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A R Ettema[1], M W P M Lenders[1], J Vliegen[1], A Slettenaar[1], M C Tjepkema-Cloostermans[1,2] and C C de Vos[3]

[1] Department of Neurology and Neurosurgery, Medisch Spectrum Twente, Enschede, The Netherlands
[2] Department of Clinical Neurophysiology, University of Twente, Enschede, The Netherlands
[3] Department of Anesthesiology, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands

E-mail: AR.Ettema@mst.nl

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Abstract

In the present study we investigated whether multiple sclerosis (MS) can be detected via exhaled breath analysis using an electronic nose (eNose). The Aeonose™ (an eNose, The eNose Company, Zutphen, the Netherlands) is a diagnostic test device to detect patterns of volatile organic compounds in exhaled breath. We evaluated whether the Aeonose™ can make a distinction between the breath patterns of patients with MS and healthy control subjects. In this mono-center, prospective, non-invasive study, 124 subjects with a confirmed diagnosis of MS and 129 control subjects each breathed into the Aeonose™ for 5 min. Exhaled breath data was used to train an artificial neural network (ANN) predictive model. To investigate the influence of medication intake we created a second predictive model with a subgroup of MS patients without medication prescribed for MS. The ANN model based on the entire dataset was able to distinguish MS patients from healthy controls with a sensitivity of 0.75 (95% CI: 0.66–0.82) and specificity of 0.60 (0.51–0.69). The model created with the subgroup of MS patients not using medication and the healthy control subjects had a sensitivity of 0.93 (0.82–0.98) and a specificity of 0.74 (0.65–0.81). The study showed that the Aeonose™ is able to make a distinction between MS patients and healthy control subjects, and could potentially provide a quick screening test to assist in diagnosing MS. Further research is needed to determine whether the Aeonose™ is able to differentiate new MS patients from subjects who will not get the diagnosis.

1. Introduction

1.1. Multiple sclerosis

Multiple sclerosis (MS) is a multifocal central nervous system disorder characterized by inflammatory demyelinating lesions affecting white and gray matter; thought to be mediated by auto reactive T cells. MS usually starts with an acute episode of neurological disturbance, often followed by a phase consisting of relapses and remissions, which may transition after several years to phase of progressive accumulation of disability without relapses. About 20% of patients however will have a primary progressive course [1]. The clinical course and the pathology of MS strongly vary between patients. The degree of macrophage activation, demyelination and axonal damage also differs between patients.

The main diagnostic criteria for MS include confirmed multifocal lesions and the onset of individual symptoms at different point in time and space. These clinical diagnostic criteria have to be supported by several investigation including MRI and lumbar puncture [2]. However, an easy and accurate diagnostic biomarker is yet to be found and a quick test for diagnosing MS or monitoring its progression is desirable.

1.2. eNose for the quick detection of MS

Volatile organic compounds (VOCs) are end products of metabolic processes in the body. Exhaled breath contains thousands of VOCs and their concentrations change with cellular metabolism and oxidative stress. It is assumed that disease-specific metabolic pathways may give rise to specific VOC patterns and could therefore aid in the diagnostic process. The

electronic nose (eNose) technology is a diagnostic test able to detect, identify and classify a pattern of VOCs in exhaled breath so that VOCs can be used as non-invasive biomarkers [3–6].

An eNose contains chemical sensors that detect measurable changes in physical properties of the sensors when exposed to a gas mixture. This way, an eNose does not look for specific compounds in exhaled breath but recognizes an unique composite breath pattern. In order to identify breath patterns, an eNose must be trained. New breath patterns can be linked to existing breath patterns through comparative pattern recognition analysis [7].

Using the eNose technology for diagnosing neurological diseases is a relatively new field of study [5, 6]. The inflammatory character of the various mechanisms of MS, will probably result in breath patterns that could function as biomarkers for disease development and severity. We therefore expect MS to be detectable by an eNose.

