Towards Chronic Liver Dysfunction Self-monitoring: a *Proof-of-Concept* Study

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***Abstract*—The liver is our very own chemical processing plant as it plays a vital role in maintaining the body's metabolic balance. Liver's health is assessed by a group of clinical tests (such as blood tests, ultrasonographic imaging, liver biopsy) most of which are invasive and burdensome for the patients.**
In the setting of severely scarred liver, toxic substances, such as ammonia, have fewer opportunities to be detoxified. Accumulation of ammonia in the systemic circulation and in the brain may result in Hepatic Encephalopathy (HE), a spectrum of neuropsychiatric abnormalities which entails changes in consciousness, intellectual functions, behavior. Minimal HE has attracted increasing attention, as it does not cause detectable changes in personality or behaviour, but the complex and sustained attention is impaired. Hence, it can be detected only by specific but biased, time-consuming and burdensome examinations, such as blood ammonia levels assessment and neuro-psychological tests.
The obstrusivity of the majority of the liver function clinical tests, and, in case of minimal HE, the lack of reliable examinations, are encouraging the scientific community to look for alternative diagnostic methods. For this purpose, the exploitation of a non-invasive technique such as breath analysis, to identify chronic liver disease, discriminate among its degree of severity and detect the onset of HE, could be a step forward for clinical diagnosis.
In this paper, we report a *proof-of-concept* study that aimed at detecting ammonia in the breath of patients suffering from chronic liver disease by means of a low-cost, easy-to-use, gas-sensors based device. Not only, we also aimed at investigating the possibility of discriminating the several severity degree of liver impairment on the basis of the detected ammonia.

***Index Terms*—Breath analysis, Liver function, Liver disease, Gas sensors, Data analysis, Biomedical Devices.**

I. Introduction

The liver is the second largest organ in the body and it is responsible for many critical functions.The loss of liver functions cause significant damage to the body. Cirrhosis (advanced chronic liver disease) refers to end stage liver disease where the damage to the organ has become irreversible. According to WHO, liver cirrhosis accounts for 1.8% of all deaths in Europe [1]. Cirrhosis can have varied clinical manifestations and complications. A serious complication of cirrhosis is Hepatic Encephalopathy (HE).

HE is characterized by personality changes, confusion, intellectual impairment and depressed level of consciousness [2]–[4]. Minimal HE has attracted increasing attention. Such stage shows an absence of detectable changes in behaviour, but the complex and sustained attention is impaired.

Although HE exact pathogenesis is unknown, accumulation of ammonia in the systemic circulation and, hence, in the brain, from poor hepatic function and portosystemic shunting has been assessed as a primary factor [5]–[7].

Clinical testing for HE includes several neurophysiologic tests such as "numbers connecting test" A and B and the "digit-symbol test" [8]. Nevertheless, such tests can be very time-consuming. Biochemical testing may consist of determination of blood ammonia level, though it is not usually used as its accuracy may vary depending on specimen handling and transport, and measurement techniques [9].

Therefore, the lack of reliable examinations for the assessment of HE, as well as the obstrusivity of the majority of the liver function clinical tests (from the blood tests to the liver biopsy), are encouraging the scientific community to look for alternative diagnostic methods.

It has been well-established that increasing levels of ammonia concentrations in blood, lead to its elevation in exhaled breath gases [5], [10]–[12]. As a consequence, if an easy-to-use, real-time, non-invasive breath ammonia test system could be used to identify chronic liver disease and evaluate its degree of severity, it would be a very positive step forward.

There are few studies on patients with chronic liver disease with e-nose [13], [14]. A large number of methods which were developed for measuring ammonia in human breath and discriminating between healthy and cirrhotic subjects, involve

ion flow tube mass spectrometry (SIFT-MS), or photoacustic laser spectrometry (PALS), [15], [16], or gas chromatography-mass spectrometry (GC-MS) [17], or proton transfer reaction time-of-flight mass spectrometry (PTR-MS) [18]; although very accurate and excellent laboratory tools, such systems are not portable, very expensive, and far from being translated into daily applications in biomedical diagnostics, where the major goal is to provide easy-to-use point-of-care systems.

