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LETTER TO THE EDITOR

SIX CONSECUTIVE DAYS REPRODUCIBILITY OF EXHALED VOLATILE ORGANIC COMPOUNDS ANALYSIS BY E-NOSE

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To the Editor,

Breathomics are defined as the comprehensive molecular analysis of exhaled breath volatile organic compounds (VOCs), including several techniques such as gas-chromatography and mass spectrometry (GC-MS), and electronic noses (e-noses) (1). In particular, E-nose technology is based on sensor arrays which react to specific molecules allowing to discriminate VOC combinations by pattern recognition software (1). This approach has shown its potential to offer quick and non-invasive biomarkers for diagnosing and monitoring a number of respiratory diseases such as lung cancer, asthma and chronic obstructive pulmonary disease (2). However, before its validation, numerous methodological concerns regarding e-nose technology still need to be addressed, among which, the assessment of repeatability of breath samplings by e-nose is essential, either in healthy subjects or in patients with respiratory conditions (3).

For the above reason, the aim of this study was to investigate the repeatability of exhaled VOCs-profile by e-nose in healthy adults during six consecutive days of sampling. Twenty-four healthy, never-smoking subjects (9 males, 15 females), with a negative anamnesis of chest symptoms and systemic diseases and not taking any medications were enrolled in the study. Mean age was 50.9 years [±15.7 standard deviation (SD)]. All had normal lung function [mean forced expiratory volume in one second (FEV1) was 103.2% of predicted (±12.4 SD) and mean forced vital capacity (FVC) was 105.4% of predicted (±14.3 SD)]. None of the subjects described upper or lower respiratory tract infection in the 28 days prior to the first measurement, nor during the days of sampling. All participants were volunteers and were enrolled from hospital staff. A sequence of exhaled breath measurements were performed on all individuals at the same hour for six consecutive days.

The current study was approved by our local ethics committee and all participants signed an informed consent to participate in the study. Our study had a longitudinal design. The measurements were performed during six separate and consecutive visits (day 0, day 1, day 2, day 3, day 4, day 5). Participants were not allowed to eat or drink anything except water in the 3 h prior to the tests, or perform physical exercise.

Exhaled breath was collected as follows: subjects breathed with a nose-clip at tidal volume for 5 min through a 2-way non-rebreathing valve (Hans Rudolph 2700, Hans Rudolph, USA) connected to an inspiratory VOC filter (A2, North Safety, Netherlands) Then, a single vital capacity volume

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was exhaled into a Tedlar bag and immediately sampled by the e-nose (Cyranose 320, Sensigent, USA: nano-composite array of 32 organic polymer sensors). Operating parameters were: sampling time 60"; purging time 200"; temperature 42°C. Flow-volume spirometry (MasterscreenPneumo, Jaeger, Wurzburg, Germany) was performed on all participants during day 0 after breath collection. FEV1 and FVC were collected.

Raw data of the exhaled breath samples were analyzed by SPSS software, version 18.0. Principal component analysis (PCA) and subsequent linear canonical discriminant analysis (CDA) were calculated, providing the Cross Validated Accuracy percentage (CVA%), which estimates how accurately a predictive model will perform in practice. Pearson correlation coefficient was used to evaluate relationships between principal components and clinical variables. We estimated sample size based on data from previous studies (4, 5). A p-value of <0.05 was considered statistically significant.

The two-dimensional principal component analysis plot showed that the exhaled VOC-patterns among six days (days 0,1,2,3,4 and 5) could not be distinguished from each other (Fig. 1). The CDA of those data showed a CVA of 24.3%, confirming the absence of differences. Moreover, no relationships between principal components and age, sex, FEV1 and FVC were reported (p=ns for all).

