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A breath ammonia analyser for monitoring patients with end-stage renal disease on haemodialysis

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ABSTRACT

Objective: Breath ammonia measurement has attracted increasing interest for clinical diagnosis and metabolic status monitoring. This pilot study aims to evaluate a simple detection method to measure breath ammonia levels in haemodialysis patients.

Materials and methods: The study group comprised 44 adults undergoing haemodialysis and a control group of 44 age- and sex-matched individuals with a glomerular filtration rate >90 mL/min. To measure breath ammonia concentration, we designed a device based on that used to monitor atmospheric air, which uses a specific colorimetric tube. A single operator took two readings from each haemodialysis patient (one predialysis and one postdialysis) and one reading from each control. The results were compared with the urea concentrations in blood and saliva.

Results: Breath ammonia concentration correlated significantly with blood urea both predialysis ($P < 0.001$; $R^2 = 0.55$) and postdialysis ($P = 0.009$; $R^2 = 0.25$), as well as with predialysis saliva urea concentration ($P < 0.001$; $R^2 = 0.24$). Ammonia was not detectable in breath of any of the control group.

Conclusions: The collection of breath samples in polyvinyl fluoride bags and their subsequent analysis using colorimetric tubes is a simple, noninvasive method that enables variations in breath ammonia concentration to be measured rapidly in haemodialysis patients. Using this method, we found that the breath ammonia concentration correlated significantly with the blood urea concentration before and after haemodialysis.

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Introduction

In healthy individuals, ammonia is produced mainly in the intestine, from where it is absorbed and passed into the blood. On reaching the liver, it is transformed into urea and excess is finally excreted in the urine. The concentration of ammonia in the blood is usually low, between 1.2 and 6.6 ppb (parts per billion).[1] Small amounts of ammonia produced in the oesophagus, stomach and saliva (due to urea degradation by the oral bacteria), together with a minute quantity from the alveoli, can be detected in the oral cavity.[2] Ammonia can cross the alveolar–capillary membrane and appear in the exhaled breath when its concentration in the blood is higher than in the air.[3,4]

The normal physiological range for ammonia in human breath is between 50 and 2000 ppb.[5] The measurement of breath ammonia has attracted increasing interest in the field of clinical diagnosis and metabolic status monitoring; it has been used to evaluate the severity of asthma,[6] to diagnose hepatic encephalopathy,[7] to detect *Helicobacter pylori* infection,[8] to study halitosis[9] and to monitor the results of haemodialysis.[3]

The presence of ammonia in the exhaled breath is well established as a diagnostic marker of renal dysfunction.[10] Breath ammonia concentration correlates closely with blood urea and creatinine concentrations. This suggests that measurement of breath ammonia concentration should be a surrogate for urea in the monitoring of dialysis and even in decision-taking regarding treatment endpoint.[3]

Most chemical devices for human breath analysis are based on gas chromatography [11] and other sophisticated methods of detection such as high-sensitivity laser spectroscopy [6] and laser-based sensors.[12] Many of these techniques are costly and involve complex systems of sample collection and pre-concentration. As a result, they are only available at specialist centres and cannot usually satisfy the need for an affordable, real-time, point-of-care clinical device.

The objectives of the present study were to measure the ammonia concentration in the exhaled breath of patients with severe chronic renal failure (CRF) before and after a haemodialysis session, using colorimetric tubes widely employed to monitor environmental gases,

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Table 1. Demographic and clinical data of patients undergoing haemodialysis and of controls.

	Patients ($n = 44$)	Controls ($n = 44$)	*P* value
Age (years)	69.8 ± 10.0	69.3 ± 9.8	0.910
Gender: Male/Female	59%/41%	59%/41%	1
Current smoker	20.4%	9.0%	0.185
Alcohol use	34.0%	52.2%	0.085
Diabetes	20.4%	–	–
Number of prescribed drugs	8 (4–12)	2 (1–5)	0.026
Most commonly prescribed drugs:			
Erythropoietin stimulating agents	34		
Folate	26		
Vitamin B	25		
Calcium	24		
Anxiolytics	20	7	
Iron	20		
Nondiuretics antihypertensive drugs	18		
Antibiotics	15		
Diuretics	12		
Omeprazole	12	23	
Antidepressants	11	10	
Vitamin D	11		
Statins	9	18	
Insulin	8		
Nonsteroidal anti-inflammatory drugs	7	28	
Time on haemodialysis (months)	42 (29–71)	–	–
Predialysis weight (kg)	64.1 ± 12.1	75.5 ± 4.2	0.037
Predialysis BMI (kg/m^2)	23.8 ± 3.4	26.4 ± 3.5	0.022
Predialysis systolic blood pressure (mm Hg)	141 ± 22	134 ± 14	0.193
Predialysis diastolic blood pressure (mm Hg)	66.5 ± 14	88.0 ± 10	0.012

Note: Data are numbers/% [analysed by chi-squared], mean (SD) [analysed by paired *t* test] or median and (IQR) [analysed by Wilcoxon's method].

and to determine whether any significant correlation existed between the ammonia concentration in exhaled breath and the urea concentration in blood and saliva.

