Comparative analysis of volatile organic compounds of breath and urine for distinguishing patients with liver cirrhosis from healthy controls by using electronic nose and voltammetric electronic tongue

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ABSTRACT

Advanced stage detection of liver cirrhosis (LCi) would lead to high mortality rates in patients. Therefore, accurate and non-invasive tools for its early detection are highly needed using human emanations that may reflect this disease. Human breath, along with urine and blood, has long been one of the three main biological media for assessing human health and environmental exposure. The primary objective of this study was to explore the potential of using volatile organic compounds (VOCs) assay of exhaled breath and urine samples for the diagnosis of patients with LCi and healthy controls (HC). For this purpose, we used a hybrid electronic nose (E-nose) combining two sensor families, consisting of an array of five commercial chemical gas sensors and six interdigitated chemical gas sensors based on pristine or metal-doped WO_3 nanowires for sensing volatile gases in exhaled breath. A voltammetric electronic tongue (VE-tongue), composed of five working electrodes, was dedicated to the analysis of urinary VOCs using cyclic voltammetry as a measurement technique. 54 patients were recruited for this study, comprising 22 patients with LCi, and 32 HC. The two-sensing systems coupled with pattern recognition methods, namely Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA), were trained to classify data clusters associated with the health status of the two groups. The diagnostic performances of the E-nose and VE-tongue systems were studied by using the receiver operating characteristic (ROC) method. The use of the E-nose or the VE-tongue separately, trained with these appropriate classifiers, showed a slight overlap indicating no clear discrimination between LCi patients and HC. To improve the performance of both electronic sensing devices, an emerging strategy, namely a multi-sensor data fusion technique, was proposed as a second aim to overcome this shortcoming. The data fusion approach of the two systems, at a medium level of abstraction, has demonstrated the ability to assess human health and disease status using non-invasive screening tools based on exhaled breath and urinary VOC analysis. This suggests that exhaled breath as well as urinary VOCs are specific to a disease state and could potentially be used as diagnostic methods.

Keywords: Liver cirrhosis; volatile organic compounds; exhaled breath analysis; urine analysis; electronic sensing system; data fusion.

1. Introduction

Liver disease has become a major global health problem. Indeed, it is responsible for the death of two million people per year worldwide. Of these, one million are due to complications of cirrhosis [1,2]. This mortality is due to symptoms that are usually detected at a very advanced stage and in a metastatic state [3]. Although many efforts have been made in recent years, liver cirrhosis (LCi) still causes many deaths worldwide. This mortality is also due to the relatively high cost of treatment in some parts of the world [4-6]. Generally, patients use drugs and surgery to hope to prolong their lives.

To counteract the progression of the disease, early detection or intervention would be of great interest. This would avoid or delay clinical interventions. For this purpose, the development of effective predictive methods would be of paramount importance.

Efforts to develop therapeutic methods are still not effective enough. There is therefore a need to improve early detection methods. Early detection and accurate diagnosis of the onset of the disease are the most promising approaches to accelerate the healing process or significantly reduce the associated mortality.

Exhaled breath contains volatile organic compounds (VOCs) that may reflect disease. The development of non-invasive methods to monitor the progression of diseases would offer more advantages than other conventional methods [7]. Several studies have even identified VOCs emitted from the breath of the affected person as indicators or markers of disease [8,9]. In other words, the onset of various diseases or metabolic changes is accompanied by a significant change in the concentration of certain VOCs in exhaled breath [10].

Besides, it is known that the human body reacts to infections and the development of diseases in various ways by producing biomarkers in biological fluids, which then act as a mirror of the diseases. For example, the composition of urine varies according to the health status of the person [11,12]. Thus, the chemical compounds in urine are good indicators because they reveal the general state of the patient's body and thus make it possible to diagnose certain dysfunctions or pathologies. Depending on the pathology, specific VOCs emanate from the human body. They provide information on the state of health of the individual. VOCs analysis would therefore be a new approach to diseases monitoring. Some studies have even established a link between urinary VOC

profiles and infectious diseases [13]. These compounds in urine, whether electro-active or not, have different electrochemical activities.

The aim is to detect the disease at an early stage so that treatment can be started quickly, which could reduce the severity and mortality of the disease. Therefore, it is advisable to combine the analysis of exhaled breath samples with non-invasive body fluids analysis [14-16].

The main disadvantage of traditional diagnostic tests is the long delay before the test can be performed. Furthermore, despite their efficiency, sensitivity and often specificity, chromatography techniques have many drawbacks and can only be used in structured laboratories. In addition, some techniques may require patient irradiation or surgery and test results are not obtained immediately. A lack of reliable tests and the opacity of many clinical tests of liver function (from imaging, blood sampling to liver biopsy) are also noticed. Then, these negative aspects of traditional methods have paved the way for the wider development of multi-sensory systems (E-Nose and VE-tongue) as useful alternatives to conventional methodologies. They are simple, reliable, cost-effective, fast and can be used in complex environments. In addition, they can be used on site, even by unskilled personnel [17-19].

For this purpose, multi-sensor-based detection systems, such as electronic noses and tongues, have become serious candidates in recent years. These devices are based on cross-selective sensor arrays. These systems have certain advantages due to their known specific characteristics [20,21]. These detection approaches are based on sensor arrays that can analyse samples with different chemical signatures [22,23]. The samples are classified by distinguishing these chemical signatures using chemometric methods. Electronic noses and tongues can be used in many fields such as the automotive industry [24], medicine and pharmaceuticals [25], military industry [26], wastewater treatment [27], and agriculture [28]. The biomedical field, such as analysis of human breath and urine, is the focus of this report. Recently, a number of works using multi-sensor systems, so-called E-nose and tongue are devoted to LCi [29,30].

However, the individual use of an electronic nose or tongue only reflects one aspect of the sample (smell or taste). In order to obtain more in-depth information while minimising the limitation of the detection systems, the data fusion technique can be used to generate a global signature associated with the sensitivity of both devices. According to some literature [31-33], data fusion technique is categorised as "low level", "medium level" and "high level", depending on the processing stage at which

fusion takes place. Therefore, the data fusion strategy is an effective tool for using compatible measurement data from devices.

When using an electronic nose and tongue, several studies have exploited data fusion. They include the prediction of the mixing ratio of old frying oil [34,35], the classification of different samples of honey and rice [36,37], the non-destructive detection of fish freshness [38], and the evaluation of tea and strawberry juice flavours [39].

Taking all these considerations into account, the aim of this study was to use an E-nose in conjunction with a VE-tongue combined with pattern recognition methods to analyse exhaled breath and human urine samples from LCi patients and HC. Indeed, the key clue was to merge the data from both measurement systems to improve their discrimination performance when taken separately.

2. Materials and methods

2.1. Exhaled breath and urinary collection

A total of 54 volunteers has participated in this study including 22 patients with LCi, and 32 HC. The patient samples, used in this study, are in the primary stage of disease. **Table 1** illustrates the general characteristics of all studied volunteers. In this study, only adult volunteers were considered. Persons who had consumed medication, drugs, alcohol, tobacco, drink or food before 12 noon were automatically excluded from the study.

In the literature there is no standard method for breath sampling [40] and there are different protocols for sample analysis [41]. Tedlar® bags have been shown to have acceptable sample storage properties compared to other bags made of different materials [42].

The volunteers were asked to rinse their mouth before collecting exhaled breath samples in Tedlar® bags with mouthpiece. Three breath samples were collected per each volunteer. Indeed, the volunteer blows into commercial 2 L Tedlar® bags (Supelco Inc., Belfonte, PA. USA) through a one-way valve. This valve prevents outside air from mixing with the collected breath. Before and after each breath sample, the Tedlar® bags are cleaned 3 times with synthetic air.

Besides, morning urine samples are collected in 25 mL vials.

An approval was issued by the Biomedical Research Ethics Committee of the Avicenne University Hospital (Mohammed V University, Rabat, Morocco). Prior to the collection of the samples, all the volunteers signed a consent agreement.

2.2. Electronic sensing systems

Electronic nose and tongue have been used to analyze volatile organic compounds (VOCs) in exhaled breath and urine samples collected from patients with LC and HC, respectively.

2.2.1. E-nose experimental set-up

Fig. 1 shows a schematic diagram of the system developed for the analyse of exhaled breath gas, which consists of a Tedlar® bag containing the samples, a micro-pump (flow rate of 200 L/min) to transport the analyte to a sensor chamber, a USB-6212 NI module for data acquisition and a man-machine interface for monitoring and recording the sensor responses. The E-nose comprises:

- Five chemical sensors (MQ-2, MQ-3, MQ-135, MQ-137 and MQ-138) purchased from Henan Hanwei Electronics Co, (Zhengzhou, China). The commercial sensors and their target compounds are listed in **Table 2.**
- Six interdigitated gas sensors (Pristine WO_3, Pt/WO_3, $Au/Pt/WO_3$, Au/WO_3, Ni/WO_3 and Fe/WO_3). These nanostructures were deposited on alumina substrates. Tungsten hexacarbonyl (WC_6O_6) was used as a precursor for preparing tungsten trioxide (WO_3) nanostructures, which were synthesized according to the process described in our previous work [43]. For the analysis of breath samples, these interdigital chemical gas sensors are used at their optimal operating temperatures as determined in a reported study of our laboratory [44]. During the measurement, the sensors were irradiated with UV light at 394 nm. The optimum temperature for good sensitivity was 100ºC (for pristine WO_3, Pt/WO_3, $Pt/Au/WO_3$), and 160ºC (for Au/WO_3, Ni/WO_3, Fe/WO_3).
- A relative humidity sensor (Honeywell HIH 4000-002), and a temperature sensor (LM35DZ) from National Semiconductor.

For each volunteer, three replica breath samples were collected. They were then transferred to the sensor chamber by pumping the analyte for 10 minutes. The responses of the gas sensors are recorded as conductance vs. time.

2.2.2. VE-tongue experimental set-up

The scheme of the experimental VE-tongue used for urine samples analysis is shown in **Fig. 2**. It is a conventional electrochemical cell based on a three-electrode configuration. Briefly, it consists of an Ag/AgCl reference electrode and an array of five working electrodes: copper (Cu), glassy carbon (GC), platinum (Pt), palladium (Pd), gold (Au), and an auxiliary electrode made of platinum.

After explaining the measurement protocol to the volunteer, a new polystyrene bottle was given to him to get his urine sample. The VE-tongue analysis of the urine is immediately performed after the urine is received without any pre-treatment. Once the measurement is done, the urine sample is stored at -4°C.

Electrochemical measurements are carried out by immersing the electrodes in a vessel containing the sample. The measurements are then initiated, and a data acquisition device (PalmSens^3 potentiostat, Netherlands) was used to automatically store the output data of the electrode responses. The CV technique was configured as follows: the potential scanning window was between - 0.2 to 0.6 V. The scanning was performed at 50 mV/s. To avoid possible measurement errors due to contamination of the electrode surfaces, the electrodes were all rinsed with piranha and polished before and after each measurement. This also helps to minimize drift.

During electrochemical measurements, the electrode responses are recorded as voltammograms. For the sake of repeatability in the measurements, 6 measurement repetitions were carried out for each urine sample. The total response obtained from the analyzed analyte was therefore 6 voltammograms x 5 electrodes per urine sample.

These experiments were carried out at room temperature (25°C). After analysis of the exhaled breath and urine samples, a very large database was obtained, which made interpretation difficult. To overcome this drawback, relevant features were extracted from the responses of both systems (E-nose and VE-tongue) to facilitate visualization, interpretation and understanding.

2.3. Data pre-processing

Sensor response drift is a general issue that is considered in this work. Numerous correction techniques have been tested on pre-treatment data to reduce it [45].

Indeed, to reduce the effect of sensor drifts, normalization procedure of each gas sensor response ($\frac{G-G_0}{G_0}$) is used where G is the measured conductance and G_0 is the baseline [45].

To simplify the processing of multivariate data, only the variables of interest are retained in the initial data. These variables retain as much information as possible from these responses. In this study, the analysis of the responses of E-nose system was carried out using the two variables cited below:

- $\boldsymbol{\Delta G} = (G_S - G_0)$: The difference between the stabilised conductance (G_s) and the initial conductance (G_0);
- $\boldsymbol{AUC}$: Area under the response curve of the sensor measured by a trapezoidal method. The area considered is between 1 and 9 minutes of the measurement time.

Furthermore, for processing the VE-tongue database, two eigenvalues related to the output current values were exacted from the cyclic voltammogram response of each working electrode, including:

- $\boldsymbol{Pt_{ox}}$**:** Maximum signal slope in the first half wave (oxidation)
- $\boldsymbol{Area}$**:** Area under the response curve of the sensor.

In this study, for the E-nose, 162 breath samples (54 volunteers * 3 Tedlar bags) were measured and 2 features (ΔG and AUC) were used. These 2 features were extracted from the response of each gas sensor. Since we have 11 gas sensors, for each breath sample, 22 features were extracted. Therefore, for all samples, we had a total of 3564 variables (i.e. 162 samples * 22). The same logic was used for the VE-tongue. Thus, 7128 variables were extracted (i.e. 324 samples*22).

2.4. Data analysis

After extraction of the variables, data processing algorithms (PCA, DFA) are used to classify the analyzed samples for better visualisation and interpretation [46]. Chemometric techniques are crucial tools for obtaining a practical system capable of characterising a wide diversity of compounds [47].

The aim of using these methods in this work is to evaluate the effectiveness of the E-nose and VE-tongue in discriminating between the two groups based on breath and urine samples using both supervised and unsupervised methods.

PCA is an unsupervised linear method that uses an orthogonal transformation to reduce the dimensionality of multivariate data [48,49]. This transformation is applied so that the principal components (PCs) have the greatest possible variance while remaining orthogonal. In this study, PCA was used to process multivariate data from the E-nose and VE-tongue to obtain a clear illustration in a low-space projection.

DFA is a supervised chemometric method whose effectiveness has been proven in many applications of electronic sensing systems [50]. With the DFA method, the discriminating functions are determined in such a way that the ratio within a group is minimised (intra-class variance). At the same time, it maximises the ratio between groups (inter-class variance).

A ROC curve is a graph that illustrates the diagnostic ability of a binary classification system as a function of the variation in its discrimination threshold. The ROC curve is obtained by plotting the true positive rate (TPR) against the false positive rate (FPR) for different thresholds. The true positive rate is called sensitivity, recall or probability of detection [51] in machine learning. The false positive rate is known as the false alarm probability [51] and can be calculated as follows (1 - specificity).

SVM is a supervised nonlinear chemometric technique based on the notion of maximum margin. The maximum margin is the distance between the separation boundary and the nearest samples [52]. SVMs allows to draw a hyperplane for this separation. There is a multitude of valid hyperplanes. However, SVMs searches among the valid hyperplanes for the one that not only passes "in the middle" of the points of the two classes but also maximizes the margin.

