Correlation of breath ammonia with blood urea nitrogen and creatinine during hemodialysis

L. R. Narasimhan*, William Goodman†, and C. Kumar N. Patel*‡

*Department of Physics and Astronomy, University of California, Los Angeles, CA 90095; and †Department of Nephrology, School of Medicine, University of California, Los Angeles, CA 90095

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We have spectroscopically determined breath ammonia levels in seven patients with end-stage renal disease while they were undergoing hemodialysis at the University of California, Los Angeles, dialysis center. We correlated these measurements against simultaneously taken blood samples that were analyzed for blood urea nitrogen (BUN) and creatinine, which are the accepted standards indicating the level of nitrogenous waste loading in a patient's bloodstream. Initial levels of breath ammonia, i.e., at the beginning of dialysis, are between 1,500 ppb and 2,000 ppb (parts per billion). These levels drop very sharply in the first 15–30 min as the dialysis proceeds. We found the reduction in breath ammonia concentration to be relatively slow from this point on to the end of dialysis treatment, at which point the levels tapered off at 150 to 200 ppb. For each breath ammonia measurement, taken at 15–30 min intervals during the dialysis, we also sampled the patient's blood for BUN and creatinine. The breath ammonia data were available in real time, whereas the BUN and creatinine data were available generally 24 h later from the laboratory. We found a good correlation between breath ammonia concentration and BUN and creatinine. For one of the patients, the correlation gave an R^2 of 0.95 for breath ammonia and BUN correlation and an R^2 of 0.83 for breath ammonia and creatinine correlation. These preliminary data indicate the possibility of using the real-time breath ammonia measurements for determining efficacy and endpoint of hemodialysis.

end-stage renal disease | breath analysis

Expired human breath has been analyzed extensively by mass spectrometric techniques for the existence of a variety of trace amounts of volatile organic compounds and several small inorganic molecules such as ammonia, nitric oxide, carbon disulfide, and carbon dioxide (1). Of these, several gases exhaled in human breath, e.g., ammonia, nitric oxide, aldehydes, and ketones, have been linked to kidney and liver malfunction, asthma, diabetes, cancer, and ulcers (2–4). Others, such as carbon disulfide, ethane, butane, and pentane have been linked to neurological disorders, including schizophrenia (5, 6). A few nascent technologies promise the ability to detect some of these compounds at the required parts-per-million (ppm) or parts-per-billion (ppb) concentration levels while in the presence of other interfering species. Recently, a chemiluminescence detector is being deployed for quantifying nitric oxide in human breath (7–9). Of the above afflictions, end-stage renal failure forces over 197,000 patients who require hemodialysis in the United States to undergo lengthy, three-times-per-week, often painful, in-clinic treatment (paid by Medicare) to compensate for the loss of their kidney functions. Another 28,000 patients undergo peritoneal- or hemodialysis in their homes. Improper, insufficient, and/or delayed treatment leads quickly to secondary organ failures and a rapid death.

Our study indicates that a breath ammonia measurement may be capable of providing patients requiring kidney dialysis and their physicians a fast, painless, and cost-effective *in situ* monitor that measures the progress of dialysis in real time, and which could potentially improve the quality of renal care. We have assessed the quantitative measure of ammonia exhaled in human breath as an instantaneous, noninvasive, and low-cost alternative to blood tests in evaluating the effectiveness of kidney dialysis. We report results of deploying a laser-based breath ammonia sensor (100 ppb ammonia-detection sensitivity) into the University of California, Los Angeles, kidney dialysis center to correlate the reduction in the accepted blood markers—creatinine (≈14 mg/dl to ≈5 mg/dl) and blood urea nitrogen (BUN) (≈90 mg/dl to ≈30 mg/dl)—with a reduction in the breath ammonia concentration from ≈2,000 ppb to ≈200 ppb. We have observed a monotonic reduction in breath ammonia as the dialysis proceeds and we present quantitative correlation between breath ammonia and BUN and creatinine measured in blood samples. Once refined and established, the use of the breath ammonia sensor can serve (*i*) as an endpoint detector for dialysis treatment, (*ii*) to measure painlessly the rate of buildup of waste products in blood after treatment, and (*iii*) as a means for physicians to rapidly tailor the dialysis regimen to the changing needs of their patients.

