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BIONOTE e-nose technology may reduce false positives in lung cancer screening programmes[†]

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Abstract

OBJECTIVES: Breath composition may be suggestive of different conditions. E-nose technology has been used to profile volatile organic compounds (VOCs) pattern in the breath of patients compared with that of healthy individuals. BIOsensor-based multisensorial system for mimicking NOse, Tongue and Eyes (BIONOTE) technology differs from Cyranose® based on a set of separate transduction features. On the basis of our previously published experience, we investigated the discriminating ability of BIONOTE in a high-risk population enrolled in a lung cancer screening programme.

METHODS: One hundred individuals were selected for BIONOTE based on the attribution to the high-risk category (i.e. age, smoking status, chronic obstructive pulmonary disease status) of the University Campus Bio-Medico lung screening programme. We used a measure chain consisting of (i) a device named Pneumopipe (EU patent: EP2641537 (A1):2013-09-25) able to catch exhaled breath by an individual normally breathing into it and collect the exhalate onto an adsorbing cartridge; (ii) an apparatus for thermal desorption of the cartridge into the sensors chamber and (iii) a gas sensor array which is part of a sensorial platform named BIONOTE for the VOCs mixture analysis. Partial least square (PLS) has been used to build up the model, with Leave-One-Out cross-validation criterion. Each breath fingerprint analysis costs €10.

RESULTS: The overall sensitivity and specificity were 86 and 95%, respectively, delineating a substantial difference between patients and healthy individuals.

CONCLUSIONS: Our preliminary data show that BIONOTE technology may be used to reduce false-positive rates resulting from lung cancer screening with low-dose computed tomography in a cost-effective fashion. The model will be tested on a larger number of patients to confirm the reliability of these results.

Keywords: E-nose • Lung cancer • Screening

INTRODUCTION

The 20% reduction in lung cancer-specific mortality reported by the investigators of the National Lung Screening Trial has sparked renewed interest for the use of low-dose computed tomography (LDCT) in the screening of populations at high risk for developing lung cancer [1]. Along with these encouraging results, the international community has also emphasized the risks and the significant costs related to the indiscriminate adoption of an LDCT screening programme [2]. Apart from radiation exposure, the occurrence of false-positive results leading to unnecessary invasive diagnostic procedures and the reported over-diagnosis of slow-growing (indolent) tumours, along with the paradoxical

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encouragement of smoking [2] represent potential arguments against the implementation of LDCT screening. In fact, in the European cost-conscious health systems, the absence of benefit from the closed trials or the lack of mortality figures from the Nelson trial has so far contributed to the delay in the activation of national screening programmes [3]. In spite of these risks, the United States Preventative Task Force recommendations paved the way for the recent approval of funding of LDCT screening under the Medicare scheme per the provisions of the Patient Protection and Affordable Care Act [4]. Strategies for minimizing false positives resulting from LDCT screening are based on the revision of threshold criteria for nodule positivity (i.e. volumetric assessment of the nodule) [5] and the addition of biomarker (i.e. MiRNA) analysis [6]. In this setting, it has been postulated that the assessment of volatile organic compounds (VOCs) in the exhaled breath can be used as a diagnostic tool for lung cancer. VOCs are small molecules whose partial evaporation leads to an equilibrium between their concentration in the air and the one in liquids and/or solids [7–9]. More than 4,000 VOCs have been analysed so far; however, only 20–30 of these can be found in each individual. Furthermore, no single VOCs have been associated with a lung cancer profile. In the past two decades, several artificial olfaction systems (i.e. e-nose technology) based on non-selective chemical sensors aimed at measuring and comparing VOC patterns have been proposed in the literature [10, 11]. The hypothesis that the e-nose technology can assist in the identification of early-stage lung cancer patients has been brought forward in recently published studies [11–14]. Among e-noses, the Cyranose® technology represents a conventional sensor array still widely used by investigators [15]. The current study reports on the use of a new sensorial platform called BIONOTE (BIOsensor-based multisensorial system for mimicking NOse, Tongue and Eyes) [16] to detect lung cancer in patients enrolled in a lung screening programme ('Un Respiro per la Vita'—University Campus Bio-Medico, Rome, Italy). BIONOTE and Cyranose® technology are different in many respects (Table 1), from the working principle to the sensing material and array composition, let alone costs. Conversely, selectivity and performance are comparable to a certain extent.

