Correlation of Breath Biomarkers with Current Gold Standards for Modeling Acute Renal Failure in Rats*

Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) is an analytical technique for the real-time quantification of Volatile Organic Compounds (VOCs) in air or breath samples. By analyzing changes in VOC concentrations in breath over time, SIFT-MS can offer fast diagnosis of various conditions and diseases non-invasively and in real-time.

Current measurements of renal function rely on daily measurements of plasma creatinine. When most glomerular filtration function is lost, the diagnosis of kidney failure is delayed by up to three to five days in clinical situations. This results in unacceptable delays in instituting treatment. Consequently, there have been no improvements in mortality in the management of Acute Renal Failure (ARF) for over 50 years, despite the availability of many useful experimentally effective treatments. This study demonstrates the potential application of breath-based diagnosis using SIFT-MS.

Experimental Method

Female Sprague-Dawley rats (280-320g, n=11) were housed at 21 °C in a 12 hour light/dark cycle, and allowed free access to food and water. Under ketamine/domitor anesthesia, an in-dwelling cannula was inserted into the jugular vein for the purposes of fast serial blood sampling. Glomerular Filtration Rate (GFR) was monitored via plasma creatinine, SIFT-MS breath sampling, and bolus inulin clearance for 5 days while the animal recovered from surgery.

In a subsequent surgery, the renal arteries were exposed via a single mid-ventral incision, and clamped for 60 minutes, creating ARF via an ischemic event. GFR was monitored for 7 days using the same three techniques, as the animal recovered and renal function returned to normal levels.

Inulin Clearance

The clearance of an exogenous marker such as inulin can be used to estimate GFR. ^{14}C-labeled inulin was injected into the jugular vein cannula of a conscious animal over 2 seconds, and flushed with heparinized saline. A 150 µL blood sample was collected via the jugular vein cannula at -1, 1.5, 3, 6, 12, 20, 40, 70 minutes post-bolus, for scintillation analysis.

Plasma Creatinine

Measuring GFR by calculating the clearance of an exogenous marker is time consuming, expensive, and thus not appropriate in most clinical settings. Hence, GFR is usually estimated using an endogenous marker, such as creatinine. Steady state serum creatinine concentration is approximately inversely proportional to GFR. However, creatinine does not act as an 'ideal' marker, so the Cockcroft-Gault equation below, is used clinically to correct for its limitations.

$$GFR = \frac{(140 - age)(wt)(0.85\ if\ \ female)}{72Cr}$$

Rats in this study are all female albino rats of approximately the same age. Hence, Figure 1 shows the weight to plasma creatinine ratio plotted against GFR obtained via inulin clearance. The result is an equation for GFR from a plasma creatinine sample, specific for rats.

SIFT-MS Breath Analysis

Direct breath was collected from conscious rats by wrapping the rat in a towel and placing it into a 300 mL bottle (Figure 2). Breath analysis was carried out using a Syft Technologies Voice100^{TM} SIFT-MS instrument.

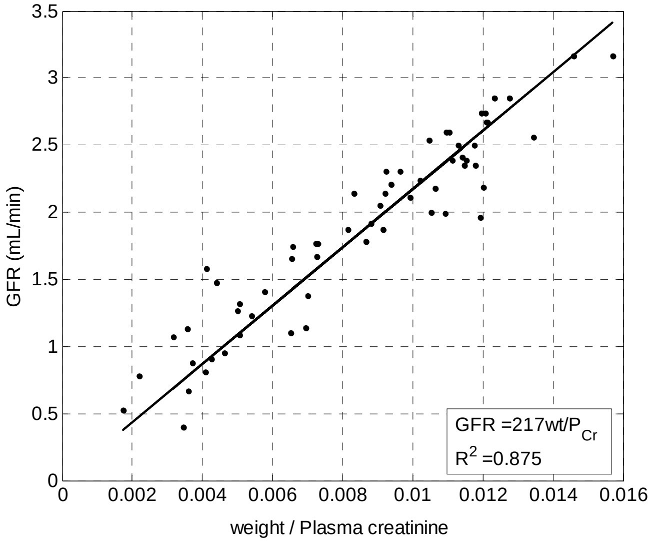

Figure 1. Relationship between GFR (derived from the inulin clearance) and the weight to plasma creatinine ratio.

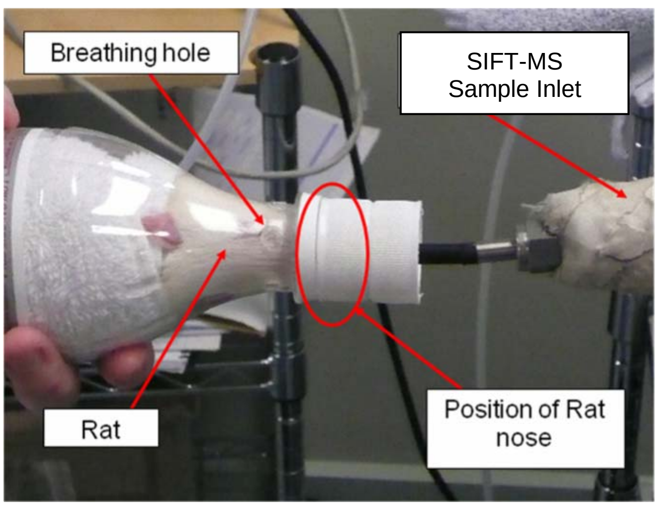

Figure 2. Sampling procedure for rat breath.