2.5. Multisensory data fusion approach

In this study, a data fusion strategy was adopted. The main objective of using this approach was to improve the classification performance obtained using the two electronic devices individually. Here, medium level data fusion was applied to compensate for the limitations in terms of classification/discrimination of HC and LCi patient samples by the used electronic sensing systems. In this approach, the fusion is done using the features extracted from the E-nose and VE-tongue data [39,53]. It is obvious that the number of features from the two systems should be similar when merging data using the medium level abstraction approach. If the number of features from one instrument is significantly higher, they could dominate the merged dataset. In

our case, the E-nose dataset is larger than that of the VE-tongue. For this reason, since only 5 electrodes were used for the VE-tongue, an appropriate method of selecting the five most discriminating gas sensors was carried out in order to obtain a similar dimensionality of both matrices. Therefore, using the medium level abstraction approach, the datasets obtained from each multi-sensor system (E-nose and VE-tongue) were merged into a single matrix of 162 samples on 4 features (ΔG and area for the E-nose; Ptox and area for the VE-tongue).

3. Results and discussion

3.1. Analysis of VOCs in exhaled breath by E-nose system

Sensor responses were recorded during the 10 minutes of breath exposure, followed by 10 minutes of return to baseline under synthetic air. **Fig. 3** displays the responses of the two families of gas sensor arrays towards exhaled breath samples of HC and LCi. As shown in **Fig. 3**, the response of the sensor array to the exhaled breath gas was directly related to the gas detection and pattern identification. The MQ-138 sensor of **Fig. 3(b)** showed higher sensitivities than the other sensors. Many studies have been performed on the analyze of breath VOC biomarkers from LCi patients and reported that volatile biomarkers in breath associated with liver cirrhosis are Methanol [54-56], Dimethyl sulfide [57,58], Ethanol and Toluene [58]. Though the other sensors showed relatively low signals, there were differences between LCi patients and HC (**Fig 3(a)**). We can notice that the gas reaction was completed after 600 s, whereas the desorption of the gas was completed after around 30 s for MQ sensors. However, this time to return to the baseline remains longer for interdigitated chemical gas sensors based on pristine or metal-doped WO_3 nanowires (**Fig. 3 (c,d)**). Besides, Au/WO_3 sensor response (**Fig 3(d)**) provided higher response toward LCi patient's breath sample than to the HC (**Fig 3(c)**). This behaviour can be justified by the difference in VOC concentrations in the breath related to HC and LCi patients [6,23].

3.2. Analysis of urinary VOCs by VE-tongue

Fig. 4 shows different electrochemical responses, depending on the health state associated with the urine samples that were analysed. These irreversible responses are different in terms of shape and amplitude. However, oxidation processes are only observed when using Cu working electrode. This oxidation effect is more pronounced in urine samples of patients with LCi. This is probably due to abnormal variation of

copper level in the body of LCi patients and released in urine [59-68]. It should be noted that the intensity of the voltammograms yield by Cu working electrode corresponding to urine samples of patients with LCi (**Fig. 4(b)**) is higher than those of HC (**Fig. 4(a)**). The oxidation peak is around 0.30 V for LCi (**Fig. 4(b)**), while the Cu electrode admits a small oxidation peak at around 0.02 V for the urine sample of HC (**Fig. 4(a)**). With the Cu electrode, the voltammograms demonstrate metal oxidation, forming copper ions in urine samples, but with different trend for LCi. According to the literature, copper primarily deposits in the liver and the lethal dose is about 10-20 g [69]. On one hand, *A. Amit et al*. and Y. *Yoshida et al*. report that liver problems modify the metabolism of trace elements. In this case, they declare the presence of either a high presence of copper or a deficiency of zinc (Zn) [63,70]. In this purpose, *D. Rahelic et al.* explains the role of copper in the redox process [71]. Consequently, the proposed VE-tongue provides findings that show that the state of health influences the chemical composition of the urine and corroborates the literature.

3.3. Radar plot results

The sensors are not specific to one compound. However, each sensor is sensitive to several compounds. The combination of the responses allows plotting a specific chemical signature. After the features' extraction step, the radar plot was used to see if there were any differences or similarities in terms of the chemical signatures of the various breath and urine samples from patients with LCi and HC. **Fig. 5** shows the radar plot results highlighting the contribution of the E-nose gas sensors. These plots were constructed using the area value as a feature. There is a clear variation in pattern between the breath-prints of the two groups.

Fig. 6 shows the radar plots of the two sets of urine samples obtained after extraction of the relevant features from the voltammograms. Each representation has a particular chemical signature in their taste profile, although there are some similarities. The majority of the electrodes showed similar behaviour for the two groups except for Pd electrode. This could strongly influence the classification performance of the VE-tongue, thus generating an overlap in the sample patterns.

3.4. PCA discrimination results

The discrimination results of the two sensing systems individually by using PCA are depicted in **Fig. 7**. Using two features (ΔG and AUC), the PCA results for the E-nose (**Fig. 7(a)**) indicate a data variance of 61.98%, which was explained by the first three

principal components (PCs). This three-dimensional PCA plot shows a classification among the breath patterns of LCi patients and HC with slight overlapping between the two clusters.

Besides, the VE-tongue discrimination results obtained by applying PCA are shown in **Fig. 7(b)**, where all three PCs contributed to a score of 81.38%. Here, the samples of the LCi patients also overlapped with those of the HC. This is probably due to the similarity of the electrochemical responses of the majority of the electrodes.

3.5. DFA discrimination results

The same dataset was used to perform a treatment using a supervised method, called DFA. It was used to distinguish between different samples of breath and urine according to their health states. The results of the DFA after its application to the responses of the two systems are shown in **Fig. 8**. The same features as for the PCA were used. The results in **Fig. 8(a)** show a discrimination between LCi patients and HC breath samples. In contrast, the discriminatory feasibility of the VE-tongue, shown in **Fig. 8(b)**, does not lead to a perfect separation between LCi and HC samples. These results confirm those obtained by the PCA analysis and suggest the use of data fusion to recognise the two health states.

3.6. Receiver operating characteristic (ROC) results

ROC curves for the E-nose and VE-tongue data were plotted. AUC values were calculated to assess the diagnostic value of each data item. The ROC curve and AUC analysis of the logistic regression were performed to explore the diagnostic performances of the E-nose and VE-tongue models. **Fig. 9(a)** shows the ROC curve of the E-nose data with an AUC of 0.965 corresponding to the analysis of LCi and HC breath samples.

Besides, **Fig. 9(b)** shows the ROC curve of the VE-tongue model data with an AUC of 0.950 for the distinction of urine samples corresponding to LCi and HC. These results show that the diagnostic performances of two multi-sensor systems is significantly significant. Furthermore, the diagnostic performance of the E-nose model is higher than that of the VE-tongue model.

3.7. SVMs classification results

The dataset trained for 20 volunteers (10 LCi and 10 HC patients) of balanced age was used to run the SVMs method. It was used to distinguish between different breath and urine samples according to their health status.

Table 3 shows the SVMs results of the classification of 60 breath samples (20 volunteers providing 3 samples each). The rows of the table indicate the actual health states and the columns the predicted states. The SVMs method achieved a success rate of 98.33% in the recognition of the two groups studied (LCi and HC). As can be seen from the table, one error was detected: a sample belonging to LCi was classified in the HC group.

Besides, Table 4 presents the SVMs results when analyzing the data from the VE-tongue for 120 urine samples. The same trend as for the E-nose is observed with the occurrence of three errors and a success rate of 97.50%.

3.8. Data fusion results

3.8.1. PCA classification results

Using data fusion, the PCA method was applied to discriminate the two types of samples. **Fig. 10** shows the discrimination results obtained by combining the data from the two systems, where all three principal components contributed with a score of 64.23%. Indeed, we can notice both groups are well discriminated, demonstrating the ability of the combination of two systems to overcome the overlap observed previously. The close linkage of biological emanations to health status provides a unique signature. This explains the good discrimination obtained by merging the data as opposed to separating them.

3.8.2. DFA classification results

DFA was also used to examine the ability of the data fusion technique to improve the classification of different samples according to their health status. **Fig. 11** shows the first two DFA functions for the classification of HC and LCi patients using data fusion of the E-nose and VE-tongue systems. The result reveals a perfect discrimination between HC and LCi patients. Therefore, the processing of the data by the DFA technique reveals the effectiveness of the proposed data fusion method in distinguishing HC and LCi patients.

3.8.3. Receiver operating characteristic (ROC) results

A ROC analysis was also performed to evaluate the diagnostic performance after data fusion to improve the classification of different samples according to their health status. **Fig. 12** shows the ROC curve corresponding to the merged data of the two devices with a very good AUC of 0.999. This result shows that the diagnostic performance of the model obtained after data fusion is superior to those found using the E-nose and VE-tongue data individually. These results confirm the effectiveness of merging data from two compatible systems to successfully distinguish HC and LCi patients.

3.8.4. SVMs classification results

~~In order to~~ To enhance the SVMs classifications obtained by taking the two measurement systems individually, SVMs was applied to the merged data. For this purpose, 20 age-balanced volunteers (10 LCi and 10 HC patients) were considered.

Table 5 shows the results of the SVMs classification for the fusion of the E-nose and VE-tongue data. A very good result by the SVMs method is achieved with a success rate of 100% for the recognition of LCi and HC volunteers. In the light of this result, it can be concluded that the data fusion technique is a good way to improve the classification of different samples.

4. Conclusion

In this work, an electronic nose and tongue were used to analyse breath and urine samples from patients with LCi and HC. With the help of pattern recognition methods, the two electronic devices, taken individually, showed limitations in terms of discrimination of the two study groups. To overcome the limitation of the individual systems, the data fusion of the E-nose and VE-tongue provided very satisfactory results in terms of discrimination and classification. Indeed, the PCA results obtained from the merged data showed a good classification of the different breath and urine samples according to their health status. In addition, the supervised method (DFA) was able to clearly differentiate between LCi patients and HC. Moreover, the diagnostic result of data fusion obtained by ROC curve is superior to those found using the E-nose and VE-tongue data individually. The advantage of this study is the proposal of electronic sensing systems as alternative tools to conventional techniques, which have many drawbacks for the analysis of this disease. This demonstrates that the two proposed

devices have a classification and predictive capability that would certainly contribute to facilitating the early diagnosis of diseases. In view of the results of this study, and thanks to their portability and speed, they could therefore be used as non-invasive diagnosis for other diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure captions

Fig. 1. Architecture of the E-nose system used for exhaled breath measurements.

Fig. 2. Schematic representation of the VE-tongue dedicated to the analysis of urine samples.

Fig. 3. E-nose responses of: MQ gas sensors when exposed to breath of (a) HC, (b) LCi patient; and interdigitated gas sensors after exposure to VOCs in the breath samples from (c) HC, (d) LCi patient.

Fig. 4. Responses of the five-electrode arrays of VE-tongue and insights without Cu electrode towards urine samples from: (a) HC, (b) patient with LCi.

Fig. 5. Radar plots of E-nose gas sensors responses towards exhaled breath from HC and patient with LCi, expressed by area as a feature.

Fig. 6. Radar plots of the responses of the VE-tongue electrode array exposed to the two different sets of urine samples, expressed by Pt_{ox} as a feature.

Fig. 7. Unsupervised PCA plot showing data points for: (a) breath samples relating to the two health states with data collected from the E-nose, (b) urine samples relating to the two health states with data collected from the VE-tongue.

Fig. 8. Supervised DFA plot displaying data points for: (a) breath samples relating to the two health states with data collected from the E-nose, (b) urine samples relating to the two health states with data collected from the VE-tongue.

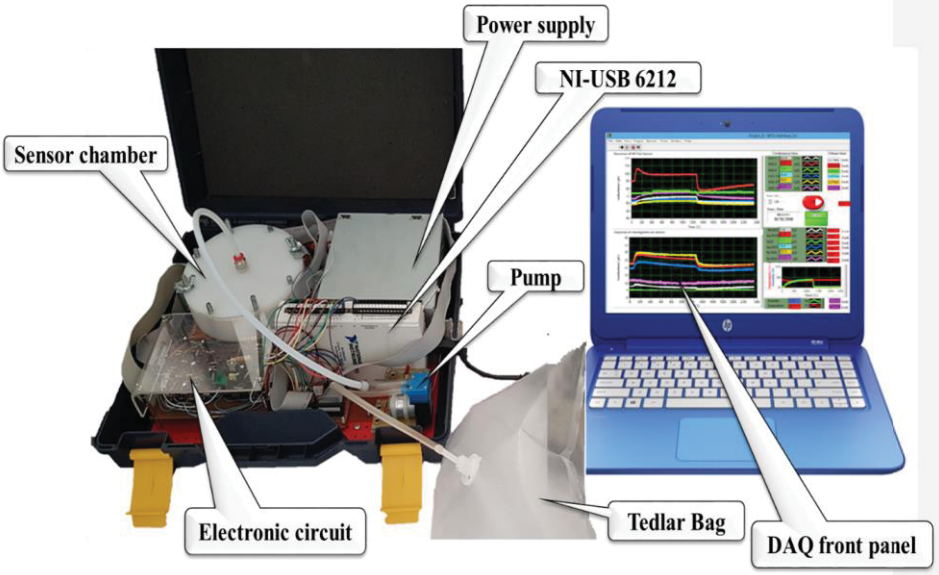
Fig. 9. ROC curve displaying data points for: (a) breath samples relating to the two health states with data collected from the E-nose, (b) urine samples relating to the two health states with data collected from the VE-tongue.

Fig. 10. Unsupervised PCA plot showing data fusion of E-nose and VE-tongue systems.

Fig. 11. Supervised DFA plot showing data fusion of E-nose and VE-tongue systems.

Fig. 12. ROC curve showing data fusion of E-nose and VE-tongue systems.

Fig. 1.


