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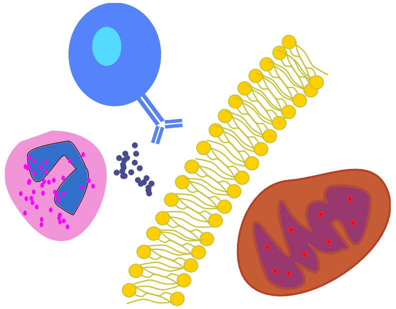
Breathomics in asthma diagnosis and monitoring

The altered volatilome in patients with airway inflammation



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JCR is funded by the European Regional Development Fund (FEDER), through the Competitiveness and Internationalization Operational Programme, and by national funding from FCT under the scope of the EXALAR 21 project: PTDC/GES-AMB/30193/2017 (POCI-01-0145-FEDER-030193).

CHARACTERIZATION OF ASTHMA

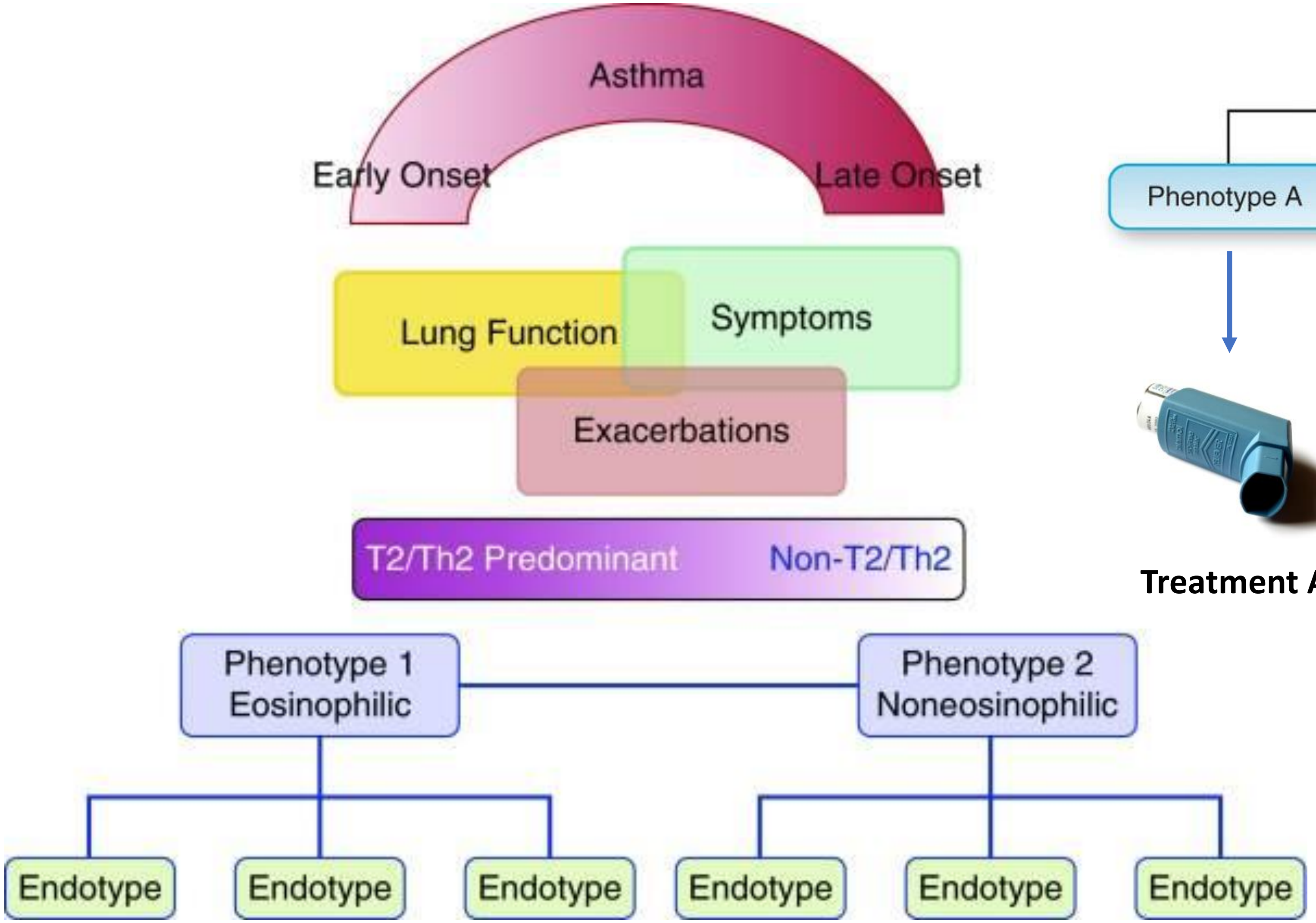
- Chronic non-communicable airway disease.
- Highly prevalent in developed countries
- Airflow obstruction, bronchial inflammation and hyperresponsiveness.
- Associated symptoms include wheezing, dyspnoea and dry cough.

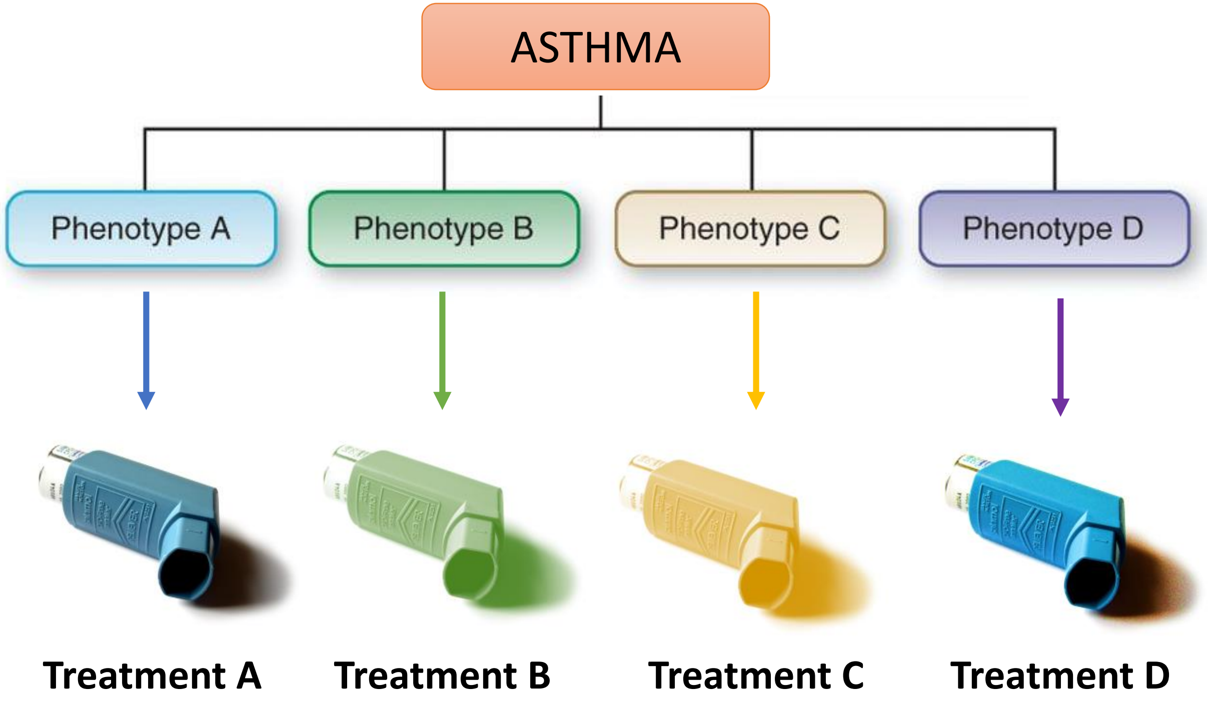
"Asthma should solely be used as a descriptive label for a collection of symptoms"

Pavord, I. D. *et al. Lancet*. 2018

Lemanske, R. F. & BUSSE, W. W. *Asthma: clinical expression and molecular mechanisms.* 2010. ***J Allergy Clin Immunol***, 125, S95-102.

