Breath ammonia concentrations were higher in CRDS-HD (914.5 ± 301.4 versus 280 ± 120 parts per billion, P < 0.0001) than in HS. We assessed real-time variations in the levels of NH₃ and showed a continuous decrease in the levels of NH₃. Expired NH₃ correlated directly with BUN levels, both in the IMS-HD (P = 0.002; r = 0.84; P = 0.009; r = 0.76) and in the CRDS-HD group (P = 0.005; r = 0.80; P = 0.008; r = 0.77), respectively, both before and at the end of dialysis. A direct correlation with Kt/V was found in both groups studied (IMS-HD: P = 0.003; r = 0.82; CRDS-HD: P = 0.006; r = 0.79).

Conclusions. Breath monitoring of NH₃ with IMS and CRDS techniques could be useful to assess the real-time clinical status of patients during HD. By using pre-dialysis ammonia values, an approximate calculation of the Kt/V urea ratio can be established.

Keywords: breath analysis; cavity ring-down spectroscopy (CRDS); haemodialysis real-time monitoring; ion mobility spectrometry (IMS)

Introduction

In recent decades, the widespread use of high-performance analytical technology and the development of miniaturized control and processing systems have opened enormous prospects for breath diagnosis as a non-invasive diagnostic tool for a variety of diseases [1–3]. It is estimated that exhaled breath contains more than a thousand compounds including nitrogen, oxygen, carbon dioxide, water, inert gases and trace components, occurring in concentrations ranging from nanomole per litre to picomole per litre [4], one relevant example being ammonia in the breath.

What is measured in the breath is the total ammonia (NH₃) plus ammonium ions (NH₄⁺). In a healthy individual, NH₃/NH₄⁺ are converted to urea in the liver through urea and citric acid cycles.

Urea is then transported through the bloodstream to be excreted into urine by the kidneys.

In fact, in concentrations exceeding physiological values, breath ammonia is reliable evidence of renal impairment [5, 6].

Haemodialytic treatment is administered at least three times a week for 4–5 h in order to ensure optimum toxin removal [7, 8].

An adequate dialysis dose has been shown to be associated with better clinical outcomes [9].

Real-time delivered dialysis dose monitoring may therefore be used to ensure adequacy of treatment.

Analysers that can detect breath ammonia in the parts per billion (ppb) range with a sufficiently fast response time would be useful not only as screening and diagnostic tools for renal diseases but also as a non-invasive instrument to monitor haemodialysis (HD) efficacy by real-time continuous monitoring of breath ammonia in the exhaled breath of patients during treatment. To the best of our knowledge, only a few studies have so far dealt with real-time monitoring of breath ammonia in patients undergoing dialysis treatment [5, 10–12].
A number of different approaches have been proposed for the development of analysers suitable for breath ammonia analysis. Breath ammonia levels in healthy people using Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) were reported by Smith [13]. Patel et al. introduced a non-invasive CO$_2$ laser-based approach to measure breath ammonia by photoacoustic spectrometry, which is a very sensitive technique [5, 6].

Our interest was aimed at the identification of promising analytical techniques, which enable accurate real-time quantitative analyses of ammonia in the exhaled breath from patients undergoing HD treatment.

This study reports an investigation into ion mobility spectrometry (IMS) and cavity ring-down spectroscopy (CRDS) techniques to analyse ammonia in breath in real time. IMS technology is quite mature and commercial instruments are available in a range of sizes and can be miniaturized for integration into hand-held units for many applications.

A review paper by Stach and Baumbach [14] summarized the detection limits for various compounds detected by means of IMS.

Cavity-enhanced direct optical frequency comb spectroscopy by using a laser source has been also reported for simultaneous detection of molecules that may be markers for a number of diseases [15].

Nishimura et al. [16] have developed a transportable, highly accurate, highly specific real-time gas monitor that detects ammonia, using CRDS.

In addition, the high-spectral resolution of CRDS makes the analyser less susceptible to interference from other gases when compared to other detection methods.

Pre- and post-dialysis breath ammonia concentrations were correlated with blood urea nitrogen (BUN) and $K_t/V$ values.