Two previous studies already explored whether VOCs in exhaled breath could be used as a diagnostic biomarker for MS [8, 9]. However, in those studies analysis of exhaled breath was not performed by a handheld pattern detecting eNose, but by gas chromatography–mass spectrometry (GC–MS) and nanomaterial-based sensor arrays. The sensor arrays revealed discrimination between MS patients and control subjects in hexanal and 5-methyl-undecane levels [9]. GC–MS analysis revealed significantly higher levels of heptadecane, nonanal, decanal and sulfur dioxide in the exhaled breath of patients with MS compared to the control group, while acetophenone levels were higher in the control group. Measurements derived from the sensor array were used to create a predictive model, using artificial neural networks (ANNs). The model created with the training set was able to distinguish MS patients and healthy controls with accuracy up to 90% [8].

The above-mentioned studies both support our hypothesis that MS can be detected by an eNose via analyzing VOC patterns. The asset of the eNose is that it is an accessible, fast, easy-to-use, hand-held device that can be directly used in the outpatient clinic. Another advantage of eNose technology is that trained models can easily be implemented into other devices, making it available for large-scale use, and that while GC–MS analysis can detect single VOCs an eNose identifies VOC patterns created by hundreds of VOCs.

1.3. Objectives

The primary objective of this mono-center, prospective, non-invasive study is investigating whether an eNose can make a distinction between the breath pattern of patients with confirmed diagnosis of MS and control subjects without any suspicion of MS. The secondary objective is determining the influence of medication intake by creating a second predictive model with a subgroup of MS patient without medication prescribed for MS.

2. Methods

All consecutive MS patients that were visiting the neurology department were recruited in a secondary care referral hospital: Medisch Spectrum Twente, the Netherlands. MS patients and healthy control subjects were asked to breathe through an eNose during a period of 5 min. Patient younger than 18 years and patients who were physically or cognitively unable to use an eNose were excluded from the study. Each participant performed a single measurement. The study protocol was approved by the medical ethics committee and the board of directors of Medisch Spectrum Twente. Informed consent was obtained from all participants.

2.1. Materials

For this study we used one Aeonose^{TM} (an eNose, The eNose Company, Zutphen, the Netherlands). The Aeonose^{TM} contains three micro hotplate metal-oxide sensors, which differ in terms of metal-oxide type and catalyzing agents. VOCs in exhaled breath can give rise to a redox reaction at the sensor surface that changes the conductivity of the sensor. These changes can be measured and quantified resulting in unique breath patterns [7].

2.2. Statistical analysis

We aimed to include at least 100 patients with MS and 100 control subjects, which would be sufficient for training the Aeonose^{TM} in the pilot phase, creating a model and determining how reliably the Aeonose^{TM} can differentiate between groups. To assess the influence of MS medication on the model, a subgroup of MS patients without medication prescribed for MS was created from the group of all patients with MS. The following medication was prescribed as MS treatment for one or more patients in this study: dimethylfumaraat, fingolimod, ocrelizumab, teriflunomide, natalizumab, interferon beta-1a and fampridine.

Continuous variables were reported as means and dichotomous variables as numbers with corresponding percentages. Baseline characteristics were compared using the independent sample t-test for normally distributed continuous variables and the Fisher's exact test for dichotomous variables. IBM SPSS Statistics for MAC, version 25.0 (IBM CORP., Armonk., NY) was used to perform statistical analysis. Baseline differences were considered statistically significant with a p value < 0.05.

Raw measurement data from the Aeonose^{TM} were used to generate a predictive model to provide estimations of diagnostic outcome. Developing an algorithm to differentiate between VOC patterns in exhaled breath required training of an ANN. For

more information about these statistical analyses, see Kort *et al* [7].

3. Results

Between February 2019 and June 2019, a total of 134 MS patients and 133 control subjects were included in the study. Ten MS patients and four control subjects were unable to breathe through the Aeonose™ due to dyspnea and were excluded from analysis. The subgroup consisted of 58 MS patients not using medication prescribed for MS. Table 1 discloses the baseline characteristics, different disease phases and data on food intake for all groups. There were no significant differences in comorbidities between the group of MS patients and the control group.