MATERIALS AND METHODS

The electronic nose

Compared with artificial olfactory systems, BIONOTE represents a multisensorial platform aimed at reproducing the human senses contamination and interaction called synaesthesia [16]. In fact, BIONOTE enables the simultaneous analysis of the vapour and liquid phase of the same sample, together with the test of its optical properties by means of a single instrument based on the same sensing interface [16]. We used a measure chain consisting of (i) a device named Pneumopipe (EU patent: EP2641537 (A1):2013-09-25) able to catch exhaled breath by an individual normally breathing into it and collect the exhalate onto an adsorbing cartridge (Fig. 1); (ii) an apparatus for thermal desorption of the cartridge into the sensors chamber and (iii) a gas sensor array. We used an e-nose device based on quartz crystal microbalance (QCM) sensors utilizing anthocyanin-coated gold electrodes to detect mass changes induced by VOCs on the sensor surfaces. In turn, mass changes induce alterations in the oscillation frequency of the quartz wafer; VOCs with different compositions have different total mass which can be measured by counting the oscillation frequency of the quartz wafer. Therefore, lung cancer signatures can be detected based on the breath pattern demonstrated by the oscillation frequency. This breath pattern is an expression of multiple VOC detection; no specific VOCs are analysed with this semi-selective electronic nose.

Test administration

The individual is kept nihil per os since midnight of the day before the e-nose. Brushing teeth is not permitted to avoid interference by toothpaste chemicals. For smokers, it is recommended to abstain from smoking for the duration of fasting. The e-nose investigations are performed early in the morning and the cartridges sent in a canister maintained at very low temperatures (−20°C) to the gas chromatography laboratory. For each patient, the test was performed in the same location with a constant temperature between 20 and 22°C. Each breath fingerprint analysis in the BIONOTE investigation costs €10 in disposable materials (i.e. cartridges and nozzles).

The Università Campus Bio-Medico low-dose computed tomography screening programme

We performed the analysis of VOCs in the exhaled breath of patients enrolled in the lung screening programme being conducted at Università Campus Bio-Medico (UCB) in Rome, Italy. This programme has been implemented by UCB following approval of the local Ethics Committee. Participating patients originated also from the Lung Cancer Screening enrolment unit of the Department of Thoracic Surgical and Medical Oncology of the Istituto Nazionale Tumori, Fondazione Pascale, IRCCS, located in

Table 1: Differences in biomechanical properties between conventional e-nose technology (i.e. Cyranose) and BIONOTE

Characteristics	Cyranose®	BIONOTE
Working principle	Conductometric sensors	Acoustic-mass sensors
Sensing material	Conducting polymers	Anthocyanins
Array composition	32 different polymers	28 different responses obtained by 7 sensors operating at four different temperatures
Selectivity	Non-selectivity of the individual sensors and the mechanisms of interaction with VOCs	
Performance at low concentrations for standard compounds such as ethanol and hexane	Comparable	

VOCs: volatile organic compounds; BIONOTE: BIOsensor-based multisensorial system for mimicking NOse, Tongue and Eyes.

