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Multi-centre prospective study on diagnosing subtypes of lung cancer by exhaled-breath analysis

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ABSTRACT

Objectives: Lung cancer is a leading cause of mortality. Exhaled-breath analysis of volatile organic compounds (VOC's) might detect lung cancer early in the course of the disease, which may improve outcomes. Subtyping lung cancers could be helpful in further clinical decisions.

Materials and methods: In a prospective, multi-centre study, using 10 electronic nose devices, 144 subjects diagnosed with NSCLC and 146 healthy subjects, including subjects considered negative for NSCLC after investigation, breathed into the Aeonose™ (The eNose Company, Zutphen, Netherlands). Also, analyses into subtypes of NSCLC, such as adenocarcinoma (AC) and squamous cell carcinoma (SCC), and analyses of patients with small cell lung cancer (SCLC) were performed.

Results: Choosing a cut-off point to predominantly rule out cancer resulted for NSCLC in a sensitivity of 94.4%, a specificity of 32.9%, a positive predictive value of 58.1%, a negative predictive value (NPV) of 85.7%, and an area under the curve (AUC) of 0.76. For AC sensitivity, PPV, NPV, and AUC were 81.5%, 56.4%, 79.5%, and 0.74, respectively, while for SCC these numbers were 80.8%, 45.7%, 93.0%, and 0.77, respectively. SCLC could be ruled out with a sensitivity of 88.9% and an NPV of 96.8% with an AUC of 0.86.

Conclusion: Electronic nose technology with the Aeonose™ can play an important role in rapidly excluding lung cancer due to the high negative predictive value for various, but not all types of lung cancer. Patients showing positive breath tests should still be subjected to further diagnostic testing.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide [1]. The main types of lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), accounting for 15% and 85% of the established cases respectively. NSCLC can be subdivided into two major subtypes: squamous cell carcinoma (SCC) and adenocarcinoma (AC), which differ in clinical, radiological, and histological characteristics [2]. The diagnosis of early-stage lung cancer is crucial for succesful curative therapy, because treatment options and prognosis directly depend on tumour size and metastatic spread at the time of diagnosis [3]. Five-year survival rates for those with stage IA NSCLC is 73%, whereas metastatic disease has a five-year overall survival rate of only 29% with local lymphatic spread and 4.5% for patients with distant metastases [4–6]. SCLC is associated with even worse survival rates where limited disease has a five-year survival of 10–20%, and metastatic disease < 1%. Unfortunately, only 16% of lung cancer cases present with localised, potentially curable disease, which explains the poor survival rates [6]. The current gold standard for diagnosing lung cancer is a histological or cytological proof, either from the primary or

Abbreviations: AC, adenocarcinoma; AUC, area under the curve; LDCT, low dose computed tomography; NLST, national lung screening trial; NPV, negative predictive value; NSCLC, non-small cell lung cancer; PPV, positive predictive value; ROC, receiver operating curve; SCC, squamous cell carcinoma; SCLC, small cell lung carcinoma; VOC, volatile organic compound

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metastatic lesion. There have been many attempts to develop screening tests in order to detect early-stage lung cancer. Currently, the only screening method implying reduced lung cancer mortality in high-risk groups is annual low-dose computed tomography (LDCT) [7,8]. However, several issues still need to be addressed, such as the high rate of false positives cases (up to 96.4%) in the National Lung Screening Trial (NLST), leading to unnecessary invasive procedures, radiation risk, and unnecessary anxiety. In most countries in Europe, results of the Dutch-Belgian lung cancer screening trial (NELSON) are awaited before a decision on implementation of screening programs will be made [7,9]. One approach could be adding a simple, non-invasive and reliable test to reduce the number of false positives and consequently unnecessary invasive interventions. Lately, sensor technologies based on pattern recognition in exhaled breath have been developed. These so-called electronic noses allow fast, low-cost, and non-invasive analysis of exhaled breath. Although this diagnostic approach seems very promising in the lung cancer field, it has not been incorporated in clinical practice so far [10–16]. This can partly be explained by the fact that in most cases, calibration models for electronic noses aren't transferrable among different devices. On the other hand, the negative predictive value (NPV) of electronic noses is still too low to allow clinical implementation.

