



# Influence of age and gender on the profile of exhaled volatile organic compounds analyzed by an electronic nose

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**Submitted:** 20 August 2015.  
**Accepted:** 30 November 2015.

Study carried out in the Disciplina di Malattie dell'Apparato Respiratorio, Dipartimento di Scienze Mediche di Base, Neuroscienze e Organi di Senso, Scuola di Medicina, Università degli Studi di Bari, Bari, Italia.

Since the discovery of electronic noses, or e-noses, and of their application in the molecular profiling of exhaled breath (i.e., creation of breathprints), great advances have been made with respect to the discrimination of diseases through the comparison of overall breathprints. Numerous studies have shown the potential for applying exhaled volatile organic compound (VOC) profiling in three classes of respiratory diseases: lung cancer, respiratory infections, and obstructive lung diseases. After the ability of e-noses to sniff out these diseases was proven, the question of what constitutes the exhaled markers of those pathologies was raised. Because e-noses assess the overall mixture of VOCs in exhaled breath, no primary discriminating markers can be specified as being suggestive of the pathophysiological pathways involved. In addition, likely sources of signal interference must be identified and corrected for, because they are potentially confounding factors.<sup>(1)</sup> The exhaled VOC profile can be influenced by disease-associated factors, such as airway caliber and airway inflammation; treatment-associated factors, such as medication use; and patient-associated factors, such as age, gender, comorbidities, pregnancy, diet, and smoking.<sup>(2)</sup> Concerning age and gender, these two factors are known to alter VOC levels.<sup>(2)</sup> Previous studies on e-nose exhaled breath profiling in several diseases have suggested that age does not affect the overall VOC profile.<sup>(3,4)</sup> However, to our knowledge, there have been no studies specifically addressing e-nose analysis of exhaled biomarkers in relation to age and gender differences in healthy subjects. Therefore, the aim of the present study was to investigate the effects of age and gender on exhaled breath VOC profiles, as analyzed by an e-nose, in a population of healthy adults.

## ABSTRACT

We aimed to investigate the effects of age and gender on the profile of exhaled volatile organic compounds. We evaluated 68 healthy adult never-smokers, comparing them by age and by gender. Exhaled breath samples were analyzed by an electronic nose (e-nose), resulting in "breathprints". Principal component analysis and canonical discriminant analysis showed that older subjects ( $\geq 50$  years of age) could not be distinguished from younger subjects on the basis of their breathprints, as well as that the breathprints of males could not distinguish from those of females (cross-validated accuracy, 60.3% and 57.4%, respectively). Therefore, age and gender do not seem to affect the overall profile of exhaled volatile organic compounds measured by an e-nose.

**Keywords:** Breath tests; Volatile organic compounds; Electronic nose.

In this cross-sectional study, exhaled breath samples were obtained from 68 healthy adults between 20 and 68 years of age. Participants were volunteers recruited from among hospital staff members. We selected an equal number of individuals  $< 50$  years of age ( $n = 34$ ) and  $\geq 50$  years of age ( $n = 34$ ). Of the 68 volunteers, 32 (47.1%) were male. All were never-smokers, none had a history of chest symptoms, and all were free of any known disease. All had an  $FEV_1 > 70\%$  of the predicted value and an  $FEV_1/FVC$  ratio  $> 80\%$ . None had experienced any upper or lower respiratory tract infections in the 4 weeks prior to the study. We evaluated the study sample by age group ( $< 50$  vs.  $\geq 50$  years of age) and by gender. The study was approved by the Research Ethics Committee of the University of Bari School of Medicine, in the city of Bari, Italy (Protocol no. 46403/15), and all participating subjects gave written informed consent.

All measurements were obtained during a single visit. Subjects were asked to refrain from eating and drinking, as well as from engaging in strenuous physical exercise, for at least for 3 h before the visit.