Power supply
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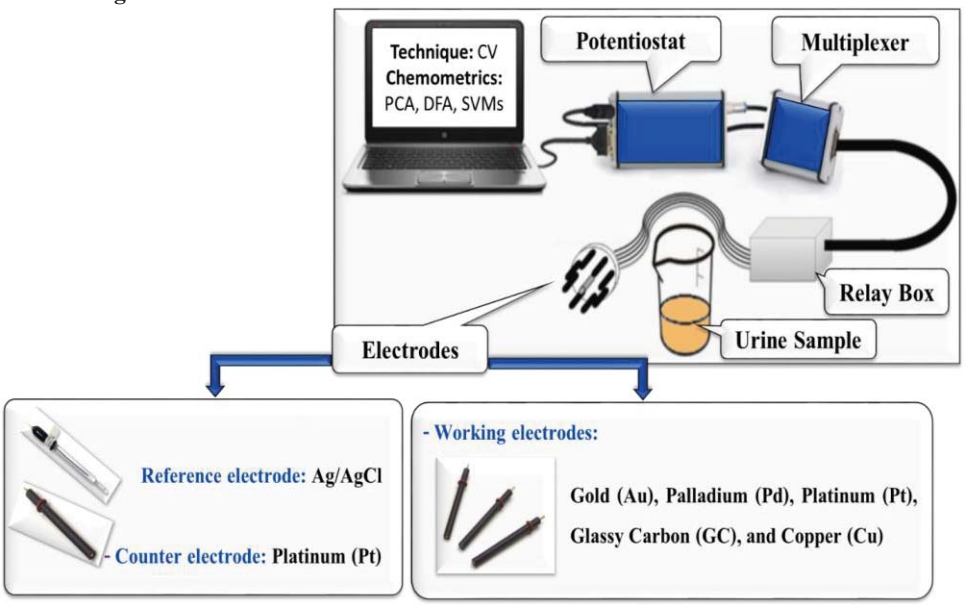
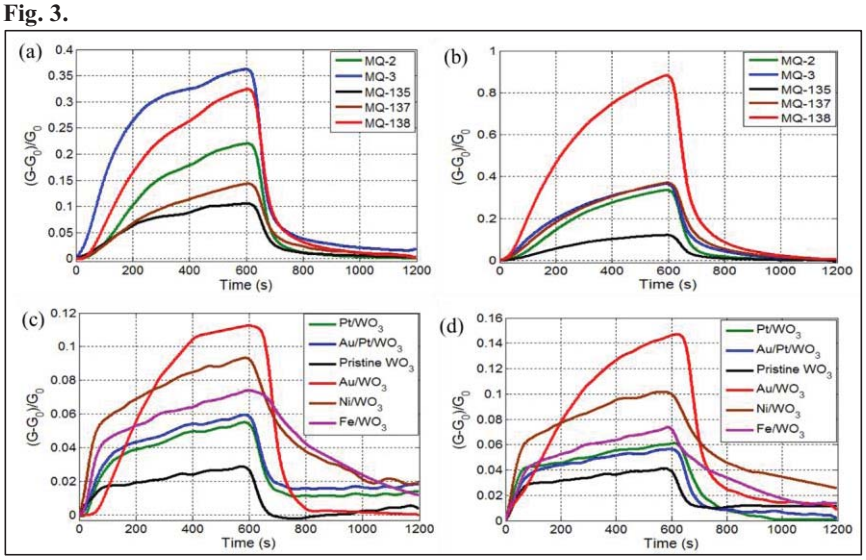
Sensor chamber
Pump
Electronic circuit
Tedlar Bag
DAQ front panel

695 **Fig. 2.**

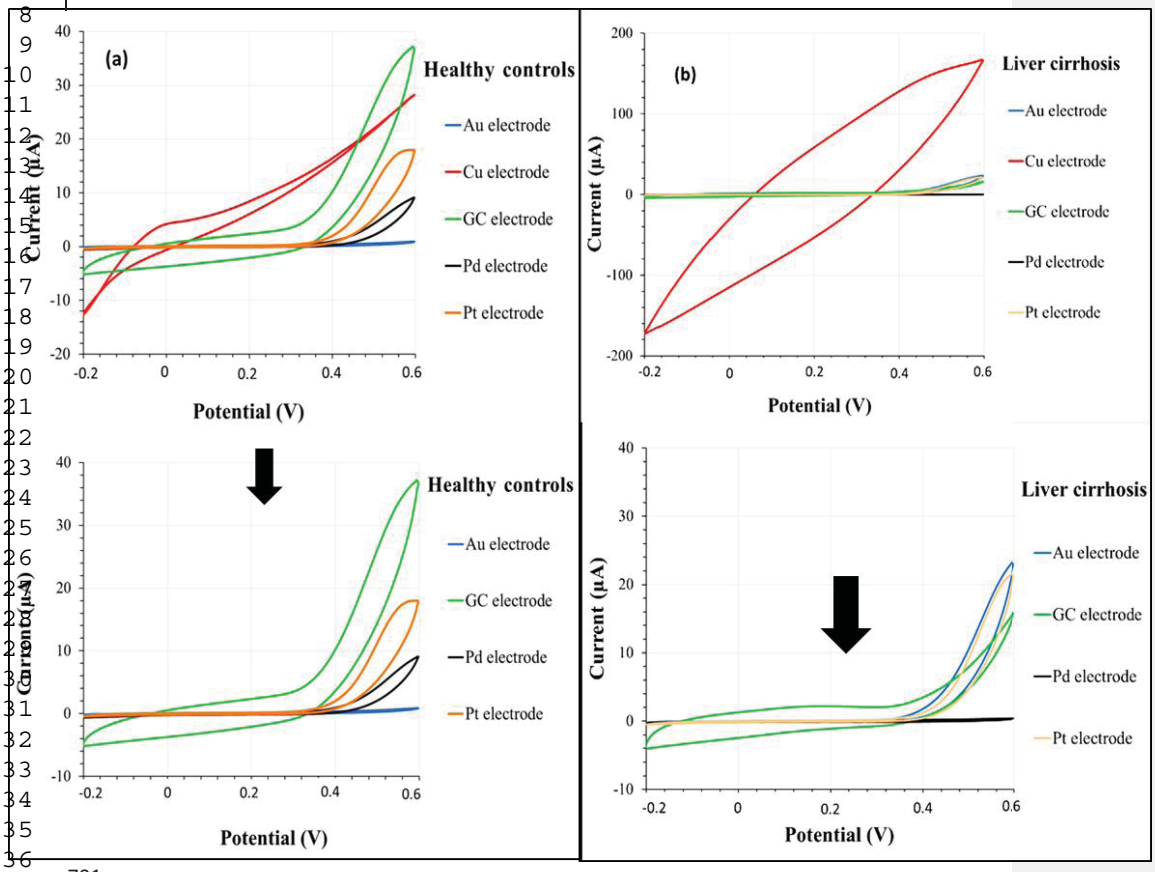

Technique: CV
Chemometrics:
PCA, DFA, SVMs
Potentiostat
Multiplexer
Relay Box
Urine Sample
Electrodes
Reference electrode: Ag/AgCl
- Counter electrode: Platinum (Pt)
- Working electrodes:
Gold (Au), Palladium (Pd), Platinum (Pt),
Glassy Carbon (GC), and Copper (Cu)

696

Fig. 3.


(a)
MQ-2
MQ-3
MQ-135
MQ-137
MQ-138
(G-G0)/G0
Time (s)
(b)
MQ-2
MQ-3
MQ-135
MQ-137
MQ-138
(G-G0)/G0
Time (s)
(c)
Pt/WO3
Au/Pt/WO3
Pristine WO3
Au/WO3
Ni/WO3
Fe/WO3
(G-G0)/G0
Time (s)
(d)
Pt/WO3
Au/Pt/WO3
Pristine WO3
Au/WO3
Ni/WO3
Fe/WO3
(G-G0)/G0
Time (s)

700 **Fig. 4.**



701

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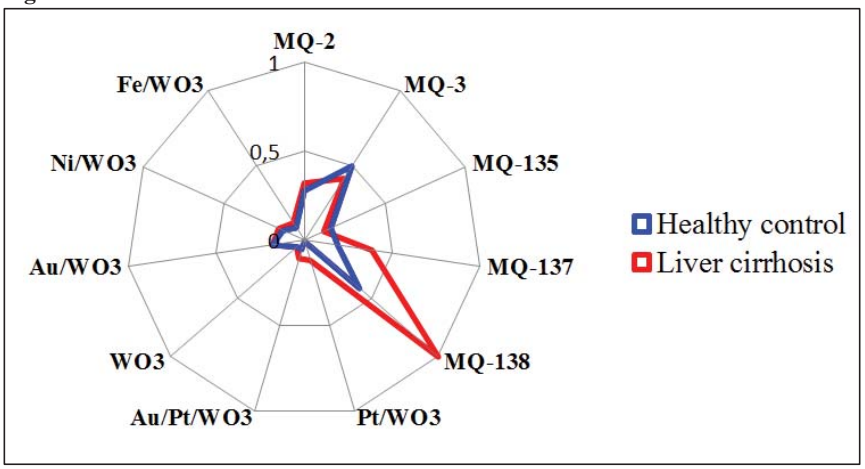
Fig. 5.


MQ-2
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WO3
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Ni/WO3
Fe/WO3
Healthy control
Liver cirrhosis

Fig. 6.

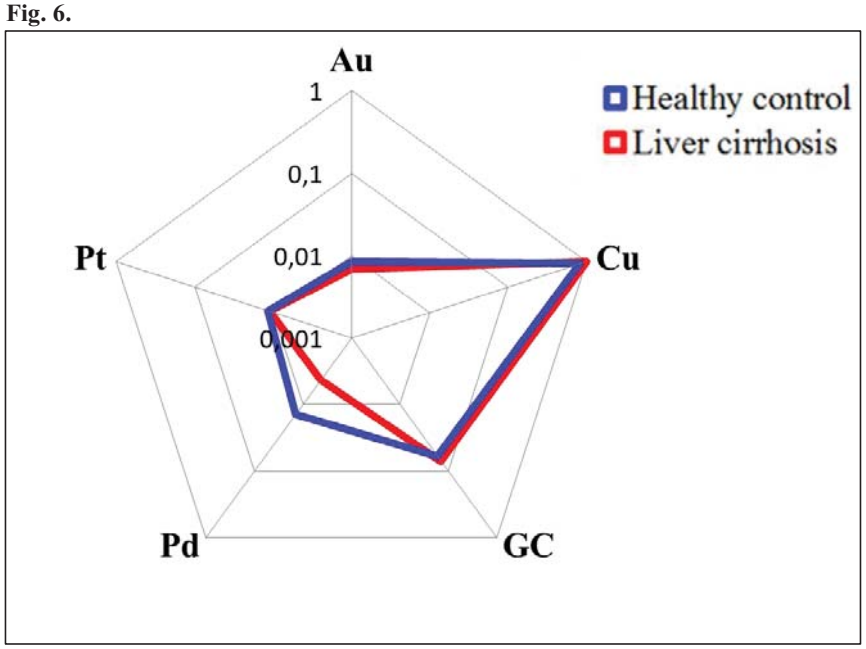


Fig. 7.

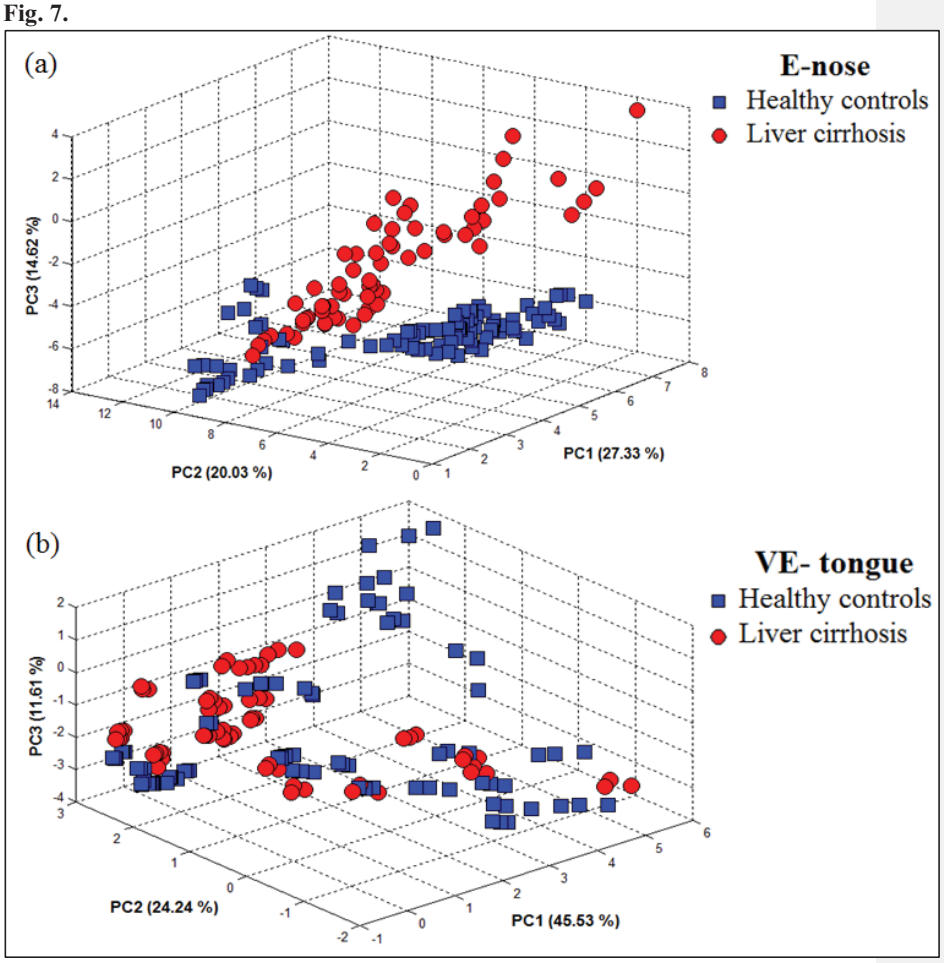

(a)
E-nose
Healthy controls
Liver cirrhosis
PC3 (14.62 %)
PC2 (20.03 %)
PC1 (27.33 %)
(b)
VE- tongue
Healthy controls
Liver cirrhosis
PC3 (11.61 %)
PC2 (24.24 %)
PC1 (45.53 %)

Fig. 8.

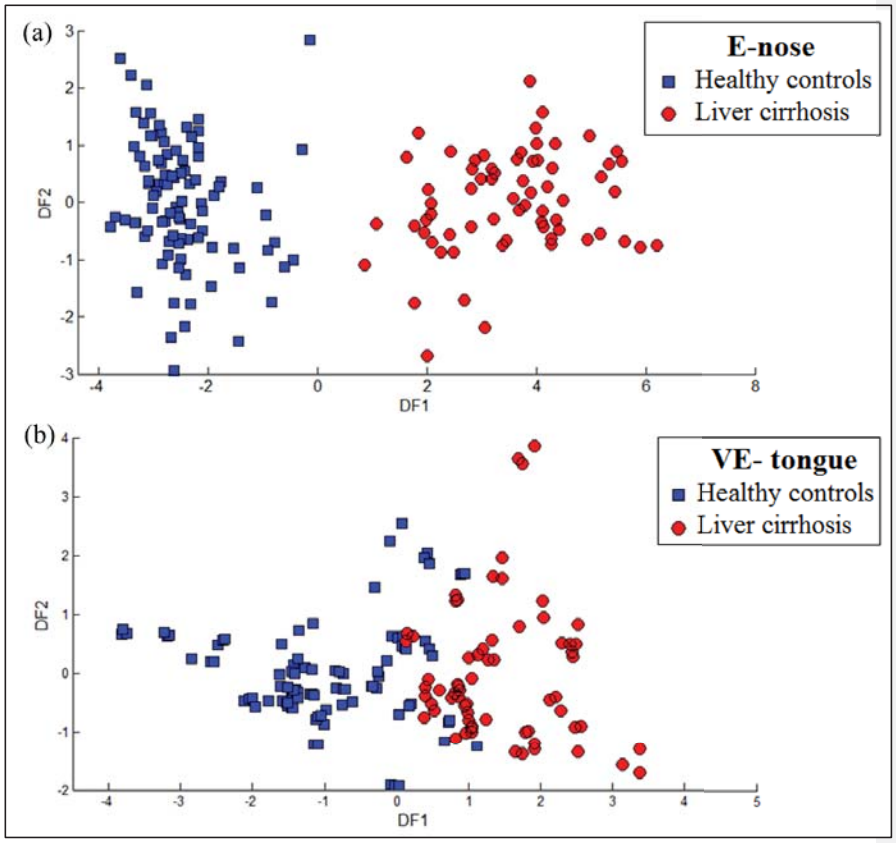
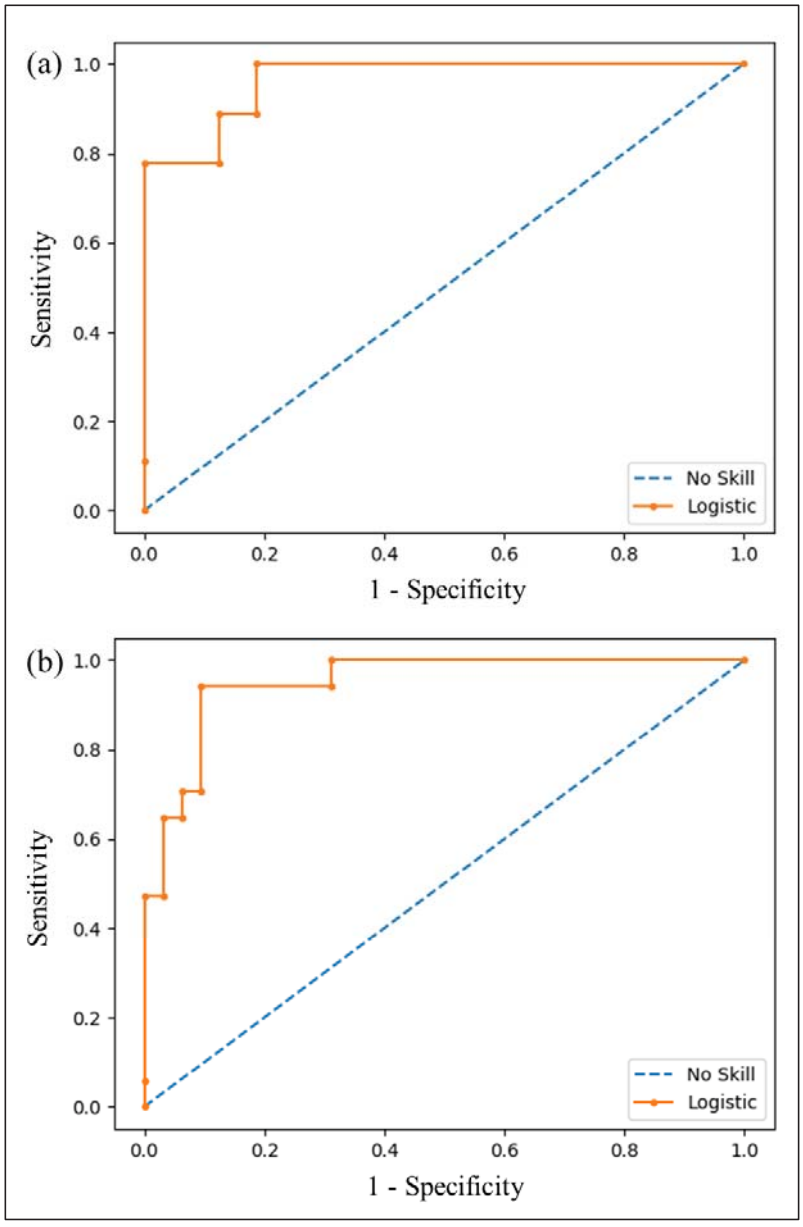