The concept of the electronic nose as described in this manuscript, the Aeonose™ (the Enose Company, Zutphen, the Netherlands), is based on the availability of powerful IT solutions, allowing the application of pattern recognition techniques to complex measurement data without the need of specific identification of individual molecules. An electronic nose can measure low concentrations of volatile organic compounds (VOC's) in exhaled breath, that represent a breath print and reflect pathological processes in the body on tissue level, such as inflammation, infection, and neoplasms [17]. In this way, a combination of VOC's can serve as a non-invasive, diagnostic biomarker for metabolic changes associated with different pathological conditions. These VOC's can be detected with multiple, highly-sensitive electro-chemical sensors. This detection method is directed at changes in physical properties of the sensors, such as surface conductivity when being exposed to VOC's [18].

Recently, a pilot study on detecting lung cancer using the Aeonose™ was reported by van de Goor et al. [19]. In this study, the Aeonose™ was used to distinguish between patients with lung cancer and healthy controls. A total of 167 subjects were included of whom 107 were diagnosed with lung cancer. They found a promising sensitivity of 83%, a specificity of 84% with an area under the curve (AUC) of 0.83. However, this study was single center, and the researchers did not distinguish between various types of lung cancer.

2. Goals and objectives

The aims of this multi-centre study were: 1) to rapidly prove or reject the diagnosis of lung cancer in a cohort of patients suspected of lung cancer and healthy controls, 2) to discriminate between the subtypes of NSCLC: adenocarcinoma and squamous cell carcinoma, and 3) to distinguish SCLC patients from non-SCLC subjects in patients suspected of lung cancer and healthy controls.

3. Material and methods

It concerns a multi-centre, prospective diagnostic study in subjects suspected for lung cancer who were referred for a histological biopsy, as well as in healthy volunteers. The four secondary teaching hospitals participating in this study were Medisch Spectrum Twente Enschede, Ziekenhuis Bernhoven Uden, Medisch Centrum Leeuwarden, and Deventer Ziekenhuis, all in the Netherlands. Each hospital weekly diagnoses approximately 2–3 patients with lung cancer. For patients who turned out to have lung cancer, staging was established according to the 7th edition of the American Joint Committee on Cancer TNM staging system [5]. For all subjects, demographic parameters (e.g age), smoking status, amount of pack-years, and comorbidities were recorded.

Participants with suspected lung cancer visiting the outpatient clinic of the pulmonology departments of the participating hospitals were included between June 2015 and December 2017. Suspected subjects were divided into a group with confirmed lung cancer and a group with a rejected diagnosis of lung cancer, based on imaging and/or derived histopathology. Subjects with a suspicion of lung cancer were not biopsied when the CT-scan showed no evidence of lung cancer, even when the chest X-ray did. Also, some subjects showed a spontaneous decrease in nodule size without any treatment, which does not fit with the suspicion of lung cancer. A few patients with a high suspicion of lung cancer did not undergo biopsy because of their weak condition, but these subjects were excluded from the analyses. Finally, subjects who had a negative biopsy, but still a very high clinical suspicion of lung cancer were directed for a re-biopsy that eventually led to a confirmed diagnosis lung cancer. Healthy volunteers with a minimum age of 50 were recruited through an advertisement at the hospitals' website. The only exclusion criterion for all subjects was being diagnosed with an other active malignancy. We compared breath patterns from patients with a proven diagnosis of lung cancer prior to initiation of treatment with subjects without lung cancer, i.e. healthy volunteers and suspected subjects with a rejected diagnosis of lung cancer. The study protocol was approved by the medical ethics committee of Medisch Spectrum Twente, and the board of directors at each participating centre. All patients provided written informed consent.

3.1. Aeonose technology and procedure of breath sampling

The Aeonose™ is a hand-held electronic nose, containing three micro-hotplate metal-oxide sensors (MOS) that are mass producible, and offer the opportunity for transferring calibration models. This means that once a calibration model for a specific indication has been developed, it can easily be transferred to other Aeonose™ devices [20]. In this study we used 10 Aeonose™ devices which were randomly applied to subjects to avoid specific device dependent variations. Patients were instructed to perform tidal breathing through the non-rebreathing Aeonose™ device for 5 minutes during a single visit. A disposable mouthpiece with a carbon active filter was used (filtering inhaled air) and the patient's nose was clipped to prevent nose breathing. A washout period during the first 2 minutes was used for clearing the lungs from ambient, possibly polluted air with a carbon filter and the nose clip, without recording any measurements. During the next 3 minutes, metal-oxide sensors were exposed to exhaled breath and conductivity values of the sensors were recorded.