3.1. Breath pattern analysis

The model calculated a classification value between −1 (negative) and 1 (positive) for each participant. The cut-off value of −0.16 in the scatterplot determined whether the values were classified as negative or positive for MS. Data analysis showed a sensitivity of 0.75 (95% CI: 0.66–0.82), a specificity of 0.60 (0.51–0.69), a positive predictive value (PPV) of 0.65 (0.56–0.72) and a negative predictive value (NPV) of 0.72 (0.62–0.80). This results in an overall diagnostic accuracy of 0.68. The Aeonose™ created a second predictive model based on the subgroup of MS patients not using medication and the control subjects with a cut-off of −0.13. This model reached an accuracy of 0.80 with a sensitivity of 0.93 (95% CI: 0.82–0.98), a specificity of 0.74 (0.65–0.81), a PPV of 0.63 (0.52–0.73) and an NPV of 0.96 (0.89–0.99). Figure 1 shows the scatterplots of the individual classifications and the receiver operating characteristics curves.

4. Discussion

The results of this pilot study showed that the Aeonose™ is able to make a distinction between the breath patterns of MS patient, without medication, and healthy control subjects with a sensitivity of 0.93 (CI 0.82–0.98) and accuracies up to 0.80.

A previous study, using GC–MS analysis revealed significantly different levels of five main VOCs in the exhaled breath of patients with MS compared to the control group [8]. Another study, using bilayers of polycyclic aromatic hydrocarbons and single-wall carbon nanotubes showed that these sensors could distinguish the exhaled breath of MS patients from healthy controls with a sensitivity of 85% [9]. This corresponds with our findings that MS can be detected in exhaled breath, but we have shown that it can also be detected with an easy-to-use, hand-held eNose. Collecting a breath sample and comparing it with a trained model can be done within 15 min, and a trained model can easily be transferred to other devices, thus allowing for large-scale use. The sensor in the eNose however will show some drift over time. The shape of the response signal however will stay constant. Thus by normalizing the signal data during the data analysis, potential drift issues are resolved. An important aspect is the exact sensor temperature, through internal calibration it is ensured that the response is reproducible. This is a prerequisite for using the same algorithm in several devices and making eNose technology available for large-scale MS screening and monitoring [10].

Comparing the two predictive models, suggests that the Aeonose™ did not just discriminate based on medication intake, but actually on disease-specific VOC patterns, as the model with the subgroup of patients without medication performed significantly better. Medication might slow down the disease activity and thereby making it harder for a model to distinguish between MS patients and healthy control subjects. According to this hypothesis, the Aeonose™ might be able to monitor the progression of MS and distinguish between patients with a good response to medication (prescribed for MS) and patients with no response to medication.

Using eNose technology could provide a non-invasive, well tolerable and quick screening test to accelerate the diagnostic process of MS. This would mean a shorter period of uncertainty for the patients and their family. Furthermore, it is preferable to diagnose the disease in an early stage allowing a better response to the available drugs for MS during the inflammatory stages of the disease [11]. Eventually it could mean less invasive tests and thus fewer risks for the patient.

4.1. Limitations

The majority of the patients were diagnosed with relapsing-remitting MS (RRMS). Knowledge on the pathogenic mechanisms underlying MS seems to be primarily based on RRMS [1]. If the pathophysiology of the various types of MS differs, then there might be a difference in the volatome. In future research we will determine whether or not the Aeonose™ can distinguish different types of MS from healthy control subjects.

There is a significant age difference between the subgroup of patients with MS without medication, and the healthy control group. However, a previous study showed that the overall breath pattern does not differ between age groups [12].

Another significant difference between the groups is in smoking habits. A previous study investigated the influences of lifestyle factors and diet on 15 common VOCs. They found statistically significant correlations between individual VOCs and 9 out of 25 analyzed food items, including coffee, leeks and garlic. However, a correlation between smoking and any of the analyzed VOCs was not found [13]. This finding

Table 1. Baseline characteristics of: participating healthy controls, patients with MS and subgroup of patients with MS who did not use medication.

