Investigating novel biomarkers associated with AKI diagnosis and risk

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Abstract – Acute kidney injury (AKI) is a common complication associated with cardiac surgery. Those patients who develop AKI have increased hospital costs, hospital LOS and odds of death. The current diagnostic criteria rely on serum creatinine measurements and urine output data. Serum creatinine is not renal specific. It is known that there are other factors that influence serum creatine concentrations other than renal injury. Serum creatinine levels are measured as concentrations which means large changes in fluid balance may alter the serum creatinine measurement. Urine output is not a reliable measurement of renal injury either. During surgery it is common to administer diuretics which influence urine output independent of renal damage. Researchers are investigating novel biomarkers that are renal specific and can monitor AKI risk in real time. Some researchers are focused on identifying chemical biomarkers to diagnose AKI more effectively. Preliminary studies have also shown that urine oxygen concentration may be a physiological biomarker that can be used to monitor AKI risk in real time. Urine oxygen concentration may reflect tissue oxygen concentration in the medullary region of the kidney. The aim of this study was to investigate the relationship between urine biomarker concentrations, urine oxygen concentration and AKI diagnosis. The results from this study suggest that is possible to monitor oxygen concentration non-invasively and it could potentially be used to monitor AKI risk in real time.

INTRODUCTION AND BACKGROUND

Acute kidney injury (AKI) occurs in approximately 30% off all patients who undergo cardiac surgery.[1] AKI is described by an acute decrease in renal function and is associated with decreased urine output and excess metabolites in the urine. One study found that mild to moderate AKI increased hospital length of stay (LOS), hospital associated costs and risk of death.[2] Severe AKI, which requires renal replacement therapy (RRT), is associated with mortality rates as high as 50-90% and an 8-fold increase in the risk of death.[3,4] Based on these data it is important to accurately diagnose AKI and further investigate therapies or treatments to protect patients from developing this syndrome.

The definition of AKI has changed over time as new standards and criteria have been developed. The Acute Dialysis Quality Initiative (ADQI) group was the first group to develop a uniform diagnostic criterion, which became known as the RIFLE criteria. This standard uses a three tiered severity system that is based on the subjects glomerular filtration rate (GFR) or urine output.[5] In 2007 the Acute Kidney Injury Network (AKIN) modified the RIFLE criteria with the hope of creating a uniform standard with higher sensitivity for detecting AKI. This definition of AKI is based on an increase in serum creatinine concentration or decreased urine output.[6] A study from Engleberger et. al found that significantly more patients were diagnosed with AKI

when using the AKIN criteria compared to the RIFLE criteria.[7] The most recent standard has been set forth by Kidney Disease Improving Global Outcomes (KDIGO) with the goal of creating a universal standard that could be uniformly applied to future research studies. This new standard is based on the previous RIFLE and AKIN criteria and uses serum creatinine concentrations or urine output data to diagnose patients with AKI.[8] While increased serum creatinine concentrations and decreased urine output have been associated with impaired renal function, these markers are not perfect indicators of renal injury because they are influenced by factors other than kidney function.

One study found that the half-life of creatine dramatically increases when there is a 5 % reduction in kidney function. This means that serum creatinine levels may not rise until several days after the initial renal injury. This does not help prevent or mitigate AKI, and shows that serum creatinine concentrations can only be used as a diagnostic tool.[9] Because serum creatinine is measured as a concentration it is affected by changes in volume. This means that patients who are given large amounts of fluid will have a lower serum creatinine concentration, independent of whether they experienced renal injury. It is also known that serum creatinine concentrations are affected by certain drugs that alter the concentration independent of renal injury or damage.[10] Another concern with using serum creatinine as a measure of renal injury is that the definitions for AKI require a baseline measurement. If there is not a baseline measurement it is often estimated through various methods which require assumptions about the patient's health and prior renal function.

The validity of urine output as a marker for renal injury has also recently been questioned. Some researchers argue that the KDIGO urine output AKI definition has not been validated, and that the definition is too liberal. One study showed that those patients who were under a threshold of 0.3 ml/kg/hr for 6 hours or longer had a higher risk of death and need for dialysis.[11] The current criteria uses 0.5 ml/kg/hr as a threshold. It is also important to consider that urine output is affected by administration of diuretics which are commonly given during surgery. For these reasons it is important to identify other physiological biomarkers that are renal specific, could be used to identify renal injury and monitor AKI risk in real time.