Materials and methods

Patients’ selection procedure

The study series consisted of 20 patients with end-stage renal disease on intermittent HD treatment [10 women (50%) and 10 men (50%), mean age 57.6 ± 14 years]. The criteria for inclusion in the study were age >18 years and dialysis vintage of at least 6 months. All had been dry-weight stable for at least 6 months before the study started and had achieved a normotensive oedema-free state. Exclusion criteria were history of bleeding, malignancy, liver, thyroid or infectious diseases, alterations in the leukocyte count or formula and/or recent treatment with steroids or immunosuppressors. The HD sessions, of 3–4 h duration, took place three times a week; the blood flow was >250 mL/min, bicarbonate infusion 2000 mL/h. The acetate-free biofiltration technique being employed, with the Integra® (Hospal, Bologna, Italy) monitor. Twenty healthy volunteers (10 women and 10 men, mean age 55.2 ± 10 years) were also recruited as controls (healthy subjects (HS)) with a clinical history negative for arterial hypertension, diabetes mellitus, cancer, cardiovascular, pulmonary, inflammatory, liver, renal and endocrine diseases.

The study was approved by the local ethics committee, and written informed consent was obtained from all participants.

Management of blood sampling

In HD patients, blood samples were taken from the arterial circuit immediately before, every hour during and immediately after the end of dialysis. In healthy controls, a venous blood sample was collected at 8.00 h following an overnight fast.

Blood samples were collected into chilled vacutainer tubes containing ethylenediaminetetraacetic acid. Tubes were instantly cooled on ice and centrifuged at 3000 r.p.m (70.56 g) for 10 min at 4°C within 30 min. Plasma was stored at –80°C until analysed.

Common biochemical parameters, including urea, creatinine, uric acid, calcium-phosphate product, serum iron, serum transferrin, serum ferritin albumin, haemoglobin, haematocrit, erythrocytes, white cells, albumin (β2-microglobulin, parathyroid hormone (PTH) and high-sensitivity C-reactive protein (hsCRP), were measured at baseline in all patients and controls, according to standard methods in the routine clinical laboratory. Intact PTH was measured by immunoradiometric assay using Elecsys 2010 autoanalyser system (Roche Diagnostics, Basel, Switzerland).

Breath ammonia tests

Randomly, the expired air of 10 patients was analysed using CRDS (CRDS-HD) and 10 using IMS (IMS-HD).

Breath samples were taken from patients undergoing HD treatment. The first breath samples were taken before the start of dialysis and further breath samples were taken every hour during the treatment and at the end of the session. Data on BUN and other parameters were collected at the same time as breath ammonia measurements were carried out. Unlike breath ammonia data that were available instantaneously, BUN data were received 4 h after the blood samples were sent for analysis.

IMS breath test measurements were performed with a continuous StackMaster-IMS emission analyser (Molecular Analytics LLC). The breath sample from the sampling system is forced over a semipermeable membrane on the outside of the cell where it is ionized by a weak plasma formed by a small 63Ni radioactive source. The ionized sample molecules drift through the cell under the influence of an electric field. An electronic shutter grid allows periodic introduction of the ions into a drift tube where they separate based on charge, mass and shape. The current created at the detector is amplified, measured as a function of time and a spectrum is generated. A microprocessor evaluates the spectrum for the target compound and determines the concentration based on the peak height.

CRDS measurements were performed with an ESP-1000 NH3 Trace Gas Analyser (Picarro Inc., CA). This employs a high-resolution laser absorption technique that uses a unique measurement cell to achieve an optical path length thousands of times longer than the physical length of the cell. This path length multiplication effect is the key to achieving ppb sensitivity levels. In addition to the NH$_3$ concentration, the analyser provides simultaneous measurements of CO$_2$ and H$_2$O concentrations, all using the same compact optical cell.

Breath sampling

Before each breath assay, the measuring cell of the analyser was flushed with ambient air. To collect breath samples, patients were asked to wear a face mask and requested to inhale deeply and then blow the entire lung volume into the sampling system maintained at 40°C. Finally, at the end of expiration, the sampling system was sealed off with a manual valve, and the alveolar sample was delivered in the detector cell of IMS or CRDS apparatus. Carbon dioxide concentration was used as a marker of alveolar sample. Samples were accepted if the exhaled CO$_2$ was >2.5 vol %.

To ensure reliability and reproducibility, all the tubes and collections were maintained at 40°C. This is because the volatile compounds such as ammonia could be lost by partitioning into the aqueous phase when collected breath is saturated with water vapour at body temperature.