Characteristics	Healthy controls	Patients with MS	MS subgroup (no medication)	*p* Value[d]	*p* Value[e]
No. of patients	129	124	58	—	—
Sex (male)	44 (34%)	41 (33%)	21 (36%)	0.89[a]	0.87[a]
Mean age (years)	43.7	44.5	48.7	0.65[b]	0.04[b]
Body mass index	25.8	26.4	26.5	0.37[b]	0.38[b]
Mean time to last meal (h)	2.5	2.7	2.5	0.53[b]	0.92[b]
Coffee intake <4 h (yes)	71 (55%)	51 (41%)	24 (41%)	0.05[c]	0.21[c]
Alcohol intake <24 h (yes)	26 (20%)	26 (21%)	12 (21%)	1.00[a]	1.00[a]
Currently smoking (yes)	8 (6%)	29 (23%)	11 (19%)	0.00[a]	0.01[a]
Disease phase					
• RRMS	—	80 (64%)	29 (50%)	—	—
• SPMS	—	32 (26%)	19 (32%)	—	—
• PPMS		2 (2%)	1 (2%)		
• CIS		1 (1%)	1 (2%)		
• Unknown		9 (7%)	8 (14%)		

RRMS: relapsing-remitting multiple sclerosis.
SPMS: secondary progressive multiple sclerosis.
PPMS: primary progressive multiple sclerosis.
CIS: clinically isolated syndrome.
[a] Fisher's exact test.
[b] Independent sample *t*-test.
[c] Pearson Chi-square test.
[d] Healthy controls vs. MS patients.
[e] Healthy controls vs. subgroup of MS patients.

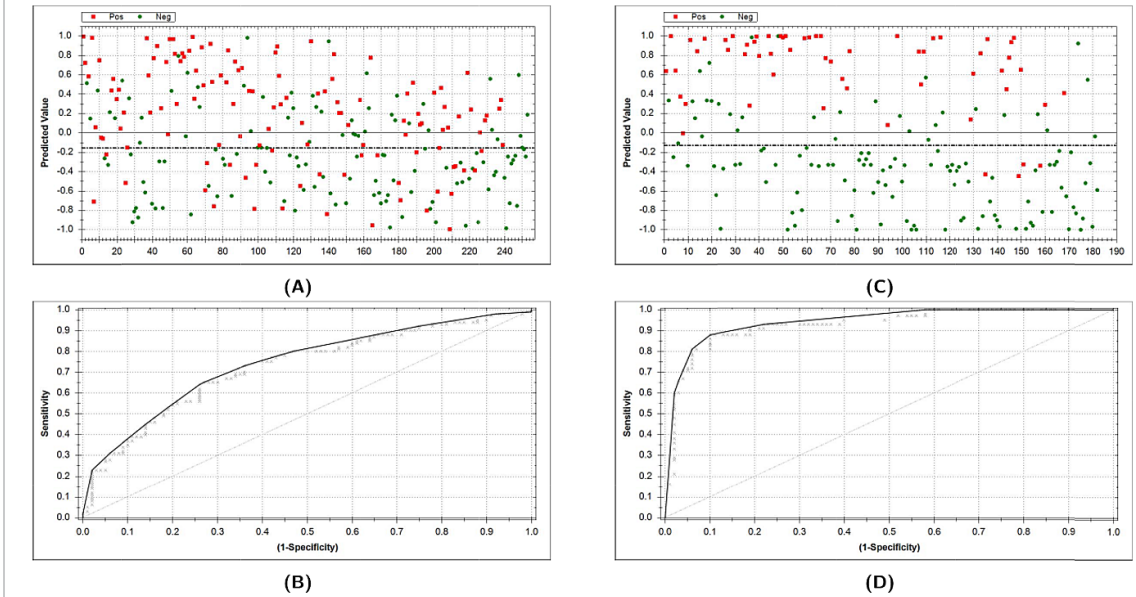

Figure 1. Predictive model of all MS patients vs. control subjects (A) and (B) and predictive model of MS patients without MS medication vs. control subjects (C) and (D). (A) Scatterplot, green dots are control subjects and red dots are patients with MS. (B) Receiver operating characteristics curve (ROC curve) with area under the curve of 0.73. (C) Scatterplot, green dots are control subjects and red dots are patients with MS without medication. (D) ROC curve with area under the curve of 0.93.

is supported by other research that also studied effects of recent food intake and diet in general on the VOC pattern [14, 15]. In our study no significant differences were found between the groups in coffee intake, alcohol intake, drug intake, or time of food intake before measurements. Although a limitation of our study could be that other parameters, such as specific diets, were not taken into account.

The validity of the PPV and NPV depends on the prevalence of a certain disease in the studied population. In the present study the prevalence of participants with MS was about 50% and for the MS patients not using medication 30%, which is not representative for the general population. It could prove useful to investigate whether the validity would be the same in a study that more resembles the general population.