Several micro-systems have been developed to detect breath ammonia. In [19] Timmer and co-workers presented a microfluidic system able for detecting ammonia by using a conductivity sensor. However, ammonia needs to be converted to ammonium and, then, back to gaseous ammonia for measurement; such conversion requires the supply of acid and water. Toda et al. detected breath ammonia by dissolving it in a droplet of concentrated sulfuric acid on top of a conductivity sensor [20]. Nevertheless, the difficulty of fabricating and replicating such devices and methods would make laborious their daily application.

In [21], a MoO_3 nanosensor is presented; the authors demonstrated that the sensor is able to selectively measure concentrations of ammonia gas in breath-simulating environments at ppb levels. The sensor was tested only in laboratory setting and not on a population.

Organic thin-film transistors (OTFTs) were reported to be inexpensive, manageable and disposable diagnostic device because of their low-cost manufacturing process and good sensitivity to gaseous compounds [22]. Zan et al. [23] developed a pentacene-based OTFT, whose sensitivity was improved by an UV irradiation treatment which modified the functional groups on the poly (methyl methacrylate) dielectric layer. The presented sensor was able to detect $0.5 \sim 5$ppm concentration ammonia gas, which is the critical range of breath ammonia levels that can discriminate between healthy subjects and patients with cirrhosis and renal impairment. Nonetheless, tests on a population were not carried out.

Optical sensing systems, though show a very good sensitivity in part-per-billions (ppm) or part-per-trillions (ppt) regime, demonstrate to require expensive set-up, as remarked in [24].

AmBeR®, an ammonia in-breath measurement device, was developed by BreathDX [25], a UK-based company founded by Prof. T. Killard. The device is based on sensors fabricated using printed functional nanomaterials which are capable of parts per billion limits of detection [26]. AmBeR®was designed to diagnose and manage a range of conditions: from stomach ulcer (e.g. H. pylori) to chronic liver disease, to non-invasive volume drug toxicity study.

In the present *proof-of-concept* study, we focused our attention on patients with liver dysfunction. We aimed to detect ammonia in the breath of a population of subjects suffering from liver disease by means of a low-cost, gas sensor-based device designed for human breath analysis. In addition, our study went beyond the state of the art as it was not limited to distinguishing healthy from diseased subjects, but it aimed at understanding the several severity degrees of liver impairment (from chronic liver impairment, to cirrhosis, to hepatic encephalopathy) on the basis of the detected breath ammonia. The paper is organized as follows. In section II, we briefly describe the semiconductor gas sensor-based device that was used for the presented study: the *Wize Sniffer*; the experimental tests are reported in Section III; in Section IV the results are presented. Finally, Section V conclude the paper.

II. The Wize Sniffer

A. The Hardware

The Wize Sniffer (WS) (Figure 1) is a portable gas sensor-based device for human breath analysis which was developed in the framework of SEMEiotic Oriented Technology for Individual's CardiOmetabolic risk self-assessmeNt and Self-monitoring (SEMEOTICONS, www.semeoticons.eu) European Project [27], [28]. The WS was firstly designed to monitor subject's noxious habits for cardio-metabolic risk (smoke, alcohol intake, wrong diet) by detecting a set of volatile compounds including hydrogen, carbon monoxide and ethanol in exhaled breath [29]. It was demonstrated [30], [31] that the WS is: *(i)* able to accurately analyse human breath composition in real time, *(ii)* able to monitor user's habits and well-being state, *(iii)* easy-to-use, also for non-specialized personnel.