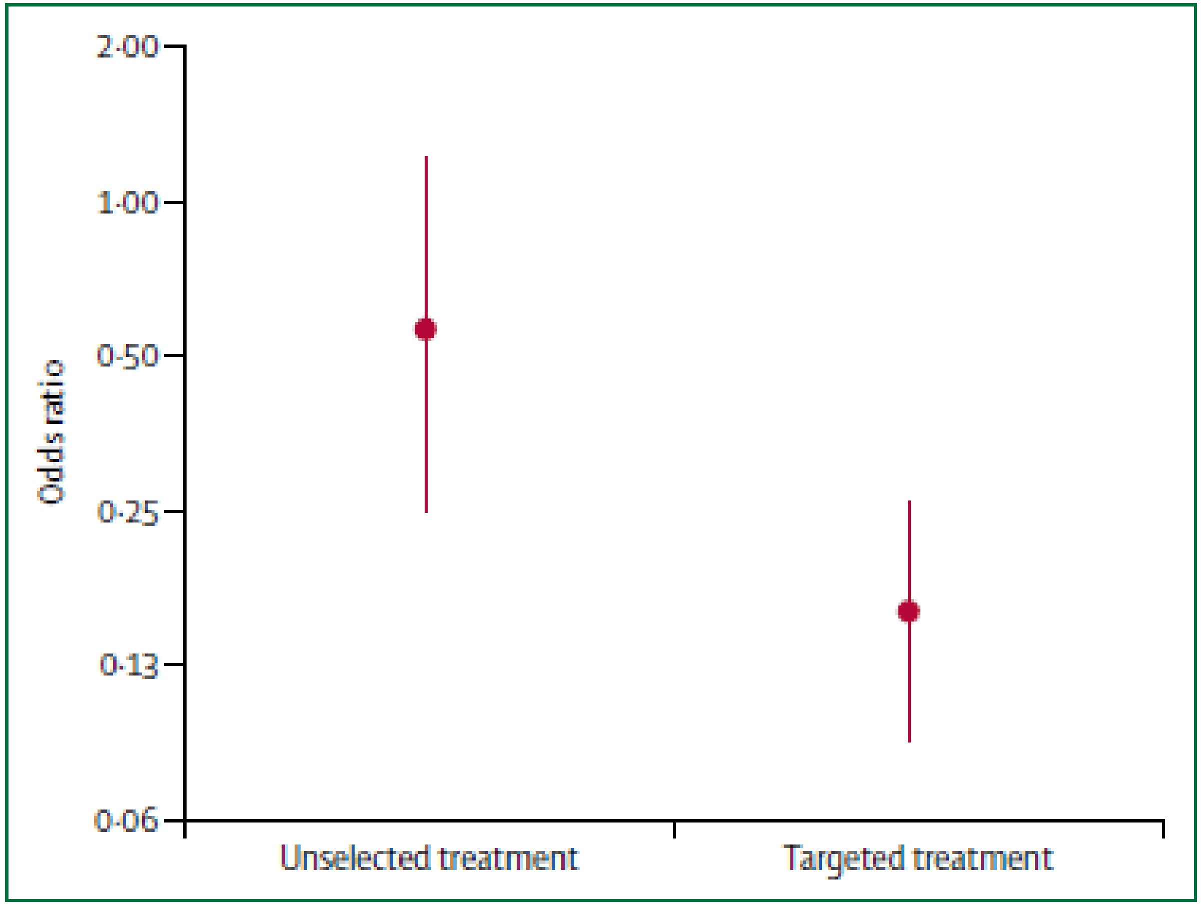
THE UMBRELLA TERM "ASTHMA"





SE Wenzel. *Asthma phenotypes: the evolution from clinical to molecular approaches.* 2012. ***Nature Medicine***, 18(5):716-25.
Tara F Carr, *et al*. *Eosinophilic and Noneosinophilic Asthma*. 2018. ***Am J Respir Crit Care Med***, 197(1): 22–37.

DIFFERENT PHENOTYPES, DIFFERENT TREATMENTS



Pavord, I. D. *et al. After asthma: redefining airways diseases*. 2018. ***Lancet***. 391(10118):350-400
SE Wenzel*. Asthma phenotypes: the evolution from clinical to molecular approaches.* 2012. ***Nature Medicine***, 18(5):716-25.

THE NEED FOR IMPROVED ASTHMA DIAGNOSIS AND MONITORING SOLUTIONS

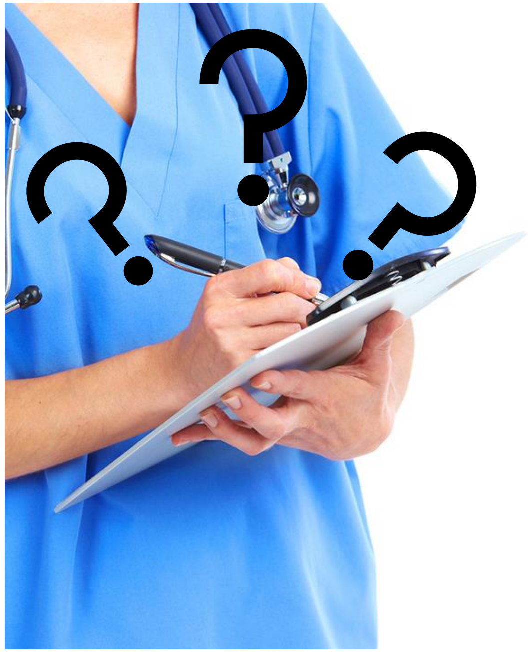
Currently available point-of-care solutions for asthma diagnosis and monitoring

<u>Spirometry with bronchodilation</u>
- Low sensitivity
- Requires patient cooperation
- Low reproducibility

<u>FeNO</u>
- Low specificity
- Highly susceptible to confounders
- Only works for eosinophilic asthma

<u>Skin-prick-tests</u>
- Meant for identifying atopic patients

<u>Self-reported history of symptoms</u>
- Unspecific and biased



WHAT BIOMARKERS WOULD WE NEED FOR AN IMPROVED ASTHMA DIAGNOSIS?

“Among the various omics technologies, those that can be measured **at point of care** are likely to **prevail in clinical practice**”.

Bos *et al.* (2016)

FAST

To collect
To analyse

NON-INVASIVE

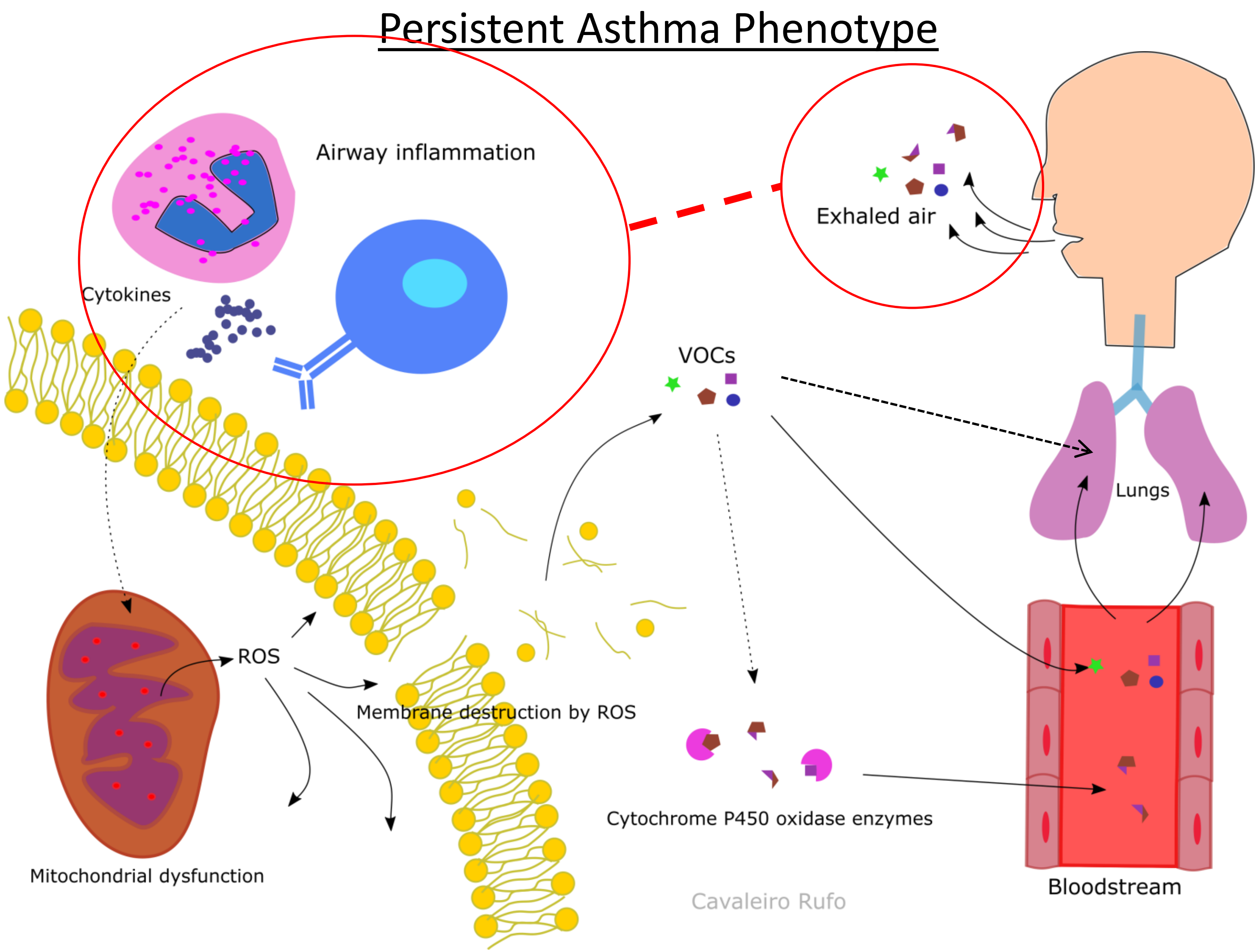
Safe to collect
Minimal discomfort to patient