Patients and methods

Participants selection

The study group comprised 44 adult patients with severe CRF on treatment with haemodialysis. A control group was 44 individuals age- and sex-matched with the study group. Inclusion criteria of the latter were a glomerular filtration rate >90 mL/min, no history of kidney disease or of any systemic disease with potential repercussions on the kidneys, no anatomic or physiological alterations or abnormalities, and no systemic disease that could affect serum ammonia concentrations. As only normotensive individuals were included, the most commonly used drugs among controls were nonsteroidal anti-inflammatory drugs, omeprazole, statins and antidepressants. The demographic and clinical data of CRF patients and controls are detailed in Table 1. All participants were recruited in the Portuguese Institute of Oncology in Oporto, Portugal. Patients with end-stage CRF came regularly to the Nephrology Department and the controls were blood donors recruited in the Immunotherapy-Hemotherapy Department.

Measurement of breath ammonia

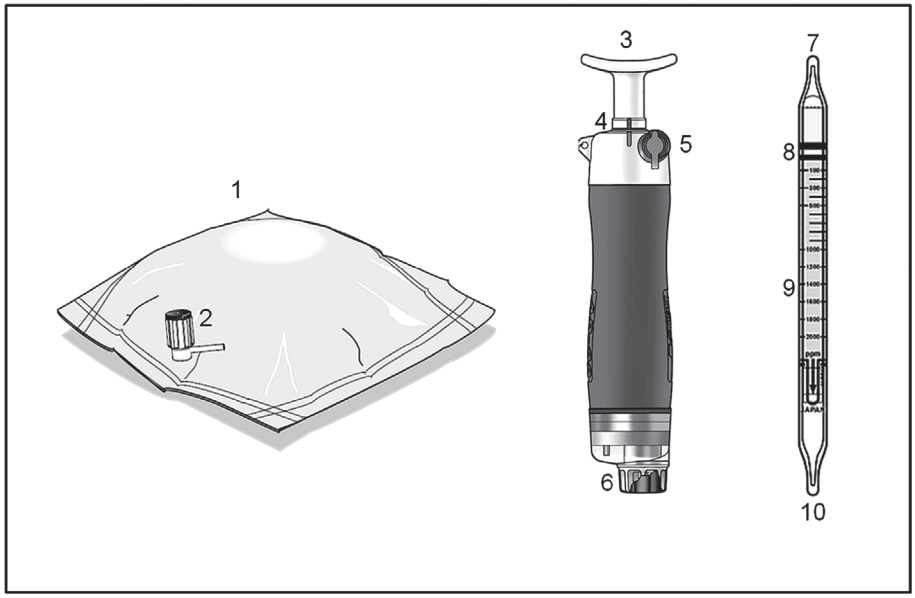
Measurement of the ammonia concentration in exhaled air was performed using a specifically designed device that was a modification of the system typically used to monitor atmospheric air. The device had the following components: a mouthpiece; an inert Tedlar® polyvinyl fluoride bag with 1 litre capacity (SKC Sample Bags, Series 232, SKC Inc., Eighty Four, PA, U.S.A), fitted with a valve to allow its filling and subsequent emptying as required for the tests; a manual vacuum pump (Kitagawa AP-20, Sensidyne, Clearwater, Florida, U.S.A); and a colorimetric tube specific for the detection of ammonia (Kitagawa Precision Gas Detector Tube No. 105DS Ammonia), with a detection range of 0.1–20 ppb (Figure 1).

Sample collection was performed by asking the patient to breathe in deeply and then make a prolonged and complete exhalation through the mouthpiece that was connected to the Tedlar® bag. One end of the colorimetric tube was then connected to the manual vacuum pump (for which it was first necessary to snap off the tip of the tube) and the other end was connected to the Tedlar® bag. The air sample was sucked from the bag through the tube (approximately 100 mL for each complete pump cycle). The pump action was repeated 5 times and time was then allowed for the reagents inside the tube to produce a colour change (from pale purple to pale yellow) as a result of the chemical reaction between ammonia and the phosphoric acid in the tube [$NH_3 + H_3PO_4 = (NH_4)_2\ PO_4$]. After a maximum of 5 min (1 min per 100 mL of sample), the ammonia concentration was read directly on the scale printed on the tube.