Redox reactions of VOC's at the sensor surfaces were recorded in terms of conductivity changes. After these 5 minutes, the Aeonose™ was put aside, and the sensors were regenerated by guiding clean air to them through another active carbon filter. Then, a build-in Tenax™-tube that collected VOC's during the measurement was heated, and these VOC's released were guided over the sensors and recorded, providing additional information on the breath profile. Finally, another regeneration step with clean air was enforced. Using this protocol, the total breath test cycle took approximately 15 minutes.

3.2. Sample size

We calculated a sample size taking into account a required sensitivity of 90% with a confidence interval of 82.5%–95%. Therefore, approximately 105 subjects diagnosed with lung cancer must be included. Presuming a 1:1 ratio of a confirmed versus a rejected diagnosis of lung cancer in suspected subjects, we also needed 105 subjects with a rejected diagnosis. We also planned to include approximately 75 'healthy' subjects without any suspicion for lung cancer.

3.3. Statistical analysis

Clinical characteristics are reported as means with standard deviations when normally distributed or as medians with interquartile range (IQR). Nominal variables are reported as numbers with corresponding percentages. To assess differences between the different groups, either the ANOVA test for normally distributed continuous variables, Kruskall Wallis non-parametric test for skewed distributed continuous or ordinal variables, or chi-squared test (x [2]) for nominal and categorical variables were applied. We used the Bonferroni Holm correction to adjust for multiple testing. Data of exhaled breath were analysed by Aethena, a proprietary big-data software package from The eNose Company [21]. In the course of the big data analysis and pattern recognition (using artificial neural networks), several steps can be distinguished such as pre-processing of data, data compression, leave-10%-out cross-validation, model selection, and combining prediction models with promising AUC's. Sensitivity, specificity, positive predictive value (PPV) and (NPV) were calculated for the diagnosis of lung cancer and it's subtypes. Receiver operating characteristics (ROC) curves were composed and AUC's were calculated with 95% confidence intervals. A scatter plot showing values between −1 and +1 was calculated for each subject indicating the degree to which the subject was classified as positive (maximum value +1) or negative (minimum value −1) for lung cancer. During the analysis, a cut-off value was chosen, which showed best separation between the two groups in terms of optimal sensitivity and NPV to exclude lung cancer early, together with an acceptable number of false positives. All analyses were based on the complete dataset after including all participating subjects.

In order to rule out any influence of device characteristics on results during the training phase, it was required for every Aeonose™ to measure at least four positive and four negative samples. If this condition was not met, some measurements from that specific device were excluded from the analysis. No Aeonose™ device was excluded during the study. All statistical tests were two-sided with a significance level at 0.05. SPSS V.22.0 was used.

4. Results

Of the 308 subjects included, 144 had confirmed NSCLC, 18 had confirmed SCLC, 61 were suspected for lung cancer due to complaints or an abnormal chest X-ray, but were considered negative after investigation, and 85 subjects were healthy volunteers (Fig. 1). No adverse effects were found when performing the breath measurements. Clinical characteristics of the subjects are described in Table 1. Healthy volunteers were significantly younger, more likely to be female and non-smoker, had smoked less pack-years, and did not have COPD (all $p < 0.001$). Suspected patients without lung cancer were more often never-smokers and had smoked less pack-years than confirmed NSCLC patients ($p < 0.001$). Out of the 144 NSCLC patients, 93 had AC, 42 had SCC, 4 had large cell carcinoma and 5 were NSCLC not otherwise specified (Fig. 1). Approximately 75% of the lung cancer patients were classified as stage III or IV disease.

Table 2 summarizes the diagnostic performance of the Aeonose™ for the different groups in terms of sensitivity, specificity, PPV, NPV, and AUC with corresponding 95% confidence intervals. Limited case sample sizes resulted in different group sizes for healthy subjects. When focusing on a high sensitivity and NPV, a high-sensitivity point was chosen, based on the ROC-curve to distinguish between NSCLC and all negatives, which led to a sensitivity of 94.4%, an NPV of 85.7%, at an AUC of 0.76.