Researchers have studied the role of neutrophil gelatinase-associated lipocalin (NGAL) as a potential biomarker for diagnosing AKI. It has been reported that following renal injury there is an increase in uptake of NGAL, which gives researchers hope that it can be used in place of the conventional biomarkers currently used.[12] Other studies have identified kidney injury mocule-1 and interleukin-18 as biomarkers that could potentially be used to detect AKI.[13] Studies have also been done to determine if urine oxygen concentration can be used a physical biomarker to detect AKI.

Several studies have shown that tissue hypoxia is a final common pathway for multiple forms of AKI.[14] Even though the kidneys receive 20-25% of the cardiac output and only extract 10-20% of the oxygen delivered to them they are susceptible to hypoxia. This is because the medullary region of the kidney accounts for approximately 80% of the oxygen extraction associated with the kidney and due to complex oxygen conservation and delivery mechanisms receives oxygen poor blood.[15] It

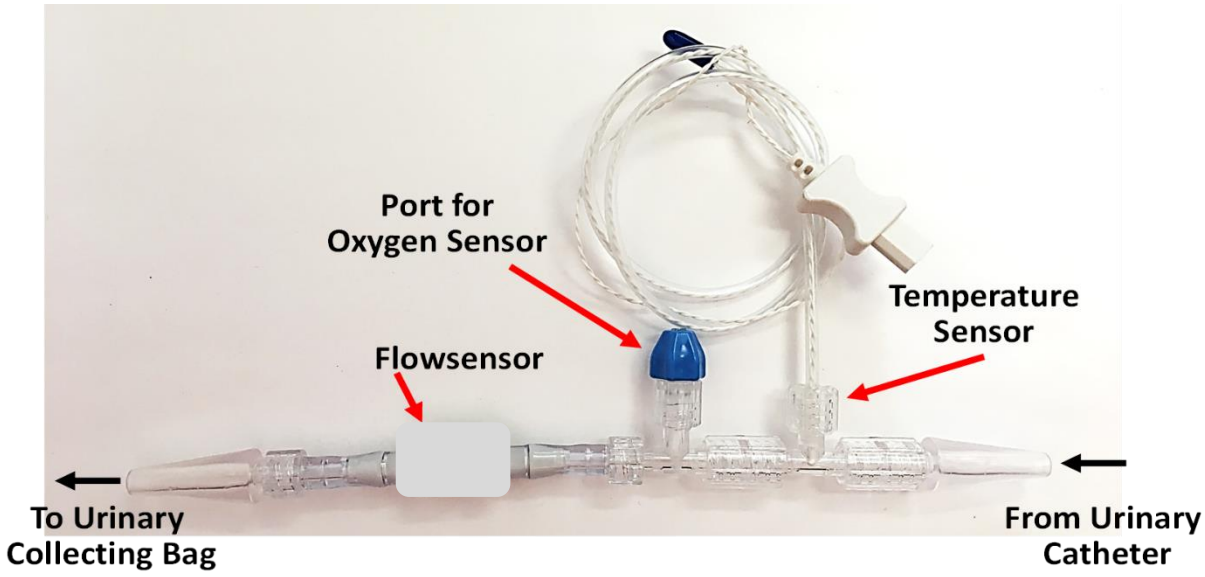

Figure 1 - An image of the device that was used to measure urine oxygen concentration and urine flow rate. The device was placed between the end of the urinary catheter and the tubing that goes to the urine collection bag.

would be ideal to directly measure the tissue oxygen concentration in the medullary region of the kidney, however this is not currently feasible in a clinical setting.