A single operator took two readings from each patient with CRF (one prior to the dialysis session and one immediately after completing the session) and one reading from each individual in the control group. The results were compared with the variations in the blood urea concentration (gold standard) and with the saliva urea concentration.

Blood urea measurement

The urea concentration was measured in blood (20 µl, undiluted) using the UV-kinetic method in an Olympus AU460 analyzer (Olympus Diagnostics Systems). This method employs the OSR6134 reagent (Olympus Life and Material Science Europa GmbH). In an aqueous medium, the urea is hydrolysed by the enzyme urease to produce ammonia and carbon dioxide. The ammonia combines with 2-oxoglutarate and with nicotinamide adenine dinucleotide (NADH), which, in the presence



Tedlar® bag
1. Tedlar® bag
2. Mouthpiece and valve

Manual vacuum pump
3. Pump plunger handle
4. Plunger axis
5. Gas (air) outlet
6. Connector for sample intake

Colorimetric tube
7. End that connects to the Tedlar® bag
8. Zero concentration of ammonia
9. Ammonia concentration scale
10. End that connects to the pump

Figure 1. Components of the device for the quantitative measurement of breath ammonia.

Table 2. Raw data of breath ammonia, blood urea and saliva urea concentrations of patients with end-stage renal disease before and after haemodialysis sessions ($n = 44$).

	Predialysis	Postdialysis	*P* value
Breath ammonia concentration (ppm)	1.6 (0.1–2.5)	0.0 (0.0–0.7)	<0.001
Blood urea concentration (mmol/L)	21.1 (16.8–25.9)	4.7 (3.7–6.2)	<0.001
Saliva urea concentration (mmol/L)	23.4 (17.8–28.7)	4.8 (3.7–7.2)	<0.001

Note: Data are median and (IQR) [analysed by Wilcoxon's method].

of glutamate dehydrogenase, produces glutamate and NAD^+. The fall in the level of absorbance of NADH per unit of time is proportional to the concentration of urea in the sample. Values are expressed in mmol/L.

Saliva urea measurement

The concentration of urea in saliva (20 µl, undiluted) was also determined using the UV-kinetic method in an Olympus AU460 analyzer (Olympus Diagnostics Systems), as previously described. Values are expressed in mmol/L.

Statistical analysis

The results were analysed using the SPSS statistical package version 12.0 for Windows (SPSS Inc., Chicago, U.S.A). The intra-assay variation in breath ammonia concentration was tested by repeating (in duplicate) the measurements of 40 patients (4 patients did not repeat the procedure as they had difficulty inflating the bag) and the 44 controls, analysing the results using a coefficient of variation (% CV). Correlations between quantitative variables (e.g. breath ammonia and blood urea concentration) were analysed using the Pearson coefficient of correlation. Data before and after dialysis were analysed by paired *t* test or Wilcoxon's test as distribution demanded.

Results

Table 1 shows clinical and demographic data of the patients and controls, who were age and sex matched. The ammonia concentration range in exhaled air was between 0 and 10 ppb (Table 2). The coefficient of variation was 0.94% in the study group before the haemodialysis session and 0.1% in the control group.