4.2. Conclusion

This study reveals that the Aeonose™ is able to distinguish breath patterns of patients with MS (not using medication) from healthy control subjects, with a sensitivity of 0.93, specificity of 0.74 and an overall accuracy of 0.80. Further research is needed to determine whether the Aeonose™ is able to distinguish between subjects who present themselves for the first time at the clinic who will be diagnosed with MS and those subjects who will not get the diagnosis MS.

Conflict of interest

M W P M has shares in The eNose Company.
A R Ettema—reports no disclosures.
J Vliegen—reports no disclosures.
A Slettenaar—reports no disclosures.
M C Tjepkema—reports no disclosures.
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Statistical analysis

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ORCID iD

A R Ettema https://orcid.org/0000-0002-8325-0755

References

[1] Tsang B K T and Macdonell R 2011 Multiple sclerosis diagnosis, management and prognosis *Aust. Fam. Physician* **40** 948–55
[2] Milo R and Miller A 2014 Revised diagnostic criteria of multiple sclerosis *Autoimmun. Rev.* **13** 518–24
[3] Bijland L R, Bomers M K and Smulders Y M 2013 Smelling the diagnosis: a review on the use of scent in diagnosing disease *Neth. J. Med.* **71** 300–7 (http://www.njmonline.nl/getpdf.php?id=1330)
[4] Fens N, Zwinderman A H, van der Schee M P, de Nijs S B, Dijkers E, Roldaan A C, Cheung D, Bel E H and Sterk P J 2009 Exhaled breath profiling enables discrimination of chronic obstructive pulmonary disease and asthma *Am. J. Respir. Crit. Care Med.* **180** 1076–82
[5] Bijl E J, Groeneweg J G, Wesselius D W, Stronks D L and Huygens F J P M 2019 Diagnosing complex regional pain syndrome using an electronic nose, a pilot study *J. Breath. Res.* **13** 036004
[6] van Dartel D, Schelhaas J, Colon A, Kho K H and de Vos C C 2020 Breath analysis in detecting epilepsy *J. Breath Res.* **14** 031001
[7] Kort S, Brusse-Keizer M, Gerritsen J W and Van Der Palen J 2017 Data analysis of electronic nose technology in lung cancer: generating prediction models by means of Aethena *J. Breath. Res.* **11** 26006
[8] Broza Y Y *et al* 2017 Exhaled breath markers for nonimaging and noninvasive measures for detection of multiple sclerosis *ACS Chem. Neurosci.* **8** 2402–13
[9] Ionescu R *et al* 2011 Detection of multiple sclerosis from exhaled breath using bilayers of polycyclic aromatic hydrocarbons and single-wall carbon nanotubes *ACS Chem. Neurosci.* **2** 687–93
[10] Bruins M, Gerritsen J, Sande van de W J, Belkum van A and Bos A 2013 Enabling a transferable calibration model for metal-oxide type electronic noses *Sensors Actuators* B **188** 1187–95
[11] Fernandez O, Fernandez V, Arbizu T, Izquierdo G, Bosca I, Arroyo R, García Merino J A and de Ramón E 2010 Characteristics of multiple sclerosis at onset and delay of diagnosis and treatment in Spain (the Novo Study) *J. Neurol.* **257** 1500–7
[12] Dragonieri S, Quaranta V N, Carratu P, Ranieri T and Resta O 2016 Influence of age and gender on the profile of exhaled volatile organic compounds analyzed by an electronic nose *J. Bras. Pneumol.* **42** 143–5
[13] Krilaviciute A *et al* 2018 Associations of diet and lifestyle factors with common volatile organic compounds in exhaled breath of average-risk individuals *J. Breath Res.* **13** 026006
[14] Baranska A, Tigchelaar E, Smolinska A, Dallinga J W, Moonen E J C, Dekens J A M, Wijmenga C, Zhernakova A and van Schooten F J 2013 Profile of volatile organic compounds in exhaled breath changes as a result of gluten-free diet *J. Breath Res.* **7** 037104
[15] Ajibola O A, Smith D, Spanel P and Ferns G A A 2013 Effects of dietary nutrients on volatile breath metabolites *J. Nutr. Sci.* **2** 1–15