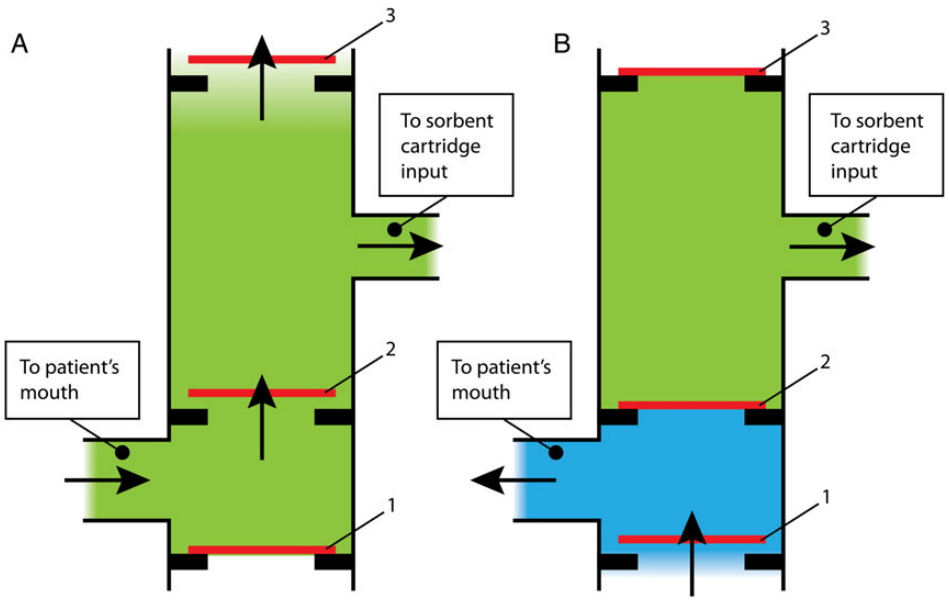

Figure 1: Schematics demonstrating the function of a Pneumopipe in inspiration (**A**) and expiration (**B**).

Naples, Italy. After BIONOTE, these patients would be managed and followed up at the originating institution.

The UCB LDCT screening programme was initiated in June 2011 and contemplates LDCT for a population in the age range of 55–75 years deemed at a risk for developing lung cancer given their age, smoking history (current smoker of at least 15 cigarettes a day or with a 10-year smoking cessation history), known exposure to carcinogens (such as asbestos, radiation, beryllium, hydrocarbons) and coexisting chronic obstructive pulmonary disease (COPD) [17]. Individuals can voluntarily participate in the screening programme free of charge since the 'Un Respiro per la Vita' programme is entirely funded by the UCB by means of successive instalments from private funding.

To assess eligibility for LDCT screening, candidates undergo a preliminary outpatient clinic where a multidisciplinary team decides on the assignment to the LDCT programme. General exclusion criteria also include current oxygen therapy and history of previous cancer apart from non-melanoma skin cancer, *in situ* uterine cervix cancer and localized prostatic tumour. For the purpose of this programme, LDCT is being performed by using 3-mm slices and by generating 2.36 mSv per rotation. A positive finding is reported if the nodule is equal to or larger than 5 mm. Below this 5-mm cut-off, the advice is to watch the nodule and repeat the LDCT after 1 year. If the nodule is in the 5–8 mm range, a repeat LDCT is prescribed within 3 months of the first LDCT. Further investigations (i.e. PET scan and transthoracic fine needle aspiration biopsy) are prescribed if the nodule is larger than 8 mm. Finally, if surgery is warranted to complete the diagnostic and the therapeutic pathway, strict adherence to minimally invasive criteria [18] is implemented.

An *ad hoc* instalment was provided by UCB to cover 18 LDCTs per week during the August 2014–June 2015 period (http://www.policlinicocampusbiomedico.it/un-respiro-per-la-vita/un-respiro-per-la-vita.html), which is the time frame of the current study on BIONOTE technology.

Statistical analysis

As in previous studies [17], the statistical analysis was performed with the MATLAB software (The MathWorks, Inc., Natick, MA, USA). Multivariate data analysis was done by using the PLS-Toolbox (Eigenvector Re-search, Inc., Wenatchee, WA, USA). Data were reported as means [and 95% confidence intervals (CIs)] for continuous variables, and as percentages for categorical variables. Partial least square discriminant analysis (PLS-DA) has been performed on the 7D data array, and the model has been cross-validated via the leave-one-out criterion and, then, the Root Mean Square Error (in Cross-Validation) (RMSECV) of the model. The RMSECV provides a measure of the robustness of the PLS-DA model.

RESULTS

Our BIONOTE study was performed between August 2014 and April 2015 and included 100 consecutive patients from the 'Un Respiro per la Vita' LDCT screening programme. In this group, the median age was 62 years (range, 55–73 years); there were 33 females and 67 males with a median body mass index of 25 (range, 19–42.8). In 49 individuals (49%), there was familiarity for cancer or COPD (in 12 patients, 12%), whereas in only 9 (9%) a diagnosis of diabetes had been established. Of the 100 individuals, 1 (1%) was a never smoker, 23 were former smokers (at least 15 cigarettes/day, 23%) and 76 were current smokers (76%). The subjects' characteristics are reported in Table 2.