When only suspected subjects with a rejected diagnosis of NSCLC were distinguished from NSCLC patients, we observed a relatively decreased performance when compared to all negatives and when compared to healthy volunteers only. This analysis revealed a sensitivity of 90.5%, an NPV of 52.4%, and an AUC of 0.73. At the same time, diagnostic performance improved when discriminating breath prints of NSCLC patients from healthy volunteers, resulting in a sensitivity of 92.2%, an NPV of 84.3%. an AUC of 0.85. The corresponding scatterplots are presented in Fig. 2A–C.

We investigated whether the most prevalent subtypes of NSCLC, being AC and SCC could be discriminated more accurately compared to the combined group of NSCLC patients. The results are presented in Table 2. The diagnostic accuracy in diagnosing AC from healthy subjects resulted into a sensitivity of 81.5% with an NPV of 79.5% and a corresponding AUC of 0.74. When discriminating SCC patients from healthy subjects, we found an interesting performance of the Aeonose™ to rule out SCC with a sensitivity of 80.8%, an NPV of 93.0% and a corresponding AUC of 0.77. The corresponding scatterplots of the analyses of the subtypes are presented in Fig. 3.

Due to the lower prevalence of SCLC, analyses could only be performed in a limited number of patients to distinguish SCLC-patients from healthy controls (Table 2). Ninety-three subjects of whom 18 had pathologically confirmed SCLC were included (Fig. 4). The diagnostic accuracy in diagnosing SCLC resulted in a sensitivity of 88.9%, a specificity of 80.0% with a PPV of 51.6%, an NPV of 96.8%, and an AUC of 0.86.

5. Discussion

This exploratory study showed that exhaled-breath analysis with the Aeonose™ can differentiate between patients with lung cancer and healthy subjects, including suspected subjects that are considered

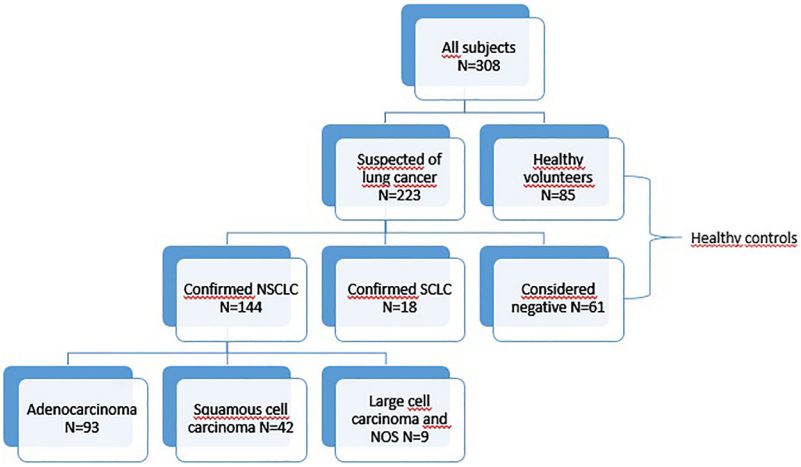

Fig. 1. Flow chart showing the different groups. NOS: not otherwise specified.

Table 1
Clinical characteristics of subjects.

	All subjects N = 308	Confirmed NSCLC N = 144	Confirmed non-NSCLC N = 61	Confirmed SCLC N = 18	Healthy N = 85	P-value
Age in years, mean (SD)	64.6 (8.5)	67.1 (9.0)	65.1 (8.8)	63.2 (8.2)	60.0 (4.4)	< 0.001[a]
Sex, number of males (%)	142 (49%)	83 (57.6)	32 (52.5)	10 (55.6)	27 (31.8)	0.001[b]
BMI, mean (SD)	25.6 (5.2)	25.3 (5.5)	27.0 (5.9)	28.0 (4.8)	25.2 (3.8)	0.056
Smoking status, N (%)						
Current smoker	71 (24.5)	51 (35.4)	13 (21.3)	7 (38.9)	7 (8.2)	< 0.001[c]
Ex-smoker	164 (56.6)	86 (59.7)	33 (54.1)	10 (55.6)	45 (52.9)	
Never smoked	55 (19)	7 (4.9)	15 (24.6)	1 (5.6)	33 (38.8)	
Pack-years[d], median (IQR)	21.5 (3.25–40.0)	35.0 (20.0–46.75)	20.0 (1.25–32.75)	45.0 (27.75–52-75)	2.0 (0.0–14.5)	< 0.001[c]
COPD, N (%)	89 (37)	66 (46.5)	21 (34.4)	8 (44.4)	1 (1.2)	< 0.001[b]