To identify a physiological biomarker to better identify renal injury researchers have investigated the relationship between urine oxygen concentration and tissue oxygen concentration in the medulla. The collection of blood vessels known as the vasa recta run parallel and lie near the urinary collecting ducts in the medullary region. Pannabecker, Datnzler and Thomas et al. showed that due to this spatial relationship the urine oxygen concentration approximated the tissue oxygen concentration in the medullary region of the kidney. In addition, Evans et al. has demonstrated that it is feasible to measure urinary oxygen concentration in the bladder and that those who later develop AKI spend more time below a designated urinary oxygen concentration threshold than those who do not develop AKI.[16] Other studies have shown that urinary oxygen concentration may be a viable surrogate measurement for monitoring renal hypoxia.[17–19]

The goal of this research was to investigate potential biomarkers that could be used to monitor AKI risk and diagnose AKI intraoperatively. A novel device that non-invasively measured urinary oxygen concentration and urine flow rate was developed. Identifying novel biomarkers that could be used intraoperatively could help mitigate or prevent the development of AKI. It would also help researchers further study different therapies and interventions to mitigate or prevent surgery associated AKI. Preventing surgery associated AKI would improve surgical outcomes, save people's lives, and reduce costs associated with hospital and ICU length of stay.

METHODS

After IRB approval and informed consent 38 patients scheduled for cardiothoracic surgery were enrolled at University of Utah Health Sciences. PuO_2, temperature, and urinary flow sensors were installed between the urinary catheter and the tubing going to the urinary collection bag. All sensors were sampled at 1 Hz. The device was removed 24

hours after the end of cardiopulmonary bypass. After removal, each device was calibrated according to a calibration protocol.

The concentration of five urinary biomarkers (NGAL, KIM-1, IL-18, MCP-1, YKL-40) were measured at baseline (shortly after placement of urinary catheter) and 12-hours post cardiopulmonary bypass. For each patient, the percent change from baseline was calculated for each biomarker. A percent change in the upper tertile for any biomarker was defined as "subclinical AKI." "Clinical AKI" was defined as meeting the KIDGO criteria for AKI based on serum creatinine measurements.[4] Patients with subclinical or clinical AKI, were in the AKI group, patients with neither subclinical nor clinical AKI were in the "No AKI" group.

An average calibration curve was calculated. The calibration curve was applied to the raw oxygen data to obtain oxygen concentration measurements. The oxygen concentration and urine flow signals were combined to calculate the oxygen mass flow rate. The average oxygen mass flow rate, average oxygen concentration, and average urine flow rate during cardiopulmonary bypass were calculated for each patient in the “No AKI” and “AKI” groups. When comparing the means of two groups a two-tailed t-test was used. Eleven patients were excluded from the analysis because they were diagnosed with clinical AKI before the procedure, they required a non-latex urinary catheter, or their baseline measurement for any of the biomarkers was in the top ten percent

RESULTS

The average oxygen mass flow rate (mean ± SD) for the AKI group (n=12) was 337.62 ± 238.57 g/hr. For the No AKI group (n=14) the average rate was 669.18 ± 381.75 g/hr.

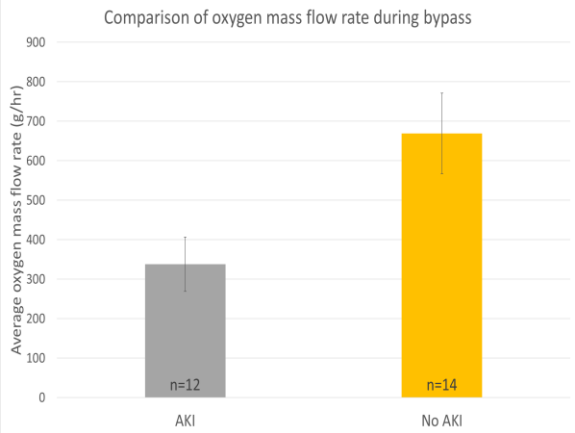

Figure 2 - The comparison of the average mass flow rate for the AKI and No AKI group. The difference was statistically significant (P = 0.019)

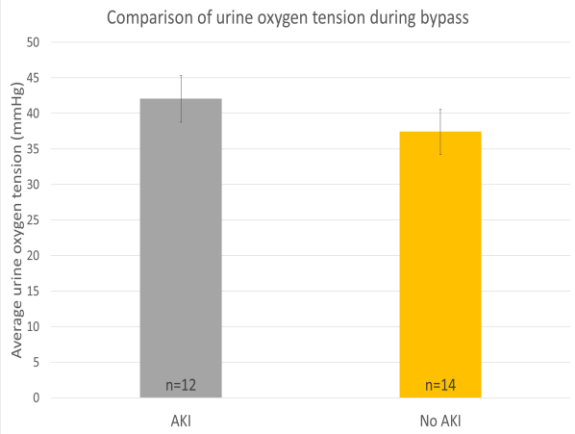

Figure 3 - The comparison of the average urine oxygen concentration for the AKI and No AKI groups. There was not a statistically significant difference between the groups.