Based on our results, it appears that the exhaled VOC-profile measured by our e-nose was stable during six consecutive days of sampling. To the best of our knowledge, this is the first study which investigated exhaled VOC-spectrum by e-nose for such a long consecutive period of time. Our data extend previous investigations about e-nose signal

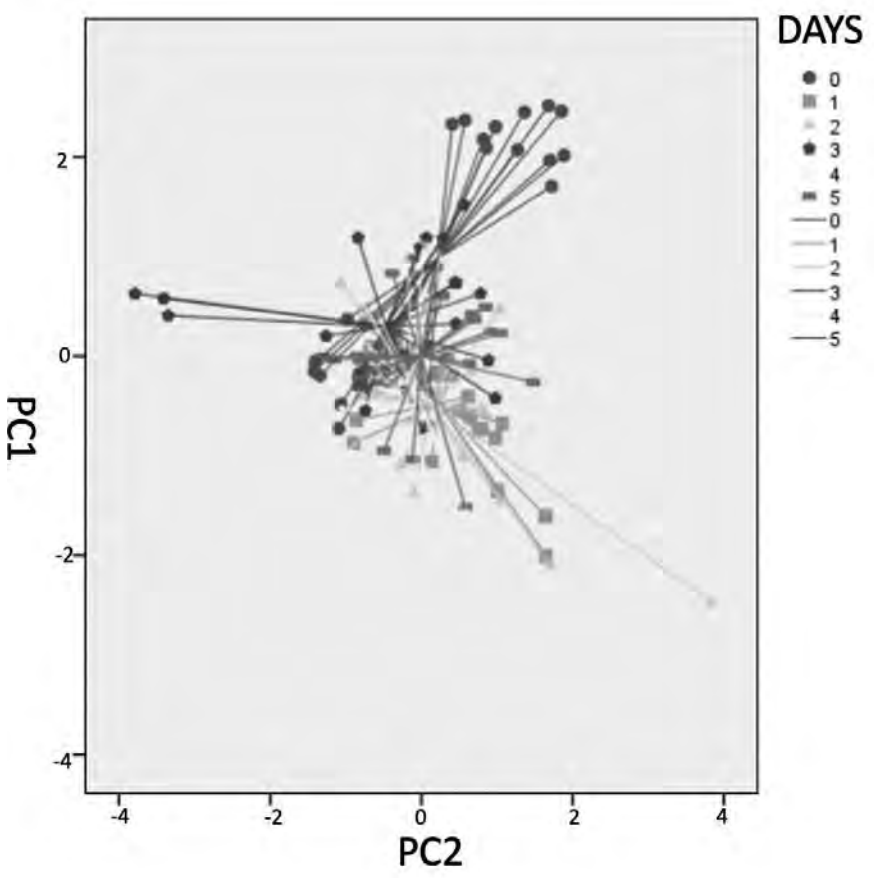

Fig. 1. *Two dimensional principal component analysis plot, showing that exhaled VOC-profiles among six days (Days 0,1,2,3,4 and 5) are indistinguishable from each other. PC: Principal Component.*

repeatability. Bofan et al. (6) found acceptable within-day and between-day repeatability for the same e-nose as ours by evaluating two breath samples collected 30 min and seven days apart, although sensors showed different repeatability (6). Similarto the above, Kovacs et al. (7) assessed temporal changes of VOC pattern in exhaled breath at baseline as well as 1 day, 1 week, 4 weeks and 8 weeks after showing no significant changes. Moreover, Fasola et al. (8), by using a recently manufactured e-nose named Pneumopipe, showed more than acceptable within-day and between-day repeatability (breath collection twice during the baseline visit, 30 min apart and after 7 days), both in stable moderate asthmatics and in healthy children. Interestingly, the within-day and between-day for all spirometry parameters and exhaled nitric oxide were significant and similar to those of the most reliable sensors (8).

The present findings warrant further investigations, aiming to assess the long-term repeatability of different e-nose technologies in patients with several respiratory disturbances both in clinical stability and exacerbation. Furthermore, wide multicentric studies should be conducted for evaluating and monitoring drug-treatment in patients with lung diseases.

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