A standardized and reproducible breath sample is also important for the quantitative breath analysis of metabolites at very low concentration. Since CO$_2$ is primarily of alveolar origin, it was chosen as an internal standard to probing the efficacy of the breath sampling. Carbon dioxide concentration in alveolar air is stable and more or less constant in resting healthy subjects [17].

In our case, a minimum CO$_2$ concentration of 2.5 vol% was required in order to ensure the collection of an appropriate alveolar breath sample. Figure 1 shows an overview of the procedures employed.

Assessment of dialysis efficacy

The dialysis dose for each patient was assessed by calculating the weekly $K_t/V_{\text{urea}}$ [18], which was calculated using a second-generation single-pool Daugirdas formula [19].
BUN was measured in the plasma by Biostat 1000® (Baxter Health-care Corporation, McGaw Park, IL) and its levels were expressed as micrograms per decilitre.

Statistical analysis
Statistical analyses were performed with NCSS for Windows (version 4.0), the MedCalc (version 8.0) software and the GraphPad Prism (version 5.0) package. Data were presented as mean ± SD for normally distributed values (at Kolmogorov–Smirnov test) and median [interquartile range] for non-normally distributed values. Differences between groups were established by unpaired *t*-test for normally distributed values and by Kruskal–Wallis analysis followed by Dunn’s test for non-parametric values. Pearson’s correlation coefficient was searched to examine the relation between variables. Before testing correlations, all non-normally distributed values were log-transformed to better approximate normal distributions. All results were considered statistically significant if *P* was <0.05.

Results

Study subject characteristics
The main characteristics of the study cohort are summarized in Table 1.

There were no statistical differences in gender and age between the populations studied. The mean age of healthy subjects was 55.2 ± 10 years; CRDS-HD group 57.6 ± 14 years, NS IMS-HD group 54 ± 7 years. Inflammatory markers, such as β₂-microglobulin, hsCRP and serum ferritin, were higher than values in healthy controls, whereas the main haematological (erythrocytes, haemoglobin and haematocrit) and iron (serum iron and transferrin) parameters were significantly lower (*P* < 0.001). The two groups of HD patients did not show any statistically significant difference with regard to the laboratory parameters listed above.

Breath ammonia

At baseline, before the start of the dialysis session, breath ammonia concentrations were significantly higher in CRDS-HD (914.5 ± 301.4 versus 280 ± 120 p.p.b., *P* < 0.0001) and IMS-HD patients (964.4 ± 402.4 versus 280 ± 120 p.p.b., *P* < 0.0001) than in HS.

There were no statistically significant differences between the concentrations of ammonia expired and analysed by the two different techniques in either group of dialysed patients at any of the four sampling times.

Figure 2 shows typical examples of ammonia breath measurements carried out with the CRDS (A) and IMS (B) techniques during dialysis treatment time. CRDS apparatus also measures real-time breath CO₂. Both apparatus appear to be suitable for the monitoring of breath ammonia concentration at p.p.b. levels. The NH₃ baseline level, which is a measure of the background ambient level of ammonia in the hospital during the experiment, was found to be in the range 25–50 p.p.b.

The real-time hour-to-hour breath ammonia decreased significantly as HD progressed.
Table 2 summarizes all values of NH₃, Kt/V and BUN in our cohort.

In particular, Figure 3 shows measured breath ammonia levels that occur during HD of patients monitored by CRDS. This observation has already been highlighted by several authors by using different analytical techniques. For example, Smith [11] reported a mean of the absolute value of ammonium expired that is analogous with the levels reported by Narasimhan [5].

Univariate correlations for IMS and CRDS breath tests

We have shown that exhaled ammonia levels correlate directly and statistically significantly with BUN levels, both in the IMS-HD (P = 0.002; r = 0.84; P = 0.009; r = 0.76) and in the CRDS-HD group (P = 0.005; r = 0.80; P = 0.008; r = 0.77), both before and at the end of the dialytic treatment, respectively, as shown in Figure 5.

To assess a possible correlation between levels of expired ammonia and Kt/V, we first calculated the NH₃/PCT ratio: NH₃ Pre-HD/NH₃Post-HD.

Then, we demonstrated that there is, in both groups studied (IMS-HD: P = 0.003; r = 0.82; CRDS-HD: P = 0.006; r = 0.79), a statistically significant direct correlation Figure 6.

Discussion

Breath ammonia is well established as a marker for medical diagnosis of renal pathologies [12]. Such interest has led many groups to investigate various aspects of breath ammonia monitoring in kidney disease.