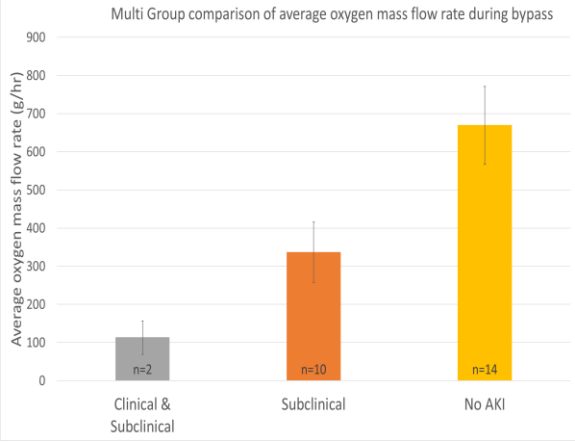

Figure 4 - The comparison of the average oxygen mass flow rate for the AKI, Subclinical AKI, and the Subclinical and Clinical AKI groups. Those who had both forms of AKI had the lowest average oxygen mass flow rate.

Patients diagnosed with AKI had a statistically significant lower average oxygen mass flow rate than those who were not diagnosed with AKI ($p = 0.019$, figure 2).
There was not a statistically significant difference between the average oxygen concentration for the AKI and No AKI groups (figure 3).
Subjects who had both clinical and subclinical AKI had a lower oxygen mass flow rate compared to the other groups. Those subjects with only subclinical AKI had a lower oxygen mass flow rate than those who did not have AKI (figure 4).

DISCUSSION

AKI is common complication associated with cardiac surgery. Those who develop AKI have increased risk of death and hospital LOS. Current diagnostic criteria are based on serum creatinine concentrations and urine output data. These measures are insufficient because they are not renal specific and can only be used for diagnostic purposes after the surgery is complete. Researchers are focused on identifying novel biomarkers to detect AKI intraoperatively. Many studies have shown that certain chemical biomarkers such as NGAL, KIM-1 and IL-18 have potential to detect AKI much earlier than the current methods. Preliminary research also shows that measuring urine oxygen concentration in the bladder may be a viable alternative to monitoring renal hypoxia which is associated with increased risk of developing AKI. The purpose of this study was to investigate the relationship between several different biomarkers, urine oxygen tension outside the body and AKI diagnosis.
This study showed the it is possible to measure urine oxygen concentration outside the body and that it could potentially be used to monitor renal hypoxia. However, based on the urine oxygen concentration data and previously published research there are other factors that confound the oxygen concentration measurement. As the urine travels through the ureter, into the bladder and then through the urinary catheter to our device outside the body oxygen may diffuse in or out of the urine. The quantity of oxygen that diffuses in or out of the urine is likely dependent on the urine flow rate. Future models may help account for the oxygen diffusion which currently confounds the signal.
In addition, this study showed that there are biomarkers that are more sensitive than creatinine for detecting renal injury. Creatinine is not renal specific and therefore may not be specific enough to detect all cases of renal injury. Another advantage to using these biomarkers is that may detect AKI sooner than current methods.
Limitations of this study include the population size and the definition of AKI. Clinical AKI was defined only using serum creatinine concentration and not urine output. It has been shown that urine output is associated with AKI diagnosis so future work should include urine output in the AKI diagnosis. Subclinical AKI was an arbitrary definition and further research is needed to determine appropriate cutoffs for diagnosing AKI.