In 23 of 100 individuals (23%), the LDCT had identified undetermined nodules (i.e. positive LDCT). The median diameter of screen-detected lesions was 6.5 mm (range, 5–53). In 16 subjects (69% of 23 subjects with positive findings), lesions larger than 5 mm were detected, whereas multiple nodules and pure GGOs were identified in 5 (21.7%) and 2 (8.6%) subjects, respectively. Eventually, all 23 patients with positive LDCT results were found to have histologically proven primary lung cancers. BIONOTE had detected true positives in 19 subjects out of 23 with histologically confirmed tumours (83%) and false positives in 4 (17%); conversely, true-negative results had been seen in 74 individuals out of 77 with negative LDCT findings (96%), and false negatives in 3 (4%). Accordingly, in this group of 100 individuals enrolled in the UCB lung screening programme, BIONOTE sensitivity and specificity

Table 2: Subject characteristics (median or counts)

Variable	Value (n = 100)
Median age	62 years
Sex (m: male; f: female)	m: 67; f: 33
Body mass index	25
Family history of cancer	49
COPD	12
Diabetes	9
Never smokers	1
Past smokers	23
Current smokers	76
Lung cancer	23
Squamous	5
Adenocarcinoma	18

COPD: chronic obstructive pulmonary disease.

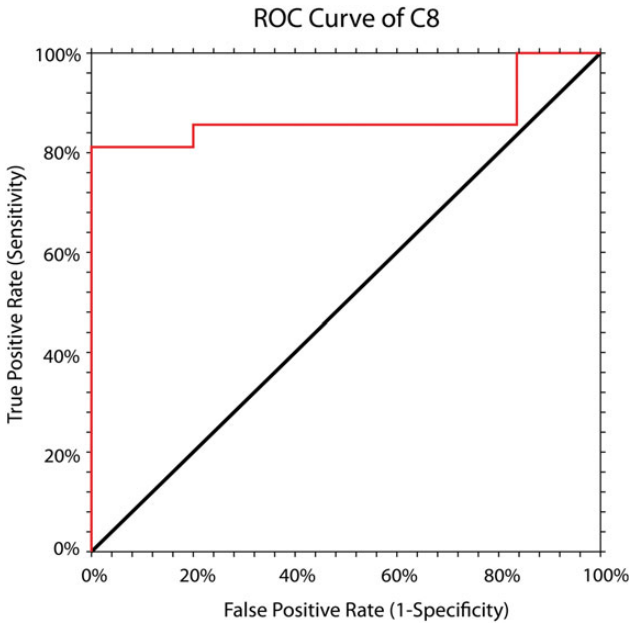

Figure 2: ROC curve plotting true-positive versus false-positive rates of BIONOTE used in 100 individuals enrolled in the UCB lung cancer screening programme (AUC = 0.87). UCB: Università Campus Bio-Medico; BIONOTE: BIOsensor-based multisensorial system for mimicking NOse, Tongue and Eyes.

were 86 and 95%, respectively (positive predictive value: 83%; negative predictive value: 96%).

The ROC analysis [area under the curve (AUC) = 0.87] showed that BIONOTE had a good predictive value for lung cancer (z = 3.14; P = 0.0008, Fig. 2).

The radar plots based on the PLS model yielded immediately visible evidence of the performance of BIONOTE in the entire subset of 100 patients (Fig. 3).

