BREATH AMMONIA REDUCTION RATIO (ARR) MEASURES DIALYSIS EFFICACY

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WHY?

Real time biomarkers which correlate with dialysis efficacy could provide clinicians with a method for individual tayloring of dialysis dose.

INTRODUCTION

Contemporary evidence supports the centuries old notion that expired breath and the headspaces above body fluids and products can serve as biomarkers of organ function. Clinical responsiveness to alterations in clinical status or therapy is dependent upon timely, accurate, relevant physiological data. Current measures of urea and creatinine to assess renal urea reduction are invasive and cannot be repeated frequently or reported quickly enough to define individual response to treatment in real time. In contrast, breath analysis is minimally invasive and can provide real time information about low molecular weight volatile organic compounds (VOCs) such as ammonia[1,2].

METHODOLOGY

We examined the relationship between Breath Ammonia (BA), Ammonia Reduction Ratio (ARR) and Urea Reduction Ration (URR), in 40 dialysis treatments in 15 patients commencing haemodialysis (HD). Blood was collected just prior to haemodialysis and 30 minutes after stopping. Thirty minutes prior to and after haemodialysis patients exhaled directly into VOICE100 (SYFT Technologies Ltd., Christchurch, New Zealand) and also inflated a Tedlar™ (polyvinyl fluoride) bag (SKC Inc. Valley View Road, Eighty Four, PA USA) then and at one hourly intervals during dialysis. Haemodialysis was performed on a Fresenius 4008B for 5 hours (Fresenius F8 polysulfone dialyser) (*Figure 1*).

VOCs were analysed by the chemical ionisation reactions of precursor ions (NO+, O2+ and H3O+) with each VOC using selected ion flow tube mass spectrometry (SIFT-MS) and a VOICE100 (Syft). The VOICE100 was operated on in both mass scan and selected ion monitoring (SIM) modes.

Mass scan analysis can be used to determine which masses change the most between two chosen time points and thus act as good biomarkers for dialysis efficacy. A classification algorithm using SIFT-MS data has been described elsewhere[3]. For each mass, a mixed distribution made up of a kernel density and a Dirac delta function is used to develop a probability density profile from each time-point group using each sample's concentration value at that mass.

BREATH ANALYSIS STUDY FLOW

Exclusion criteria
1.No consent
2.Patients too ill
3.Patients receiving dialysis anywhere other than in the acute haemodialysis unit
4.<16 years of age
5.Patients unable to answer initial assessment questions
6.Patients with severe respiratory disease

Inclusion criteria
1.Patients receiving dialysis in the acute haemodialysis unit, Christchurch Hospital
2. >16 years of age

Potential patients identified on Friday of the previous week

STAGE 1 — Consent discussion with patient at first session of the week.

STAGE 2 — Patient met at HD unit at 08:00. Complete initial screening sheet

STAGE 3 — To Syft rooms by wheelchair for 1 direct to machine and 1 bag breath sample (PreBag)

STAGE 4 — Return to HD unit by wheelchair. Blood collected for: BUN – via lampson tube
Ammonia – on wet ice and to lab within 20 minutes of collection
Head space analysis – to Syft room
Remind patient of any dietry or other conditions

STAGE 5 — Bag breath collection at 1 hourly intervals from the time of dialysis starting. Bags directly to the Syft room within 5 minutes of collection

STAGE 6 — At completion of dialysis blood collected as in stage 4

STAGE 7 — 30 minutes after completion of dialysis stage 3 repeated (PostBag)

Figure 1

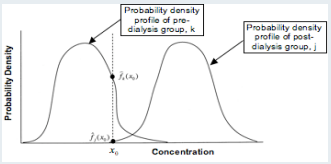


Figure 2: Probability densities for a given mass

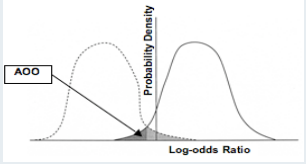


Figure 3: Biomarker determination

For each mass (figure 2), the log-odds ratio is defined as the natural log of the probability of a group j classification divided by a group k classification. Biomarkers are found by determining which masses have log-odds density profiles with minimal area-of-overlap (AOO) (figure 3).

With significant inter-patient variability, intra-patient variability and small datasets, a paired analysis approach with normalisation was used to pre-process the datasets. The mass scan analysis is interested simply in the magnitude of the relative change in VOC concentration between the pre- and post-dialysis state, as thus the concentration data is normalised to half of the average of these two concentrations. As such, the data is bounded between [0 1], with values greater than 0.5 representing a decrease in concentration over the course of the dialysis treatment, and values less than 0.5 representing an increase in concentration. Biomarkers are displayed visually on an image plot using the difference between the pre- and post-dialysis groups. Good biomarkers are recognised as those masses with a narrow distribution centered far from the increase/decrease interface. These can then be selected for Selected Ion Monitoring (SIM).