CONCLUSION

In summary, the current methods for detecting AKI are not renal specific and cannot be used as a diagnostic tool in real time. To prevent AKI, it is important to detect AKI risk in real time. Urine oxygen concentration and chemical biomarkers are two potential methods that could be used to monitor AKI risk. However, more research is

needed to validate preliminary results and refine the technology to obtain measurements that reflect medullary tissue oxygen concentration.

REFERENCES

1. Rosner, M. H. & Okusa, M. D. Acute Kidney Injury Associated with Cardiac Surgery. 19–32 (2006) doi:10.2215/CJN.00240605.
2. Chertow, G. M., Burdick, E., Honour, M., Bonventre, J. V. & Bates, D. W. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J. Am. Soc. Nephrol.* **16**, 3365–3370 (2005).
3. Dasta, J. F., Kane-gill, S. L., Durtschi, A. J., Pathak, D. S. & Kellum, J. A. Costs and outcomes of acute kidney injury (AKI) following cardiac surgery. 1970–1974 (2008) doi:10.1093/ndt/gfm908.
4. Karkouti, K. *et al.* Acute Kidney Injury After Cardiac Surgery Focus on Modifiable Risk Factors. (2009) doi:10.1161/CIRCULATIONAHA.108.786913.
5. Bellomo, R., Ronco, C., Kellum, J. A., Mehta, R. L. & Palevsky, P. Acute renal failure – definition , outcome measures , animal models , fluid therapy and information technology needs : the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. **8**, (2004).
6. Mehta, R. L. *et al.* Acute Kidney Injury Network : report of an initiative to improve outcomes in acute kidney injury. **11**, 1–8 (2007).
7. Englberger, L. *et al.* Clinical accuracy of RIFLE and Acute Kidney Injury Network (AKIN) criteria for acute kidney injury in patients undergoing cardiac surgery. 1–9 (2011).
8. KDIGO. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Int. Suppl.* **2**, (2012).
9. Chiou, W. L. & Hsu, F. H. Pharmacokinetics of Creatinine in Man and Its Implications in the Monitoring of Renal Function and in Dosage Regimen Modifications in Patients with Renal Insufficiency. *J. Clin. Pharacology* **15**, 427–434 (1975).
10. Desouza, C., Keebler, M., Mcnamara, D. B. & Fonseca, V. Homocysteine Metabolism Impact on Cardiovascular Risk. *Drugs* **62**, 605–616 (2002).
11. Ralib, A., Pickering, J. W., Shaw, G. M. & Endre, Z. H. The urine output definition of acute kidney injury is too liberal. *Crit. Care* **17**, R112 (2013).
12. Mishra, J. *et al.* Identification of Neutrophil Gelatinase-Associated Lipocalin as a Novel Early Urinary Biomarker for Ischemic Renal Injury. *J. Am. Soc. Nephrol.* **14**, 2534–2543 (2003).
13. Mendoza, J. D. Biomarkers in Acute Kidney Injury. in *Acute Kidney Injury: Causes, Diagnoses, Treatment* 111–140 (2011).
14. Heyman, S. N., Evans, R. G., Rosen, S. & Rosenberger, C. Cellular adaptive changes in AKI : mitigating renal hypoxic injury. *Nephrol. Dial. Transplant.* **27**, 1721–1728 (2012).
15. Evans, R. G., Smith, D. W., Lee, C. J., Ngo, J. P. & Gardiner, B. S. What Makes the Kidney Susceptible to Hypoxia? *Anat. Rec.* (2019) doi:10.1002/ar.24260.
16. Zhu, M. Z. L. *et al.* Urinary hypoxia: An intraoperative marker of risk of

cardiac surgery-associated acute kidney injury. *Nephrol. Dial. Transplant.* **33**, 2191–2201 (2018).

17. Kainuma, M., Yamada, M. & Miyake, T. Continuous urine oxygen tension monitoring in patients undergoing cardiac surgery. *J. Cardiothorac. Vasc. Anesth.* **10**, 603–608 (1996).
18. Aukland, K. & Krog, J. Renal Oxygen Tension. *Nature* **188**, 671 (1960).
19. Sgouralis, I. *et al.* Bladder urine oxygen tension for assessing renal medullary oxygenation in rabbits: Experimental and modeling studies. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **311**, R532–R544 (2016).