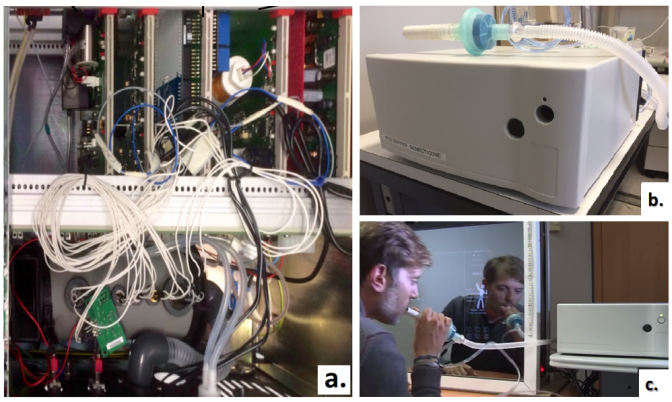

Fig. 1. The Wize Sniffer. a) internal configuration: the gas sensors in the gas sampling chamber are shown, as well as the electronics and the data pre-processing board. b) external configuration: WS dimensions are: 30x30x14cm. c) The Wize Sniffer while performing a breath test.

The WS is composed of a gas sampling chamber (whose dimensions were fixed at about 600ml, according to the human resting tidal volume [32]), where the exhaled breath is collected, and an array of six Taguchi metal-oxide semiconductor gas sensors sensitive to ammonia and other VOCs. A signal acquisition module filters and manipulates sensor raw signals. A flow-meter allows for monitoring the user's flow rate and for calculating the exhaled gas volume. A signal conditioning module transfers sensors raw output from the measurement module to the controller board, an Arduino Mega2560. A data analysis algorithm, based on multivariate statistics, allows for features extraction and individuals breath-print identification.

In [30], [31], further details on WS hardware are reported.

B. The pre-processing stage

Pre-processing stage is somewhat tied to the underlying sensor technology: therefore we aimed, on one hand, at compensating the factors which can influence the measure; on the other hand, we aimed at selecting significant descriptive parameters from the sensor array output curves, which better represented both quantitative and qualitative sensor response.

- the value at curve plateau ($\Delta X_s(\infty)$), as it better describes the chemical balance between sensors sensing element and target gases [33]–[36];
- the response time T_r, as it is characteristic of each vapour/sensor pair [37];
- the maximum slope of the curve.

Also humidity values (within the gas sampling chamber) are read before and after each breath test, in order to eventually compensate sensors' drift due to humidity.

III. Experimental tests

In this *proof-of-concept* study we exploited WS potentialities and aimed at:

- detecting ammonia in the breath of patients suffering from chronic liver dysfunction;
- discriminating the several severity degrees of liver impairment on the basis of the detected breath ammonia.

The study included 64 subjects: 20 women (mean age: 52, with a range of 31 to 78) and 44 men (mean age: 55, with a range of 32 to 84), among which 20 non-cirrhotics with chronic liver disease (NC-CLD), 22 cirrhotics (CIRRH), 6 cirrhotics with recent episode of HE (CHE) and 16 healthy controls (HC). Individuals were defined as healthy subjects if they did not present symptoms and/or signs of either acute or chronic illness, did not have chronic illness, and were not consuming medications on a regular basis.

Exclusion criteria were: alcohol/psychoactive drugs at baseline, neurological disease, lack of compliance with psychometric evaluation, and other chronic illness (cardiac or renal insufficiency, diabetes, COPD, celiac disease).

The diagnosis of cirrhosis was based on liver biopsy and on clinical, biochemical and ultrasonographic findings. Portal-systemic shunts were searched for by US and CT scan. The Child-Pugh and the Model for End-Stage Liver Disease (MELD) scores were calculated. Blood sample was drown for each subject for a complete blood count, prothrombin time (PT), bilirubin, international normalized ratio (INR), and liver panel, albumin and creatinine determinations.

The presence and the degree of HE were evaluated by focused neurologic exams and psychometric tests, including trail-making test A (TMT-A) and B (TMT-B) and the digit-symbol-test (DST) [38], [39].