Kidney dialysis adequacy is determined presently through the use of a dimensionless parameter called the urea reduction ratio (URR) that compares the pre- and postdialysis levels of BUN as determined through laboratory analyses of blood samples taken at the beginning and at the end of dialysis treatment:

$$\mathrm{URR} \equiv \frac{[\mathrm{BUN}]_{\text{before treatment}} - [\mathrm{BUN}]_{\text{after treatment}}}{[\mathrm{BUN}]_{\text{before treatment}}} \times 100\%. \quad [1]$$

A URR of at least 65% is the current standard of care in the US. According to the National Institute of Diabetes, Digestive, and Kidney Diseases (10, 11), the ". . . URR is normally measured only once every 12 to 14 treatments (i.e., once a month). URR 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 URR for patients requiring hemodialysis during each of their three-times-per-week dialysis sessions. Note that URR, by itself, measures the removal of BUN as the result of a dialysis session but says nothing about the absolute level of BUN that may be acceptable for assuring long-term health of the patient.§

Abbreviations: ESRD, end-stage renal disease; BUN, blood urea nitrogen; URR, urea reduction ratio.

‡To whom reprint requests should be addressed: Department of Physics and Astronomy, 6-130H Knudsen Hall, University of California, Los Angeles, CA 90095-1547. E-mail: patel@physics.ucla.edu.

§Consider two scenarios for a desired URR of 70%. The first example is that of a patient whose predialysis BUN is 100 mg/dl and whose postdialysis BUN is 30 mg/dl. A second example involves a predialysis BUN of 200 mg/dl and a postdialysis BUN of 60 mg/dl. Both of these treatments will yield a URR of 70% but for the second case, the patient could be at a significant long-term risk if the BUN stays above 60 mg/dl for long periods of time.

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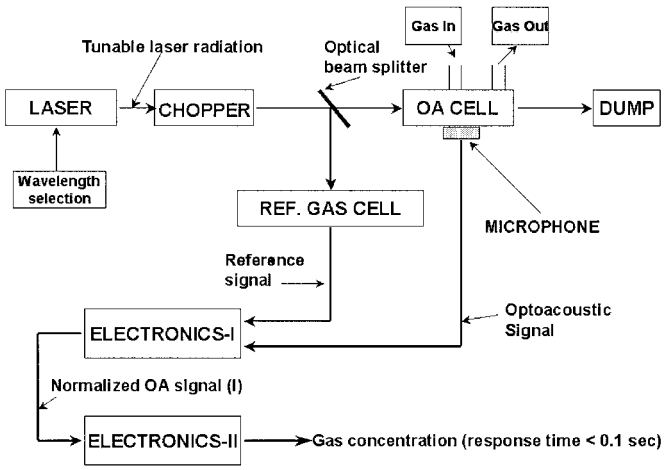

Fig. 1. Schematic of the sensitive optoacoustic (OA) measurement system.

Experimental Methods

Spectroscopic Measurements of Ammonia. Absorption and emission of light by molecules has long been used as a means of identifying which molecules are present in a mixture (qualitative analysis) and in what concentration (quantitative analysis). Most molecules with two or more atoms show distinct absorptions in the infrared region of the spectrum, generally defined as light with a wavelength between 1 μm and 15 μm (1 μm = 10^{-6} m). These features can be extremely sharp for molecules that are in the gas phase and at low pressure, enabling both the qualitative and quantitative assays with very high selectivity of species through their so-called "fingerprint" absorptions.

Laser spectroscopy has been used extensively for detection of a large number of industrially produced pollutant gases such as NO, NO_2, NH_3, SO_2, and CH_4. Many of these gases are found in large concentrations at their sources, e.g., nitric oxide at the tailpipe of an automobile (12), and at very low concentrations in ambient atmosphere and stratosphere (13). The concentration of an unknown sample is determined by characterizing the optical absorptivity of a sample of known concentration. A number of techniques have been developed to obtain the necessary spectroscopic parameters for a wide variety of molecular gases. These methods include conventional measurements of light throughput, calorimetry, cavity-ring down spectroscopy (refs. 14 and 15 and references cited in ref. 15), and thermal distortion spectroscopy (ref. 16 and references cited therein). Of these, calorimetric techniques have been shown to be widely applicable for ultra low-absorption measurements, leading to sub-ppb detection of many gaseous components (17).