[a] After Games-Howell correction, there was a significant difference between healthy volunteers and confirmed NSCLC and healthy volunteers and confirmed non-NSCLC.
[b] After Holm-Bonferroni correction there was a significant difference between healthy volunteers and confirmed NSCLC, confirmed non-NSCLC and confirmed SCLC.
[c] Between all 4 groups.
[d] 5 subjects missing pack-years. Abbreviations: BMI, body mass index; COPD: chronic obstructive pulmonary disease.

Table 2
Diagnostic performance of the Aeonose™.

Groups	N	Cut-off chosen	TP	TN	FP	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95% CI)
NSCLC vs. all negatives[a]	144 vs. 146	−0.265	136	48	98	8	94.4	32.9	58.1	85.7	0.76 (0.71–0.82)
NSCLC vs considered negative after investigation	105 vs. 43*	−0.350	95	11	32	10	90.5	25.6	74.8	52.4	0.73 (0.64–0.82)
NSCLC vs healthy volunteers	103 vs. 84[b]	−0.295	95	43	41	8	92.2	51.2	69.9	84.3	0.85 (0.79–0.90)
Adenocarcinoma vs all negatives[a]	81 vs 109*	−0.365	66	58	51	15	81.5	53.2	56.4	79.5	0.74 (0.67–0.82)
Squamous cell carcinoma vs all negatives[a]	26 vs 91*	−0.015	21	66	25	5	80.8	72.5	45.7	93.0	0.78 (0.67–0.88)
SCLC vs. all negatives[a]	18 vs. 75	−0.575	16	60	15	2	88.9	80.0	51.6	96.8	0.86 (0.78–0.95)

TP, true positive; TN, true negative; FP, false positive; FN, false negative; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.
[a] All negatives include suspected subjects considered negative after investigation and healthy volunteers.
[b] Limited case sample sizes resulted in different group sizes.

negative after investigation. The Aeonose™ could discriminate between breath prints of NSCLC patients and all negatives with a high sensitivity and high NPV for various, but not all types of lung cancer, implying that many subjects could be prevented from undergoing unnecessary invasive diagnostic procedures. These results are in agreement with results published previously [13,19,22–24]. The Aeonose™ was also able to distinguish NSCLC patients from patients who were suspected for lung cancer. However, the diagnostic performance of the Aeonose™ differed when negative subjects were split in suspected, but considered negative after investigation, and healthy volunteers with an AUC of 0.73 and 0.85 respectively, showing a remarkable decline in performance for the suspected subjects. This could be explained by the fact that not lung cancer caused complaints, leading to referral, but other diseases such as COPD or pneumonia. These other diseases could lead to different breath prints, possibly more resembling lung cancer patterns, and could therefore not properly be distinguished by the pattern recognition software. The Aeonose™ could likely be trained to distinghuish these other diseases as well, when the number of participants in these groups are sufficiently large. Another explanation could be the overlap in smoking behaviour between the suspected patients without lung cancer and patients with lung cancer, which could lead to a considerable resemblance in metabolism and breath pattern. From Table 1 it can be seen that the healthy volunteers are more often female and never smokers. What effect this might have on the diagnostic parameters of the Aeonose™ is unknown and needs to be investigated in a larger study. We found better sensitivity (94.4%), at a noticeable lower specificity (32.9%) in our analyses than reported in other eNose studies [13,25]. This might be explained by the fact that in clinical use high NPV and sensitivity are essential when using Enose technology in an early diagnostic stage, on which we based our position at the ROC-curve. As a consequence, this leads to a lower specificity in our study.