This is the first study in literature that uses IMS and CRDS techniques for expired NH₃ assessment. Our data show that breath ammonia levels correlated with decreasing BUN as dialysis progressed. This observation has already been highlighted by several authors by using different analytical techniques.

SIFT-MS has been applied in a clinical setting to differentiate between patient breath samples before and after dialysis treatment [10].

By using this technique, Smith [11] found 5400 p.p.b. of breath NH₃ in a patient suffering from end-stage renal failure prior to HD treatment and a dramatic reduction of the ammonia concentration, down to 420 p.p.b., for the same patient following dialysis treatment of ~4 h.

Using photoacoustic spectrometry, Narasimhan [5] has clearly established that breath NH₃ levels decrease during HD and can be used as surrogate measures for BUN and creatinine, thus providing a non-invasive indicator of dialysis progress and a decision-making tool as to when a patient can be taken off dialysis.

We found a mean of the absolute value of ammonium expired that is analogous with the levels reported by Narasimhan, while Smith and Shiori have found lower levels [5, 11, 12]. These discrepancies may be due to different factors. Firstly, we have shown that levels of expired NH₃ correlate with BUN levels and Kt/V, so in these three different studies, it is not possible to standardize the study population according to these two variables, except for one common element, the reduction in BUN obtained by the dialysis session.
Fig. 2. Typical examples of ammonia breath measurements carried out with the CRDS (A) and IMS (B) techniques during dialysis treatment time.

Table 2. Kt/V, BUN and NH₃ expired values of the patients involved in the study.

<table>
<thead>
<tr>
<th></th>
<th>IMS group</th>
<th>CRDS group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kt/V</td>
<td>1.22 ± 0.22</td>
<td>1.15 ± 0.06</td>
</tr>
<tr>
<td>BUN pre</td>
<td>138.7 ± 8.6</td>
<td>140.3 ± 10.5</td>
</tr>
<tr>
<td>BUN + 1 h</td>
<td>86.5 ± 6.2</td>
<td>85.8 ± 3.9</td>
</tr>
<tr>
<td>BUN + 2 h</td>
<td>66.7 ± 6.1</td>
<td>65.7 ± 5.0</td>
</tr>
<tr>
<td>BUN + 3 h</td>
<td>56.2 ± 5.4</td>
<td>55.7 ± 3.4</td>
</tr>
<tr>
<td>BUN post</td>
<td>43.9 ± 9.2</td>
<td>44.6 ± 4.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>IMS group</th>
<th>CRDS group</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃ pre</td>
<td>964.4 ± 402.4</td>
<td>914.5 ± 301.4</td>
</tr>
<tr>
<td>NH₃ + 1 h</td>
<td>502.2 ± 267.7</td>
<td>472.5 ± 287.0</td>
</tr>
<tr>
<td>NH₃ + 2 h</td>
<td>378.9 ± 236.7</td>
<td>332.9 ± 214.2</td>
</tr>
<tr>
<td>NH₃ + 3 h</td>
<td>234.3 ± 190.3</td>
<td>261.3 ± 191.4</td>
</tr>
<tr>
<td>NH₃ post</td>
<td>168.3 ± 140.6</td>
<td>203.3 ± 170.4</td>
</tr>
</tbody>
</table>

Statistical comparisons: (A) both groups are well matched for the variables Kt/V and BUN (mg/dL), evaluated at the start of dialysis and every hour during the treatment until its end (P > 0.05). In IMS group and CRDS group, there is a reduction in the levels of BUN with statistically significant differences (P < 0.0001) in each analysis time. (B) In both groups, there is a reduction in the levels of NH₃ (p.p.b.) with statistically significant differences (P < 0.0001) in each analysis time.
Secondly, the inherent sensitivity and reliability of analytical techniques at these low ammonia levels should be taken into account. In a laboratory comparison among different analytical techniques, very large differences have been found compared to NH3 concentration values delivered [20].

Furthermore, since a volatile compound in the breath may have originated either from the body or the inspired air, the analysis method should allow the determination only of the endogenous part (the amount of ammonia originating from the body) or calculate it, taking into account the ammonia concentration in room air. From this perspective, ammonia concentration in room air is dependent on the presence of NH3-emission sources, for example of agricultural or industrial origin, and varies considerably with distance from them [21].

It is expected that the same situation can occur in a hospital environment, due to the presence of potential sources of gaseous NH3 such as many ammonia-based chemicals.