There is increasing evidence that the chemical composition of expired breath can be an accurate, timely, and painless indicator of the health of an individual (1). The exhaled gases can be used as surrogates for inferring the makeup of blood and the functioning of vital organs. Here we describe our early results on the application of optoacoustic spectroscopy for the detection of minor constituents of human breath of patients undergoing hemodialysis (see ref. 18 for a preliminary report).

The experimental scheme is shown in Fig. 1. It consists of a tunable laser and an optoacoustic cell. The patient's breath is conducted to the optoacoustic cell and is illuminated by the chopped laser beam. Any light absorbed by the gases in breath is converted into heat, thereby generating an acoustic signal that is detected by sensitive microphones. The optoacoustic signal is processed to yield an absolute absorption coefficient and therefore yields the concentration of the absorbing species of molecules. This technique has been applied to the measurement of a variety of gases (19). Detection of a specific gas, even in the presence of other absorbing species, is possible because of its distinct and characteristic fingerprint absorption. Thus, tuning the laser frequency permits us to discriminate among different gases of interest that may be present simultaneously in the optoacoustic cell. Even when continuous frequency tuning of the laser is not possible, there are a number of laser systems in the infrared—CO_2, CO, HF, DF, etc.—that possess discrete laser transitions, and it is possible to tune in to any one of these lines.

Ammonia Detection. We use a sealed-off, radiofrequency excited CO_2 laser whose operating wavelength can be line switched from R40 of the 9-μm band to P50 of the 10-μm band (by using an intracavity grating), giving us laser operation on more than 120 discrete frequencies. These transitions are separated by 1–2 cm^{-1} and the laser frequency, therefore, is not continuously tunable. Nonetheless, we take advantage of pressure broadening of the absorbing species by choosing the gas pressure appropriately (see ref. 18 for additional details). In particular, we operate the optoacoustic cell at nearly atmospheric pressure. At this pressure, ammonia presents a large absorption at a particular CO_2 laser wavelength and is transparent at another nearby laser line. We divert a small portion of the laser beam into another optoacoustic cell, which contains a reference mixture of ammonia in air, and normalize the breath measurements to this calibrated mixture. At the selected wavelength of the CO_2 laser, there are two additional components in human breath that could interfere with NH_3 absorption. These are saturated water vapor at human body temperature (37°C) and CO_2 ($\approx$4% by volume). We have found that water vapor interference is negligible at the wavelength chosen for NH_3 absorption measurements (20). Independent measurements of breath on another laser line show that the CO_2 level is relatively constant. Hence, the background absorption signal caused by breath CO_2 and H_2O is assumed to be relatively constant, and this amount is subtracted as a constant offset for all measurements. Patients requiring dialysis do dehydrate during treatment. We examined the effect of moisture content through a "synthetic patient" protocol as part of the system calibration procedure (*vide infra*). These data indicate that moisture content is a second-order effect in the measured signal, and hence we believe the assumption of a constant (and small) water-vapor contribution to be valid. This scheme allowed us to measure NH_3 levels as low as 100 ppb in human breath by using a 3-second data integration time.

The breath ammonia measurements involve the patient breathing into a lightweight disposable mouthpiece (or a face mask) and hose that conveys the breath to the instrument. The instrument continuously analyzes the sample and displays an absolute measure of the ammonia concentration in ppb. An accurate measurement can be obtained in well under a minute, the time required for the breath sample to reach the measurement chamber.

Clinical Background

Kidney Failure and Malfunction. Kidney failure can be a result of diseases such as diabetes, glomerulonephritis, certain viral infections, and/or direct trauma to the organ. Nephrons, the filtering agents that remove nitrogen-bearing wastes from the blood, are damaged either partially or fully during kidney failure. Renal disease is perforce signaled by a rise in the nitrogen-bearing compounds in the patient's blood stream, with serious consequences to other organs and to the patient's lifespan. Two of the important compounds are BUN [$CO(NH_2)_2$] and creatinine (2-amino-1,5-dihydro-1-methyl-4*H*-imidazol-4-one). Patients with end-stage renal disease (ESRD) must have their blood filtered through reverse osmosis every other day for several hours. In the U.S., dialysis times range from 2 to 5 h. Standard practice during a dialysis session involves withdrawing

