

Can the electronic nose diagnose chronic rhinosinusitis? A new experimental study

E. Bruno · M. Alessandrini · F. Ottaviani · A. Delfini ·
D. Di Piero · A. Camillo · A. De Lorenzo

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Abstract In otorhinolaryngologist's experience the nasal out-breath of people affected by chronic nasal or paranasal infections may be characterized by peculiar odours. In a previous study we showed that an electronic nose (EN), examining nasal out breath was able to distinguish subjects affected by chronic rhinosinusitis from healthy subjects. The present study is aimed at analysing the intensity and the quality of the odorous components present in the air expired by patients affected by rhinosinusitis, using a new EN based on gas-chromatography and surface acoustic wave analysis. In the gas-chromatographic tracings of the pathologic subjects there were six peaks, which were not present in control group cases. These peaks correspond to odorous components, whose chemical composition ranges from C6 to C14. Peaks obtained were compared with other tracings revealed from specific bacterial and fungal cultures analyses and we appreciated some analogies.

Keywords Electronic nose · Chronic rhinosinusitis

Introduction

In common otorhinolaryngologist's experience, the nasal out-breath of people affected by chronic nasal or paranasal infections may be characterized by evident and peculiar odours. Although the role of viral, bacterial and fungal infections in sinusitis remains controversial, in our experience these odours are in most cases related to bacterial and/or fungal infections of the sinuses [4, 9, 14]. Therefore, we believe that the number, the type and the metabolism of these microorganisms may influence the intensity and quality of eminent odours.

Anatomic characteristics and clinical conditions of nasal and paranasal cavities may significantly influence the infective bacterial and fungal burden and its persistence, allowing the survival of particular bacterial species with peculiar metabolism and, eventually, the production of typical odours whose characteristics have been recently analysed by the electronic nose (EN) [14].

In a previous preliminary study, we compared the nasal outbreath of patients affected by chronic rhinosinusitis (CRS) versus healthy controls and we showed that EN was able to identify and classify typical patterns of the disease [8]. We employed a simple EN with eight quartz microbalance sensors, developed for research purposes only (Libranose, University of Rome Tor Vergata and Technobiochip) [3, 5]. Results were qualitatively classified by using the principal components analysis (PCA) and artificial neural network (ANN) [3, 6].

In the present study, however, a new and more sophisticated EN was employed. This new EN (zNose™) is based on gas-chromatography (GC) and surface acoustic wave technology (SAW) and it can detect up to picograms of volatile compounds in 10 s [11–13].

E. Bruno (✉) · M. Alessandrini · F. Ottaviani · A. Delfini ·
A. Camillo
Department of Otolaryngology, Policlinico "Tor Vergata",
Viale Oxford, 81, 00133 Rome, Italy
e-mail: bnapolitano@inwind.it

D. Di Piero
Department of Experimental Medicine,
"Tor Vergata" University, Rome, Italy

A. De Lorenzo
Department of Human Nutrition,
"Tor Vergata" University, Rome, Italy

Therefore, this study is aimed at analysing the intensity and the quality of the odorous components present in the air expired by patients affected by rhinosinusitis, always considering their eventual correlation with particular microorganisms. Some bacterial (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*) and fungal (*Candida albicans*) cultured species have already been analysed by the zNose™, which allows to examine the vapours obtained during the growth of microorganisms and the possibility of identifying them [1, 7].

Clinical presentation

The zNose™ consists of a heated inlet, vapour preconcentrator, temperature-programmed GC column and a solid state SAW detector, [10]. The SAW detector is a temperature-controlled quartz crystal, which absorbs vapours as they exit the GC capillary column. The fundamental acoustic frequency of the crystal changes according to the mass of each condensed analyte. Any chemical substance can be calibrated according to the retention times of standard odour mixture of linear chain *n*-alkanes and a chemical library can be created indexing other hydrocarbon compounds. Finally, a chromatogram showing retention times and total counts per second provides a qualitative and quantitative analysis of each specific chemical in the odour. Moreover, polar olfactory images of specific vapour mixtures can be obtained (VapourPrint™ images) plotting the sensor frequency change versus elution time; such images can be viewed and recognized as part of a previously learnt image set [11].

The right interpretation of the gas-chromatographic profile so obtained enables to point out the diversity of the single volatile compounds and, therefore, to carry out a not only quantitative but also, and above all, qualitative analysis.

We selected 14 subjects affected by CRS and 14 apparently healthy subjects in order to establish a control group. All the patients were recruited from the Department of the University of Rome Tor Vergata. The average age of patients affected by CRS was 43, while 35 was the average age of healthy volunteers.