Regarding the breath tests, there is no consensus in literature about how measurement of ammonia in exhaled breath should be measured [12]. Exhaled breath composition is strongly influenced by breath sampling method [40], as well as by breath flow rate [41], posture [42], ambient air [43], etc.

Among the different sampling methods (end-tidal sampling, mixed expiratory sampling, time-controlled sampling [40]), we chose the most used technique, that is mixed expiratory breath sampling [40], [44], [45] given, in addition, its easily manageable and cost-efficient applicability.

In the mixed expiratory breath sampling technique, the volunteer has to breath the whole exhaled volume. Nevertheless, mixed expired air consists of dead space, transition phase and alveolar phase. Only the latter contains the VOCs resulting from alveolar exchanges, which better represent the individual's metabolic conditions. Several studies have demonstrated possible advantages when applying specific manoeuvres, such as breath holding [46], high exhaled volume, lower exhalation flow rate [47]–[49] and single exhalation [50], which may lead to an increase in alveolar VOCs concentrations in breath samples. Therefore, the volunteers were instructed to: *(i)* first, take a deep breath in;*(ii)* then, hold the breath for $10\,\mathrm{sec}$;*(iii)* finally, breath out once through the WS mouthpiece trying to keep the expiratory flow low (about 160L/min $\pm 10\%$) and constant, and to completely empty their lungs.

All the participating subjects were under the same conditions of environmental temperature and humidity when breath test was performed, in a seated position, at morning, fasting and several hour after brushing their teeth.Breath carbon dioxide was monitored in real time as its profile defines the quality of the breath sample [51], as well as the breath flow rate. The breath ammonia was detected with TGS2444 and TGS2602 gas sensors present in WS gas sensor array. finally, breath out once through the WS mouthpiece trying to keep the expiratory flow low (about 160L/min $\pm 10\%$) and constant, and to completely empty their lungs.

The tests were conducted at the Hepatology Unit of the University Hospital of Pisa, Italy. The methods and the protocol were submitted to the Ethical Committee of the Azienda Ospedaliero Universitaria Pisana for approval. All subject provided a signed informed consent before enrolment.

IV. Results

The following statistical analysis were performed in MATLAB®and R3.2.4®environments.

First, descriptive statistics were used to quantitatively describe and summarizes both breath and clinical data.

In Tables I and II the features extracted from TGS2444 and TGS2602 output curves are summarized. For each class of subjects (healthy controls HC, non-cirrhotics with chronic liver disease NC-CLD, cirrhotics CIRRH, and cirrhotics with recent episode of hepatic encephalopathy CHE) the mean value (and the relative confidence interval C.I.) of each feature is reported. The used gas sensors, as can be observed in Tables I and II, gave good results in detecting breath ammonia. In particular, the sensors maximum output increased with increasing liver impairment, as we expected. Such result can be graphically observed also in Figure 2.

Also, in Figure 3 the outputs relative to all the WS sensors are shown for three subjects taken, just as example, from each class. Visual analysis of these radar-plot profiles showed

TABLE I

MEAN VALUES OF TGS2444 MAX VALUE, RISING TIME AND MAXIMUM SLOPE FOR EACH CLASS: HC, NC-CLD, CIRRH AND CHE. A VALUE OF P<0.05 WAS CONSIDERED TO BE STATISTICALLY SIGNIFICANT.

	TGS2444 *max value (V)* ±C.I.95%	**TGS2444** T_r *(msec)* ±C.I.95%	**TGS2444** *max dV/dt* ±C.I.95%
HC	0.39±0.06 *p-val.:* 1.61e-09	843.75±82.47 *p-val.:* 8.99e-13	0.06±0.01 *p-val.:* 6.91e-10
NC-CLD	0.69±0.13 *p-val.:* 9.05e-10	1476.19±502.39 *p-val.:* 5.46e-06	0.08±0.02 *p-val.:* 1.45e-06
CIRRH	0.87±0.16 *p-val.:* 1.45e-11	973.21±189.04 *p-val.:* 4.21e-11	0.14±0.05 *p-val.:* 3.69e-06
CHE	1.12±0.47 *p-val.:* 1.09e-3	714.28±280-91 *p-val.:* 7.95e-4	0.20±0.16 *p-val.:* 2.02e-2

