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TECHNICAL ADVANCES

The use of the PEN3 e-nose in the screening of colorectal cancer and polyps

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Introduction

Colorectal cancer (CRC) still afflicts a large number of patients worldwide and is the third most common cancer diagnosed in Western countries as well as the third most common cause of cancer deaths. Colonoscopy is the gold standard for CRC diagnosis but is unsuitable for mass screening, while fecal immunochemical testing (FIT) for occult blood in the feces suffers from low specificity (particularly for polyps) and insufficient patient compliance.

Recent studies using a metabolomic approach with gas chromatography-mass spectrometry (GC–MS) analysis of the exhaled breath of these patients have demonstrated the occurrence of significant changes in the pattern of their volatile organic compounds (VOCs) compared to healthy controls. Therefore, detection of an altered pattern of VOCs has been proposed as a potential screening tool in CRC [1]. However, the identification and analysis of these molecules by GC/MS is complex and time consuming.

Several commercial and/or custom electronic noses (e-nose) that try to reproduce human senses using sensor arrays and systems have been developed and are currently used in several research fields including medicine.

The aim of this study was to test the reliability of a commercial e-nose as screening tool for patients with CRC and polyps.

Materials and methods

After Ethics Committee approval, 3 groups of 15 patients were enrolled in the study (Table 1). The CRC cancer group patients (CRC) had an adenocarcinoma of the colon/rectum; the polyps group (POL) patients had at least 1 benign adenomatous polyp $\geq$1 cm in diameter and the healthy control group (HC) consisted of patients with a negative colonoscopy. Patients with previous or concomitant extra-colonic cancer or inflammatory bowel disease were excluded.

Exhaled breath collections were performed in the same room under similar environmental conditions in order to minimize the influence of indoor air contaminants.

The alveolar portions of the breath were collected using a commercially available breath-sampler (Loccioni, Italy) coupled to a self-assembled device for the automatic suction of breath in Tedlar® bags (Sigma-Aldrich) (3 l) (Fig. 1).

Patients refrained from eating and drinking for at least 3 h and were asked to breathe through a mouthpiece for 90 s. The sample was automatically sucked into the bag when the CO_2 level in the breath exceeded 3 %.

E-nose analysis

Analyses were performed using the electronic nose PEN3 (Airsense Analytics GmbH, Schwerin, Germany), a

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Table 1 Clinical characteristics of the study population

Groups in study	CRC	POL	HC
No. patients	15	15	15
Age (mean/range)	67.7 ± 12.5 (43–90) years	74.7 ± 7 (67–84) years	61.6 ± 12 (63–84) years
Male/female ratio	1.3	2	1.5
Disease location	8 rectum, 4 left colon, 3 right colon	2 rectum, 6 left colon, 1 transverse colon, 6 right colon	n.a.
TNM stage	1:pT1N0M0	n.a	n.a.
	5:pT3N0M0		
	6:pT3N1M0		
	1:pT3N2M0		
	2:pT4N1M0		

CRC Colorectal cancer, *POL* Polyps, *HC* Healthy controls, *n.a.* Not available

Fig. 1 Sampler employed for breath collection

compact (92 × 190 × 255 mm) and lightweight (2.3 kg) portable olfactory system, consisting of a gas sampling unit and a sensor array. Using a pattern recognition software (Win Muster v. 1.6.2), the PEN3 allows visualization and analysis of the data and works using filtered ambient air during the cleaning step or dilute gas during sampling with a maximum flow rate of 600 ml/min.

The sensor array is composed of 10 different thermos-regulated (200–500 °C) metal oxide thick film sensors (MOS) positioned in a very small stainless steel chamber (volume: 1.8 ml, temperature: 110 °C) and sensitive to different classes of chemical compounds (Table 2). The selectivity of the sensors is influenced by the sensing and the dopant materials employed, and by the working temperature and sensor geometry.