The study protocol was approved by the University's Ethics Committee and each subject provided signed informed consent.

The study took place in two phases. We carried out an accurate anamnesis about the patients' personal data (age, height and weight) and about the pathologies correlated with CRS.

In particular, we formed some subgroups of patients affected, beyond CRS, by cystic fibrosis (three patients), ASA syndrome (one patient), allergic rhinitis (four patients) and Kartagener's syndrome (one patient).

Then we carried out a complete objective ENT (ear–nose–throat) exam, at the end of which we inserted a cotton swab in the middle meatus of one of the nasal fossae for about 5 min in order to allow its imbibition with the nasal secretion and then we inserted it in a sterilized hermetic seal test tube. In the same way we collected also the 14 tampons of the healthy subjects in order to establish the control group.

Swabs were extracted from Test tubes at uniform room temperature and were analysed by the EN. The test tubes were previously heated in a thermostat for 10 min so that the odorous particles could get more volatile. Before the analysis of the samples taken from pathologic and healthy subjects, the EN analysed the contents of a test tube in which a sterile nasal tampon had been inserted and this was done in order to exclude from our study the analysis of the components peculiar to the cotton of which the tampon is made. Then we carried out the analysis of each sample inserting a probe of the EN in every single test tube and aspirated the air that had to be examined.

Soon after the analysis time (10–20 s), we displayed the results on a computer diagram showing the peaks and the time of migration of each mole.

We performed the same procedure three times per sample, so that the results obtained in the first analysis could be confirmed.

Discussion

The analysis of the swabs belonging to subjects affected by CRS has given results different from the results obtained while observing the swabs of the 14 healthy subjects.

Such difference can be noticed by observing the GC tracings shown on a Cartesian coordinate system, where the time of study and the entity of the single peaks are represented on the *x* and *y* coordinates, respectively (Figs. 1, 2).

The GC tracings of the pathologic subjects show the presence of six peaks, which are not present in the group of the control cases and correspond to various odorous components, whose chemical composition ranges from C6 to C14.

Such peaks vary according to the observation time, being present, respectively at 2.9, 5, 6.9, 8.1, 8.5, 9.3 (Fig. 3).

The six peaks are present in the 14 pathologic subjects at different percentages:

- 6.9 peak present in 12 patients out of 14 (85,7%);
- 8.1 peak present in 11 patients out of 14 (78,6%);
- 8.5 and 5 peaks present in ten patients out of 14 (71,4%);

