity	PPV	NPV
8 VOCs	0.898 (0.821-0.975)	75.00%	78.57%	72.50%	66.67%	82.86%
8 VOCs + PRO <sub>s</sub>	0.933 (0.874-0.992)	80.88%	75.00%	85.00%	77.78%	82.93%

Discriminant model	AUC (95% CI)	Accuracy	Sensitivity	Specificity	PPV	NPV
PRO <sub>s</sub>	0.737 (0.607-867)	77.94%	50.00%	97.50%	93.33%	73.58%

Legend: PPV=positive predictive value, NPV= negative predictive value

**Figures:**



**Figure 1** Two dimensional scatterplot based on Ethylenimine and methenamine from GC-MS data of recovered, mild rattling, moderate rattling and severe rattling infants. Each data point represents one patient; the center of the dot cloud represents the mean value of the components.