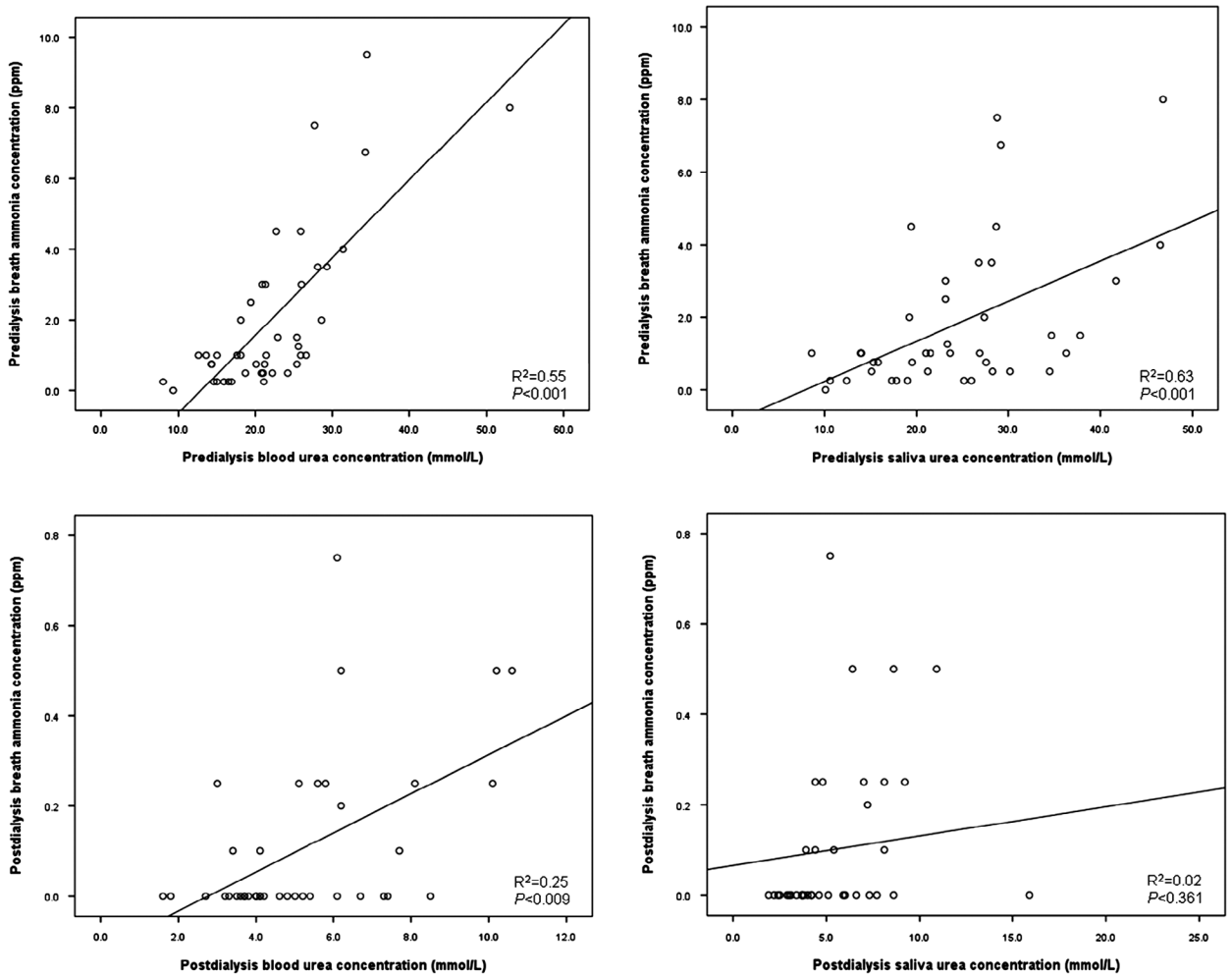


Figure 2. Simple correlation dot plots between breath ammonia concentration and the blood urea and saliva urea concentrations (the line is the 'line of best fit' from the software).

Prior to dialysis, the breath ammonia concentration showed a statistically significant relationship with the blood urea concentration ($P < 0.001$; $R^2 = 0.55$) as well as with the saliva urea concentration ($P < 0.001$; $R^2 = 0.63$) in patients with CRF (Figure 2). Breath ammonia was undetectable in the participants in the control group.

In patients with CRF, the detection of breath ammonia after the haemodialysis session showed a statistically significant relationship with the blood urea concentration ($P = 0.009$; $R^2 = 0.25$) (Figure 2). In the postdialysis period, however, no significant correlation was found between the breath ammonia concentration and the saliva urea concentration ($P = 0.36$; $R^2 = 0.02$) (Figure 2).

Discussion

We describe a method for the collection of air samples and their subsequent analysis using colorimetric tubes to measure the breath ammonia concentration. A simpler form of this method (in which the Tedlar® bag is not used) has been used, with scant results, for the noninvasive diagnosis of *H. pylori* infection (HELIC ABT®, Association of Medicine and Analytics-AMA Co. Ltd, Russia).[13] The ideal device must be sensitive to the gas to be analysed and be able to detect physiologically relevant concentrations in the ppb range accurately, not be susceptible to interference (in particular, to the effects of temperature and moisture), be portable for occasional use, easy to use, and provide measurements in real time and at low cost. This very demanding set of criteria means that there is still no ideal technique for the measurement of breath ammonia in the clinical setting. [14] The main advantages of the method we propose are the immediacy of the results, the high sensitivity and specificity, its ease of use, and the fact that it is noninvasive, providing biosafety.