During the analysis when the VOCs react with the sensing film of the sensor surface, there is an oxygen exchange with a decrease in electrical conductivity,

Table 2 MOS sensors in the sensor array used in the experiment and sensitivities and detection limits for specific organic and inorganic gases

Sensor number	Sensor name	Sensor description and sensitivities	Detection limits
1	W1C	Aromatic organic compounds	Toluene, 10 mg kg 1
2	W5S	Very sensitive, broad range sensitivity, reacts to nitrogen oxides, very sensitive with negative signal	NO_2, 1 mg kg 1
3	W3C	Ammonia, also used as sensor for aromatic compounds	Benzene, 10 mg kg 1
4	W6S	Detects mainly hydrogen gas	H_2, 0.1 mg kg 1
5	W5C	Alkanes, aromatic compounds, and non-polar organic compounds	Propane, 1 mg kg 1
6	W1S	Sensitive to methane. Broad range of organic compounds detected	CH_3, 100 mg kg 1
7	W1W	Detects inorganic sulfur compounds, e.g. H_2S. Also sensitive to many terpenes and sulfur containing organic compounds	H_2S, 1 mg kg 1
8	W2S	Detects alcohol, partially sensitive to aromatic compounds, broad range	CO, 100 mg kg 1
9	W2W	Aromatic compounds, inorganic sulfur and organic compounds	H_2S, 1 mg kg−1
10	W3S	Reacts to high concentrations (>100 mg/kg) of methane and aliphatic organic compounds	n.d.

MOS Metal oxide thick film sensors, *n.d.* Not detected

Table 3 PEN3 operating conditions

Pre sampling time	10 s
Measurement time	90 s
Flush time	300 s
Zero point count auto	10 s
Chamber flow	400 ml/min
Injection flow	400 ml/min
Dilution	0

detectable by a transducer element (electrode) attached to each sensor.

The software makes it possible to set up the experimental conditions to adopt during the analysis and in real time it gives a graphic display of relative sensor values and also a numerical table of the same data.

The operating conditions used during the experimental study carried out are shown in Table 3.

The sampling run time and the flush time (cleaning step programmed after each analysis) were defined during previous tests aimed at setting up the best experimental conditions. Two replicate samples were collected to verify the signal stability and to get a sufficient number of data that considers all sample variations. All samples were processed immediately after the collection in bags.

Data analysis

All samples were analyzed with the PEN3 e-nose and mean values used for statistical analyses. Considering the absence of peaks in the sensor-array response outputs, an e-nose data set was built by calculating the sensor signal means in the signal constant range of 65–84 s recorded during analysis.

Box and Whisker plots were prepared to evaluate the distribution of the data between the 3 groups. Principal component analysis (PCA) was also performed by using the data analysis software system Statistica (version 8.0, StatSoft, Italy, www.statsoft.com) in order to extract useful information from the data and to explore their structure and therefore to evaluate the possibility of discriminating between the different classes of samples. The possibility of discriminating between the 3 groups involved in the study was also checked by the Probabilistic Neural Network (PNN) (DTREG software, version 10.8.0, Phillip H. Sherrod, Brentwood, Tennessee, USA). The statistical method was internally validated by the leave-one-out method.

Results

Figure 2 shows an example of the PEN3 output graph relative to a CRC patient. In each plot every line is relative to the response of a specific sensor to the VOCs in the sample, while the ratio of the conductivity response of the sensors to the sample gas (G) relative to the carrier gas (G0) and the running time (s) are displayed on the *y*- and *x*-axes, respectively.

In the PCA 2 principal components able to explain 99.91 % of variance were extracted, but the absence of