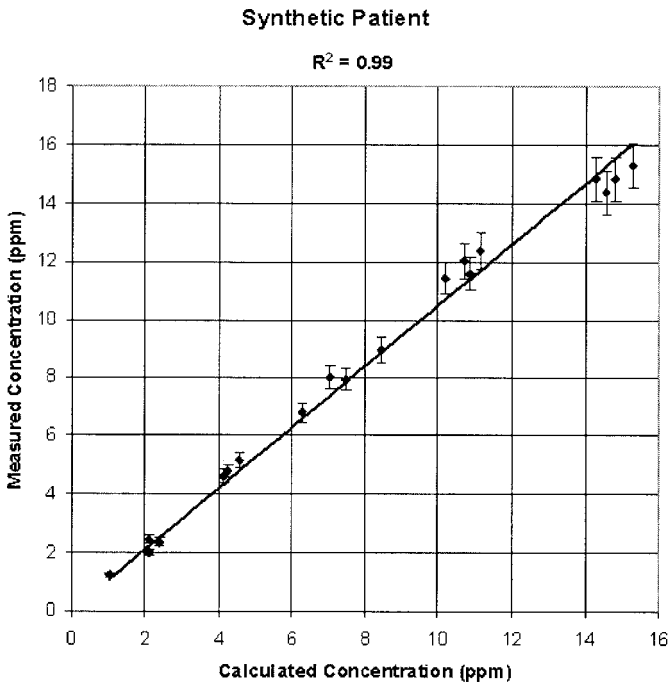

Fig. 2. Linearity of breath ammonia measurement instrument as determined by diluting calibrated ammonia with human breath (solid line shows a least-squares fit to the data; $R^2 = 0.99$).

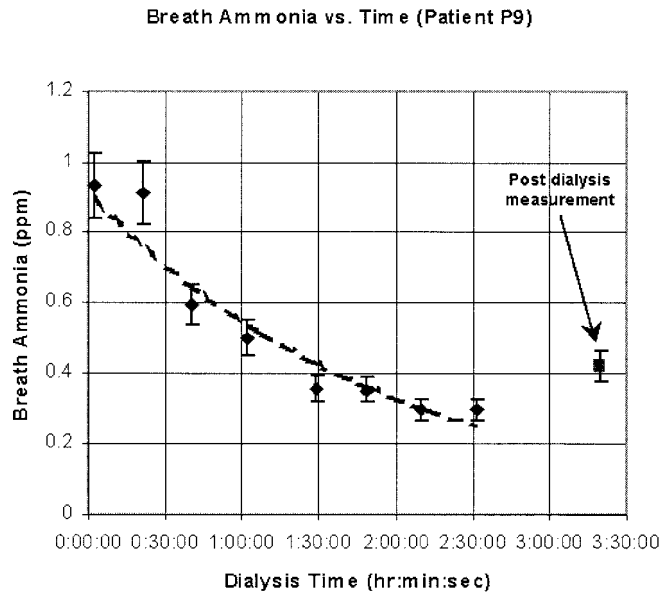

Fig. 3. Reduction in breath ammonia vs. dialysis time for patient P9. Note that the total dialysis time is 2 h and 30 min. (Dashed line through the data points is meant as a guide for the eye.)

3–5 ml of blood immediately before and immediately after treatment, and then sending the samples for analysis with typically a 1-day turnaround time. The decrease in concentration of BUN is used to compute the URR, as defined earlier.

Under normal circumstances, the predetermined period of hemodialysis functions reasonably well but it does not account for the patient's change in lifestyle or any change of diet. There is, however, substantial agreement among nephrologists that the present methods of determining dialysis times and sufficiency are too empirical. The blood workups do provide useful long-term information about anemia and other conditions but they are not a source of timely information on the progress during any particular session. Dialysis is a chemical titration that presently has no effective real-time endpoint detector.

Nitrogenous Wastes and Ammonia. In a healthy individual, ammonia and ammonium ions are converted to urea in the liver through the enzymatically moderated and energetically expensive linked urea and citric acid cycles identified by Krebs and Henseleit (21). The urea is then transported through the bloodstream to be excreted into urine by the kidneys. The reversibility of the process requires an equilibrium concentration of ammonia related to the BUN loading of the blood. As small molecules, ammonia and ammonium ions can penetrate the blood–lung barrier, mix, loft, and appear in exhaled breath. Given a reliable correlation between breath ammonia and blood markers, we can use breath ammonia concentration as an instantaneous tracer of nitrogen-bearing wastes in the human body and provide (*i*) an important real-time indicator of the efficacy of the dialysis treatment and (*ii*) a reliable and real-time endpoint detector of the level of BUN in the blood of the patient with ESRD to determine an acceptable termination of the dialysis session. The before and after measurements also provide URRs for comparison with the accepted standards.

