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Short communication

An electronic nose may sniff out amyotrophic lateral sclerosis

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ABSTRACT

Amyothrophic lateral Sclerosis (ALS) is a neurodegenerative disease characterized by a progressive degeneration of the cortical and spinal motor neuron. Exhaled molecular profiles that have potential in the diagnosis of several respiratory and systemic diseases can be obtained by analyzing human breath with an electronic nose.

We hypothesized that exhaled molecular profiling may discriminate well-characterized patients with ALS from controls.

20 ALS patients (age: 63.5 ± 12.3), and 20 healthy controls (age: 58.1 ± 4.4) participated in a cross-sectional study. A Tedlar bag was used to collect exhaled breath by using a validated method. Bags were then sampled by an electronic nose (Cyranose 320). Statistical analysis on sensor responses was performed off-line by principal component analysis, linear discriminant analysis and ROC curves.

Breathprints from patients with ALS were discriminated from healthy controls (CVA: 75.0%; p = 0.003; AUC 0.795).

Based on our results, patients with ALS can be discriminated from healthy controls. This suggests that exhaled breath analysis has potential for screening and/or diagnosis of this neuromuscular disease.

1. Introduction

Amyothrophic lateral Sclerosis (ALS) is a neurodegenerative disease characterized by a progressive degeneration of the first (cortical) and second (spinal) motor neuron.

During advanced degrees of ALS, due to the degeneration of motor neurons innervating respiratory muscles, a progressive lung function impairment occurs. Therefore it may have serious and irreversible consequences on respiratory system, which very often brings to hypercapnic respiratory failure and death (Shaw and Eggett, 2000).

ALS is known to be a multifactorial pathology (Shaw and Eggett, 2000). However, to date, no specific etiological factors which explain the evolution towards a neuronal degeneration has been identified (Shaw and Eggett, 2000).

The diagnosis of ALS requires a combination of complex and burdensome procedures, primarily focused on excluding other diseases. Thus far, a test which provides a non-invasive and cost-effective diagnosis of ALS is not available.

Recently, three proteins in the cerebrospinal fluid have been proposed as markers of ALS presence (Pasinetti et al., 2006). Moreover, a number of blood volatile organic compounds (VOCs) were identified at the early stage of disease in a murine model (Jiang et al., 2015).

Research of new diagnostic markers for ALS is ongoing, in the attempt to establish an early diagnosis and treatment of patients affected by this disease and subsequently to extend their survival.

It is well known that exhaled breath contains thousands of VOCs in gaseous form which may be used as markers of airway inflammation and respiratory diseases (Pauling et al., 1971; Moser et al., 2005). The use of an electronic nose could allow a rapid analysis of VOCs pattern in real time (Briglin et al., 2002).

Recent studies have shown that an electronic nose has the potentials to become a diagnostic tool for various pulmonary and systemic pathologies (Röck et al., 2008), including Alzheimer disease (Mazzatenta et al., 2015) and schizophrenia (Di Natale et al., 2005). Therefore, it is likely that the exhaled VOCs profile may have a role in ALS diagnosis.

Based on the above, the aim of our study was to investigate whether an electronic nose can distinguish the exhaled breath of well-characterized patients with ALS from healthy controls in terms of VOCs composition.

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2. Materials and methods

2.1. Subjects

A total number of 40 subjects participated to the current study. All the subjects were never-smoking adults (38–83 years). The study population included 2 groups of subjects: patients with an established diagnosis of ALS and a healthy control group. Patients were recruited among those visiting the outpatient clinic of our Respiratory Diseases Unit, whereas controls were enrolled among personal contacts. None of them had any upper or lower respiratory tract infection in the 4 weeks before the study.

The study group was composed by 20 patients with diagnosis of clinically defined ALS (n = 13) and/or clinically probable ALS (n = 7), according to El Escorial revised criteria (Brooks et al., 2000). 16 individuals out of 20 had spinal onset, whereas 4 had bulbar onset. Patients with any other acute or chronic disease than ALS were excluded from the study.

The control group was composed by 20 subjects with a negative history of chest symptoms and without of any known disease. The study was approved by the local ethics committee (protocol approval number 46403) and a written informed consent was signed by all participants.

2.2. Study design

We performed a cross-sectional case-control study. One visit was sufficient to complete all measurements. Subjects were asked to refrain from eating, drinking and performing vigorous exercise at least for 3 h before the study. We collected exhaled breath from all participants and sampled by the electronic nose straightaway.