In a small series of 7 patients with CRF on haemodialysis, Narasimhan et al. [3] used mass spectrometry to demonstrate that the levels of breath ammonia varied between 1500 and 2000 ppb at the start of haemodialysis and between 150 and 200 ppb at the end of the session; recently, in another small series of 6 patients with CRF, Wang et al. [15], using photoacoustic spectroscopy, reported very similar figures, in which breath ammonia fell progressively from 1600–2200 ppb to 200–600 ppb over the course of the haemodialysis session. The range of detection of ammonia with the colorimetric tubes employed (Kitagawa Precision Gas Detectors Tubes

No. 105DS Ammonia) is 100–20,000 ppb; the use of the method described in this study thus enabled healthy controls to be differentiated from patients with end-stage CRF and, in these patients, to differentiate between the pre and postdialysis periods.

In the present study, the breath ammonia concentration correlated with the blood urea concentration in the predialysis period; it explained 55% of the breath ammonia level, a correlation already reported by other authors,[3] although this percentage was noticeably lower than in previous studies using mass spectrophotometry (71%) [16] or ion mobility spectrometry (84%). [17] Those more sophisticated techniques are presumably more sensitive than the method employed in the present study for the detection of breath ammonia; furthermore, such differences require us to consider that the detection of other nitrogenated substances that participate in the halitosis may not be detected [18] and that they could even be distorting the results of some colorimetric tubes.

Davies et al. [19] used ion flow tube mass spectrometry to analyse the breath of 26 patients in an ambulatory continuous peritoneal dialysis programme. Those authors reported that over the course of a dialysis session the concentration of breath ammonia fell to levels similar to those of healthy individuals and the blood urea concentration fell in parallel.[19] Our results support those findings, and Neri et al. [17] recently provided confirmation using latest-generation spectrometry and spectroscopy, finding that the breath ammonia concentration correlated significantly with the blood urea concentration after haemodialysis, as well as with the dialysis treatment adequacy (*Kt/V*; where '*K*' is dialyser clearance of urea, '*t*' is the dialysis time, and '*V*' represents the patient's total body water).

The presence of urea in the saliva reflects the passage of this molecule by passive diffusion from the serum into the salivary glands. During haemodialysis, the saliva urea concentration falls [20] by as much as 60% compared with predialysis levels [21]; it has therefore been suggested that measurement of the saliva urea concentration could be used as an index of renal function.[22] However, the results of our study indicate that, at the end of dialysis, there is no significant correlation between the ammonia concentration in expired air and the saliva urea concentration, as has been stated previously by other authors.[18]

The present pilot study has certain limitations that must be mentioned. Experience with these devices has come under criticism at low concentrations. The use of Tedlar® bags has been discredited in relation to the accurate measurement of breath ammonia due to problems with ammonia surface interactions and their exacerbation over time. We did not investigate some specific challenges with measurement in breath, particularly given the significant levels of moisture in breath and its effect on the equilibrium of the ammonia in the sample, or the effect of interfering amines in the breath. We failed to take into account the effect of the presence of orally generated ammonia vs. systemic ammonia. In summary, to perform a more rigorous evaluation of the method described, it probably needs to be applied in an artificial breath matrix, and a standard curve has to be provided by comparing our preliminary results with an established measurement system.

We conclude that, despite the limitations associated with any preliminary study, the collection of air samples in polyvinyl fluoride bags and their subsequent analysis using colorimetric tubes is a simple, noninvasive method that enables variations in the breath ammonia concentration to be measured rapidly in the pre and postdialysis periods in patients with end-stage CRF, without the need for health care infrastructure. Using this method, we have found that breath ammonia concentration correlated significantly with blood urea concentration before and after haemodialysis, but not with postdialysis saliva urea concentration. We believe this method to be an advance in biomedical science because it could be employed to follow-up patients with end-stage CRF in the inter-dialysis period and to monitor the response to dialysis (Table 3).

Table 3. Summary

What is known about this subject

- Ammonia can cross the alveolar–capillary membrane and appear in the exhaled breath when its concentration in the blood is higher than in the air.
- The presence of ammonia in the exhaled breath is well established as a diagnostic marker of renal dysfunction.
- Most chemical techniques for human breath analysis are costly and involve complex systems of sample collection and pre-concentration.

What this communication adds

- Collection of air samples in polyvinyl fluoride bags and their analysis using colorimetric tubes enable breath ammonia measurement.
- Breath ammonia concentration correlated significantly with blood but not with saliva urea concentrations after haemodialysis.
- This method could be employed to follow-up patients undergoing haemodialysis in the inter-dialysis period and to monitor the response to dialysis.

Disclosure statement

No potential conflict of interest was reported by the authors.

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