TABLE II

MEAN VALUES OF TGS2602 MAX VALUE, RISING TIME AND MAXIMUM SLOPE FOR EACH CLASS: HC, NC-CLD, CIRRH AND CHE. A VALUE OF P<0.05 WAS CONSIDERED TO BE STATISTICALLY SIGNIFICANT.

	TGS2602 *max value (V)* ±C.I.95%	**TGS2602** T_r *(msec)* ±C.I.95%	**TGS2602** *max dV/dt* ±C.I.95%
HC	0.33±0.08 *p-val.:* 6.21e-07	1718.75±569.09 *p-val.:* 1.12e-5	0.03±0.01 *p-val.:* 7.36e-4
NC-CLD	0.57±0.11 *p-val.:* 2.96e-09	3511.90±460.11 *p-val.:* 7.97e-13	0.02±0.007 *p-val.:* 1.16e-06
CIRRH	0.74±0.16 *p-val.:* 7.23e-10	2794.64±432.76 *p-val.:* 2.48e-13	0.05±0.03 *p-val.:* 1.4e-3
CHE	1.02±0.52 *p-val.:* 3.1e-3	2214.28±909.58 *p-val.:* 1.00e-3	0.10±0.13 *p-val.:* 1.06e-1

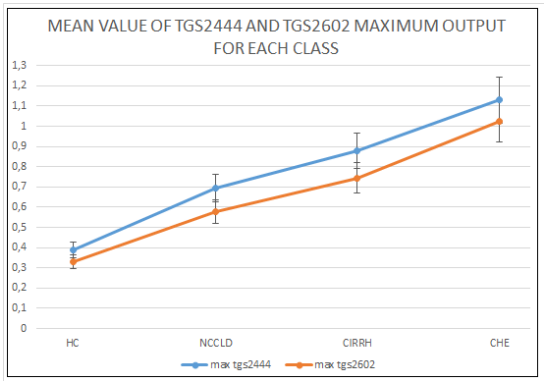

Fig. 2. Mean values of TGS2444 and TGS2602 maximum outputs relative to each class of patients: HC, NC-CLD, CIRRH and CHE. Standard deviation (of about 10%) is also shown.

a progressive concordant rise in value for TGS2444 and TGS2602 maximum output, from healthy to cirrhotics with HE subjects. However, except for TGS4161 (carbon dioxide sensor) and O_2 sensors which showed a similar profile for all subjects, a change in the whole sensors output pattern was observed. Indeed, cirrhotics with HE showed, in general, a wider radar plot profile.