RESULTS

As seen in the image plot (*Figure 4*) generated from mass scan data obtained using the H3O+ precursor, masses 18, 36, and 54, all relating to ammonia and its water clusters, are good biomarkers of kidney function.

Changed analysis of density profiles of mass scan VOC pre and post haemodialysis showed masses from ammonia were the predominant VOC (eg with H_3O^+ the ROC area was 0.983 for masses 18, 36, 54).

SIM scan monitoring of breath analysis during haemodialysis showed 3 patterns:

- **A. Progressive decline *(Figure 5)*;**
- **B. Increase at 1 hour then a progressive decline *(Figure 6)*;**
- **C. An oscillatory pattern *(Figure 7)*.**

The patterns may reflect initial vs equilibrium breath analysis values.

The Ammonia Reduction Ratio (ARR) averaged 52.5 ± 5.5% (mean ± SEM) and Urea Reduction Ratio (URR) averaged 63.4 ± 1.2% taken over all studies (n=40). ARR scatter was reduced in patients with more efficient dialysis (URR > 65%, *Figures 8a and 8b*), when ARR was better correlated with decline in Plasma Creatinine (r= 0.578, p=0.024, n=15) than with URR (r= 0.432, p=0.095, n=16).

Modelling a prior haemodialysis allowed prediction of timing of maximal decline in Breath Analysis with pattern A. This allows a prediction of optimal dialysis time (*Figure 9*).

Figure 4

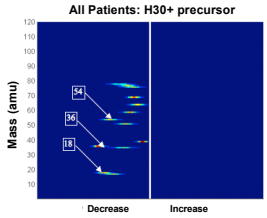


Figure 5

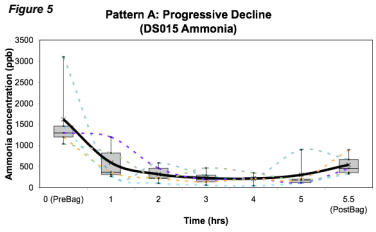


Figure 6

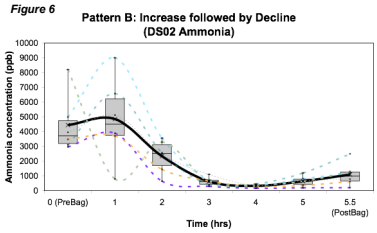


Figure 8a

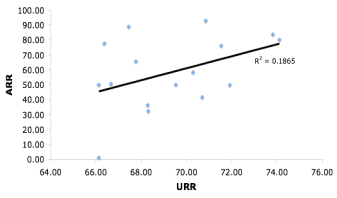


Figure 8b

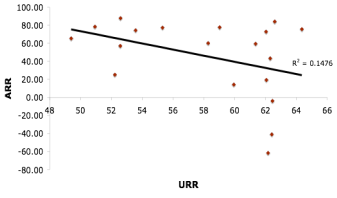
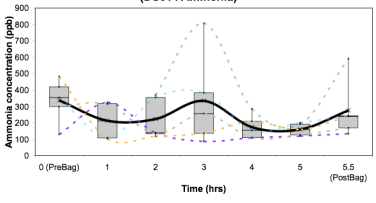


Figure 7



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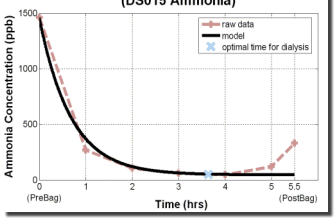
2. S. Davies, P. Spanel, and D.Smith (1997) "Quantitative analysis of ammonia on the breath of patients in end-stage renal failure." Kidney Intl. 52, pp223-228
3. K. Moorhead, D. Lee, J. G. Chase, A. Moot, K. Ledingham, J. Scotter, R. Allardyce, S. Senthilmohan and Z. Endre (2007) "Classification Algorithms for SIFT-MS Medical Diagnosis" 29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Lyon, August 23-2

Figure 9



CONCLUSION

Breath Analysis allows real-time monitoring of haemodialysis efficacy.

Thank you ….
Syft Technologies Ltd.

This may not seem very important I know.
But it is!
So, I'm bothering telling you so.
Dr Suess, The Sleep Book

HOW ARE VOCs MEASURED FROM BREATH?

A number of spectrometric techniques have been employed to measure trace gases in complex mixtures such as ambient air, breath and headspaces above liquids. Selected ion flow tube mass spectrometry (SIFT-MS) real time quantification of trace gases is based on chemical inonisation and is capable of measurements to low parts per billion (ppbv) independent of the humidity of the sample[2].