Spirometry was performed by a trained lung function technician, in accordance with the latest European Respiratory Society recommendations,<sup>(5)</sup> and the equipment (MasterScreen Pneumo; Jaeger; Würzburg, Germany) was calibrated daily. For all subjects,  $FEV_1$  and FVC were measured. Exhaled breath analysis was performed as previously described.<sup>(3)</sup> In brief, after 5 min of tidal breathing through a 3-way non-rebreathing valve connected to an inspiratory VOC filter (A2; North Safety, Middelburg, the Netherlands), subjects exhaled a single vital capacity volume into a Tedlar bag connected to an e-nose.

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Financial support: None.

We used a commercially available, handheld e-nose (CyranoSE 320; Smith Detection, Pasadena, CA, USA) with a nanocomposite array of 32 organic polymer sensors. When the sensors are exposed to a mixture of VOCs, the polymers swell, inducing a change in their electrical resistance. The raw data are captured as the changes in resistance of each of the 32 sensors in an onboard database, producing a distribution profile (breathprint) that describes the VOC mixture and can be analyzed with pattern-recognition algorithms.<sup>(6)</sup>

The estimated sample size was based on data from previous studies.<sup>(3,4,7)</sup> We calculated the sample size by estimating the standard error of the percentage of correctly classified patients:

$$SE = \sqrt{C(100 - C)/n}$$

where *SE* is the standard error, *C* is the percentage of patients classified correctly, and *n* is the estimated sample size. The reliability of the percentage correct classification is dependent on the standard error, which is itself a function of *p*. If the percentage of patients classified correctly is between 50% and 75%, the current sample sizes per subgroup provide standard errors between 8% and 9%. The raw data were analyzed with the Statistical Package for the Social Sciences, version 16.0 (SPSS Inc., Chicago, IL, USA). Data were reduced to a set of principal components capturing the largest amount of variance of the original 32 sensors. To select the principal components which best discriminated among the groups, we used one-way ANOVA. Afterwards, these principal components were then used in a canonical discriminant analysis (CDA), in order to classify cases into a categorical partition. Using the "leave-one-out" method, we calculated the cross-validated accuracy (CVA), which is expressed as a percentage. The CVA provides an estimate of how accurately a predictive model will perform in practice. For each case, the probability of a positive diagnosis was calculated on the basis of the linear canonical discriminant function.

The characteristics of the study population, as a whole and by age group, are described in Table 1. No significant differences were found for FEV<sub>1</sub>, although there was a slight difference between the two age groups in terms of the BMI. The two-dimensional principal component analysis plot showed that the breathprints of older subjects could not be distinguished from those of younger subjects (Figure 1). The CDA of those data showed a CVA of 60.3%, indicating that the difference was not significant. Similarly, the breathprints of males could not be distinguished from

those of females (Figure 1), the CDA showing a CVA of 57.4%, also indicating a less than significant difference.

Our results suggest that, although aging modifies the individual components of exhaled breath, the overall VOC profile, as measured by an e-nose, does not differ between age groups. Likewise, gender seems to have no influence on the exhaled VOC spectrum.

To our knowledge, this is the first study specifically addressing e-nose-analyzed exhaled biomarkers in relation to age and gender in well-characterized healthy subjects. Research on age- and gender-specific metabolic dissimilarities is essential for understanding the physiological and metabolic phenotype of healthy subjects. It is known that the number of neutrophils in induced sputum increase with advancing age,<sup>(8,9)</sup> as does the CD4+/CD8+ lymphocyte ratio in BAL fluid.<sup>(10)</sup> These data are consistent with those of studies showing that, with aging, oxidative stress increases and clearance of cytochrome p450 decreases.<sup>(11)</sup> In addition, various studies have shown gender-specific metabolomic profiles in the urine and serum of healthy subjects.<sup>(12)</sup> However, there have been few studies focusing on exhaled human breath. Furthermore, studies employing gas chromatography-mass spectrometry analysis have demonstrated that there are age-related changes in the VOC profile of exhaled air in healthy individuals.<sup>(13)</sup> Bikov et al. found a significant correlation between e-nose-analyzed breathprints and age only in lung cancer patients.<sup>(7)</sup> Conversely, studies have shown that the ability of an e-nose to distinguish among healthy controls, individuals with asthma, and individuals with COPD is not influenced by differences in age.<sup>(3,4)</sup> Only a few studies have identified gender-specific VOCs in human exhaled breath, as analyzed by gas chromatography-mass spectrometry.<sup>(14,15)</sup> A very recent study using an e-nose showed that gender has an effect on the classification of breathprints in high-risk smokers.<sup>(16)</sup>