SIMPLE

Easy to use


scotland.police.uk

VOC PROFILES ASSOCIATED WITH ASTHMA PATHOPHYSIOLOGY



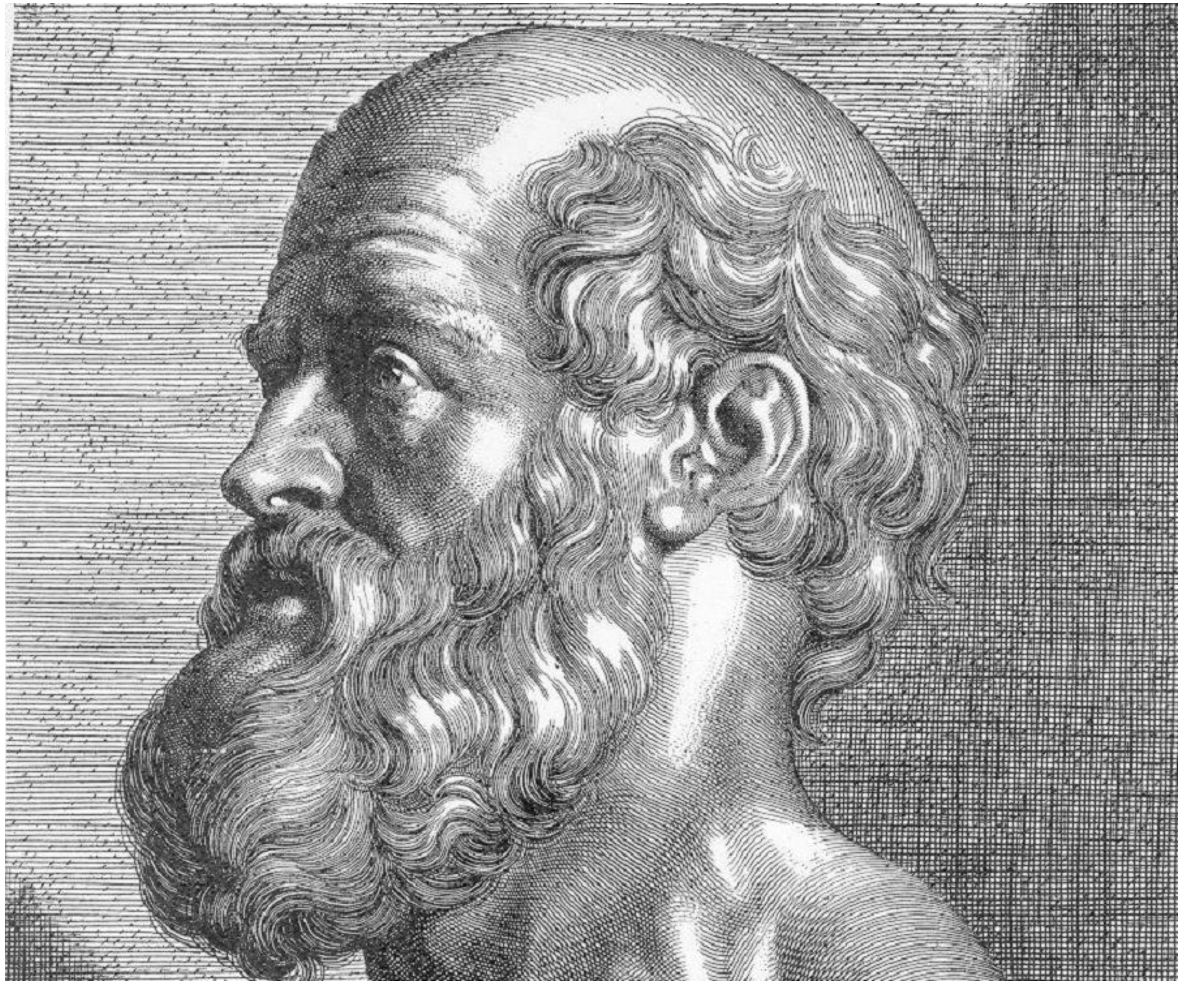
J. Cavaleiro Rufo. 2018. ***Paediatric asthma: from environmental determinants towards diagnostic breathomics***.
Bos, L. D., *et al*. 2016. ***J Allergy Clin Immunol*, **138**,** 970-976.

BREATHOMICS FOR CLINICAL APPLICATIONS

Oscar, the breathomics cat

DOSA, D. M. 2007. *A day in the life of Oscar the cat*. **N Engl J Med**, 357, 328-9.

BREATHOMICS FOR CLINICAL APPLICATIONS



- **Odours** from pathological origins.
- Faecal breath characteristic of **liver disease**.
- Stale beer smell exhaled by patients with **tuberculosis**.

Loudon, I., 1994. ***Medical History, 38, 226-227***.

BREATHOMICS FOR CLINICAL APPLICATIONS

Parkinson's smell test explained by science

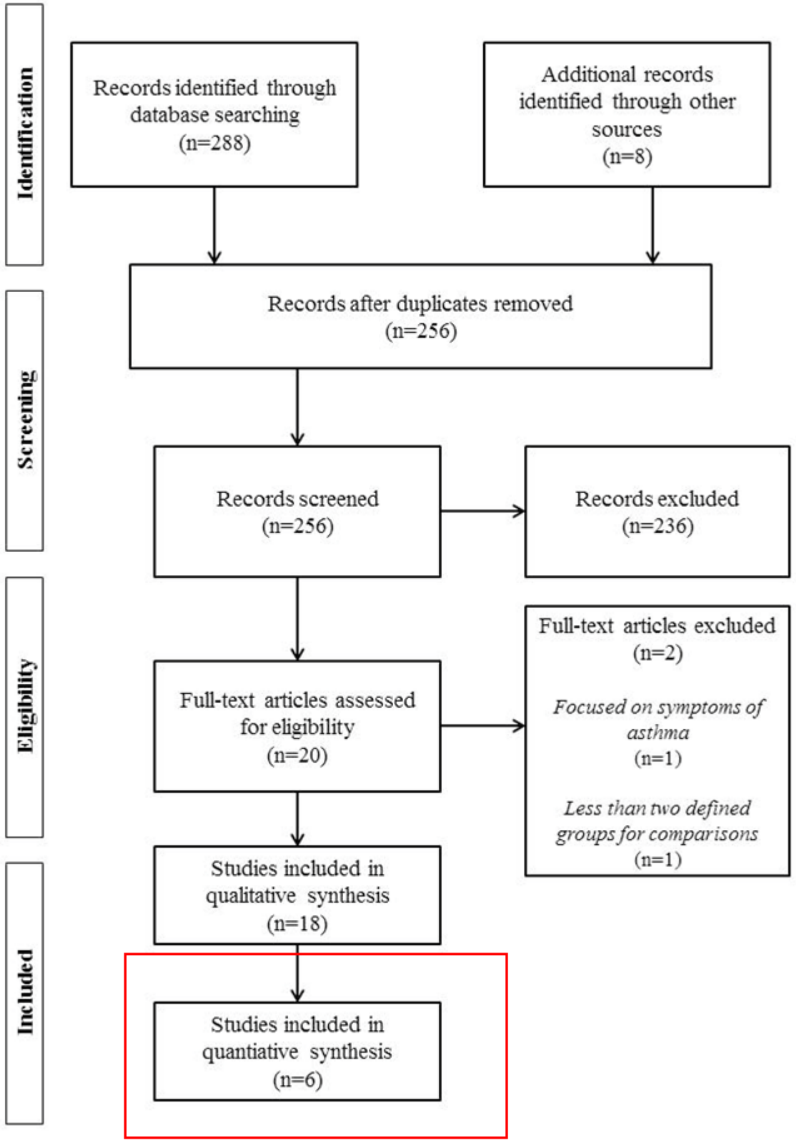
By Elizabeth Quigley
BBC Scotland news

20 March 2019

Share

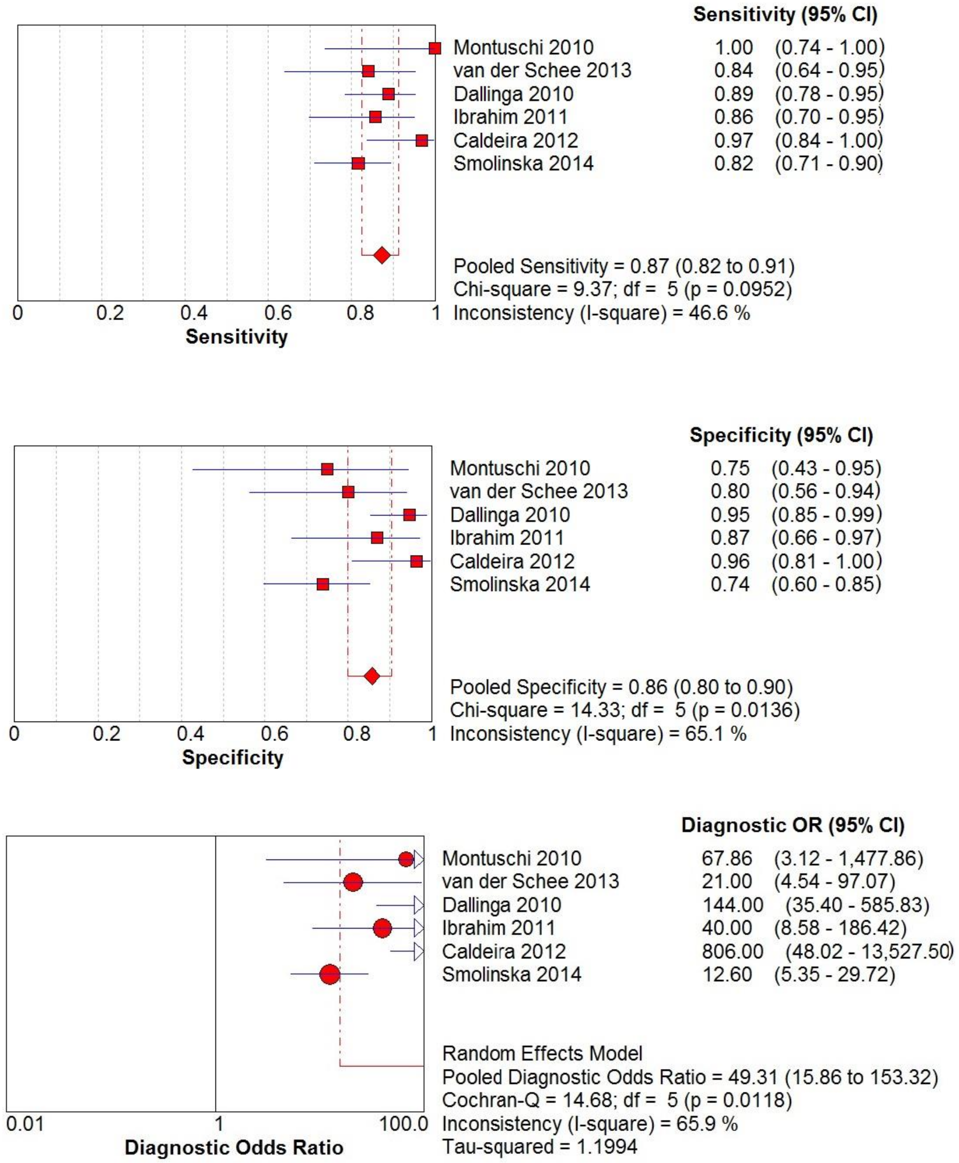
Joy Milne can smell Parkinson's disease before it is medically diagnosed

BREATHOMICS IN ASTHMA DIAGNOSIS



Cavaleiro Rufo J. et al. 2016. *Volatile organic compounds in asthma diagnosis: a systematic review and meta-analysis*. **Allergy**, 71, 175-88.