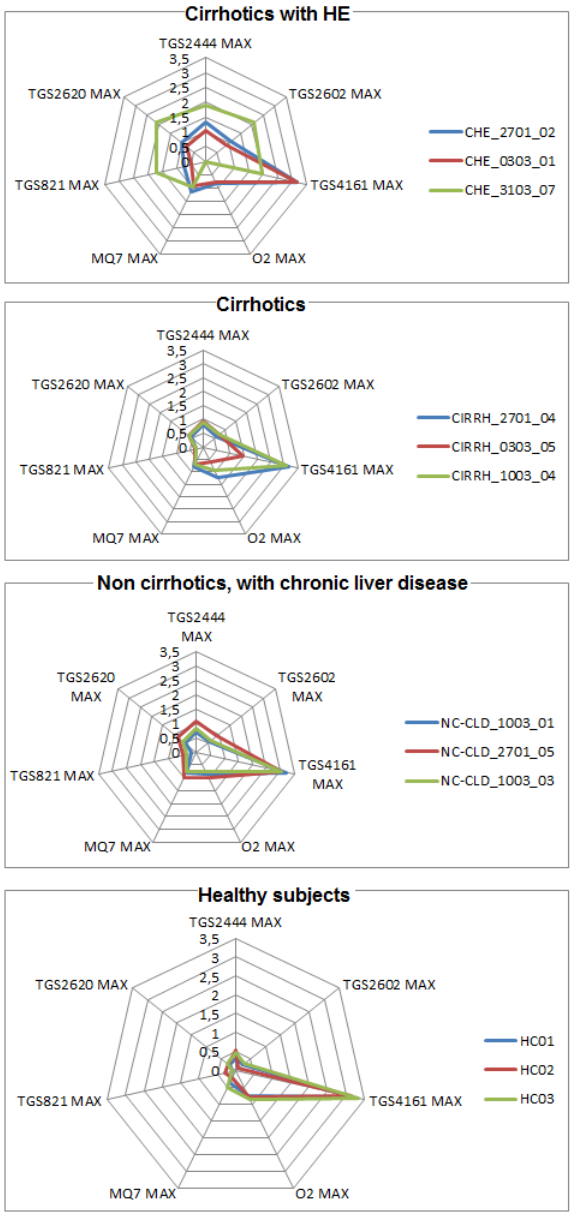

Fig. 3. Comparison of radar plot profiles relative to HC, NC-CLD, CIRRH and CHE. The radar plots showed a concordant rise in value for TGS2444 and TGS2602 (sensitive to ammonia) max. output, from HC to CHE subjects. Nevertheless, a change in the whole sensors' outputs pattern was observed.

Then, a bivariate analysis allowed for quantitatively describing the relationship between breath data and liver function tests. In particular, Pearson's correlation coefficient ρ was calculated between the variables. Spleen dimensions showed significant positive correlation with both TGS2444

and TGS2602 max. output (Pearson's correlation coefficient ρ= 0.53 *p-value*[1]= 0.0001939 and ρ= 0.42 *p-value*= 0.001814, respectively), as shown in Figure 4. Negative correlations were found between PT and TGS2444 max. output (ρ= -0.29 *p-value*= 0.02785), TGS2444 max. slope (ρ= -0.27 *p-value*= 0.0336), TGS2602 max. output (ρ= -0.30 *p-value*= 0.01767), TGS2602 max. slope (ρ= -0.29 *p-value*= 0.02057). Also, TGS2444 max. output (ρ= 0.40 *p-value*= 0.01294) and TGS2602 max. output (ρ= 0.36 *p-value*= 0,02569) showed positive correlation with serum bilirubin.

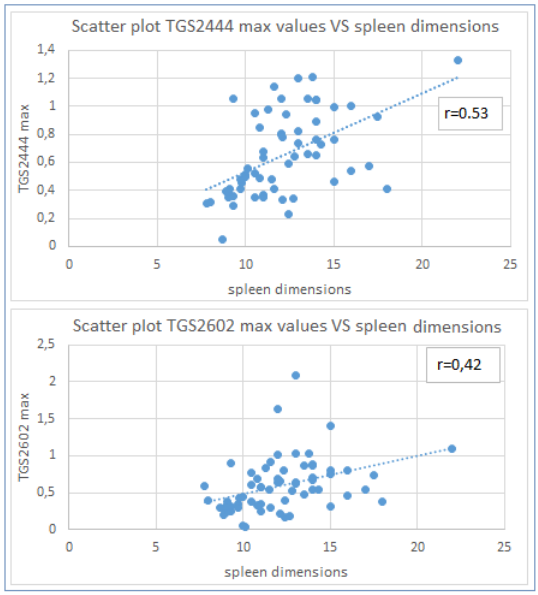

Fig. 4. The two scatter plots visually show the relationship between subjects spleen dimensions and ammonia sensors maximum values.