Fig. 1 Trace of healthy subject

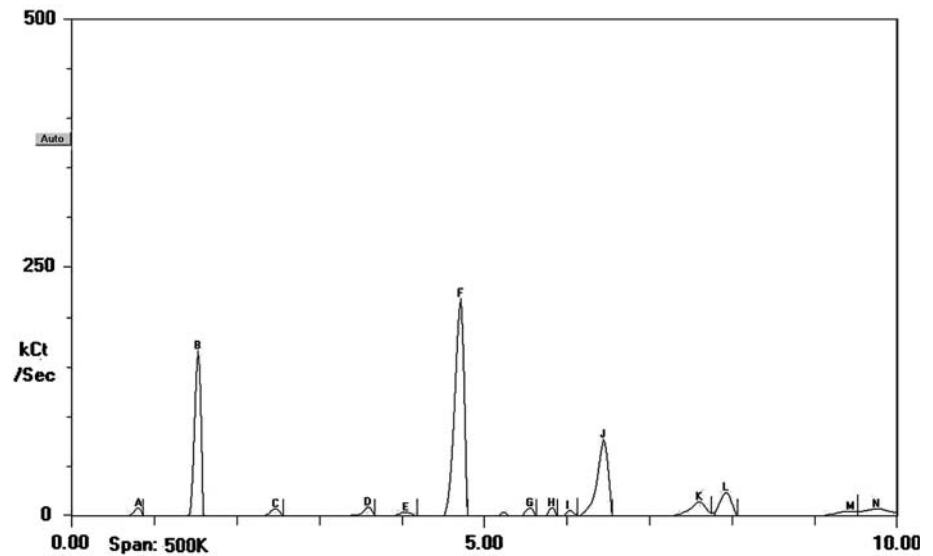


Fig. 2 Trace of patient with CRS

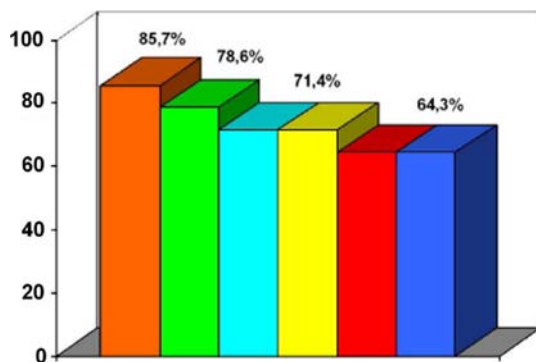
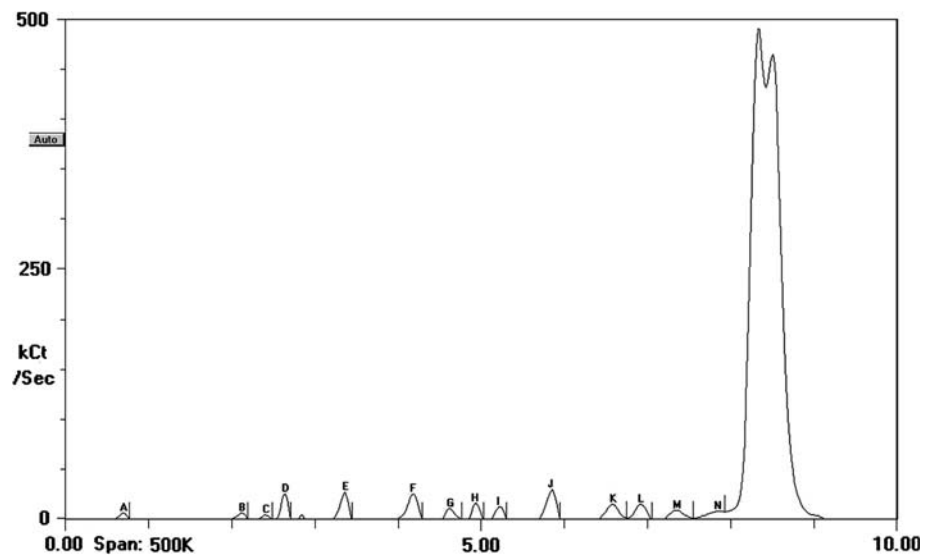


Fig. 3 Percentage of peaks present in pathologic subjects, (orange) 6.9 peak, (green) 8.1 peak, (sky blue) 8.5 peak, (yellow) 5 peak, (red) 2.9 peak, (blue) 9.3 peak

2.9 and 9.3 peaks present in nine patients out of 14 (64,3%).

Our study examined the possibility of using zNose™ in the diagnosis of the chronic nose-sinusal pathologies and the results strengthen such hypothesis.

In fact, the data obtained in the analysis carried out by the zNose™ on the samples of all subjects show the presence in the pathological cases of peaks normally absent in the control cases.

The difference between the healthy subjects' and the pathologic subjects' peaks suggests, in the future, a further study in order to identify the substances, analysed with the zNose™, corresponding to the six peaks present in the GC tracings of the pathologic subjects and to correlate each of

them with a particular etiologic agent. In fact, we can suppose that the odorous components present in the patients' secretions correspond to the final product of the bacterial and/or fungal metabolism [4, 9, 14].

In fact, comparing the GC tracings relative to the group of the pathologic subjects to the ones obtained in the analysis carried out by the zNose™ on *E. coli*, *P. aeruginosa* and *C. albicans* cultures we can deduce the following analogies [1–7]:

- the 6.9 peak, which is present in 12 patients out of 14 affected by CRS, is also present in the tracings obtained by the analysis of the odorous components produced by the cultured *E. coli*;
- the 2.9 and 8.1 peaks, present respectively in 10 and 11 patients out of 14 affected by CRS, are present also in the tracings obtained by the analysis of the odorous components produced both by the cultured *E. coli* and by the cultured *P. aeruginosa*;
- the 9.3 peak, present in 9 patients out of 14 affected by CRS is present also in the tracings obtained by the analysis of the odorous components produced by the cultured *C. albicans*.

Yet, the interpretation of the data reported above present a limit: the GC tracings of the microorganism are the results of an analysis carried out on cells cultured “in vitro” and therefore cannot be surely correlated with the tracings of our 14 pathologic subjects.

To conclude, we suppose that mass spectrometry might have an important role in the identification of the substances corresponding to the six peaks present in the tracings of the pathologic subjects, since it might enable us to determine the mass of the odorous moles already separated by GC during the analysis of the zNose™.

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