BREATHOMICS IN ASTHMA DIAGNOSIS



Reference	Sensitivity	Specificity
(Montuschi *et al.*, 2010)	100%	75%
(van der Schee *et al.*, 2013)	84%	80%
(Dallinga *et al.*, 2010)	89%	95%
(Ibrahim *et al.*, 2011)	85%	89%
(Caldeira *et al.*, 2012)	96%	95%
(Smolinska *et al.*, 2014)	82%	74%

Method	Sensitivity (%)	Specificity (%)
Spirometry	16	100
Spirometry + Bronchodilator reversibility > 12%	36	90

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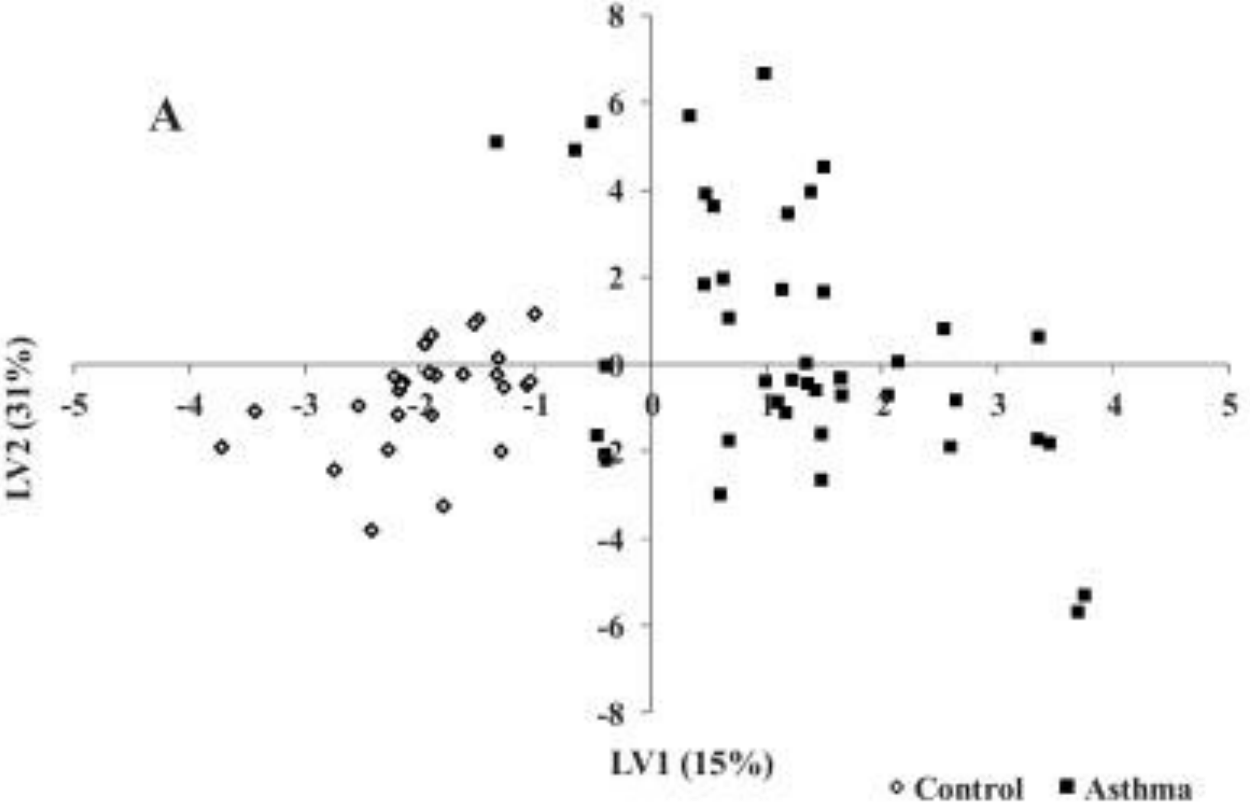
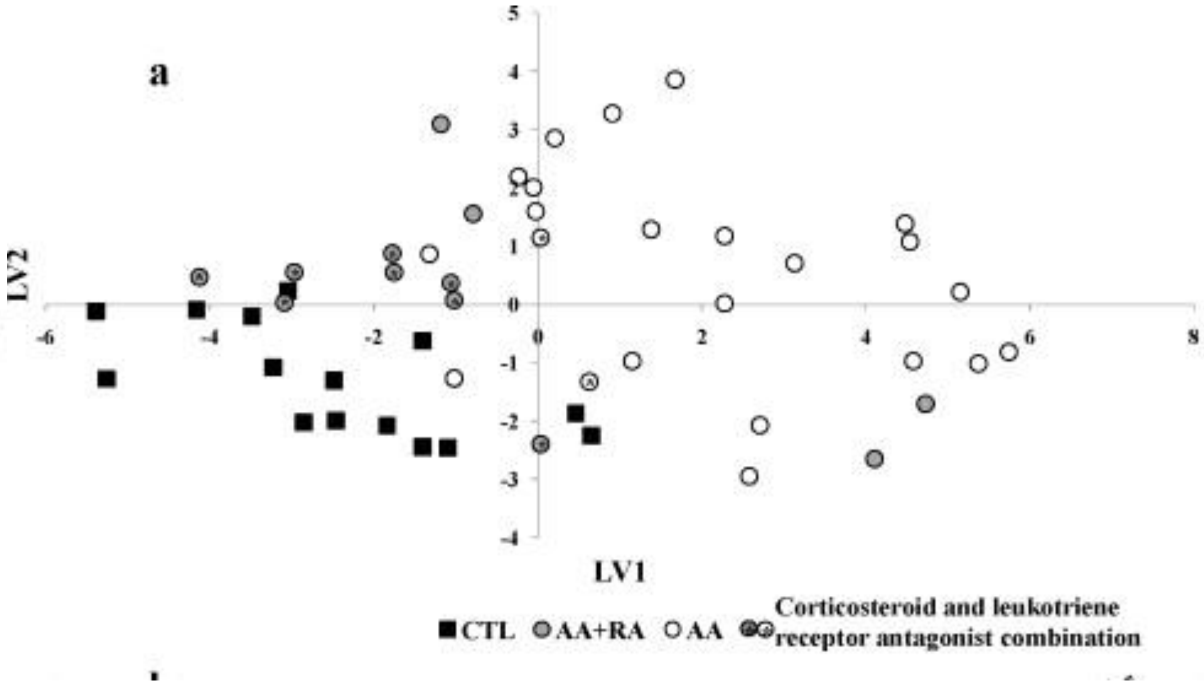
BREATHOMICS IN ASTHMA DIAGNOSIS