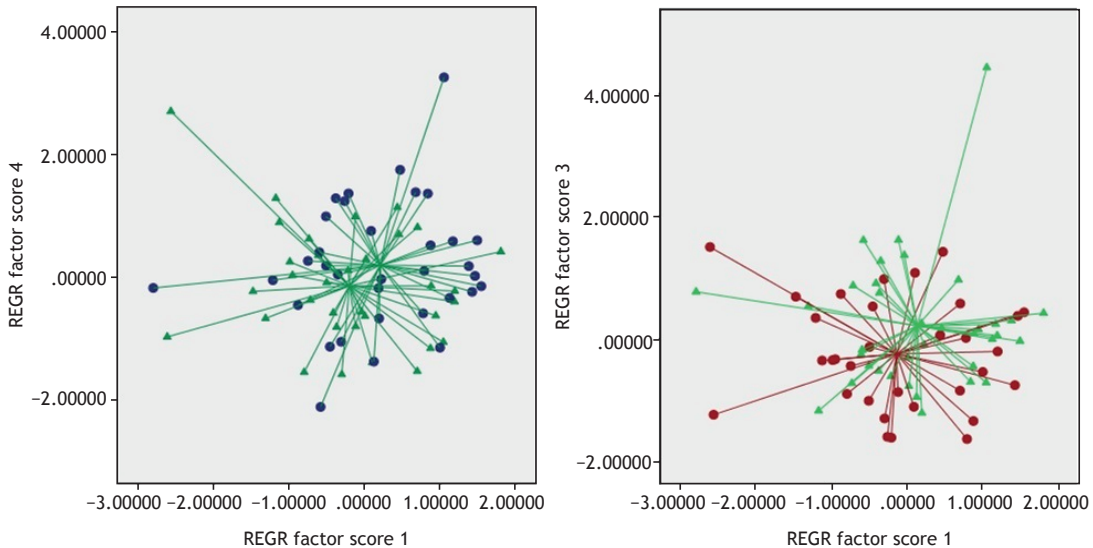
How can we explain our results? Human exhaled breath contains more than 3,000 VOCs deriving from physiologic and pathophysiological mechanisms operating via metabolic pathways.<sup>(8)</sup> In accordance with the findings of previous studies, our data suggest that, despite the presence of age- and gender-specific VOCs in healthy human exhaled breath, the overall VOC profile does not seem to be influenced by either age or gender.

What are the implications of our findings? Our results indicate that careful age- and gender-matching might not be necessary in future comparative studies.

**Table 1.** Clinical characteristics of a sample of healthy adult never-smokers.<sup>a</sup>

Characteristic	All subjects (n = 68)	< 50 years of age (n = 34)	≥ 50 years of age (n = 34)	p*
Female gender, n (%)	36 (52.9)	7 (20.6)	12 (35.3)	-
Age (years)	43.2 ± 11.3	33.1 ± 8.0	55.6 ± 4.7	< 0.01
FEV <sub>1</sub> (% of predicted)	104.7 ± 11.8	106.2 ± 11.3	103.3 ± 12.3	ns
BMI (kg/m <sup>2</sup> )	25.25 ± 3.3	24.8 ± 3.8	25.7 ± 2.9	< 0.05

<sup>a</sup>Values are expressed as mean ± SD, except where otherwise indicated. \*ANOVA between the two groups.



**Figure 1.** Two dimensional principal component analysis plot, showing that exhaled breath profiles (breathprints) of subjects  $\geq 50$  years of age (left, triangles) are indistinguishable from those of subjects  $< 50$  years of age (left, circles). Similarly the breathprints of male subjects (right, circles) could not be distinguished from those of female subjects (right, triangles). REGR: relative elemental growth rate.

Nevertheless, further studies with larger populations are needed in order to confirm our findings and to

investigate other possible confounding factors, such as pregnancy, medication, diet, and smoking.

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