The Aeonose™ was able to exclude SCC with an NPV of 93%, which accounts for a clinical relevant diagnostic power. This could be explained by the often central origin of this type of tumour [2]. However, the incidence of SCC was lower than in the other groups. Therefore, including more subjects should prove the validity of this high NPV. AC itself could also be distinguished significantly from non-adenocarcinoma, but with lesser performance than SCC. Since AC's are known for their histological heterogeneity, these tumours could probably be subclassified further into tumours with similar characteristics and consequently improved performance in Aeonose™ diagnostics [26]. This hypothesis is supported further by findings of Shlomi et al. who showed a diagnostic accuracy of 83% to discriminate between AC patients with and without an EGFR mutation [27]. Findings of improved performance of exhaled-breath analysis in lung cancer when subdividing tumours on histological or molecular biological grounds have also been presented by Barash et al [28]. They reported an accuracy of 96% when discriminating adenocarcinoma from squamous cell carcinoma when using gold nanoparticle sensors, albeit with fewer number of patients and the need to use multiple different sensors.

Next to this, we found promising results in excluding SCLC from healthy controls with a high NPV of 96.8%, taking into consideration that this analysis was performed with a relatively low number of subjects due to the lower prevalence of SCLC.

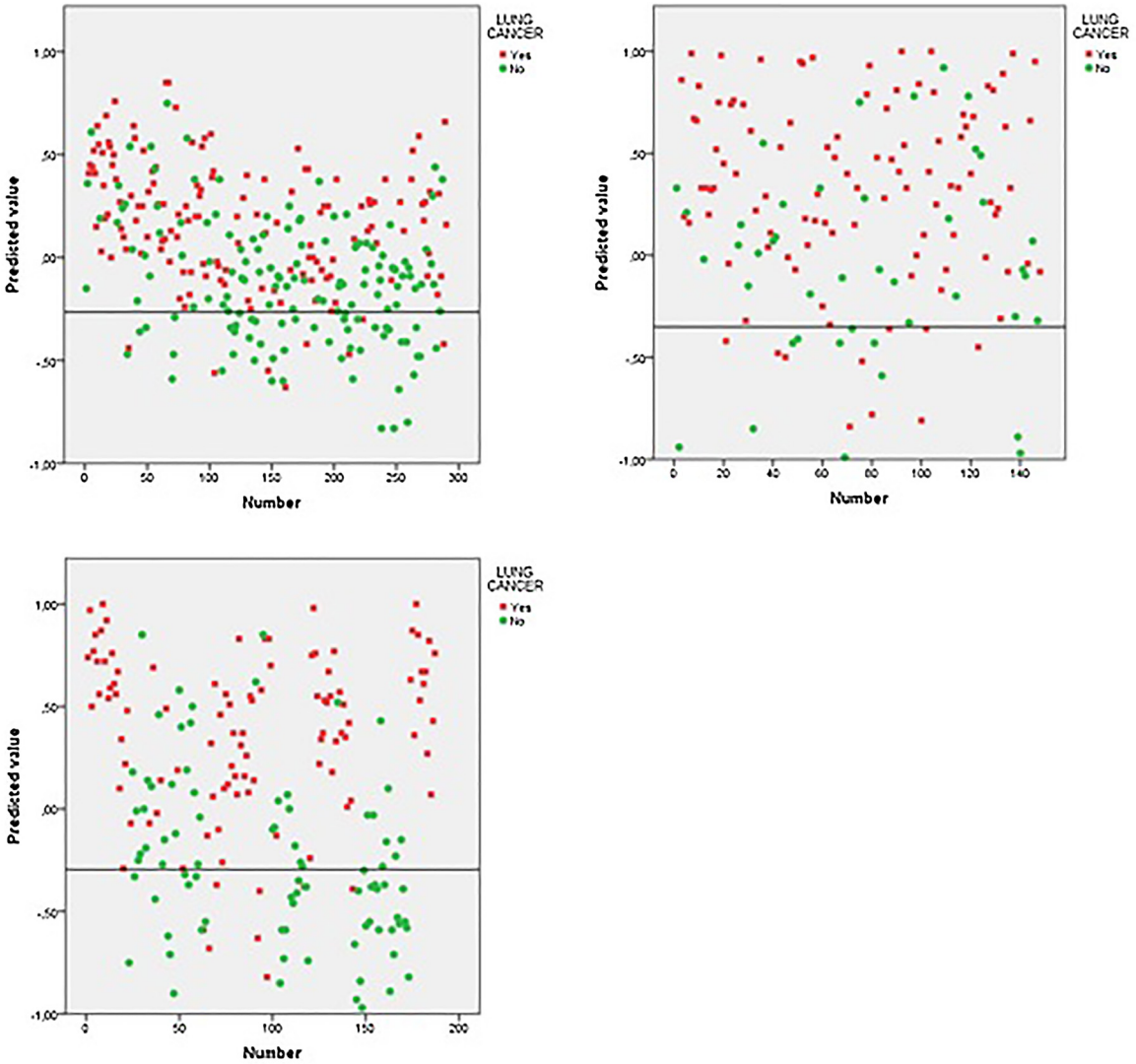

Fig. 2. Scatterplots with chosen optimal cut-off values. 2 A. NSCLC vs all negatives (AUC 0.76). 2B. NSCLC vs proven negatives (AUC 0.73). 2C. NSCLC vs healthy volunteers (AUC 0.85).

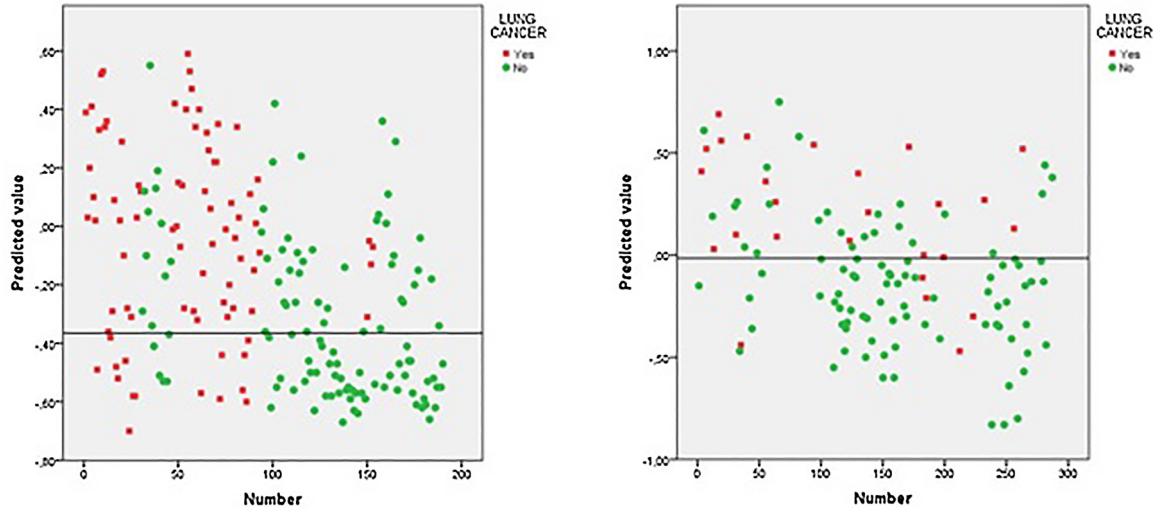

Fig. 3. Scatterplots with chosen optimal cut-off values. 3 A. Adenocarcinoma vs. all negatives (AUC 0.74). 3B. Squamous cell carcinoma vs all negatives (AUC 0.77).

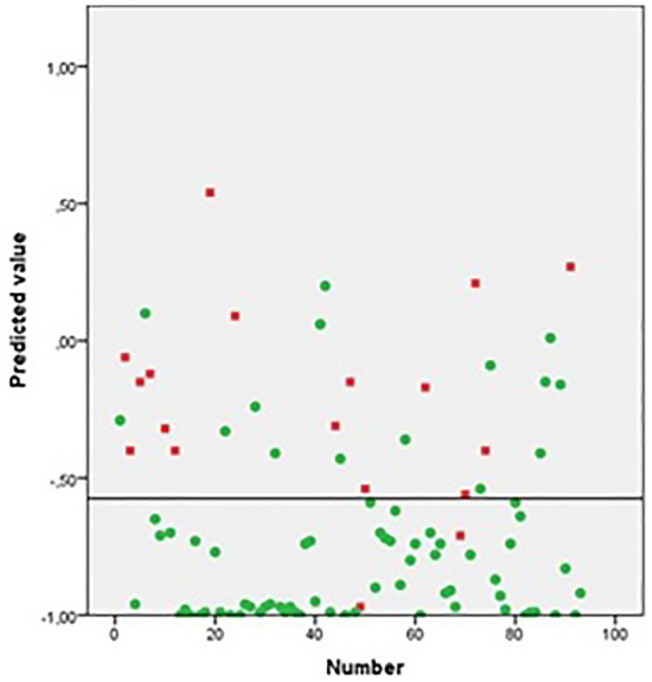

Fig. 4. Scatterplot with a chosen optimal cut-off value. SCLC vs. all negatives.