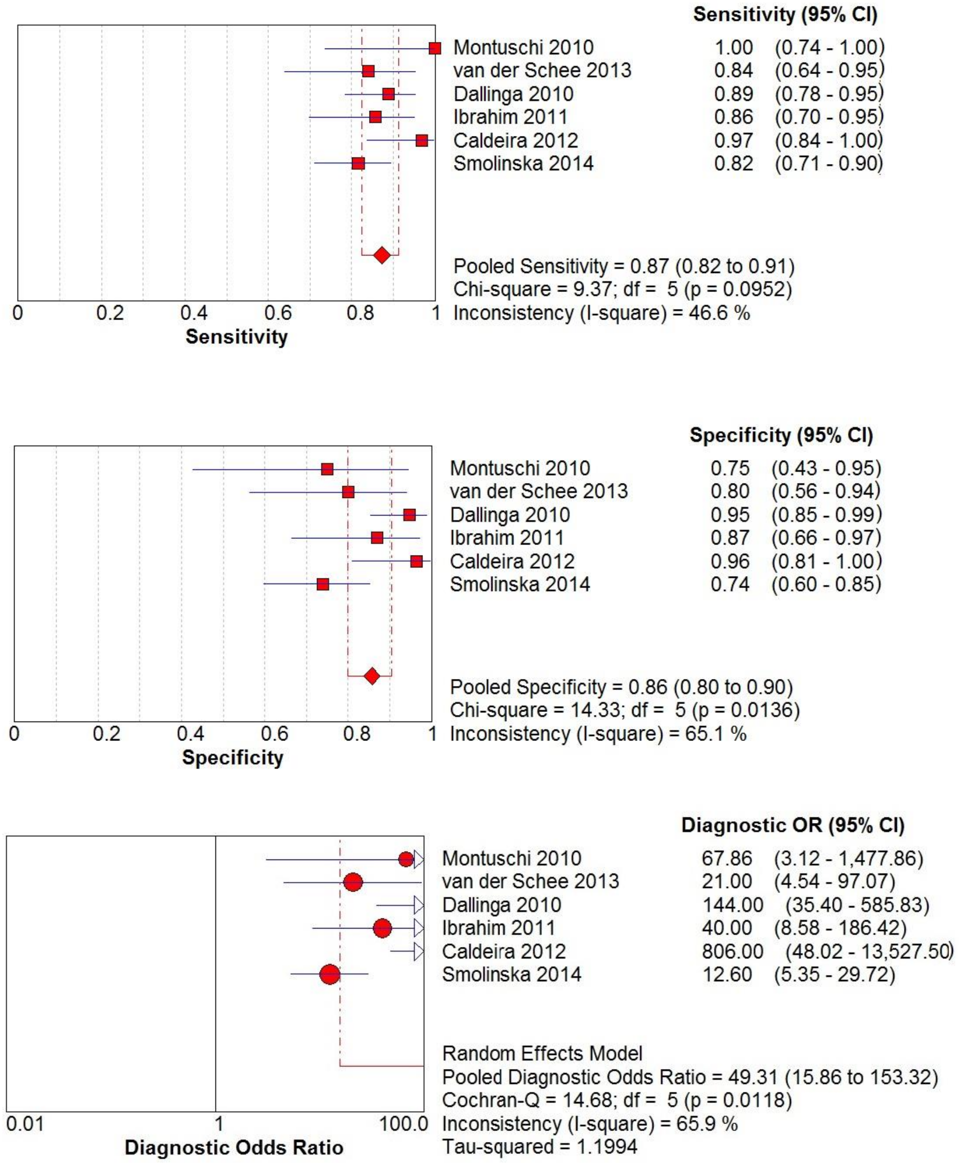


Caldeira M, et al. 2012. *Allergic asthma exhaled breath metabolome: a challenge for comprehensive two-dimensional gas chromatography*. **J Chromatogr A.** 7:87–97.
Caldeira M, et al. 2011. *Profiling allergic asthma volatile metabolic patterns using a headspace-solid phase microextraction/gas chromatography based methodology*. **J Chromatogr A.** 17:3771–3780.

BREATHOMICS IN ASTHMA DIAGNOSIS

Chromatographic lab at Wageningen University & Research, Netherlands

BREATHOMICS IN ASTHMA DIAGNOSIS



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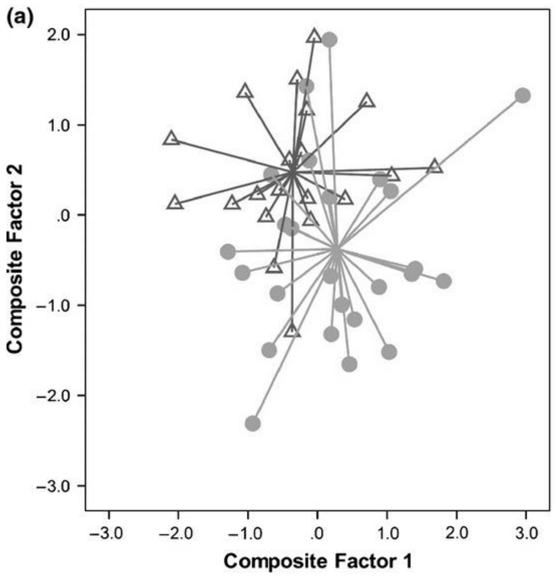
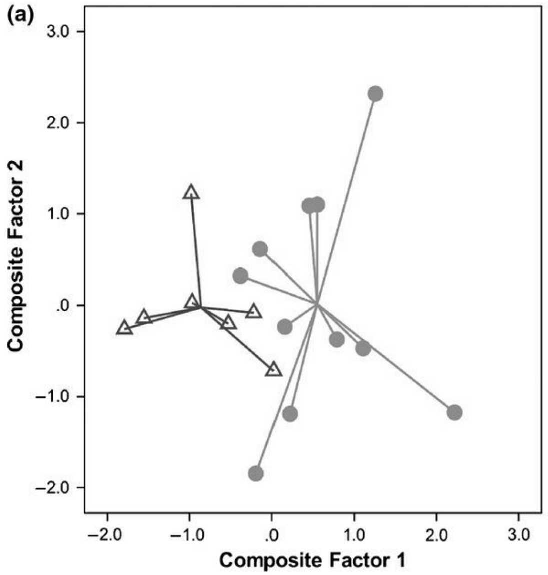
PORTABLE ELECTRONIC NOSES

Table 4—*Diagnostic Classification With 95% of CIs in Training and Testing Phase for Data Related to Combination of Electronic Nose, FENO, and Spirometry*

Technique	Classification Rate, %	Testing
E-nose (alveolar exhaled air)	92	86.2
E-nose (total exhaled air)	96	72.5
FENO	98.5	77.1
Spirometry	96	70.5
FENO and e-nose (alveolar exhaled air)	98.7	92.7
FENO and e-nose (total exhaled air)	99.1	86.6
Spirometry and e-nose (alveolar exhaled air)	99.3	81.8
FENO and spirometry	98.5	81.3
Spirometry and e-nose (total exhaled air)	99.1	81.1



Discrimination of steroid free patients with asthma (full circles) from healthy controls (empty triangles) by electronic nose: sensitivity = 80.0%; specificity = 65.0%



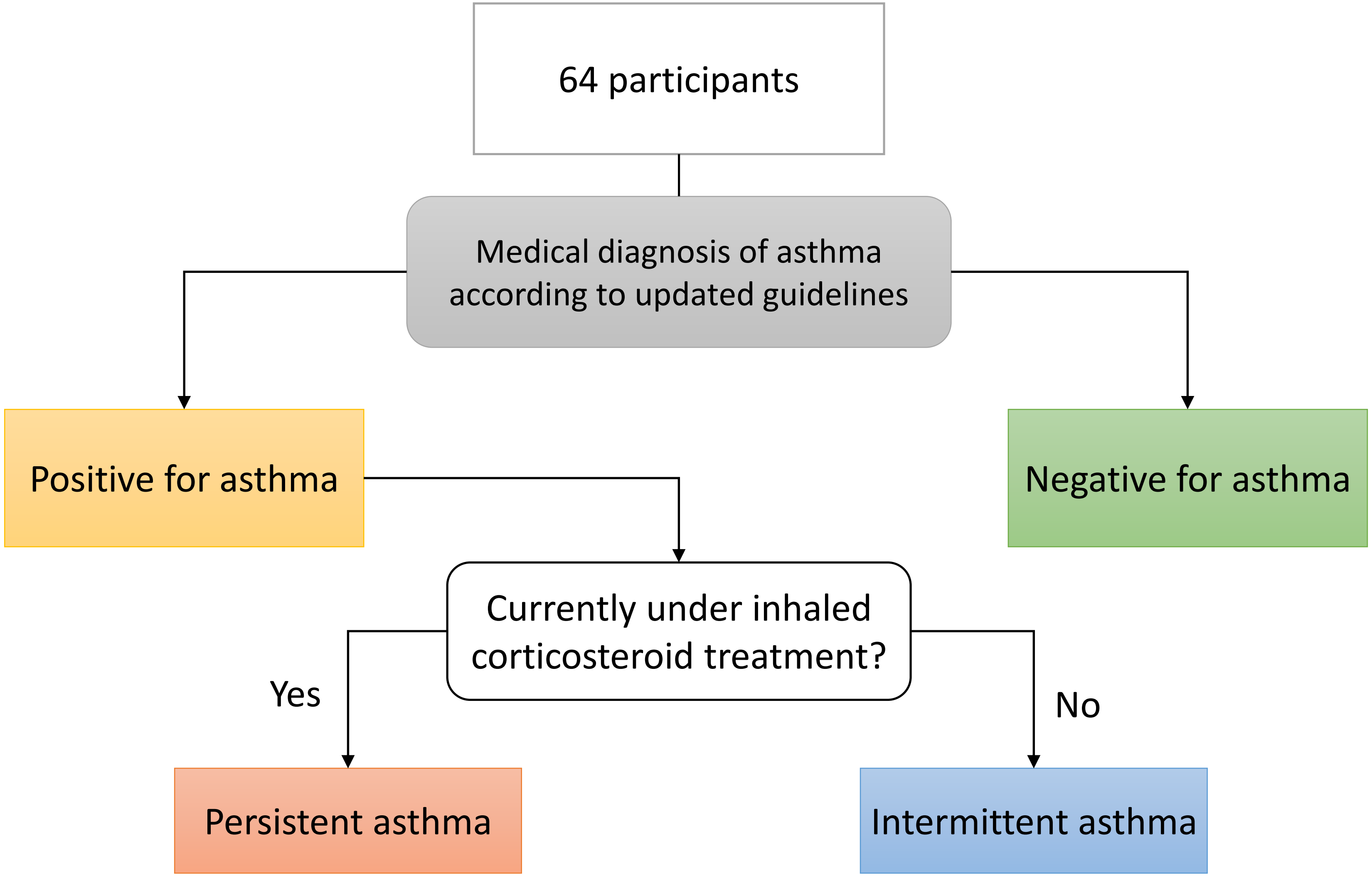
Prediction of steroid responsiveness (responsive = full circles; unresponsive = empty triangles) to oral prednisone, 30 mg daily for 14 days in steroid free patients with asthma by electronic nose. Sensitivity = 90.9%; specificity = 71.4%

Montuschi, P, et al. 2010. *Diagnostic performance of an electronic nose, fractional exhaled nitric oxide, and lung function testing in asthma*. **Chest.** 137(4):790-6.

Van der Schee, et al. 2013. *Predicting steroid responsiveness in patients with asthma using exhaled breath profiling*. **Clin Exp Allergy.** 43(11) 1217-1225.