2.3. Exhaled breath collection and sampling

We collected exhaled breath according to a previously validated method (Dragonieri et al., 2007). Briefly, all subjects inhaled VOC-filtered air for 5 min with a specifically designed equipment. Then, subjects exhaled a vital capacity volume into an inert bag, connected to the electronic nose (Cyranose 320, IOS, Pasadena, CA, USA).

2.4. Data analysis

Statistical analysis was performed by using SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA). Principal Component Analysis (PCA) was used to capture the variance of the raw data into a set of principal components. Univariate ANOVA analysis was used to select the best discriminating components. Subsequently, the selected principal components were included into a linear Canonical Discriminant Analysis (CDA), to classify cases accordingly. The "leave-one-out method" was used to calculate the Cross Validated Accuracy percentage (CVA, %). Based on the probability of a positive diagnosis for each case, we created a receiver operator curve (ROC-curve) with 95% confidence limits. The sample size calculation was based on limiting the standard error of estimated diagnostic measures to 10% at most. Assuming 75% accuracy, a sample size of 20 patients per group was sufficient. A p-value of <0.05 was considered significant.

3. Results

Table 1 describes the clinical characteristics of the two groups. Patients with ALS were somewhat older than controls ($p < 0.05$). FEV1 and FVC were significantly lower in ALS patients compared to controls ($p < 0.01$).

Table 1
Subject characteristics.

	ALS	Healthy Controls
Subjects (n)	20	20
Age (yrs,mean ± SD)*	63.5 ± 12.3	58.1 ± 4.4
Males	9	11
Females	11	9
FEV1%pred.**	78.6 ± 22.3	100.8 ± 11.7
FVC%pred.**	79.8 ± 18.4	104.0 ± 10.5

Values are indicated as mean ± SD.
* $p < 0.05$.
** $p < 0.01$ by independent samples *t*-test.

The two-dimensional Principal Component Analysis graph showed that patients with ALS could be discriminated from healthy controls (Fig. 1). Subsequent Canonical Discriminant Analysis resulted in a Cross Validated Accuracy% of 75.0 ($p = 0.003$). The area under the curve of the ROC-curve for the discrimination between ALS and healthy controls was 0.795 (Fig. 2).

4. Discussion

This study shows that an electronic nose is capable to distinguish the exhaled breath of patients with ALS from healthy controls. Our findings indicate that the VOC-spectrum in human exhaled breath is different between subjects with and without ALS. This deserves further diagnostic validation of e-nose technology in ALS.

To the best of our knowledge this is the first study which applies exhaled breath molecular pattern recognition by electronic noses in ALS. Besides, no data are available for exhaled breath VOCs analysis in neuromuscular diseases. As yet, the potential of electronic nose technology has already been tested for detecting a variety of other diseases, in the field of microbiology, otolaryngology, nephrology, endocrinology, neurology, oncology and pulmonology (Röck et al., 2008; van der Schee et al., 2015). Our findings suggest that the recognition of ALS by electronic nose is also a realistic option that deserves further clinical validation.

The current study has a number of strong points. All selected patients were well-characterized by worldwide accepted guidelines (Brooks et al., 2000) and were recruited by the same operator and from the same outpatient clinic. We also obtained a well-defined control group. Moreover, previously validated sampling techniques and breathing maneuvers were used to reduce any influence on the exhaled VOC-profile by environmental VOCs (Dragonieri et al., 2007). Finally we excluded smokers and ex-smokers, as well as patients with comorbidities, such as diabetes mellitus and renal failure which are known to alter the VOCs spectrum (Röck et al., 2008).

In contrast, our study has two major limitations. First, the current sample size was relatively small due to the small incidence of ALS. Nevertheless, it appeared to be sufficient for obtaining a distinction between breathprints of the two groups. This is supported by the 95% confidence limits of the ROC-curve. Indisputably, it is mandatory to set up further investigations with larger and independent population for analyzing exhaled breath profiling in different stages of ALS.

Second, it might be argued that patients with ALS were assuming specific therapy for their illness. The treatment of our patients with ALS included neurological medications such as riluzole, amitriptyline and phenytoin. We cannot exclude that these drugs may have influenced the VOC-profile, potentially reducing or increasing the accuracy in detecting the disease.

How can our results be interpreted? Despite researchers' efforts, no direct mechanism for ALS has been identified yet. During the last years, several etiopathogenetic hypothesis have been proposed for ALS, including oxidative stress due to free radical development,