Pattern recognition of a large amount of VOC's leading to a breath signature is only one of the methods used in electronic nose technology. Other methods used for breath sampling in lung cancer, such as gas chromatography-mass spectrometry (GCMS) or multicapillary column-ion mobility spectrometry aim for the detection, identification and quantification of specific, individual chemical compounds in exhaled breath [29–31]. In principle, these complex methods are sensitive, but more expensive and time-consuming, and require a specialized operator for the system. When looking for a convenient and low-cost tool to detect lung cancer, point-of-care VOC pattern recognition techniques are favourable.

We performed a study with a relative large study population in a multi-centre setting where we observed an acceptable difference in breath prints of lung cancer patients versus subjects without lung cancer, despite different environments. Next to this, we showed that subdivision of NSCLC types can improve performance and requires further investigation, as earlier shown by Peled et al. [32]. This was however analysed with GCMS. Our findings further support the transferability of calibration models between different Aeonose™ devices, which supports the results of a smaller, single-centre study of van de Goor et al [22].

Results from this multi-centre study are promising. The technique seems especially valuable in addition to a screening trial based on periodical low dose CT scanning. Electronic nose technology could be able to diminish the number of false positive cases by choosing a cut-off point resulting in an NPV of nearly 100%. Subjects with a false positive diagnosis according to LDCT can subsequently be excluded without having to undergo an invasive bronchoscopy. However, it must be noted that our study population with subjects suspected of lung cancer differs from the high-risk subjects included in LDCT-screening. This point of view can be seen as a limitation of our study since the majority of the included NSCLC patients was classified as stage III and stage IV disease. These are not the patients that would benefit most from screening programs. However, screening is mostly aimed at patients without symptoms, so when introducing a screening program, probably more cases of stage I and stage II disease can be detected. In this study, the prevalence of stage I and II lung cancer was too low to draw firm conclusions about the detection rates in early stage lung cancer. In future, larger studies, it should become clear if stage I and II tumours could be detected by exhaled-breath analysis as well. In such studies, really large numbers of participants will be required, including supposingly healthy persons. In this study we showed the training phase of the Aeonose™ to detect or exclude NSCLC with promising results. Including more subjects for training the artificial neural network will likely lead to improved stability, and a better prediction model. Especially differentiation of lung cancer from other lung diseases is expected to improve when data of more patients are analysed, and breath profiles relating to other lung diseases can be taken into account.

Next to further training of the predictive performance, external validation of the obtained results needs to take place in a new study population, preferably in a multi-centre setting as well. It should be noted, however, that all results presented in this study were obtained using leave-10%-out cross validation. This implies in fact that -in 10 consecutive steps- all data were predicted as if they were blind data, based on a training model built from the remaining 90% of data. So, it is to be expected, true blind data will be predicted with similar results as in the cross validation, provided the cohorts are similar.

6. Conclusion

Exhaled breath analysis is a rapidly developing field. Electronic nose technology with the Aeonose™ is a non-invasive diagnostic tool that can discriminate between patients with lung cancer and healthy subjects, including subjects suspected of lung cancer with a rejected diagnosis and healthy volunteers. The Aeonose™ is also able to discriminate between lung cancer patients with different subtypes of NSCLC, such as adenocarcinoma and squamous cell carcinoma from healthy subjects, and SCLC from healthy subjects. The data suggest that the Aeonose™ can contribute to the early diagnostic workup of lung cancer where it could provide added value in screening for lung cancer. However, the results must first be validated externally in a new multi-centre study with a larger study population.

Conflicts of interest and source of funding

Miss S. Kort was partly financed by an unrestricted research grant from The eNose Company. J.W. Gerritsen is employed by the company producing the e-nose devices used. The other authors report no conflict of interest.

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