DEVELOP A BREATHOMICS MODEL FOR PERSISTENT ASTHMA DIAGNOSIS IN PAEDIATRIC INDIVIDUALS

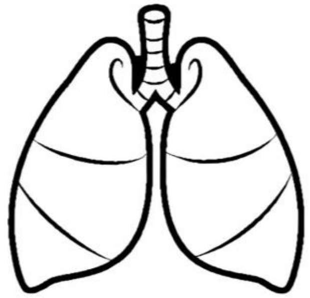
DEFINITION OF PERSISTANT ASTHMA



Cavaleiro Rufo J. *et al.* 2019. *Exhaled breath condensate volatilome allows sensitive diagnosis of persistent asthma.* ***Allergy.*** 74(3):527-534

CLINICAL ASSESSMENT AND SAMPLE COLLECTION

Lung function



Spirometry with bronchodilation

Atopy

Skin-prick tests

Human volatilome

Exhaled breath condensate collection & analysis

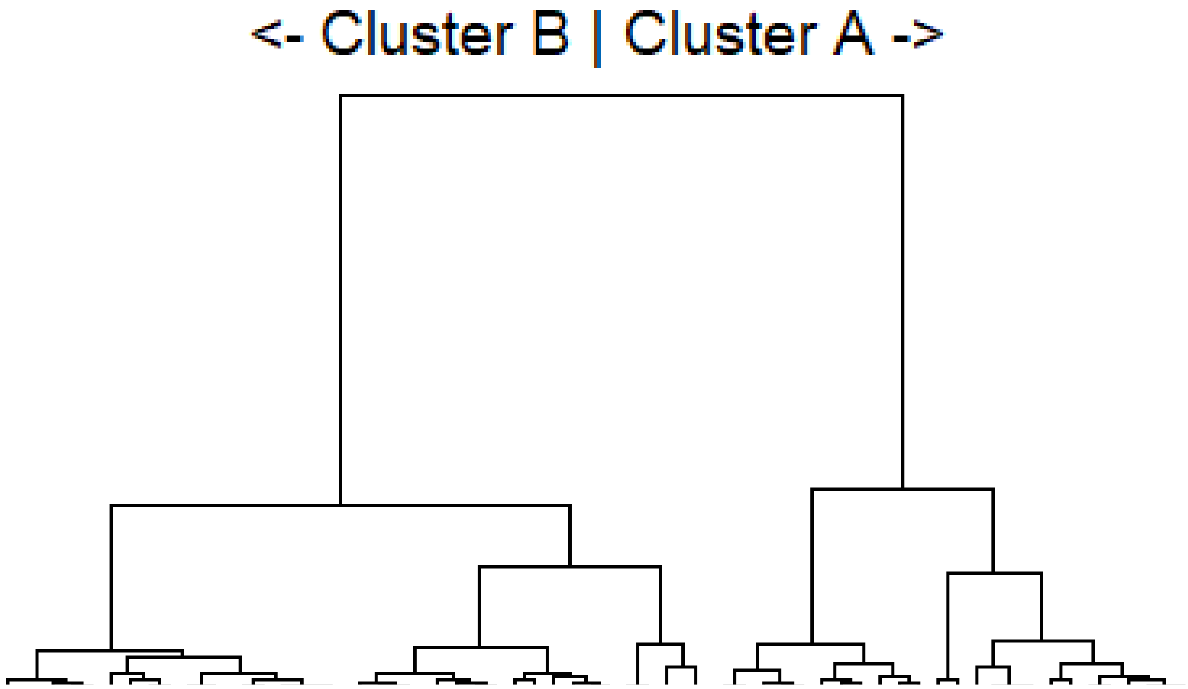
Cavaleiro Rufo J. *et al.* 2019. *Exhaled breath condensate volatilome allows sensitive diagnosis of persistent asthma.* ***Allergy.*** 74(3):527-534

PARTICIPANTS

Characteristics	
N (males)	64 (41)
Age (years, mean ± sd)	11.4 (±3.3)
Positive bronchodilation	31.3%
Atopy	79.7%
Intermittent asthma	25.0%
Persistent asthma	45.3%
Allergic Rhinitis	76.6%
Atopic dermatitis	9.4%

Cavaleiro Rufo J. *et al.* 2019. *Exhaled breath condensate volatilome allows sensitive diagnosis of persistent asthma.* ***Allergy.*** 74(3):527-534

CONSTRUCTION OF A BREATHOMICS MODEL



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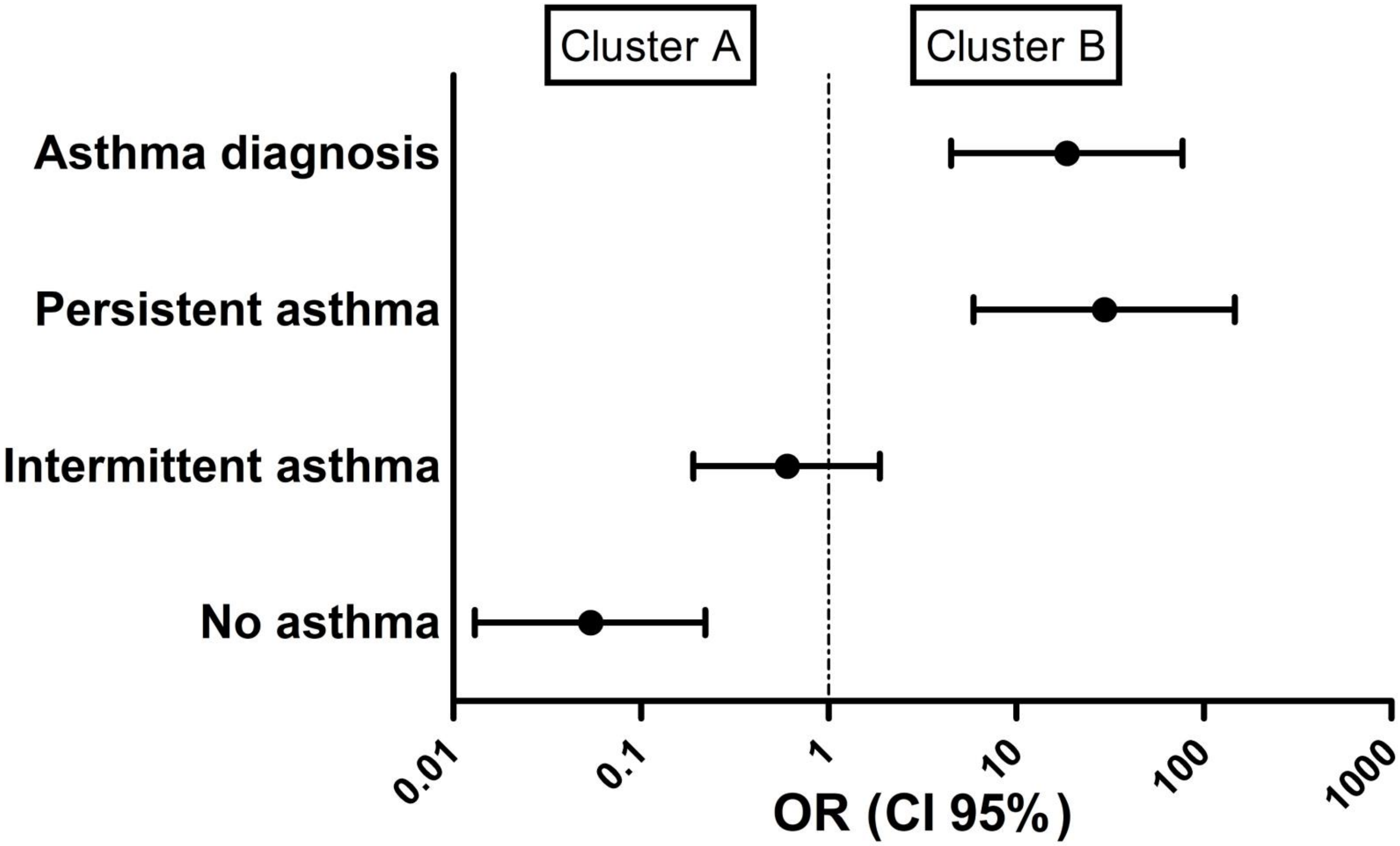
DIAGNOSTIC ACCURACY OF ENOSE-BASED EXHALED VOC ANALYSIS FOR PAEDIATRIC ASTHMA

DISCRIMINANT ANALYSIS	RHO
Persistent asthma	**0.602**
Bronchodilation	**0.544**
Weight	0.156
Age	0.146
Height	0.134
Intermittent asthma	-0.077
Skin prick tests	-0.052
Allergic rhinitis	-0.042
Sex	0.035
Atopic dermatitis	-0.028
Recruitment setting	-0.009

Importance in the model

Cavaleiro Rufo J. *et al.* 2019. *Exhaled breath condensate volatilome allows sensitive diagnosis of persistent asthma.* ***Allergy.*** 74(3):527-534