(a)
E-nose
Healthy controls
Liver cirrhosis
DF2
DF1
(b)
VE- tongue
Healthy controls
Liver cirrhosis
DF2
DF1

Fig. 9.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Sensitivity
1 - Specificity
No Skill
Logistic
(b)
Sensitivity
1 - Specificity
No Skill
Logistic

Fig. 10.

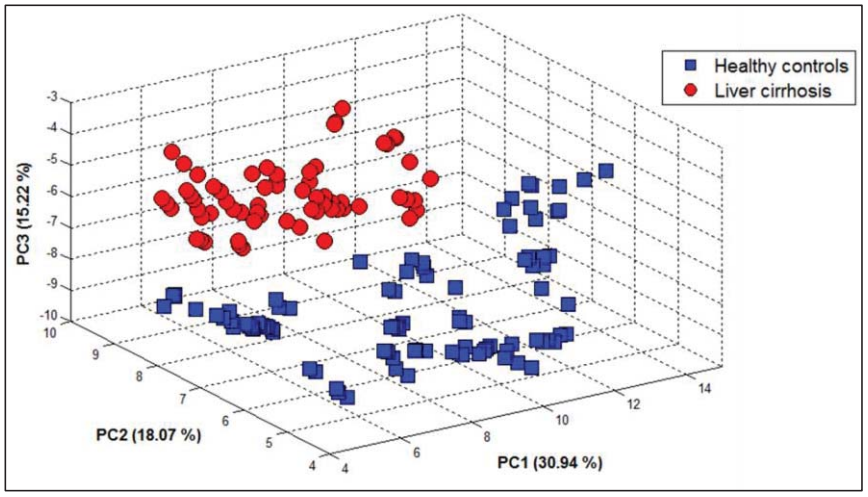
Healthy controls
Liver cirrhosis
PC3 (15.22 %)
PC2 (18.07 %)
PC1 (30.94 %)

Fig. 11.

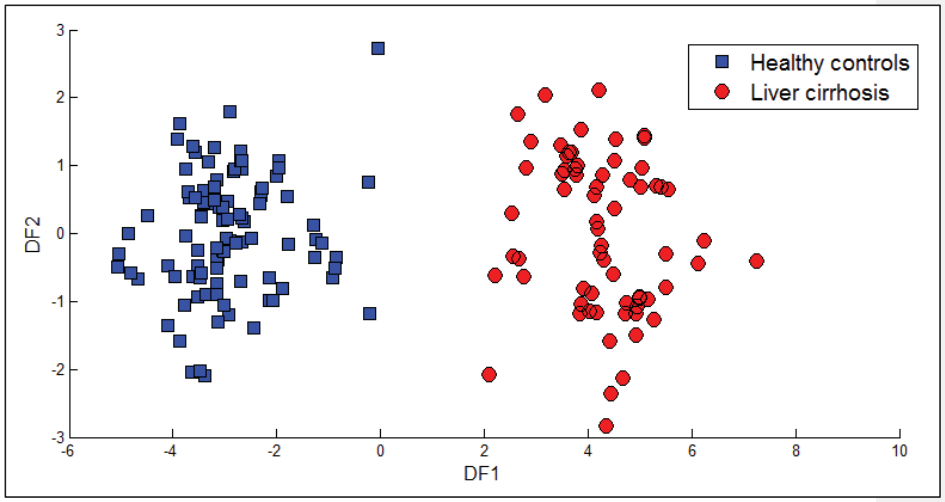
Healthy controls
Liver cirrhosis
DF2
DF1
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Fig. 12.

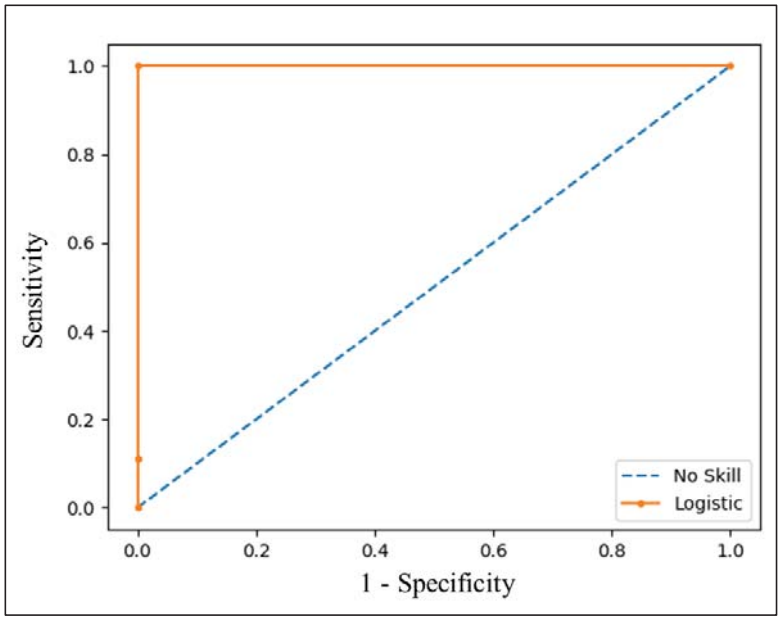
Sensitivity
1 - Specificity
No Skill
Logistic
0.0
0.2
0.4
0.6
0.8
1.0

Table 1

General characteristics of all studied volunteers.

Volunteers					
Groups	**Number**	**Age range (years)** **Age ± SD***	**Male, number (%)**	**Smoking habit+**	**Medication**
Liver cirrhosis (LCi)	22	24-79 46 ± 15	10 (45%)	1 S, 3 Ex.S, 18 NS	- Ciprofloxcicine - Aldactone - Autocardyl - Tefovin
Healthy controls (HC)	32	21-64 37 ± 17	22 (69%)	8 S, 24 NS	———

*** SD:** Standard Deviation.

+ Smoking habit: S: Smoker, Ex.S: Ex-Smoker, NS: Non-Smoker.

Table 2

Commercial gas sensors used in the e-nose system.

Gas Sensors	Target Compounds
MQ-2	Propane, Hydrogen, Methane
MQ-3	Alcohol
MQ-135	Benzene, Ammonia, Carbon dioxide and Nitric oxide
MQ-137	Ammonia
MQ-138	Toluene, Acetone, Methanol, Ethanol and Formaldehyde

Table 3

SVMs results for the classification of 60 breath samples regarding their health states by using the E-nose system with a success rate of 98.33%.

Actual	Predicted	
	LCi patients	**HC**
LCi patients	29	1
HC	0	30

Table 4

SVMs results for the classification of 120 urine samples regarding their health states by using the VE-tongue system with a success rate of 97.50%.

Actual	Predicted	
	LCi patients	HC
LCi patients	57	3
HC	0	60

Table 5

SVMs results for data fusion of E-nose and VE-tongue systems with a success rate of 100%.

Actual	Predicted	
	LCi patients	HC
LCi patients	30	0
HC	0	30

Comparative analysis of volatile organic compounds of breath and urine for distinguishing patients with liver cirrhosis from healthy controls by using electronic nose and voltammetric electronic tongue

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ABSTRACT

Advanced stage detection of liver cirrhosis (LCi) would lead to high mortality rates in patients. Therefore, accurate and non-invasive tools for its early detection are highly needed using human emanations that may reflect this disease. Human breath, along with urine and blood, has long been one of the three main biological media for assessing human health and environmental exposure. The primary objective of this study was to explore the potential of using volatile organic compounds (VOCs) assay of exhaled breath and urine samples for the diagnosis of patients with LCi and healthy controls (HC). For this purpose, we used a hybrid electronic nose (E-nose) combining two sensor families, consisting of an array of five commercial chemical gas sensors and six interdigitated chemical gas sensors based on pristine or metal-doped WO_3 nanowires for sensing volatile gases in exhaled breath. A voltammetric electronic tongue (VE-tongue), composed of five working electrodes, was dedicated to the analysis of urinary VOCs using cyclic voltammetry as a measurement technique. 54 patients were recruited for this study, comprising 22 patients with LCi, and 32 HC. The two-sensing systems coupled with pattern recognition methods, namely Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA), were trained to classify data clusters associated with the health status of the two groups. The diagnostic performances of the E-nose and VE-tongue systems were studied by using the receiver operating characteristic (ROC) method. The use of the E-nose or the VE-tongue separately, trained with these appropriate classifiers, showed a slight overlap indicating no clear discrimination between LCi patients and HC. To improve the performance of both electronic sensing devices, an emerging strategy, namely a multi-sensor data fusion technique, was proposed as a second aim to overcome this shortcoming. The data fusion approach of the two systems, at a medium level of abstraction, has demonstrated the ability to assess human health and disease status using non-invasive screening tools based on exhaled breath and urinary VOC analysis. This suggests that exhaled breath as well as urinary VOCs are specific to a disease state and could potentially be used as diagnostic methods.

Keywords: Liver cirrhosis; volatile organic compounds; exhaled breath analysis; urine analysis; electronic sensing system; data fusion.

1. Introduction

Liver disease has become a major global health problem. Indeed, it is responsible for the death of two million people per year worldwide. Of these, one million are due to complications of cirrhosis [1,2]. This mortality is due to symptoms that are usually detected at a very advanced stage and in a metastatic state [3]. Although many efforts have been made in recent years, liver cirrhosis (LCi) still causes many deaths worldwide. This mortality is also due to the relatively high cost of treatment in some parts of the world [4-6]. Generally, patients use drugs and surgery to hope to prolong their lives.

To counteract the progression of the disease, early detection or intervention would be of great interest. This would avoid or delay clinical interventions. For this purpose, the development of effective predictive methods would be of paramount importance.

Efforts to develop therapeutic methods are still not effective enough. There is therefore a need to improve early detection methods. Early detection and accurate diagnosis of the onset of the disease are the most promising approaches to accelerate the healing process or significantly reduce the associated mortality.

Exhaled breath contains volatile organic compounds (VOCs) that may reflect disease. The development of non-invasive methods to monitor the progression of diseases would offer more advantages than other conventional methods [7]. Several studies have even identified VOCs emitted from the breath of the affected person as indicators or markers of disease [8,9]. In other words, the onset of various diseases or metabolic changes is accompanied by a significant change in the concentration of certain VOCs in exhaled breath [10].

Besides, it is known that the human body reacts to infections and the development of diseases in various ways by producing biomarkers in biological fluids, which then act as a mirror of the diseases. For example, the composition of urine varies according to the health status of the person [11,12]. Thus, the chemical compounds in urine are good indicators because they reveal the general state of the patient's body and thus make it possible to diagnose certain dysfunctions or pathologies. Depending on the pathology, specific VOCs emanate from the human body. They provide information on the state of health of the individual. VOCs analysis would therefore be a new approach to diseases monitoring. Some studies have even established a link between urinary VOC

profiles and infectious diseases [13]. These compounds in urine, whether electro-active or not, have different electrochemical activities.

The aim is to detect the disease at an early stage so that treatment can be started quickly, which could reduce the severity and mortality of the disease. Therefore, it is advisable to combine the analysis of exhaled breath samples with non-invasive body fluids analysis [14-16].

The main disadvantage of traditional diagnostic tests is the long delay before the test can be performed. Furthermore, despite their efficiency, sensitivity and often specificity, chromatography techniques have many drawbacks and can only be used in structured laboratories. In addition, some techniques may require patient irradiation or surgery and test results are not obtained immediately. A lack of reliable tests and the opacity of many clinical tests of liver function (from imaging, blood sampling to liver biopsy) are also noticed. Then, these negative aspects of traditional methods have paved the way for the wider development of multi-sensory systems (E-Nose and VE-tongue) as useful alternatives to conventional methodologies. They are simple, reliable, cost-effective, fast and can be used in complex environments. In addition, they can be used on site, even by unskilled personnel [17-19].

For this purpose, multi-sensor-based detection systems, such as electronic noses and tongues, have become serious candidates in recent years. These devices are based on cross-selective sensor arrays. These systems have certain advantages due to their known specific characteristics [20,21]. These detection approaches are based on sensor arrays that can analyse samples with different chemical signatures [22,23]. The samples are classified by distinguishing these chemical signatures using chemometric methods. Electronic noses and tongues can be used in many fields such as the automotive industry [24], medicine and pharmaceuticals [25], military industry [26], wastewater treatment [27], and agriculture [28]. The biomedical field, such as analysis of human breath and urine, is the focus of this report. Recently, a number of works using multi-sensor systems, so-called E-nose and tongue are devoted to LCi [29,30].

However, the individual use of an electronic nose or tongue only reflects one aspect of the sample (smell or taste). In order to obtain more in-depth information while minimising the limitation of the detection systems, the data fusion technique can be used to generate a global signature associated with the sensitivity of both devices. According to some literature [31-33], data fusion technique is categorised as "low level", "medium level" and "high level", depending on the processing stage at which

fusion takes place. Therefore, the data fusion strategy is an effective tool for using compatible measurement data from devices.