Results and Discussion

Synthetic Patient. Fig. 2 shows an *in vitro* ammonia concentration measurement as detected by the instrument on a synthetic patient. We dilute known concentrations of ammonia in air (10 or 20 ppm, calibration gases certified by GCMS and Fourier transform infrared spectroscopy) with a healthy person's breath (containing water vapor, oxygen, and CO_2) in a gas manifold connected to the gas analyzer. Stepwise and random dilutions confirm the high linearity of the system from 15,000 ppb to 200 ppb. We have verified this linear relationship on over 100 measurements of seven healthy individuals who were part of the development team. From our data it seems, at this time, that the slope of this line is likely to be specific to each individual. The ammonia level at a given dilution is recorded for 2 min at a 1-sec sampling interval and averaged. The uncertainty (standard deviation) was $\pm$ 10% of the reading, independent of the actual reading.

Patients with ESRD. By using the instrument described above, we successfully measured the breath ammonia levels of seven patients undergoing dialysis (in the University of California, Los Angeles, dialysis center) while taking a fiduciary blood sample concomitant with each breath measurement. Fig. 3 shows the measured breath ammonia as a function of dialysis time for patient P9. As expected, and as shown by others using the selected ion flow tube (SIFT) technique (22), we see a reduction in the ammonia concentration in expired breath of patient P9 as dialysis proceeds. Our measured absolute values of ammonia levels are somewhat lower than those reported by Davies *et al.* (22), but do show a general agreement in the reduction of breath ammonia with dialysis time. Again, the error bars on the ammonia measurements are $\pm$10%, as determined by averaging the output of the detector over 2 min at a 1-sec sampling interval. We obtained similar breath ammonia reduction results in six other patients with various session times. We show data for two more patients, P3 and P8, in Figs. 4 and 5, respectively. The curves in Figs. 3–5 are one-parameter exponentials that are meant as a guide for the eye. The exponential fits assume that dialysis follows first-order kinetics. The fits in Figs. 2, 6, and 7 are from linear regression.

The most critical test is the correlation of breath ammonia with constituents of blood that are used traditionally as the measures of kidney failure in patients with ESRD. Therefore, we

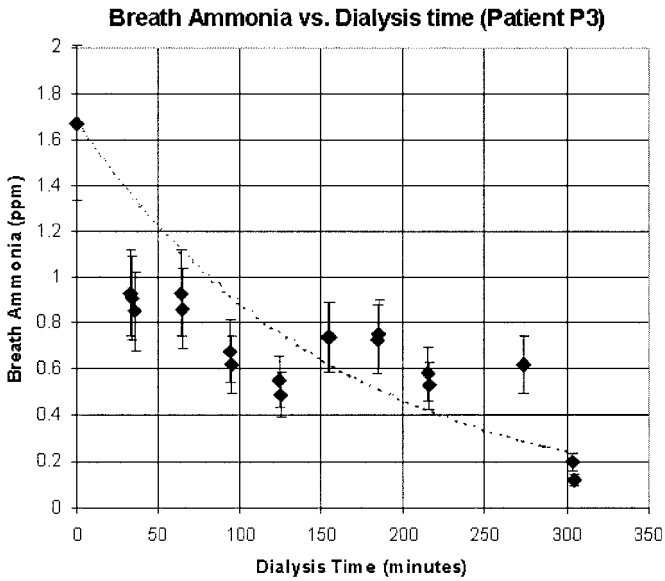

Fig. 4. Breath ammonia vs. dialysis time for patient P3. Note that the dialysis session time is 5 h. (Dashed line through the data points is meant as a guide for the eye.)