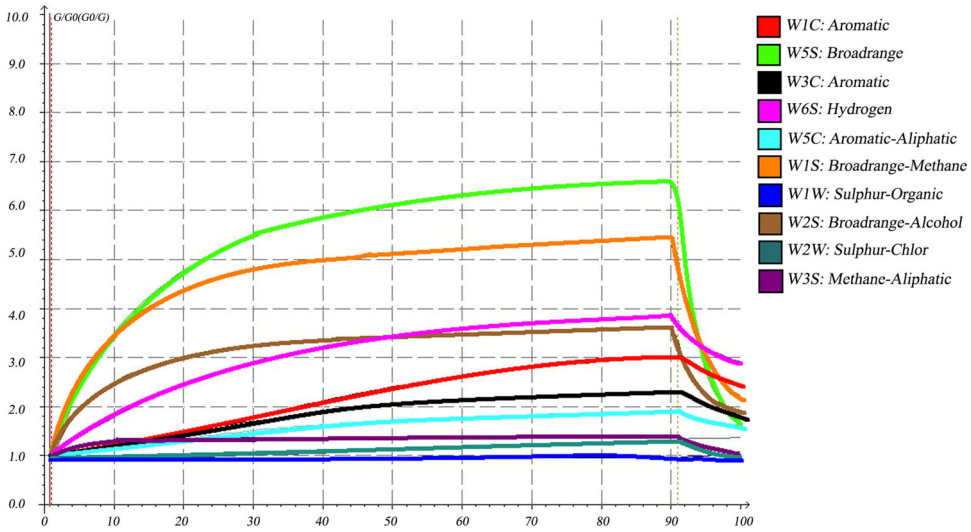

Fig. 2 Sensors response outputs of a monitored CRC patient

cluster was revealed by plotting the scores (data not shown).

The PNN optimized and validated considering as target the CRC patients resulted in a sensitivity of 93.33 %, but a specificity of only 10 % and an accuracy of 37.78 %. The same method with healthy controls as target showed a sensitivity of 0 %, a specificity of 100 % and an accuracy of 66.67 %, while in patients with benign polyps it showed a sensitivity of 20 %, a specificity of 96.67 % and an accuracy of 71.11 % (data not shown).

These results highlighted the impossibility of discriminating between the 3 groups by using a statistical method both supervised or not, which is probably due to the unspecific response of the sensors to the presence in the breath of defined VOCs resulting in a random classification of the subjects to each group.

Discussion

The availability of a sensitive, easy-to-use and reliable screening tool with rapid outcome response for CRC and colonic polyps is a major goal for the healthcare system of the industrialized countries, and the e-nose could ideally fulfill these requirements.

Experience of using e-noses for detecting CRC and polyps by analyzing the patients' breath is still limited, but has already been successfully reported in two studies where the sensors to assembly the e-nose were customized for specific groups of metabolites.

Peng et al. [2] use a tailor-made 14 nanosensors array based on organically functionalized gold nanoparticles (GNPs), and a SPME–GC–MS was able to get a very good discrimination between 26 CRC patients and 22 HC.

More recently, Amal et al. [3] reported a sensitivity of 85 %, a specificity of 94 % and an accuracy of 91 % in discriminating CRC and HC by analyzing the exhaled VOCs of 65 CRC patients, 22 patients with colonic polyps and 122 HC using a GC/MS in combination with a cross-reactive nanoarrays, even if the small number of patients in this group limits the reliability of these results. In these studies, however, the VOCs identified were different probably because of the use of different analytical platforms.

Our study has tested for the first time, the possibility of using a commercial array of sensors (PEN3 e-nose) to detect CRC and its precancerous forms by analyzing VOCs in exhaled breath, highlighting that the PEN3 does not contain a single sensor able to discriminate efficiently between the 3 groups monitored.

However, patients with polyps showed a higher signal for sensor 2, though it is not possible to understand if there are specific metabolites involved in these metabolic derangements. Of some interest is the profile relative to sensors 6, 8 and 10 which resulted in a higher signal in CRC patients than in HC and in POL. According to the manufacturer, these sensors are sensitive to methane, methane derivatives, organic and aromatic compounds, chemical classes of compounds to which belong most of the VOCs identified in our previous studies [4].

The PEN3 contains sensors apparently sensitive to the same chemical classes of compounds (i.e. aromatic compounds); however, the experimental profile shows that this does not generate similar signal. This is because the selectivity of the sensors is influenced not only by the sensing and the dopant materials, but also by the working temperature and the sensor geometry.

Conclusions

Despite these limitations, the set up of a dedicated e-nose based on sensors specifically designed to identify the types of exhaled VOCs involved in CRC metabolism will be of great importance in the future development a noninvasive and reliable screening tool.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the local Ethics Committee.

Informed consent All the patients and healthy controls gave oral informed consent to participate to the study.

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