When using an electronic nose and tongue, several studies have exploited data fusion. They include the prediction of the mixing ratio of old frying oil [34,35], the classification of different samples of honey and rice [36,37], the non-destructive detection of fish freshness [38], and the evaluation of tea and strawberry juice flavours [39].

Taking all these considerations into account, the aim of this study was to use an E-nose in conjunction with a VE-tongue combined with pattern recognition methods to analyse exhaled breath and human urine samples from LCi patients and HC. Indeed, the key clue was to merge the data from both measurement systems to improve their discrimination performance when taken separately.

2. Materials and methods

2.1. Exhaled breath and urinary collection

A total of 54 volunteers has participated in this study including 22 patients with LCi, and 32 HC. The patient samples, used in this study, are in the primary stage of disease. **Table 1** illustrates the general characteristics of all studied volunteers. In this study, only adult volunteers were considered. Persons who had consumed medication, drugs, alcohol, tobacco, drink or food before 12 noon were automatically excluded from the study.

In the literature there is no standard method for breath sampling [40] and there are different protocols for sample analysis [41]. Tedlar® bags have been shown to have acceptable sample storage properties compared to other bags made of different materials [42].

The volunteers were asked to rinse their mouth before collecting exhaled breath samples in Tedlar® bags with mouthpiece. Three breath samples were collected per each volunteer. Indeed, the volunteer blows into commercial 2 L Tedlar® bags (Supelco Inc., Belfonte, PA. USA) through a one-way valve. This valve prevents outside air from mixing with the collected breath. Before and after each breath sample, the Tedlar® bags are cleaned 3 times with synthetic air.

Besides, morning urine samples are collected in 25 mL vials.

An approval was issued by the Biomedical Research Ethics Committee of the Avicenne University Hospital (Mohammed V University, Rabat, Morocco). Prior to the collection of the samples, all the volunteers signed a consent agreement.

2.2. Electronic sensing systems

Electronic nose and tongue have been used to analyze volatile organic compounds (VOCs) in exhaled breath and urine samples collected from patients with LC and HC, respectively.

2.2.1. E-nose experimental set-up

Fig. 1 shows a schematic diagram of the system developed for the analyse of exhaled breath gas, which consists of a Tedlar® bag containing the samples, a micro-pump (flow rate of 200 L/min) to transport the analyte to a sensor chamber, a USB-6212 NI module for data acquisition and a man-machine interface for monitoring and recording the sensor responses. The E-nose comprises:

- Five chemical sensors (MQ-2, MQ-3, MQ-135, MQ-137 and MQ-138) purchased from Henan Hanwei Electronics Co, (Zhengzhou, China). The commercial sensors and their target compounds are listed in **Table 2.**
- Six interdigitated gas sensors (Pristine WO_3, Pt/WO_3, $Au/Pt/WO_3$, Au/WO_3, Ni/WO_3 and Fe/WO_3). These nanostructures were deposited on alumina substrates. Tungsten hexacarbonyl (WC_6O_6) was used as a precursor for preparing tungsten trioxide (WO_3) nanostructures, which were synthesized according to the process described in our previous work [43]. For the analysis of breath samples, these interdigital chemical gas sensors are used at their optimal operating temperatures as determined in a reported study of our laboratory [44]. During the measurement, the sensors were irradiated with UV light at 394 nm. The optimum temperature for good sensitivity was 100ºC (for pristine WO_3, Pt/WO_3, $Pt/Au/WO_3$), and 160ºC (for Au/WO_3, Ni/WO_3, Fe/WO_3).
- A relative humidity sensor (Honeywell HIH 4000-002), and a temperature sensor (LM35DZ) from National Semiconductor.

For each volunteer, three replica breath samples were collected. They were then transferred to the sensor chamber by pumping the analyte for 10 minutes. The responses of the gas sensors are recorded as conductance vs. time.

2.2.2. VE-tongue experimental set-up

The scheme of the experimental VE-tongue used for urine samples analysis is shown in **Fig. 2**. It is a conventional electrochemical cell based on a three-electrode configuration. Briefly, it consists of an Ag/AgCl reference electrode and an array of five working electrodes: copper (Cu), glassy carbon (GC), platinum (Pt), palladium (Pd), gold (Au), and an auxiliary electrode made of platinum.

After explaining the measurement protocol to the volunteer, a new polystyrene bottle was given to him to get his urine sample. The VE-tongue analysis of the urine is immediately performed after the urine is received without any pre-treatment. Once the measurement is done, the urine sample is stored at -4°C.

Electrochemical measurements are carried out by immersing the electrodes in a vessel containing the sample. The measurements are then initiated, and a data acquisition device (PalmSens^3 potentiostat, Netherlands) was used to automatically store the output data of the electrode responses. The CV technique was configured as follows: the potential scanning window was between - 0.2 to 0.6 V. The scanning was performed at 50 mV/s. To avoid possible measurement errors due to contamination of the electrode surfaces, the electrodes were all rinsed with piranha and polished before and after each measurement. This also helps to minimize drift.

During electrochemical measurements, the electrode responses are recorded as voltammograms. For the sake of repeatability in the measurements, 6 measurement repetitions were carried out for each urine sample. The total response obtained from the analyzed analyte was therefore 6 voltammograms x 5 electrodes per urine sample.

These experiments were carried out at room temperature (25°C). After analysis of the exhaled breath and urine samples, a very large database was obtained, which made interpretation difficult. To overcome this drawback, relevant features were extracted from the responses of both systems (E-nose and VE-tongue) to facilitate visualization, interpretation and understanding.

2.3. Data pre-processing

Sensor response drift is a general issue that is considered in this work. Numerous correction techniques have been tested on pre-treatment data to reduce it [45].

Indeed, to reduce the effect of sensor drifts, normalization procedure of each gas sensor response ($\frac{G-G_0}{G_0}$) is used where G is the measured conductance and G_0 is the baseline [45].

To simplify the processing of multivariate data, only the variables of interest are retained in the initial data. These variables retain as much information as possible from these responses. In this study, the analysis of the responses of E-nose system was carried out using the two variables cited below:

- $\boldsymbol{\Delta G} = (G_S - G_0)$: The difference between the stabilised conductance (G_s) and the initial conductance (G_0);
- $\boldsymbol{AUC}$: Area under the response curve of the sensor measured by a trapezoidal method. The area considered is between 1 and 9 minutes of the measurement time.

Furthermore, for processing the VE-tongue database, two eigenvalues related to the output current values were exacted from the cyclic voltammogram response of each working electrode, including:

- $\boldsymbol{Pt_{Ox}}$**:** Maximum signal slope in the first half wave (oxidation)
- $\boldsymbol{Area}$**:** Area under the response curve of the sensor.

In this study, for the E-nose, 162 breath samples (54 volunteers * 3 Tedlar bags) were measured and 2 features (ΔG and AUC) were used. These 2 features were extracted from the response of each gas sensor. Since we have 11 gas sensors, for each breath sample, 22 features were extracted. Therefore, for all samples, we had a total of 3564 variables (i.e. 162 samples * 22). The same logic was used for the VE-tongue. Thus, 7128 variables were extracted (i.e. 324 samples*22).

2.4. Data analysis

After extraction of the variables, data processing algorithms (PCA, DFA) are used to classify the analyzed samples for better visualisation and interpretation [46]. Chemometric techniques are crucial tools for obtaining a practical system capable of characterising a wide diversity of compounds [47].

The aim of using these methods in this work is to evaluate the effectiveness of the E-nose and VE-tongue in discriminating between the two groups based on breath and urine samples using both supervised and unsupervised methods.

PCA is an unsupervised linear method that uses an orthogonal transformation to reduce the dimensionality of multivariate data [48,49]. This transformation is applied so that the principal components (PCs) have the greatest possible variance while remaining orthogonal. In this study, PCA was used to process multivariate data from the E-nose and VE-tongue to obtain a clear illustration in a low-space projection.

DFA is a supervised chemometric method whose effectiveness has been proven in many applications of electronic sensing systems [50]. With the DFA method, the discriminating functions are determined in such a way that the ratio within a group is minimised (intra-class variance). At the same time, it maximises the ratio between groups (inter-class variance).

A ROC curve is a graph that illustrates the diagnostic ability of a binary classification system as a function of the variation in its discrimination threshold. The ROC curve is obtained by plotting the true positive rate (TPR) against the false positive rate (FPR) for different thresholds. The true positive rate is called sensitivity, recall or probability of detection [51] in machine learning. The false positive rate is known as the false alarm probability [51] and can be calculated as follows (1 - specificity).

SVM is a supervised nonlinear chemometric technique based on the notion of maximum margin. The maximum margin is the distance between the separation boundary and the nearest samples [52]. SVMs allows to draw a hyperplane for this separation. There is a multitude of valid hyperplanes. However, SVMs searches among the valid hyperplanes for the one that not only passes "in the middle" of the points of the two classes but also maximizes the margin.

2.5. Multisensory data fusion approach

In this study, a data fusion strategy was adopted. The main objective of using this approach was to improve the classification performance obtained using the two electronic devices individually. Here, medium level data fusion was applied to compensate for the limitations in terms of classification/discrimination of HC and LCi patient samples by the used electronic sensing systems. In this approach, the fusion is done using the features extracted from the E-nose and VE-tongue data [39,53]. It is obvious that the number of features from the two systems should be similar when merging data using the medium level abstraction approach. If the number of features from one instrument is significantly higher, they could dominate the merged dataset. In

our case, the E-nose dataset is larger than that of the VE-tongue. For this reason, since only 5 electrodes were used for the VE-tongue, an appropriate method of selecting the five most discriminating gas sensors was carried out in order to obtain a similar dimensionality of both matrices. Therefore, using the medium level abstraction approach, the datasets obtained from each multi-sensor system (E-nose and VE-tongue) were merged into a single matrix of 162 samples on 4 features (ΔG and area for the E-nose; Ptox and area for the VE-tongue).

3. Results and discussion

3.1. Analysis of VOCs in exhaled breath by E-nose system

Sensor responses were recorded during the 10 minutes of breath exposure, followed by 10 minutes of return to baseline under synthetic air. **Fig. 3** displays the responses of the two families of gas sensor arrays towards exhaled breath samples of HC and LCi. As shown in **Fig. 3**, the response of the sensor array to the exhaled breath gas was directly related to the gas detection and pattern identification. The MQ-138 sensor of **Fig. 3(b)** showed higher sensitivities than the other sensors. Many studies have been performed on the analyze of breath VOC biomarkers from LCi patients and reported that volatile biomarkers in breath associated with liver cirrhosis are Methanol [54-56], Dimethyl sulfide [57,58], Ethanol and Toluene [58]. Though the other sensors showed relatively low signals, there were differences between LCi patients and HC (**Fig 3(a)**). We can notice that the gas reaction was completed after 600 s, whereas the desorption of the gas was completed after around 30 s for MQ sensors. However, this time to return to the baseline remains longer for interdigitated chemical gas sensors based on pristine or metal-doped WO_3 nanowires (**Fig. 3 (c,d)**). Besides, Au/WO_3 sensor response (**Fig 3(d)**) provided higher response toward LCi patient's breath sample than to the HC (**Fig 3(c)**). This behaviour can be justified by the difference in VOC concentrations in the breath related to HC and LCi patients [6,23].

3.2. Analysis of urinary VOCs by VE-tongue

Fig. 4 shows different electrochemical responses, depending on the health state associated with the urine samples that were analysed. These irreversible responses are different in terms of shape and amplitude. However, oxidation processes are only observed when using Cu working electrode. This oxidation effect is more pronounced in urine samples of patients with LCi. This is probably due to abnormal variation of

copper level in the body of LCi patients and released in urine [59-68]. It should be noted that the intensity of the voltammograms yield by Cu working electrode corresponding to urine samples of patients with LCi (**Fig. 4(b)**) is higher than those of HC (**Fig. 4(a)**). The oxidation peak is around 0.30 V for LCi (**Fig. 4(b)**), while the Cu electrode admits a small oxidation peak at around 0.02 V for the urine sample of HC (**Fig. 4(a)**). With the Cu electrode, the voltammograms demonstrate metal oxidation, forming copper ions in urine samples, but with different trend for LCi. According to the literature, copper primarily deposits in the liver and the lethal dose is about 10-20 g [69]. On one hand, *A. Amit et al*. and Y. *Yoshida et al*. report that liver problems modify the metabolism of trace elements. In this case, they declare the presence of either a high presence of copper or a deficiency of zinc (Zn) [63,70]. In this purpose, *D. Rahelic et al.* explains the role of copper in the redox process [71]. Consequently, the proposed VE-tongue provides findings that show that the state of health influences the chemical composition of the urine and corroborates the literature.

3.3. Radar plot results

The sensors are not specific to one compound. However, each sensor is sensitive to several compounds. The combination of the responses allows plotting a specific chemical signature. After the features' extraction step, the radar plot was used to see if there were any differences or similarities in terms of the chemical signatures of the various breath and urine samples from patients with LCi and HC. **Fig. 5** shows the radar plot results highlighting the contribution of the E-nose gas sensors. These plots were constructed using the area value as a feature. There is a clear variation in pattern between the breath-prints of the two groups.

Fig. 6 shows the radar plots of the two sets of urine samples obtained after extraction of the relevant features from the voltammograms. Each representation has a particular chemical signature in their taste profile, although there are some similarities. The majority of the electrodes showed similar behaviour for the two groups except for Pd electrode. This could strongly influence the classification performance of the VE-tongue, thus generating an overlap in the sample patterns.

3.4. PCA discrimination results

The discrimination results of the two sensing systems individually by using PCA are depicted in **Fig. 7**. Using two features (ΔG and AUC), the PCA results for the E-nose (**Fig. 7(a)**) indicate a data variance of 61.98%, which was explained by the first three

principal components (PCs). This three-dimensional PCA plot shows a classification among the breath patterns of LCi patients and HC with slight overlapping between the two clusters.

Besides, the VE-tongue discrimination results obtained by applying PCA are shown in **Fig. 7(b)**, where all three PCs contributed to a score of 81.38%. Here, the samples of the LCi patients also overlapped with those of the HC. This is probably due to the similarity of the electrochemical responses of the majority of the electrodes.

3.5. DFA discrimination results

The same dataset was used to perform a treatment using a supervised method, called DFA. It was used to distinguish between different samples of breath and urine according to their health states. The results of the DFA after its application to the responses of the two systems are shown in **Fig. 8**. The same features as for the PCA were used. The results in **Fig. 8(a)** show a discrimination between LCi patients and HC breath samples. In contrast, the discriminatory feasibility of the VE-tongue, shown in **Fig. 8(b)**, does not lead to a perfect separation between LCi and HC samples. These results confirm those obtained by the PCA analysis and suggest the use of data fusion to recognise the two health states.

3.6. Receiver operating characteristic (ROC) results

ROC curves for the E-nose and VE-tongue data were plotted. AUC values were calculated to assess the diagnostic value of each data item. The ROC curve and AUC analysis of the logistic regression were performed to explore the diagnostic performances of the E-nose and VE-tongue models. **Fig. 9(a)** shows the ROC curve of the E-nose data with an AUC of 0.965 corresponding to the analysis of LCi and HC breath samples.