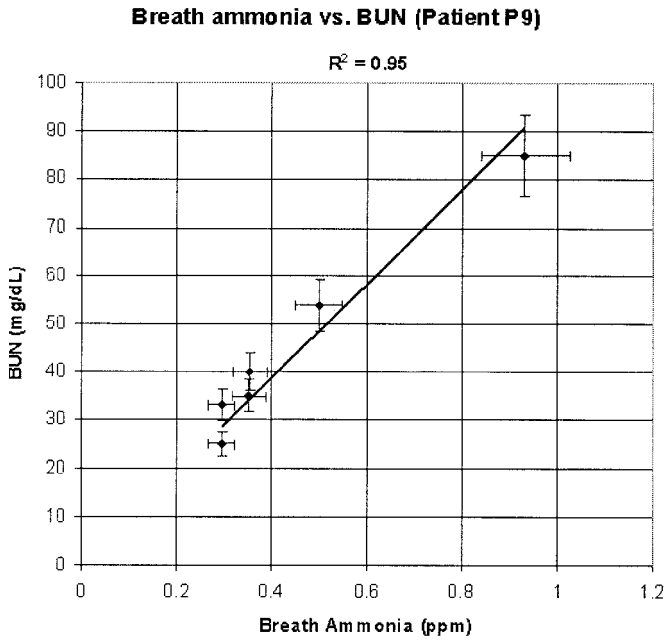

Fig. 6. Breath ammonia vs. BUN correlation for patient P9. (Solid line shows a least-squares fit to the data; $R^2 = 0.95$.)

collected data on BUN and creatinine at the same time as breath ammonia measurements were carried out. Unlike breath ammonia data that were available instantaneously, the BUN and creatinine data were received 12–24 h after the blood samples were sent for analysis. Figs. 6 and 7 show the breath ammonia and BUN and creatinine data for patient P9. We see an encouraging correlation. The uncertainty in the blood measurements (y-axis error bars) reflects the $\pm 10\%$ measurement accuracy specified by the blood-testing laboratory. The breath ammonia uncertainties (x-axis error bars) are determined as before. We independently verified the $\pm 10\%$ quoted uncertainty in blood measurements by sending samples from one patient to two different laboratories. We have confirmed both the time-dependent decrease of breath ammonia and the correlation with BUN and creatinine on six other patients. These data that show that noninvasive breath ammonia measurements can be used as a real-time surrogate measure of the status of patients with ESRD.

Returning to Fig. 3, we direct the reader to the last breath ammonia data point occurring at $t \approx 3$ h, 21 min, showing an ammonia concentration of about 420 ppb. This patient completed dialysis at 2 h, 30 min, at which point the measured ammonia concentration in the patient's breath was about 300 ppb. The bounce back in ammonia concentration from 300 to 410 ppb (and the corresponding increase in BUN from 30 to 40 mg/dl) and increase in creatinine from 5.5 to 6.5 mg/dl is one of the important observations in understanding the dynamics and partitioning of BUN and creatinine between the bloodstream and body tissue. At present, no quantitative data exist that indicate how BUN and creatinine accumulate in the blood

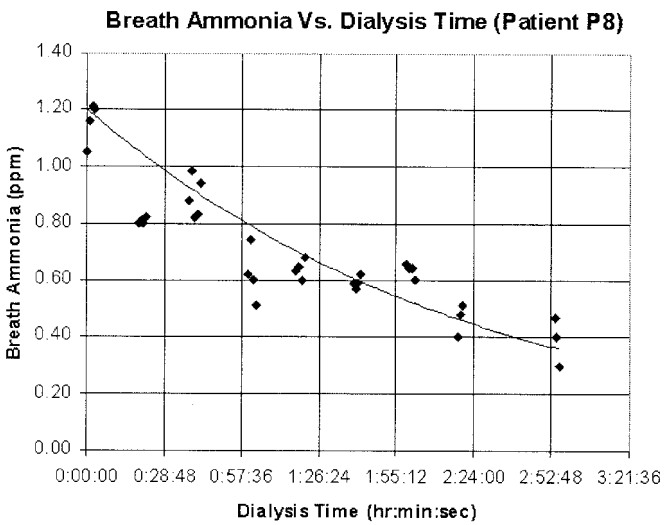

Fig. 5. Breath ammonia vs. dialysis time for patient P8. Note that the dialysis session time is 3 h. (Dashed line through the data points is meant as a guide for the eye.)