Finally, the concentration of breath ammonia is subject to diurnal and nocturnal variations and following meals, these variations are somewhat different for each individual [13]. In fact, our results for breath ammonia show very large SDs, indicating a wide variation between individual measurements.

However, despite these differences, a general agreement in the reduction of breath ammonia with dialysis time has been demonstrated.

These are limitations that must be considered. Further studies should be conducted in order to assess a standardized NH3 breath range among dialysed patients and to compare the different procedures to evaluate which of them has a better diagnostic sensitivity and specificity.

The fairly good linearity in the semi-log plot demonstrates that there is an exponential decay in breath ammonia for all patients examined. Reduction of breath ammonia can be expressed by the relation:

$$[\text{NH}_3] = [\text{NH}_3]_0 e^{-t/\tau},$$

where $t$ and $\tau$ represent dialysis time and time constant, respectively.

A plot of $\ln [\text{NH}_3]$ versus dialysis time gives a straight line, as experimentally observed.

From Figure 2, the half-life $t_{1/2}$, i.e. the time occurring to reduce ammonia breath concentration to 50% of its initial value, can be easily calculated. The half-life time is
an indicator of ammonia removal and could be useful to determine the dialysis treatment time.

To date, the main parameter used by nephrologists to evaluate the dialytic effectiveness is $K_t/V$. It is normally measured only once every 12–14 treatments (i.e. once a month); it is often averaged over several months and, therefore, it may vary considerably from treatment to treatment. Thus, with the establishment of breath ammonia as the real-time quantitative indicator of BUN, it could become a reliable real-time surrogate of the $K_t/V$ for patients requiring HD during each of their three times per week dialysis sessions. Note that $K_t/V$, by itself, measures the removal of BUN as the result of a dialysis session but makes no indication regarding the absolute level of BUN that may be acceptable for ensur-ing the long-term health of the patient.

Furthermore, the opportunity to analyse the levels of ammonium in real-time will enable the nephrologist to adjust dialysis parameters instantly. This will optimize the dialytic therapy, without using a parameter based on the monthly average of the individual sessions, which is the $K_t/V$, but by analysing a marker of efficiency, real time and non-invasive, of a single session, as breath ammonia, with obvious implications for the health of the patient.

Urea is considered to be a putative uraemic toxin. There is some evidence in the literature that would indicate a toxic effect of urea. It was shown that urea increases the synthesis of guanidine succinate, which
contributes to the pathogenesis of defects in platelet aggregation [22].

Zhang [23], in a study of cell cultures, showed that urea induces the generation of reactive oxygen species causing oxidative stress.

Recently, D’Apolito [24] reported how high concentrations of urea can promote the modification of proteins involved in insulin metabolism and play a role in the mechanisms of insulin resistance increasing the risk of developing cardiovascular disease.

However, these are only experimental observations made in vitro or in animal models.

Urea is mainly a measurable surrogate of several identified and unidentified small water-soluble uraemic toxins and its adequate removal indicates clearance of all these toxins.

Our aim is to use this marker in real-time to determine the effectiveness of the dialysis treatment. So, if decreased ammonia levels do not correspond to reduced damaged tissue/organ, this indicates an adequate dialysis session, a primary condition to improve the quality of life and the life expectancy of uraemic patients.

Breath ammonia monitoring can potentially be used to ensure that the minimum acceptable Kt/V urea is achieved each session ideally by prolonging time t (with obvious logistical implications) or optimizing K (change of dialyser, achieving optimal blood flow rates for instance). In conclusion, IMS and CRDS techniques were used to analyse ammonia in the breath of patients undergoing HD treatment in real time. Both apparatus appear to be suitable for the monitoring of breath ammonia concentration at p. p.b. levels. The results reported show that breath NH3 decreased gradually as the dialysis treatment proceeded. A direct correlation was also observed between changes in the breath NH3 and BUN and Kt/V. On the basis of data reported, it can be suggested that breath monitoring of NH3 could be useful to assess the real-time clinical status of patients during HD and it can be used to determine the exact time necessary for the desired degree of dialysis for a patient with end-stage renal disease at every session.

Further studies are needed to better define the potential of ammonia detection in breath as a biomarker of dialysis effectiveness, so that it could be applied as a non-invasive screen for kidney failure as well as a monitor of kidney function in at-risk populations.

Conflict of interest statement. None declared.

References


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