Besides, **Fig. 9(b)** shows the ROC curve of the VE-tongue model data with an AUC of 0.950 for the distinction of urine samples corresponding to LCi and HC. These results show that the diagnostic performances of two multi-sensor systems is significantly significant. Furthermore, the diagnostic performance of the E-nose model is higher than that of the VE-tongue model.

3.7. SVMs classification results

The dataset trained for 20 volunteers (10 LCi and 10 HC patients) of balanced age was used to run the SVMs method. It was used to distinguish between different breath and urine samples according to their health status.

Table 3 shows the SVMs results of the classification of 60 breath samples (20 volunteers providing 3 samples each). The rows of the table indicate the actual health states and the columns the predicted states. The SVMs method achieved a success rate of 98.33% in the recognition of the two groups studied (LCi and HC). As can be seen from the table, one error was detected: a sample belonging to LCi was classified in the HC group.

Besides, Table 4 presents the SVMs results when analyzing the data from the VE-tongue for 120 urine samples. The same trend as for the E-nose is observed with the occurrence of three errors and a success rate of 97.50%.

3.8. Data fusion results

3.8.1. PCA classification results

Using data fusion, the PCA method was applied to discriminate the two types of samples. **Fig. 10** shows the discrimination results obtained by combining the data from the two systems, where all three principal components contributed with a score of 64.23%. Indeed, we can notice both groups are well discriminated, demonstrating the ability of the combination of two systems to overcome the overlap observed previously. The close linkage of biological emanations to health status provides a unique signature. This explains the good discrimination obtained by merging the data as opposed to separating them.

3.8.2. DFA classification results

DFA was also used to examine the ability of the data fusion technique to improve the classification of different samples according to their health status. **Fig. 11** shows the first two DFA functions for the classification of HC and LCi patients using data fusion of the E-nose and VE-tongue systems. The result reveals a perfect discrimination between HC and LCi patients. Therefore, the processing of the data by the DFA technique reveals the effectiveness of the proposed data fusion method in distinguishing HC and LCi patients.

3.8.3. Receiver operating characteristic (ROC) results

A ROC analysis was also performed to evaluate the diagnostic performance after data fusion to improve the classification of different samples according to their health status. **Fig. 12** shows the ROC curve corresponding to the merged data of the two devices with a very good AUC of 0.999. This result shows that the diagnostic performance of the model obtained after data fusion is superior to those found using the E-nose and VE-tongue data individually. These results confirm the effectiveness of merging data from two compatible systems to successfully distinguish HC and LCi patients.

3.8.4. SVMs classification results

In order to enhance the SVMs classifications obtained by taking the two measurement systems individually, SVMs was applied to the merged data. For this purpose, 20 age-balanced volunteers (10 LCi and 10 HC patients) were considered.

Table 5 shows the results of the SVMs classification for the fusion of the E-nose and VE-tongue data. A very good result by the SVMs method is achieved with a success rate of 100% for the recognition of LCi and HC volunteers. In the light of this result, it can be concluded that the data fusion technique is a good way to improve the classification of different samples.

4. Conclusion

In this work, an electronic nose and tongue were used to analyse breath and urine samples from patients with LCi and HC. With the help of pattern recognition methods, the two electronic devices, taken individually, showed limitations in terms of discrimination of the two study groups. To overcome the limitation of the individual systems, the data fusion of the E-nose and VE-tongue provided very satisfactory results in terms of discrimination and classification. Indeed, the PCA results obtained from the merged data showed a good classification of the different breath and urine samples according to their health status. In addition, the supervised method (DFA) was able to clearly differentiate between LCi patients and HC. Moreover, the diagnostic result of data fusion obtained by ROC curve is superior to those found using the E-nose and VE-tongue data individually. The advantage of this study is the proposal of electronic sensing systems as alternative tools to conventional techniques, which have many drawbacks for the analysis of this disease. This demonstrates that the two proposed

devices have a classification and predictive capability that would certainly contribute to facilitating the early diagnosis of diseases. In view of the results of this study, and thanks to their portability and speed, they could therefore be used as non-invasive diagnosis for other diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure captions

Fig. 1. Architecture of the E-nose system used for exhaled breath measurements.

Fig. 2. Schematic representation of the VE-tongue dedicated to the analysis of urine samples.

Fig. 3. E-nose responses of: MQ gas sensors when exposed to breath of (a) HC, (b) LCi patient; and interdigitated gas sensors after exposure to VOCs in the breath samples from (c) HC, (d) LCi patient.

Fig. 4. Responses of the five-electrode arrays of VE-tongue and insights without Cu electrode towards urine samples from: (a) HC, (b) patient with LCi.

Fig. 5. Radar plots of E-nose gas sensors responses towards exhaled breath from HC and patient with LCi, expressed by area as a feature.

Fig. 6. Radar plots of the responses of the VE-tongue electrode array exposed to the two different sets of urine samples, expressed by Pt_{ox} as a feature.

Fig. 7. Unsupervised PCA plot showing data points for: (a) breath samples relating to the two health states with data collected from the E-nose, (b) urine samples relating to the two health states with data collected from the VE-tongue.

Fig. 8. Supervised DFA plot displaying data points for: (a) breath samples relating to the two health states with data collected from the E-nose, (b) urine samples relating to the two health states with data collected from the VE-tongue.

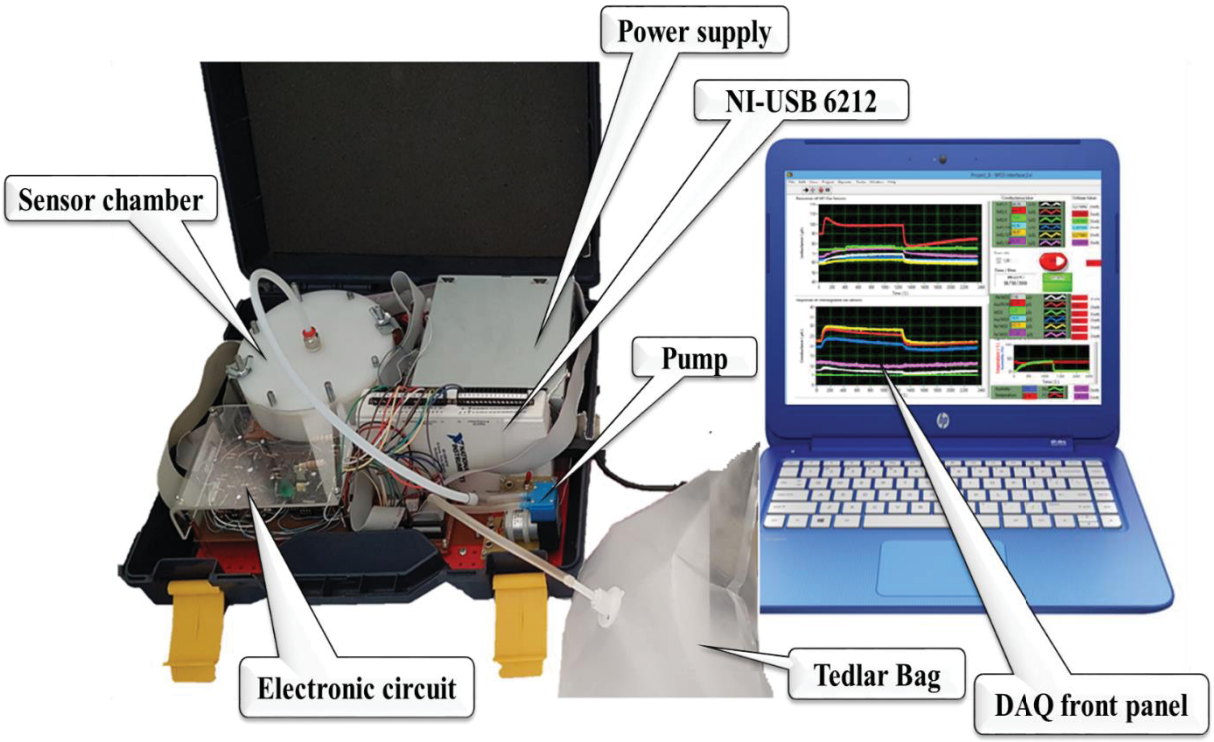
Fig. 9. ROC curve displaying data points for: (a) breath samples relating to the two health states with data collected from the E-nose, (b) urine samples relating to the two health states with data collected from the VE-tongue.

Fig. 10. Unsupervised PCA plot showing data fusion of E-nose and VE-tongue systems.

Fig. 11. Supervised DFA plot showing data fusion of E-nose and VE-tongue systems.

Fig. 12. ROC curve showing data fusion of E-nose and VE-tongue systems.

693 **Fig. 1.**


Power supply
NI-USB 6212
Sensor chamber
Pump
Electronic circuit
Tedlar Bag
DAQ front panel

694

Fig. 2.

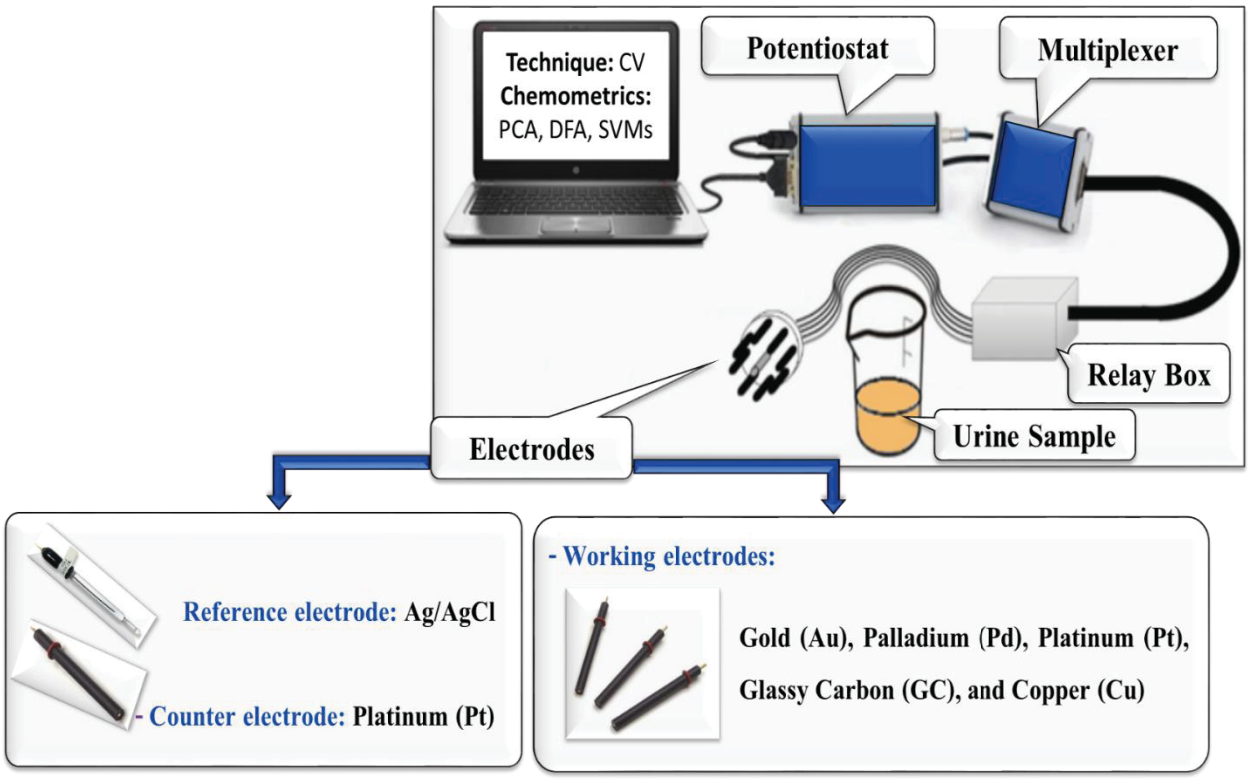

Technique: CV
Chemometrics:
PCA, DFA, SVMs
Potentiostat
Multiplexer
Relay Box
Urine Sample
Electrodes
Reference electrode: Ag/AgCl
- Counter electrode: Platinum (Pt)
- Working electrodes:
Gold (Au), Palladium (Pd), Platinum (Pt),
Glassy Carbon (GC), and Copper (Cu)

Fig. 3.

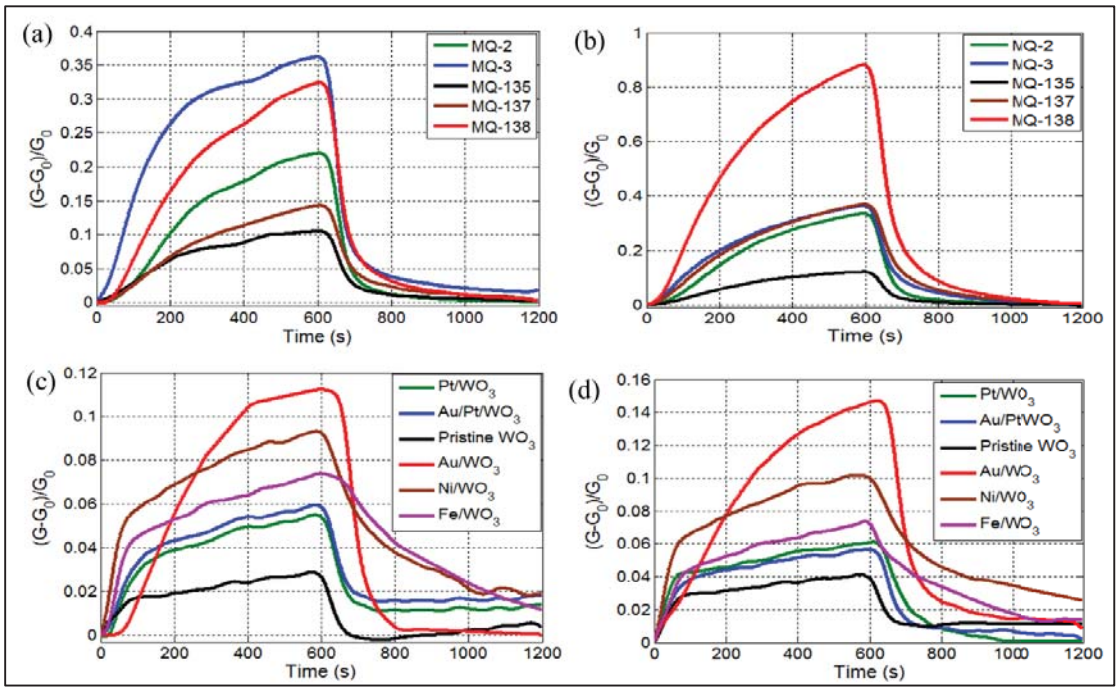
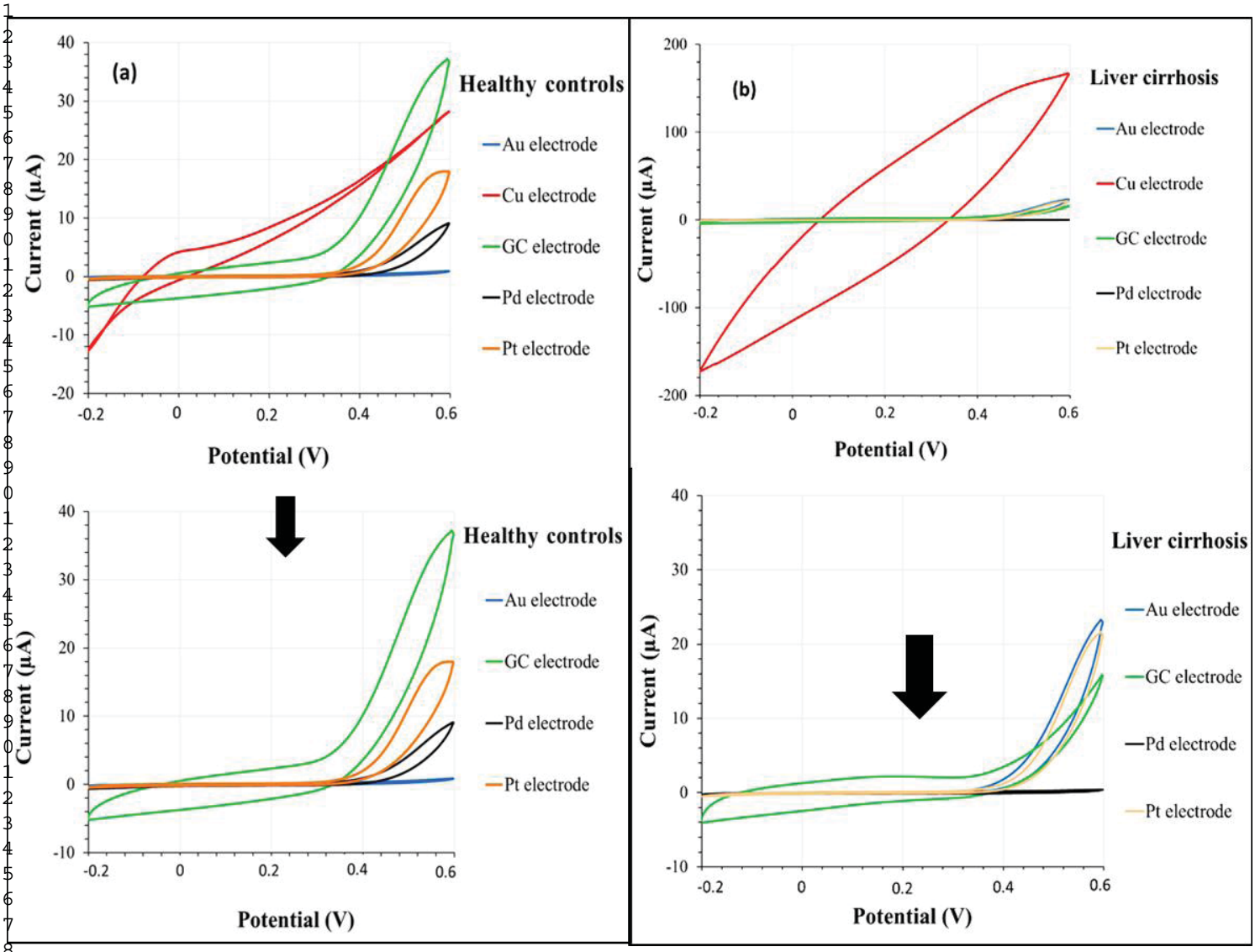