Given firstly sensor outputs coherently increasing with the severity of liver function impairment (Tables I and II) and, secondly, significant correlations between sensor outputs and a set of liver function-related parameters, a further step consisted of evaluating WS diagnostic capability by means of receiver operating characteristic (ROC) curves analysis. We looked for cut-off values in sensor output features that allowed to differentiate healthy subjects from patients with liver disease, and, among the latter, those with and without cirrhosis. In addition, among the cirrhotics, sensors cut-off values were looked for to differentiate those with and without recent episode of HE.

As shown in Figure 5 a TGS2444 maximum value of 0.572V permitted to differentiate healthy subjects from patients with liver impairment in general (HC versus LD, AUC= 0.867, 95%CI:0.783-0.952, *p-value*: <0.0001, Sensitivity: 77%, Specificity: 94%).

Among the patients with liver impairment, the boundary between CIRHH and NC-CLD was more difficult to establish. The wider AUC was observed for TGS2444 maximum slope (AUC= 0.642, 95%CI:0.486-0.798, *p-value*: <0.037, Sensitivity: 60.1%, Specificity: 65%): a value of 0.093 discriminated between CIRHH and NC-CLD.

In cirrhotic patients, the boundary between CIRRH and CHE was more clear. Indeed, as widely reported before, the hyperammonemia in patients with HE is more pronounced. A value of 0.065 for TGS2602 maximum slope (AUC= 0.864, 95%CI:0.662-1, *p-value*= 0), as well as a value of 0.8 for TGS2602 maximum output (AUC= 0.848, 95%CI:0.649-1, *p-value*= 0, Sensitivity: 88%, Specificity: 73%) permitted to differentiate between cirrhotics with and without HE.

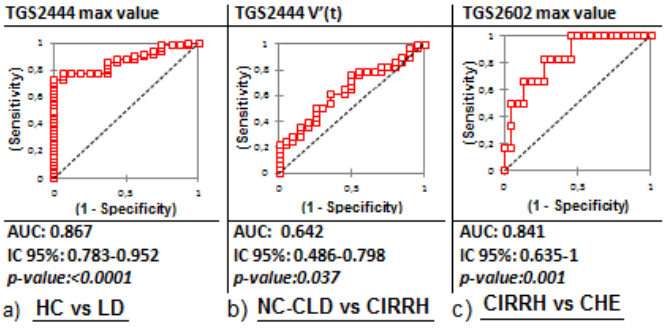

Fig. 5. Most meaningful ROC curves for TGS2444 and TGS2602 output features (maximum output and first derivative) a) in the total evaluated population: healthy subjects versus subjects with liver disease (HC vs LD), b) in the population of patients with liver impairment: NC-CLD vs CIRRH, c) in the population of cirrhotic patients: CIRRH vs CHE.

V. Conclusion

In this paper, we presented a *proof-of-concept* study which aimed at investigating the possibility to discriminate the severity degree of liver impairment based on breath ammonia levels.

Although this study did not involve measurements of blood-ammonia levels, or even the exact assessment of breath ammonia concentration levels, by means of the Wize Sniffer we were able to discriminate well not only between healthy subjects and patients with liver impairment, but also between cirrhotics with and without HE. By using both the dynamic and the steady-state features of a subgroup of sensors selective to ammonia, we obtained a clear picture about systemic ammonia levels.

A larger series of patients may permit to confirm such results. Not only, a larger number of recruited patients, including patients with suspected liver impairment, may allow for implementing a learning algorithm able to identify the patients, recognize the severity of liver impairment and eventually detect hepatic encephalopathy at its early stage (minimal hepatic encephalopathy, MHE).

Breath analysis is still in its infancy, and many challenge remain. However, it is attracting increasing interest as a non-invasive means of diagnosis that has the potential to give a clear picture about individuals' state of health. In addition, the application of suitable, low-cost, easy-to-use technologies, such as e-noses, may have the potential to lead a broad range of screening, monitoring and diagnostic solutions.

[1]A value of p<0.05 was considered to be statistically significant.

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