DISCUSSION

These preliminary results suggest that the BIONOTE sensory platform might contribute to substantiate the diagnosis of lung cancer, thereby potentially reducing the false positives resulting

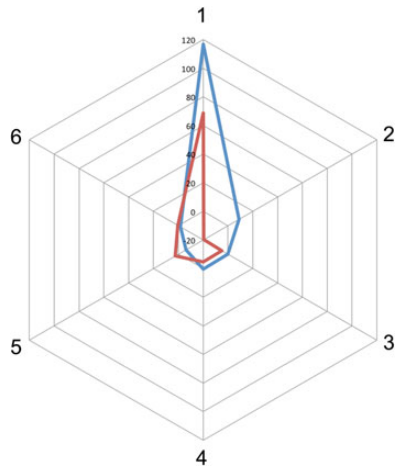

Figure 3: Breathprints of lung cancer versus control populations obtained by the partial least square model calculated on the 100 patients with 6 latent variables. In this 6D space, positive–negative discrimination is well performed. Each plot is the mean value of all the patients of the relative group. An immediate graphical representation is given by the two 3D radar plots relative to a negative patient (red) and a positive patient (blue).

from LDCT. Indeed, over the last few years, non-invasive analysis of human body fluids (exhaled breath, sweat, skin transpiration products, urine) has emerged as an innovative tool with potential for diagnosis of several conditions [19–21]. The electronic nose is an instrument able to perform measurements on gaseous samples such as the headspace of biological fluids, normally released by the human body [20]. As a consequence, the analysis of breath VOCs could be a valuable addition for the early detection of lung cancer, because it is non-invasive, sensitive, simple and relatively inexpensive [19, 21]. In this setting, the possibility of early detection of lung cancer on exhaled breath has been demonstrated by means of canine scent detection, analysis of headspace of lung cancer cell lines and utilization of sensors and spectroscopic techniques [11]. The e-nose technology represents a multifaceted spectrum of techniques that have in common the analysis of VOCs in the exhaled breath [11]. Using the e-nose based on QCM sensors in samples from 28 patients with lung cancer and 36 healthy controls, the sensitivity and specificity rates in lung cancer patients and controls were 89 and 79%, respectively [20]. A more recent study yielded overall accuracies of 90 and 85% for two different techniques of breath collection [21]. An alternative model of the e-nose technology based on surface acoustic wave sensors [11] was associated to a sensitivity of 94% and a selectivity of 83%.

With these premises, the potential advantage of using the e-nose technology in lung cancer screening programmes is associated with its use in positive patients at LDCT—in our study representing 36% of the total number of screened individuals. In the National Lung Screening Trial (NLST), the percentage of positive findings was 24% for LDCT compared with 7% for the chest X-ray arm [1, 22]. Overall, 96.4% of these positive results were not confirmed as malignant at subsequent investigations [1].

Different strategies have been proposed to reduce the false-positive rates associated with LDCT screening [23, 24]. In the Nelson trial, the volumetric assessment of lung nodules has led to a refinement of the criteria for positivity. In fact, lung nodules <100 mm^3 were not considered predictive of lung cancer in the following 2 years (i.e. 2-year lung cancer risk 0.6% compared with 0.4% of subjects without nodules [25]). Conversely, nodules of

100–300 mm^3 (intermediate risk) or >300 mm^3 (high risk) were associated with a 2-year lung cancer risk of 2.4 or 16.9%, respectively. Accordingly, the Nelson investigators proposed assessment of the volume-doubling time (VDT) for nodules of 100–300 mm^3 and further diagnostic discrimination for nodules >300 mm^3 based on the observation of the 2-year risk of lung cancer of 4% for VDT 400–600 and 9.9% for VDT ≤400 days [25].

Sozzi *et al*. recently demonstrated that by adding plasma-based miRNA signatures to LDCT a 5-fold reduction of false positives within nodules ≥5 mm (from 19.4 to 3.7%) could be obtained [6, 25].

Another strategy to reduce false positives is based on the refinement of demographic criteria of inclusion in the LDCT trials by designing more restrictive risk models [25]. As an example, United Kingdom Lung Screening (UKLS) has adopted the model of the Liverpool Lung Project which contemplates a composite score combining several risk factors for the development of lung cancer in order to calculate for each individual to be screened a lung cancer risk of at least 5% in 5 years [25]. In addition, the age range adopted by UKLS would be far more restrictive, including only individuals aged between 60 and 74 years who would be screened biannually [23]. This strategy would lead to the identification of 9 000 000 individuals at risk in the UK with only a fraction to be considered eligible according to UKLS criteria (between 270 000 and 450 000 [3]). The prospective benefit of biannual screening is calculated to be between 800 and 1400 of saved lives per year in the UK [3, 23].