DIAGNOSTIC ACCURACY OF ENOSE-BASED EXHALED VOC ANALYSIS FOR PAEDIATRIC ASTHMA



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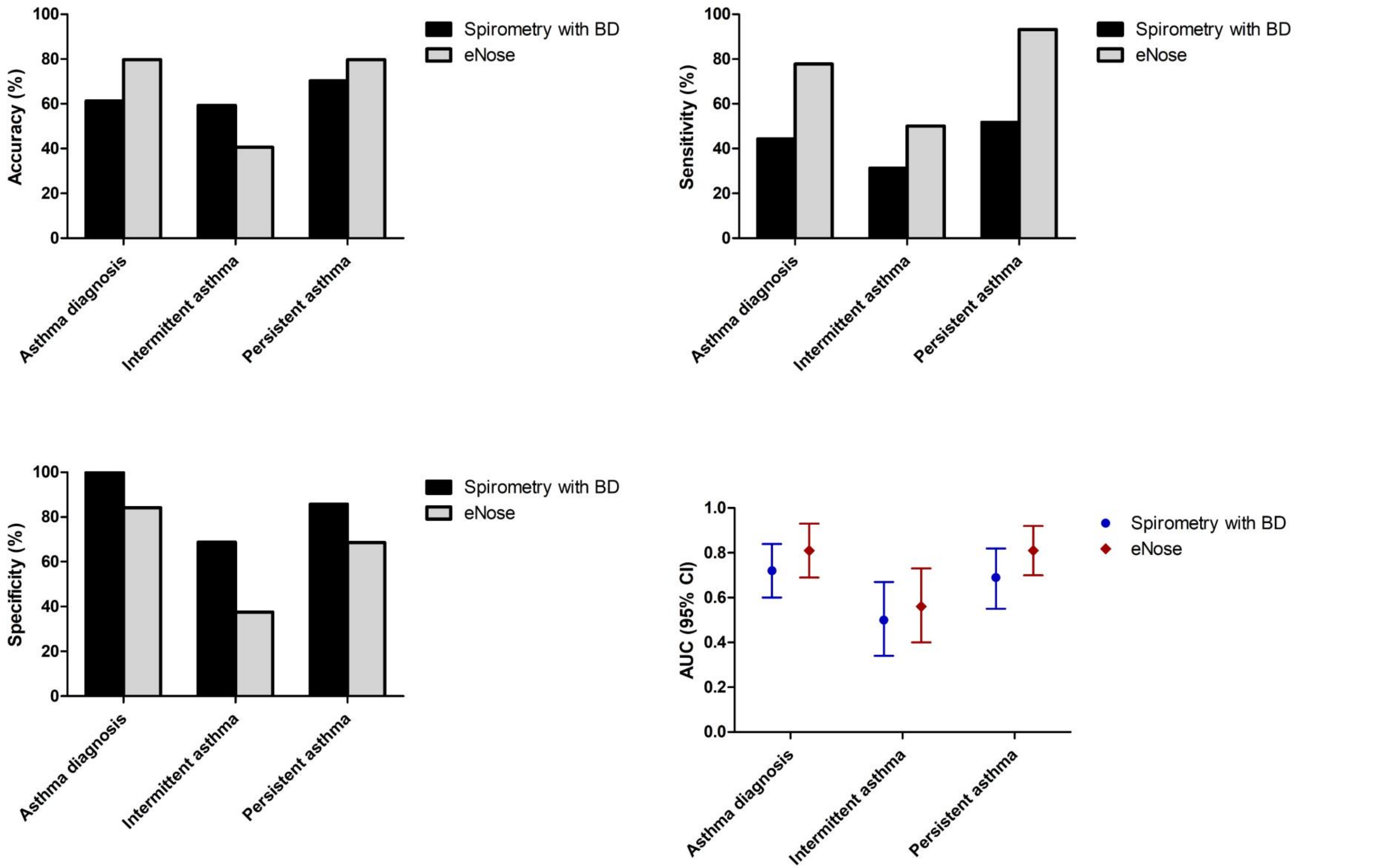

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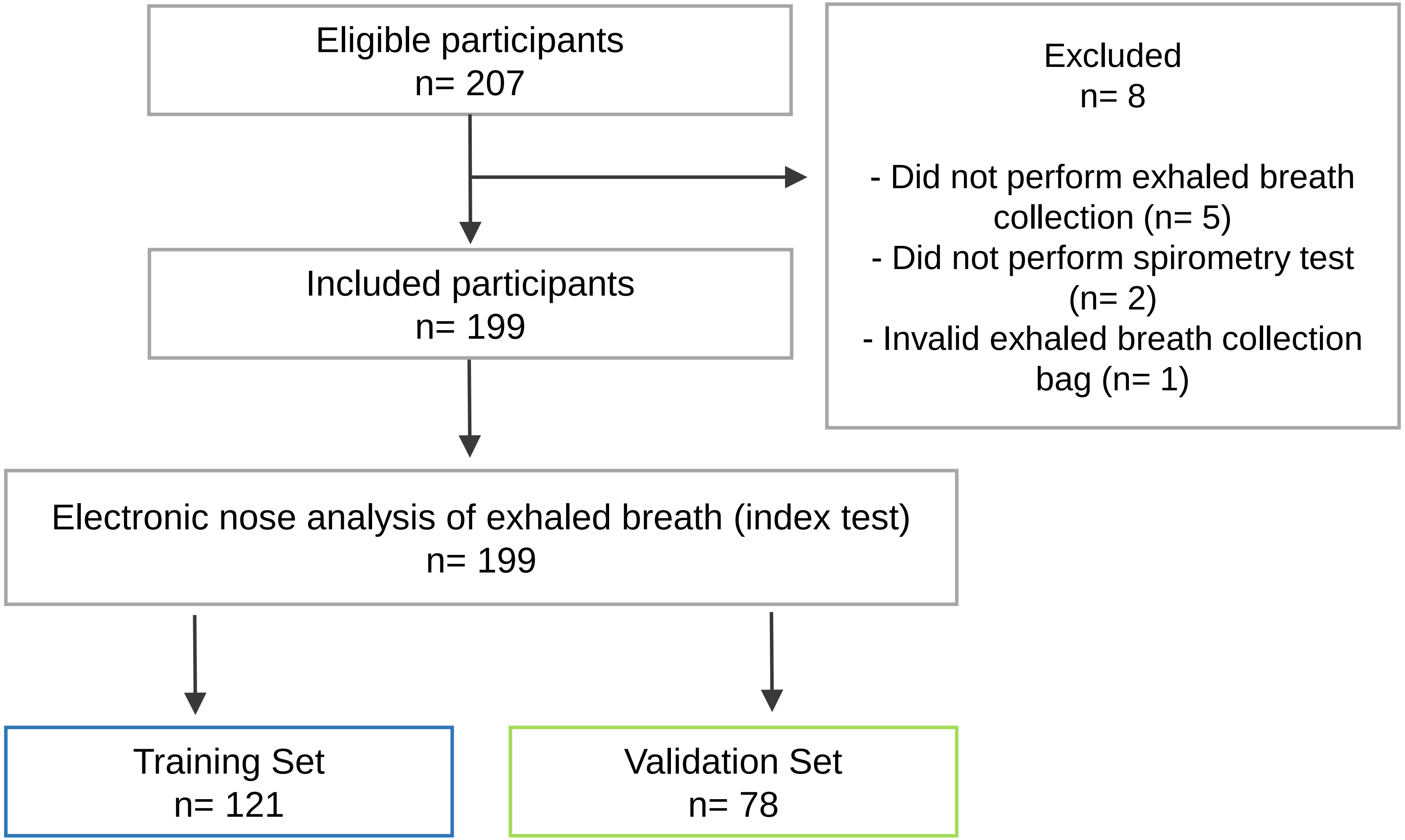
DIAGNOSTIC ACCURACY OF ENOSE-BASED EXHALED VOC ANALYSIS FOR PAEDIATRIC ASTHMA

- The developed breathomics model was able to distinguish individuals with asthma under the need of inhaled corticosteroid therapy.
- Diagnostic results surpassed those from spirometry with bronchodilation.

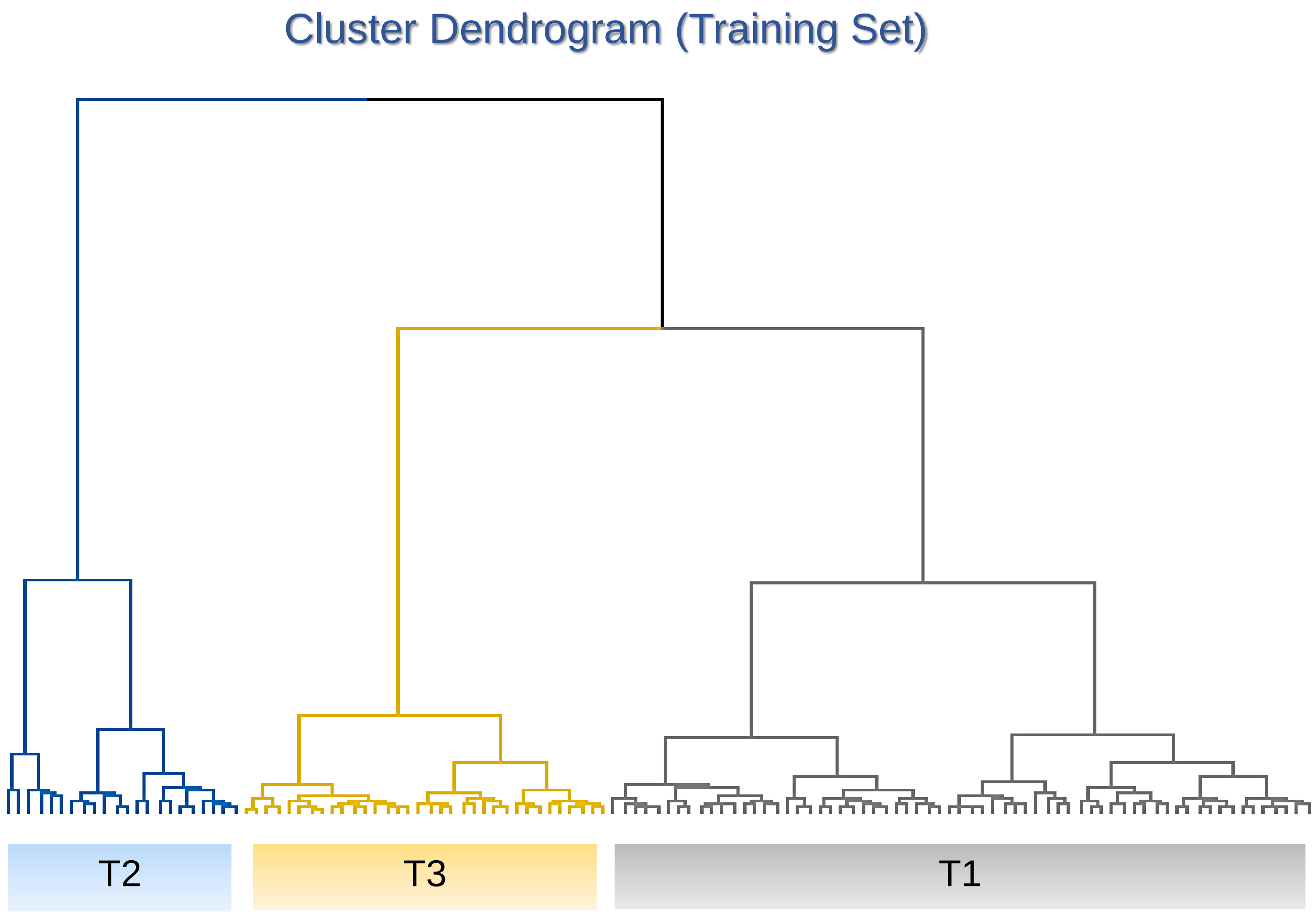
HOWEVER:

- No external validation was performed
- Only pediatric patients
- Some patients with persistent asthma were already under corticosteroid therapy
- Exhaled breath condensate was used

BREATHOMICS IN ASTHMA DIAGNOSIS (PART II): Participants



BREATHOMICS IN ASTHMA DIAGNOSIS (PART II): Hierarchical clustering of VOC profiles



BREATHOMICS IN ASTHMA DIAGNOSIS (PART II): Results for clinical parameters

	Cluster T1	Cluster T2	Cluster T3	p
N	65	22	34	
Sex (male %)	32.31	59.09	38.24	0.08
Age (years)	33.48 (±17.26)	26.23 (±14.10)	30.53 (±16.00)	0.18
<12 years old (%)	6.15	27.27	17.65	**0.03**
<18 years old (%)	23.08	36.36	32.35	0.40
BMI (kg/m^2)	24.60 (±4.96)	24.64 (±6.35)	24.05 (±5.20)	0.93
Medical diagnosis of				
Asthma (%)	75.00	76.19	51.52	**0.04**
Rhinitis (%)	90.48	85.00	84.85	0.66
Exhaled NO (ppb)	48.02 (±51.37)	44.62 (±51.08)	32.66 (±23.61)	0.54
Smoker (Yes %)	9.23	4.55	5.88	0.71
Intake of Food/drinks 2 hours prior test (Yes %)	61.54	40.91	79.41	**0.01**

BREATHOMICS IN ASTHMA DIAGNOSIS (PART II): Results for lung function

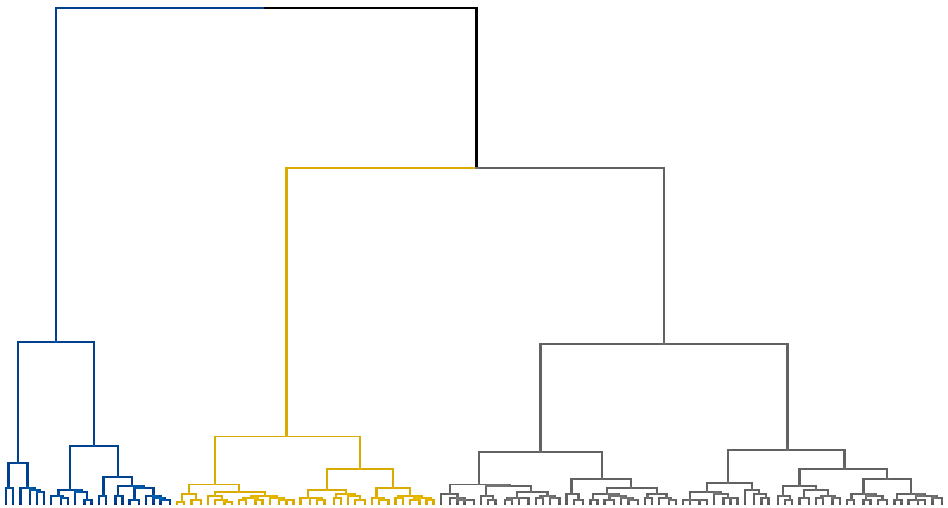
	Cluster T1	Cluster T2	Cluster T3	p
Baseline				
FEV1 (%)	103.91 (±15.36)	103.77 (±16.52)	97.97 (±15.08)	0.17
FEV1 (L)	3.14 (±0.97)	3.22 (±1.02)	2.88 (±0.92)	0.54
FVC (%)	111.06 (±13.05)	112.41 (±14.20)	106.24 (±12.82)	0.15
FEV1/FVC (%)	80.77 (±9.58)	78.82 (±9.80)	78.13 (±7.85)	0.36
FEF 25-75 (%)	81.62 (±30.61)	79.09 (±31.38)	70.68 (±26.10)	0.28
PEF (%)	102.22 (±17.85)	100.64 (±19.58)	92.03 (±16.83)	**0.03**
FEV1 reversibility (L)	0.20 (±0.23)	0.14 (±0.13)	0.16 (±0.15)	0.56
FEV1 reversibility (%)	6.32 (±6.63)	4.45 (±4.19)	6.41 (±6.82)	053
Positive BD (%)	26.15	9.09	20.59	0.24

BREATHOMICS IN ASTHMA DIAGNOSIS (PART II): Results for respiratory symptoms

Respiratory symptoms	Cluster T1	Cluster T2	Cluster T3	p
Young and Adults (age: 13-78)				
Chest tightness during exercise (Yes, %)	52.46	**25.00**	57.14	T2/T3, **0.04** T1/T2, **0.05**
Chest tightness during exercise (frequency, mean: 0 - 3)	0.98 (±1.14)	**0.37 (±0.81)**	1.00 (±1.09)	T2/T3, **0.04** T1/T2, **0.04**
Children, young and adults (age: 6-78)				
Shortness of breath/dyspnoea (Yes, %)	50.77	**31.82**	58.82	T2/T3, **0.04** T1/T2, 0.12

Symptoms in last 4 weeks were auto-reported in a questionnaire. In young and adults, the frequency of symptoms was additionally reported (0=never; 1=up to 2 days in a week; 2= more than 2 days in a week; 3= almost every day).

BREATHOMICS IN ASTHMA DIAGNOSIS (PART II): Different phenotypes, different VOC profiles?



T2	T3	T1
Early onset controlled asthma?	COPD? Exercise-induced asthma?	Uncontrolled asthma? Persistent asthma?

T2

- Less asthma-like symptoms (dyspnoea and chest tightness during exercise);
- More children (age under 12).

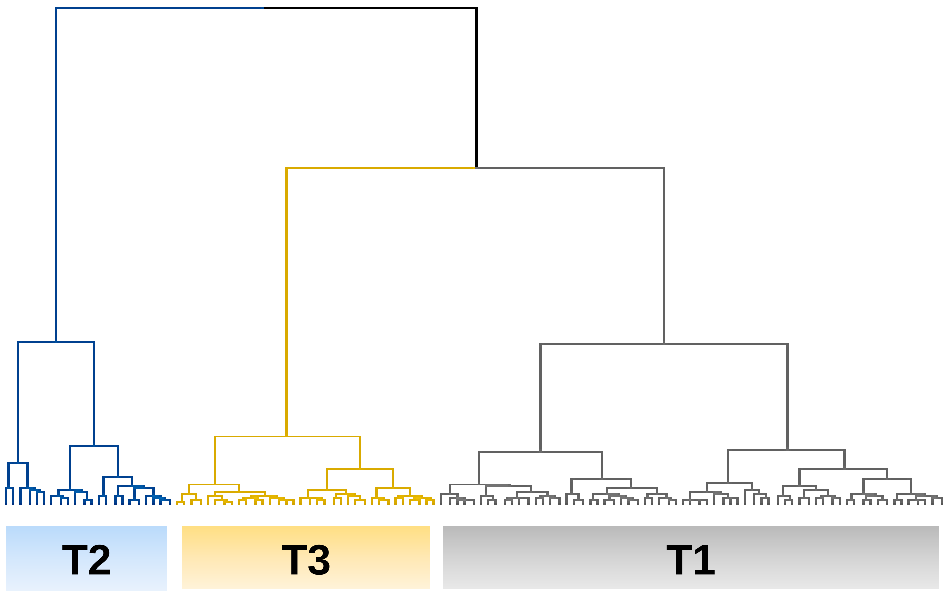
T3

- Less individuals with asthma (medical diagnosis);
- More asthma like symptoms than T2;
- Decreased PEF (%).

T1

- More symptoms than T2.
- More individuals with asthma than T3.

BREATHOMICS IN ASTHMA DIAGNOSIS (PART II): Different phenotypes, different VOC profiles?



Corticosteroid therapy

T2

- Less asthma-like symptoms (dyspnoea and chest tightness during exercise);
- More children (age under 12).

T3

- Less individuals with asthma (medical diagnosis);
- More asthma like symptoms than T2;
- Decreased PEF (%).

T1

- More symptoms than T2.
- More individuals with asthma than T3.

TAKE HOME MESSAGE

- Exhaled VOC profiles may be used as easily accessible biomarkers for asthma phenotyping and disease monitoring in point-of-care scenarios.

- May be applied in combination with other diagnostic parameters (FeNO, lung function).

- Screening approach for therapy responsiveness.

- For asthma screening:
  - Fast and practical identification of VOC profiles is a must
  - Identification of specific VOCs should be a secondary concern


I am afraid I will have to prescribe you some corticosteroid therapy

Thank you!

jcrufo@gmail.com