Results and Discussion

GFR results obtained via bolus inulin clearance, plasma creatinine, and breath ammonia are shown in Figure 3 for three rats over the course of both surgeries in the trial. Very good correlation was observed. In addition, it is seen that GFR decreases following cannulation surgery, although not to the extent observed in acute renal failure.

Uncertainty in the breath GFR estimate arises largely from sample dilution, because the Voice100 samples at a flow rate of approximately 150 mL min^{-1}. The newer Syft Voice200® SIFT-MS instrument is more sensitive than the Voice100 and samples at a significantly lower rate (~25 mL min^{-1}), so will reduce the measurement uncertainty.

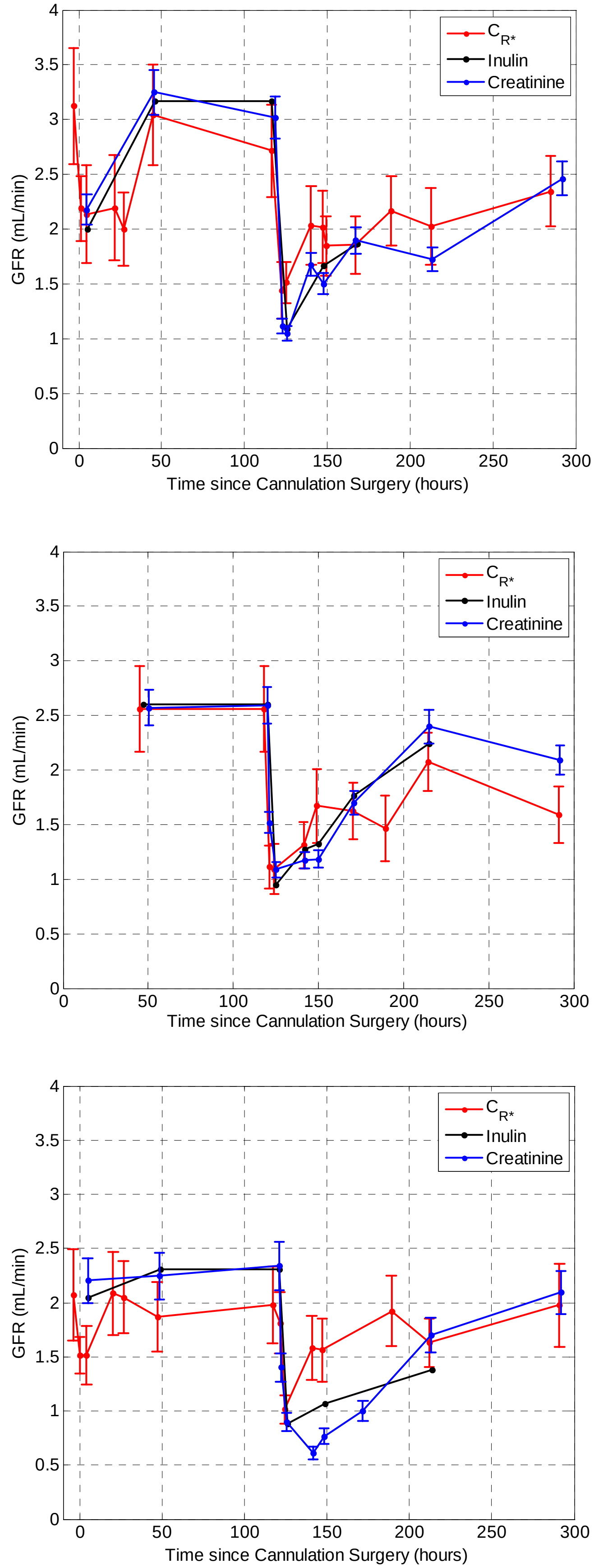

Figure 3. Comparison of the GFR results obtained using the three methods for three rats.

Conclusion

Breath ammonia, as measured by SIFT-MS, can be used to determine renal function following an acute renal injury, in rats. Relative decreases in renal function show excellent correlation with gold standard measures, and indicate promise for fast, non-invasive determination of renal function via breath testing.

For more information about the unique SIFT-MS technology or this research, please contact your nearest Syft Technologies office or visit www.syft.com.

** Adapted from a poster presented by K.T. Moorhead, J.V. Hill, J.G. Chase, C.E. Hann, J.M. Scotter, Z.H. Endre at the 6th IFAC Symposium on Modeling and Control in Biomedical Systems (MCBMS09), Aalborg, Denmark, August 12-14, 2009.*

Ethics approval for this study was granted by the New Zealand Animal Ethics Committee (C09/07601).

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