The NLST demonstrated that 320 individuals needed to be screened to prevent one cancer death with a corresponding cost of about $240 000 per lung cancer death avoided with LDCT screening in the USA [22]. By applying the UKLS risk model, a cost of $24 000 per quality-adjusted life year could be anticipated [3]. Comparing the costs of exhaled breath sampling based on bags (used to collect exhaled gases in conventional e-nose devices) and on Pneumopipe, it is worth remarking that each disposable sampling bag costs around €24, while each collection performed via Pneumopipe costs only €10 (including cartridge and device consumables).

Needless to say, this preliminary study presents several limitations. The evaluated series is small and this is in line with the paucity of data in the literature; similar to our previous publication on the use of e-nose technology which allowed for stratification of COPD severity by analysing the breath fingerprints of 25 patients [17], the current could be considered a proof-of-concept study requiring confirmation on larger numbered series. Additional recruitment of individuals to be subjected to BIONOTE could also be crucial to the assessment of possible confounding factors (COPD and other comorbidities). Finally, prospective, randomized studies are needed to substantiate the value of this technology in reducing false positives following LDCT.

In conclusion, the system consisting of BIONOTE and Pneumopipe is an e-nose technology supported by a novel exhaled breath collection device, which represents an important innovation and has shown considerable promise in contributing to the detection of lung cancer. Several characteristics of this device favour its use in LDCT screening programmes and, possibly, in the follow-up of lung cancer patients after radical treatment in order to avoid an indiscriminate use of CT and PET imaging. Among these characteristics, it is worth mentioning that exhaled breath sampling via Pneumopipe is completely non-invasive, enabling a realistic and effective utilization in a prescreening programme.

Conflict of interest: none declared.

REFERENCES

[1] Aberle DR, Adams AM, Berg CD, Black WC, Clapp JD, Fagerstrom RM *et al*. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365:395–409.

[2] Krantz SB, Meyers BF. Health risks from computed tomographic screening. Thorac Surg Clin 2015;25:155–60.

[3] Field JK. The screening imperative. Nature 2014;513:S7.

[4] Blackmon SH, Feinglass SR. The United States Preventative Services Task Force recommendations for lung cancer screening. Thorac Surg Clin 2015; 25:199–203.

[5] Nair A, Hansell DM. European and North American lung cancer screening experience and implications for pulmonary nodule management. Eur Radiol 2011;21:2445–54.

[6] Sozzi G, Boeri M, Rossi M, Verri C, Suatoni P, Bravi F *et al*. Clinical utility of a plasma-based miRNA signature classifier within computed tomography lung cancer screening: a correlative MILD trial study. J Clin Oncol 2014;32: 768–73.

[7] Phillips M, Herrera J, Krishnan S, Krishnan S, Zain M, Greenberg J *et al*. Variation in volatile organic compounds in the breath of normal humans. J Chromatogr B Biomed Sci Appl 1999;729:75–88.

[8] Hunter GW, Xu JC, Biaggi-Labiosa AM, Laskowski D, Dutta PK, Mondal SP *et al*. Smart sensor systems for human health breath monitoring applications. J Breath Res 2011. doi:10.1088/1752–7155/5/3/037111.

[9] D'Amico A, Di Natale C, Falconi C, Martinelli E, Paolesse R, Pennazza G *et al*. Detection and identification of cancers by the electronic nose. Expert Opin Med Diagn 2012;6:175–85.

[10] Pennazza G, Santonico M, FinazziAgrò A. Narrowing the gap between breathprinting and disease diagnosis, a sensor perspective. Sens Actuators B Chem 2012. doi:10.1016/j.snb.2012.09.103.

[11] Li W, Liu HY, Jia ZR, Qiao PP, Pi XT, Chen J *et al*. Advances in the early detection of lung cancer using analysis of volatile organic compounds: from imaging to sensors. Asian Pac J Cancer Prev 2014;15:4377–84.