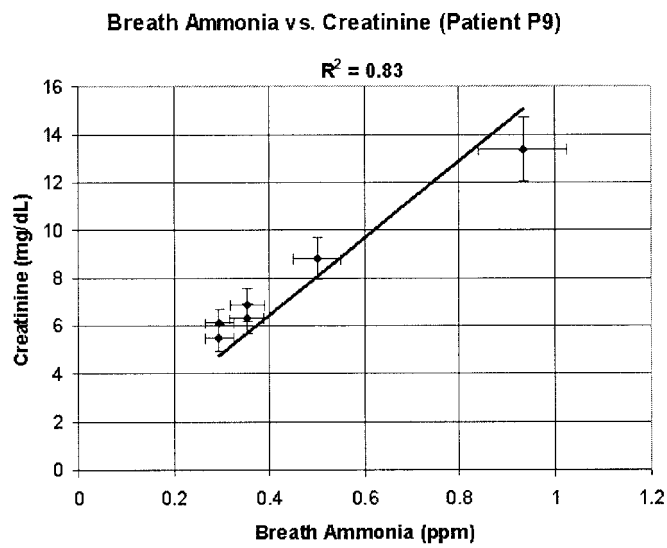

Fig. 7. Breath ammonia vs. creatinine correlation for patient P9. (Solid line shows a least-squares fit to the data; $R^2 = 0.83$.)

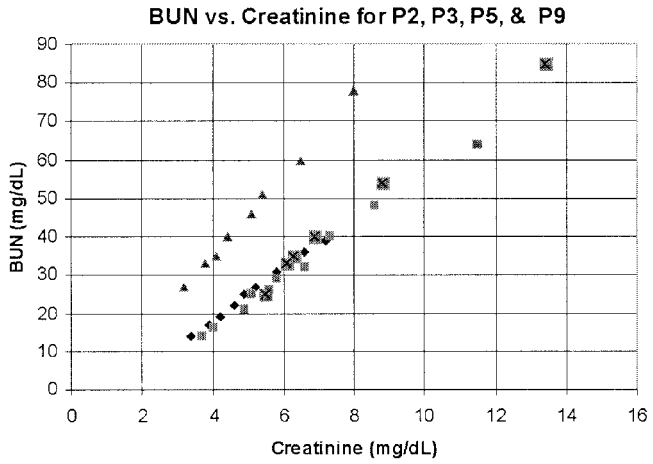

Fig. 8. BUN vs. creatinine levels as determined by periodic blood sampling of four patients undergoing dialysis, P2, P3, P5, and P9. One patient (triangles) shows a slope different from the other three.

of the patients with ESRD between dialysis treatments. This is an exceedingly important question, as dialysis patients are on restricted diets and their blood wastes can dramatically increase if they do not follow the specified regimen.

Fig. 8 dramatically illustrates the need for patient diagnostics during dialysis. We plot BUN levels against creatinine levels as determined by the blood sampling taken during breath measurements of four patients. Three patients (diamonds and large and small squares) show excellent correlation with an average URR as defined above ≈80%. The fourth patient (triangles) shows a drastically different slope than the others with a URR of only 65%. Clearly, this patient's body processes BUN and creatinine in a markedly different way than the others, and at the end of his/her treatment, s/he had substantially higher levels of BUN than the other patients. A breath measurement is of clear value here. Even assuming an inexpensive chairside real-time blood analyzer, constant blood draws to establish these slopes are not feasible. Withdrawing 5 ml every 15 min over 3 h removes 60 ml of blood from the patient. Over a three-treatments-per-week cycle, this amounts to an unacceptable 180 ml of blood lost. The questions remain, however, of why this patient is apparently under-dialyzed with respect to the others and what the consequences are to his/her long-term health.

Conclusion: Ammonia Detection in Breath. Quantifying the level of ammonia in exhaled breath serves two vital functions:

(*i*) It can be used as a surrogate for elevated BUN, which, along with the creatinine level in a patient's blood, is the accepted indicator of kidney malfunction. Our preliminary measurements are among the very few quantitative data sets correlating breath ammonia with BUN and creatinine. No correlations have been made yet with glomerular filtration rates, but these will be acquired once the breath ammonia instrument is deployed in planned clinical studies of individuals at-risk for kidney failure.

(*ii*) The breath ammonia level can be used for determining the exact time necessary for the desired degree of dialysis for a patient with ESRD at every session. The breath ammonia monitor will provide crucial information about when a dialysis treatment may be stopped, i.e., detect an endpoint. The ability of such instrumentation to detect partial kidney failure will depend on quantitative correlation between breath ammonia and BUN, creatinine, and glomerular filtration rates. In the long run, breath ammonia measurement could serve as a broad noninvasive screen for incipient kidney failure, as well as a monitor of kidney functions in at-risk populations such as diabetics and hypertensives.

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