(a)
(b)
(c)
(d)
(G-G0)/G0
Time (s)
MQ-2
MQ-3
MQ-135
MQ-137
MQ-138
Pt/WO3
Au/Pt/WO3
Pristine WO3
Au/WO3
Ni/WO3
Fe/WO3

Fig. 4.


(a)
Healthy controls
Au electrode
Cu electrode
GC electrode
Pd electrode
Pt electrode
Current (µA)
Potential (V)
(b)
Liver cirrhosis
Au electrode
Cu electrode
GC electrode
Pd electrode
Pt electrode
Current (µA)
Potential (V)
Healthy controls
Au electrode
GC electrode
Pd electrode
Pt electrode
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Pd electrode
Pt electrode
Current (µA)
Potential (V)

Fig. 5.

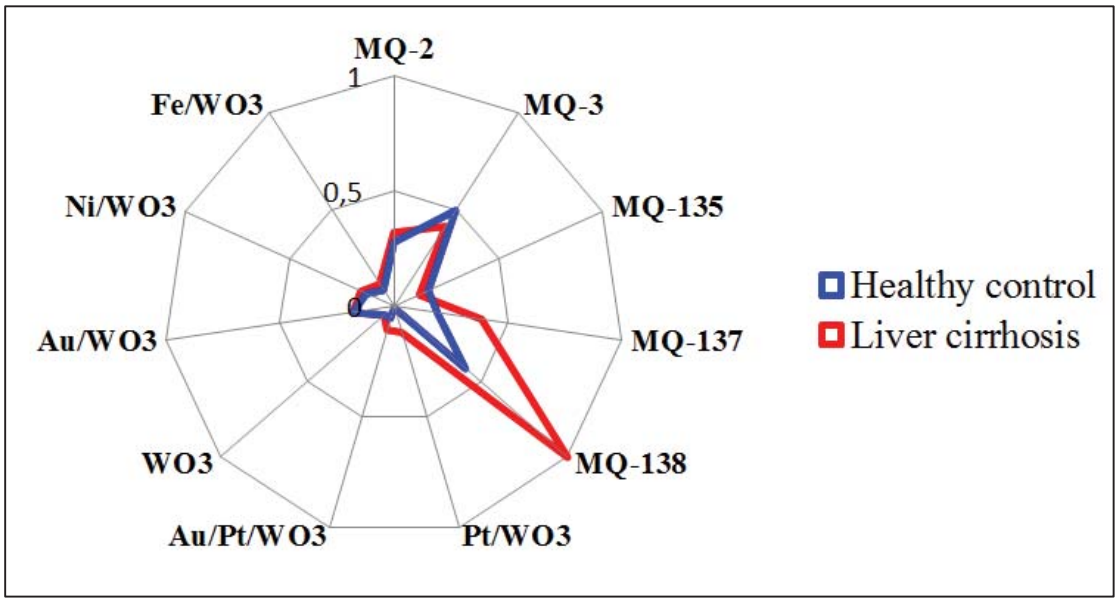
MQ-2
1
MQ-3
MQ-135
MQ-137
MQ-138
Pt/WO3
Au/Pt/WO3
WO3
Au/WO3
Ni/WO3
Fe/WO3
0,5
0
Healthy control
Liver cirrhosis

Fig. 6.

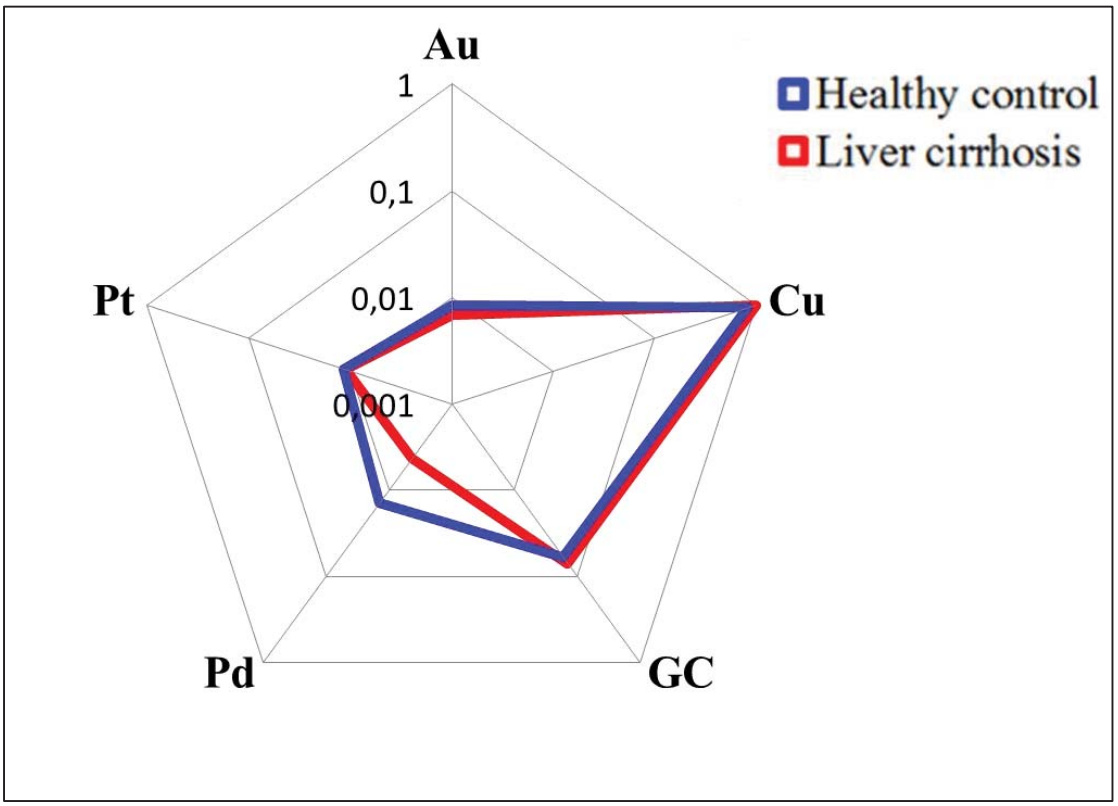

Fig. 7.

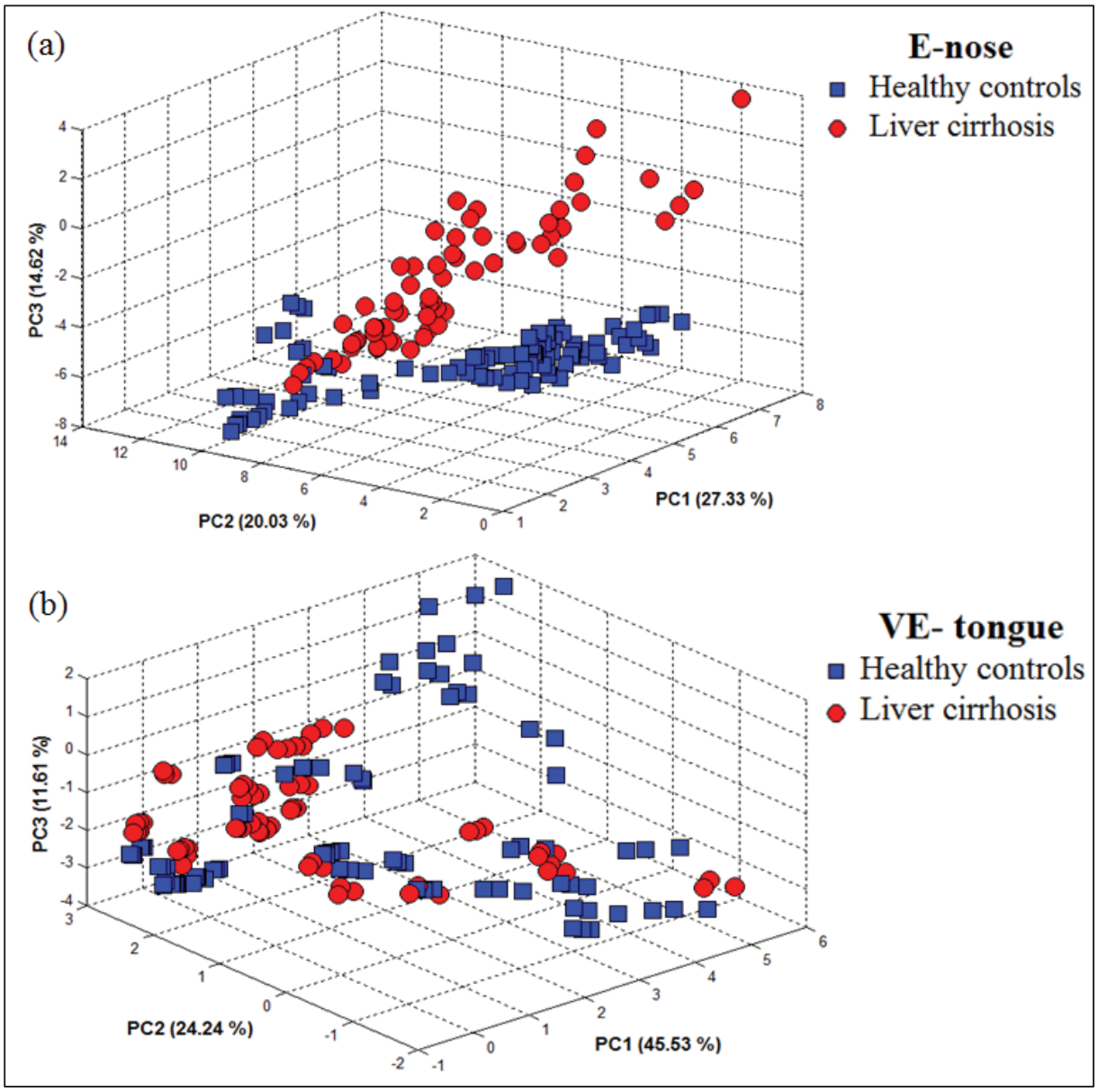
(a)
E-nose
Healthy controls
Liver cirrhosis
PC3 (14.62 %)
PC2 (20.03 %)
PC1 (27.33 %)
(b)
VE- tongue
Healthy controls
Liver cirrhosis
PC3 (11.61 %)
PC2 (24.24 %)
PC1 (45.53 %)

Fig. 8.

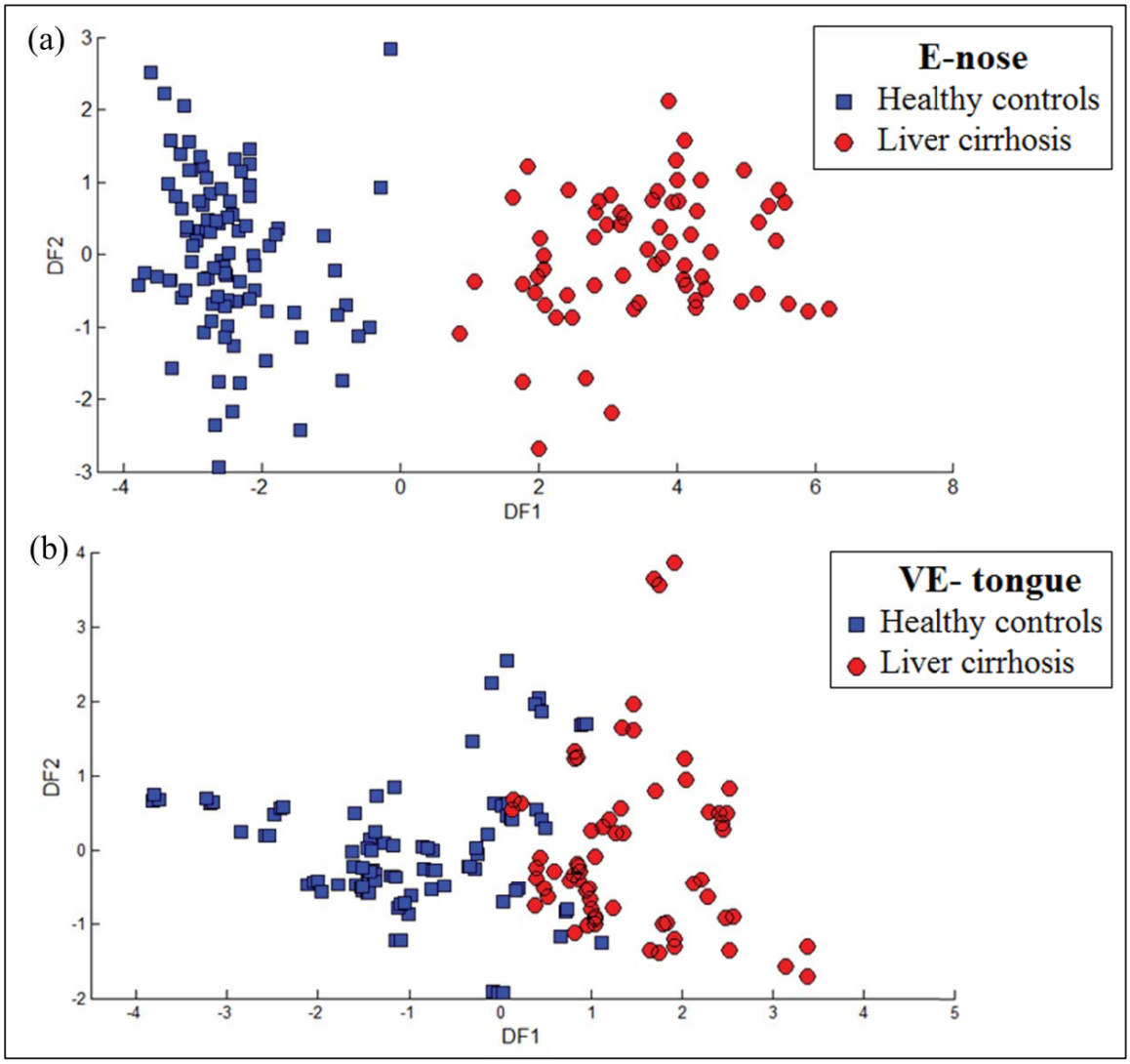
(a)
E-nose
Healthy controls
Liver cirrhosis
DF2
DF1
(b)
VE- tongue
Healthy controls
Liver cirrhosis
DF2
DF1

Fig. 9.