[12] Di Natale C, Macagnano A, Martinelli E, Paolesse R, D'Arcangelo G, Roscioni C *et al*. Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. Biosens Bioelectron 2003; 18:1209–18.

[13] Phillips M, Gleeson K, Hughes JM, Greenberg J, Cataneo RN, Baker L *et al*. Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. Lancet 1999;353:1930–3.

[14] Horváth I, Lázár Z, Gyulai N, Kollai M, Losonczy G. Exhaled biomarkers in lung cancer. Eur Respir J 2009;34:261–75.

[15] McWilliams A, Beigi P, Srinidhi A, Lam S, MacAulay C. Sex and smoking status effects on the early detection of early lung cancer in high-risk smokers using an electronic nose. IEEE Trans Biomed Eng 2015;62: 2044–54.

[16] Santonico M, Pennazza G, Grasso S, D'Amico A, Bizzarri M. Design and test of a biosensor-based multisensorial system: A proof of concept study. Sensors 2013;13:16625–40.

[17] Incalzi RA, Pennazza G, Scarlata S, Santonico M, Petriaggi M, Chiurco D *et al*. Reproducibility and respiratory function correlates of exhaled breath fingerprint in chronic obstructive pulmonary disease. PLoS One 2012;7: 1–8.

[18] Rocco G, Allen MS, Altorki NK, Asamura H, Blum MG, Detterbeck FC *et al*. Clinical statement on the role of the surgeon and surgical issues relating to computed tomography screening programs for lung cancer. Ann Thorac Surg 2013;96:357–60.

[19] Phillips M, Altorki N, Austin JH, Cameron RB, Cataneo RN, Kloss R *et al*. Detection of lung cancer using weighted digital analysis of breath biomarkers. Clin Chim Acta 2008;393:76–84.

[20] D'Amico A, Pennazza G, Santonico M, Martinelli E, Roscioni C, Galluccio G *et al*. An investigation on electronic nose diagnosis of lung cancer. Lung Cancer 2010;68:170–6.

[21] Santonico M, Lucantoni G, Pennazza G, Capuano R, Galluccio G, Roscioni C *et al*. In situ detection of lung cancer volatile fingerprints using bronchoscopic air-sampling. Lung Cancer 2012;77:46–50.

[22] Chudgar NP, Bucciarelli PR, Jeffries EM, Rizk NP, Park BJ, Adusumilli PS *et al*. Results of the National Lung Screening Trial: where are we now? Thorac Surg Clin 2015;25:145–53.

[23] Field JK, Oudkerk M, Pedersen JH, Duffy SW. Prospects for population screening and diagnosis of lung cancer. Lancet 2013;382:732–41.
[24] Heuvelmans MA, Oudkerk M, de Bock GH, de Koning HJ, Xie X, van Ooijen PMA *et al*. Optimisation of volume-doubling time cutoff for fast-growing lung nodules in CT lung cancer screening reduces false-positive referrals. Eur Radiol 2013;23:1836–45.
[25] Pastorino M, Silva M. Refining strategies to identify population to be screened for lung cancer. Thorac Surg Clin 2015;25:217–21.

APPENDIX. CONFERENCE DISCUSSION

Dr C. Pompili *(Leeds, UK)*: Dr. Rocco, are you planning to use this technology for the follow-up of cancer patients and are you hoping to obtain the same sensitivity and specificity rate?

Dr Rocco: We have not yet evaluated patients from a post-surgical point of view. However, it is definitely a thought that we have for the future. We could also somehow establish an incontrovertible nexus maybe between PET scans integrated with the e-nose. That could be another issue to look at in the future.

Dr S. Grondin *(Calgary, AB, Canada)*: Your excellent presentation embodies what the young investigator's novel thinking should be. In Calgary we have been working with a canine model with a similar type of idea, but I like the idea of using your technology that is going to be coming out. Can you tell me, is there any information on the canine model sensitivity versus your instrument as to what might be more sensitive?

Dr Rocco: With regard to the literature, we did analyse a little bit the outcomes from the canine technology, and we found that the results were not as encouraging as in our e-nose model. Of course the numbers, even in that technology, were not very large; in fact they are not big numbers, but in terms of sensitivity and specificity we have had better results so far.