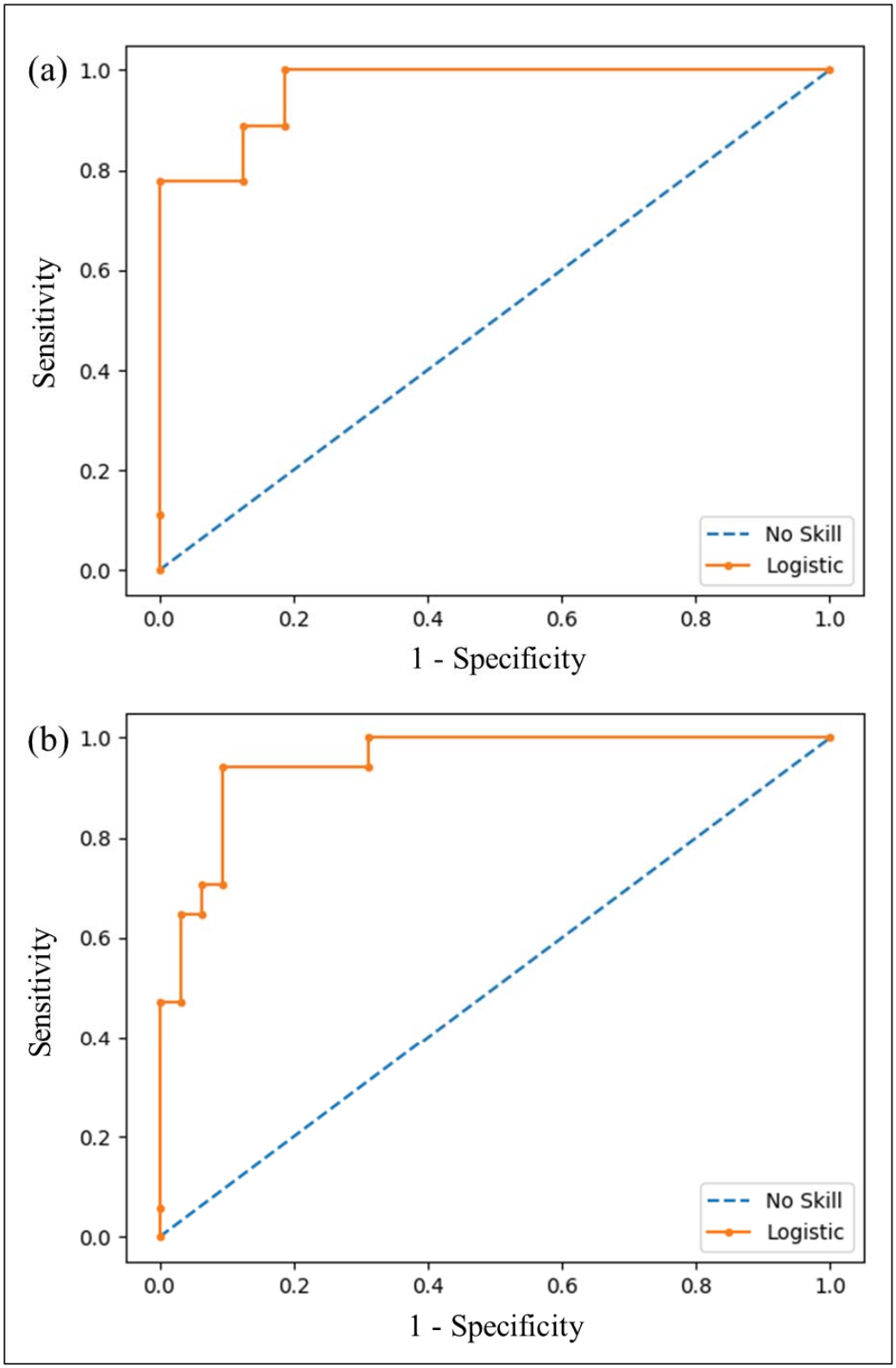
(a)
Sensitivity
1 - Specificity
No Skill
Logistic
0.0
0.2
0.4
0.6
0.8
1.0
(b)
Sensitivity
1 - Specificity
No Skill
Logistic

Fig. 10.

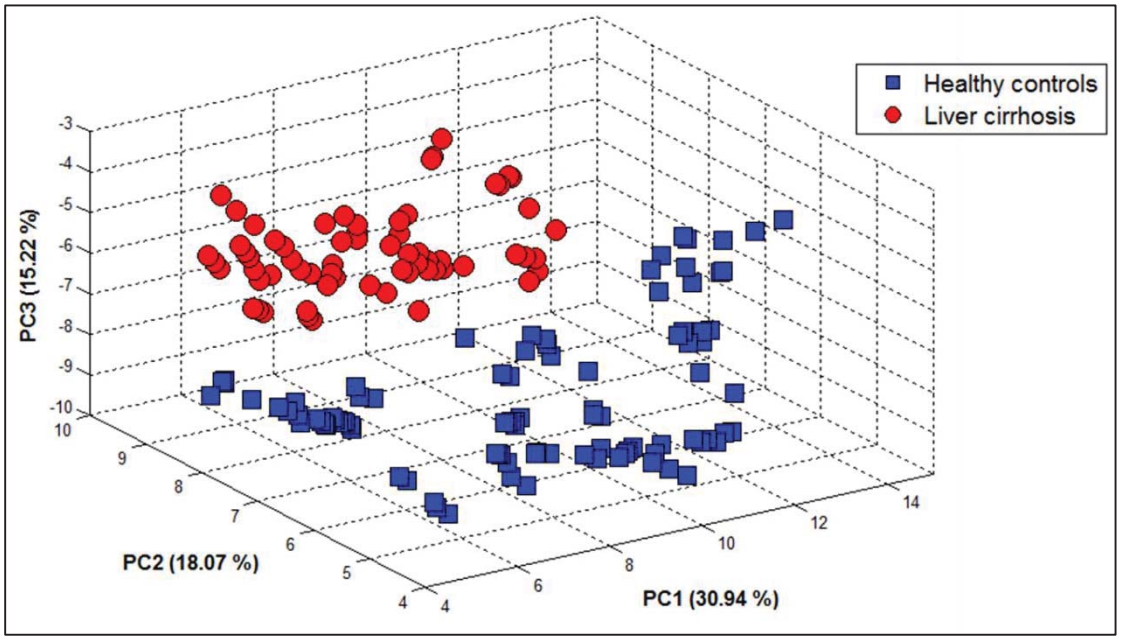
Healthy controls
Liver cirrhosis
PC3 (15.22 %)
PC2 (18.07 %)
PC1 (30.94 %)

Fig. 11.

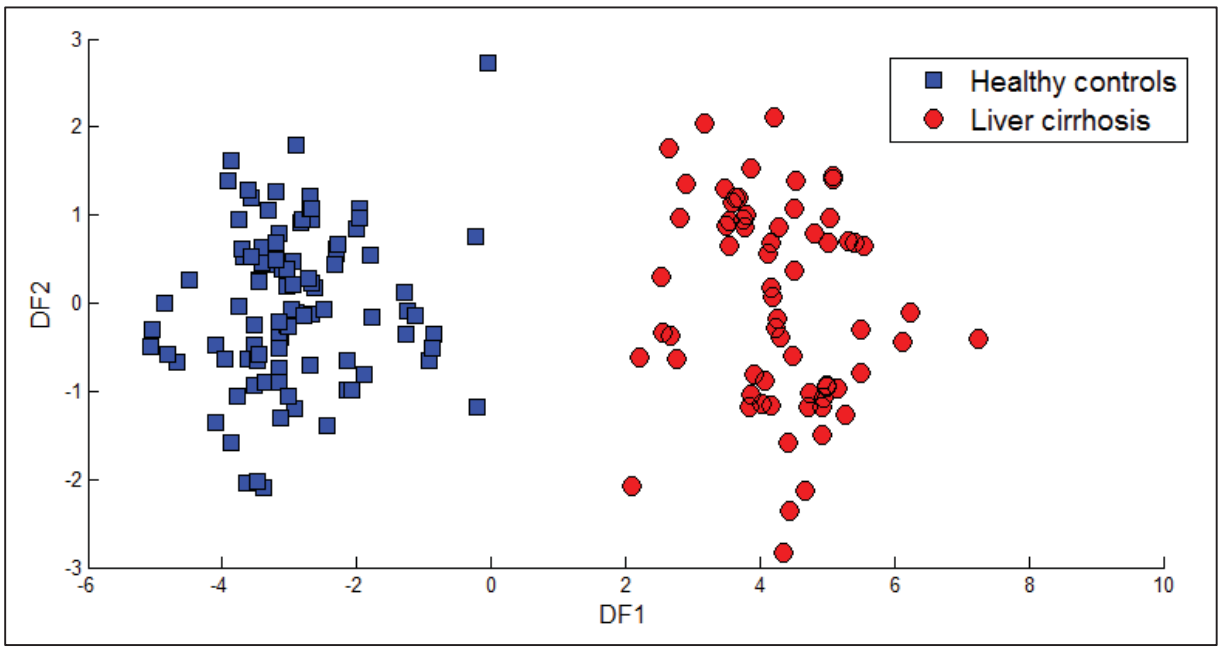
Healthy controls
Liver cirrhosis
DF2
DF1
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4
6
8
10

Fig. 12.

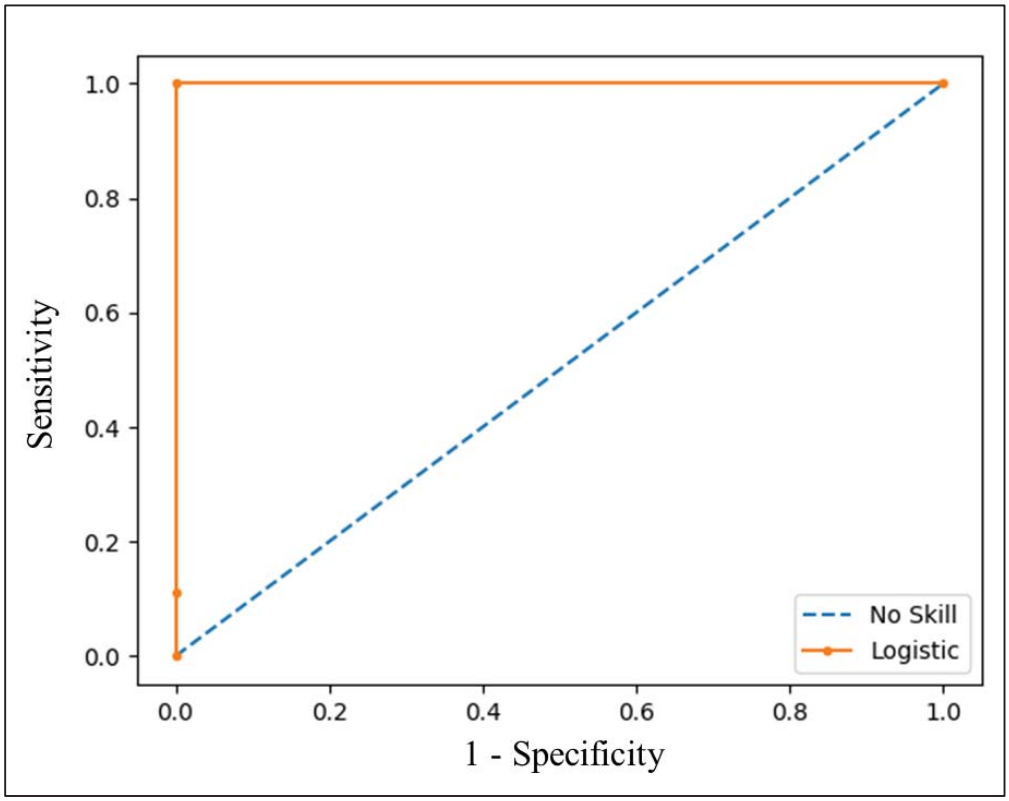
Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
No Skill
Logistic

Table 1

General characteristics of all studied volunteers.

Volunteers					
Groups	**Number**	**Age range (years)** **Age ± SD***	**Male, number (%)**	**Smoking habit+**	**Medication**
Liver cirrhosis (LCi)	22	24-79 46 ± 15	10 (45%)	1 S, 3 Ex.S, 18 NS	- Ciprofloxcicine - Aldactone - Autocardyl - Tefovin
Healthy controls (HC)	32	21-64 37 ± 17	22 (69%)	8 S, 24 NS	———

*** SD:** Standard Deviation.

+ Smoking habit: S: Smoker, Ex.S: Ex-Smoker, NS: Non-Smoker.

Table 2

Commercial gas sensors used in the e-nose system.

Gas Sensors	Target Compounds
MQ-2	Propane, Hydrogen, Methane
MQ-3	Alcohol
MQ-135	Benzene, Ammonia, Carbon dioxide and Nitric oxide
MQ-137	Ammonia
MQ-138	Toluene, Acetone, Methanol, Ethanol and Formaldehyde

Table 3

SVMs results for the classification of 60 breath samples regarding their health states by using the E-nose system with a success rate of 98.33%.

Actual	Predicted	
	LCi patients	**HC**
LCi patients	29	1
HC	0	30

Table 4

SVMs results for the classification of 120 urine samples regarding their health states by using the VE-tongue system with a success rate of 97.50%.

Actual	Predicted	
	LCi patients	**HC**
LCi patients	57	3
HC	0	60

Table 5

SVMs results for data fusion of E-nose and VE-tongue systems with a success rate of 100%.

Actual	Predicted	
	LCi patients	**HC**
LCi patients	30	0
HC	0	30