Olfactomedin 4 as a Novel Loop of Henle-Specific Acute Kidney Injury Biomarker

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Introduction

- Acute kidney injury (AKI) occurs frequently in critically ill children and adults and is associated with significant morbidity and mortality.
- Different etiologies, severity, duration, and timing of AKI impact patient-specific outcomes. Novel AKI biomarkers have the potential to disentangle clinical heterogeneity.
- To our knowledge, no novel AKI biomarker is expressed solely in the loop of Henle (LOH) although the furosemide stress test is a functional test that assesses LOH integrity.
- Olfactomedin 4 (OLFM4) is a secreted glycoprotein expressed in mature neutrophils and epithelial cells in prostate and gut epithelium following stress.
- Wild type murine pups challenged with sepsis showed increased OLFM4 expression that localized to the kidney, specifically to the LOH. OLFM4 null murine pups with sepsis were protected from renal cell apoptosis and plasma creatinine elevation seen in their wild-type septic counterparts. Furthermore, OLFM4 was detectable in murine urine only following septic challenge.

Purpose

We hypothesized that urine OLFM4 (uOLFM4) will be increased in children with AKI and septic shock, and that it will localize to the LOH in humans.

Methods

- Urine samples from patients enrolled in the single center prospective observational study, "AKI in Children Expected by Renal angina and Urinary Biomarkers" (AKI-CHERUB, NCT1735162) study at Cincinnati Children's Hospital Medical Center were analyzed.
- AKI was diagnosed as severe and persistent by Kidney Disease Improving Global Outcomes (KDIGO) serum creatinine criteria. Septic shock diagnoses were extracted from the medical record based on ICU admitting diagnosis from the ICU admission note.
- 8 patients had no AKI and no septic shock, 10 had AKI with no septic shock, 10 had no AKI with septic shock, and 8 had AKI with septic shock.
- uOLFM4 was assayed with a custom bead-based Luminex immunoassay.
- Immunofluorescence was performed on 4 healthy human control samples and 10 human samples with varying kidney injury (5 with acute tubular necrosis, 3 transplants with tubular injury, and 2 with chronic tubulointerstitial injury).
- All groups were non-normally distributed; comparisons between groups were performed using the Mann-Whitney U Test.

Results

- Patients with AKI had higher uOLFM4, median 288.4 ng/mL [IQR 59.3-995.2] than those without, median 81.7 ng/mL [IQR 30.4-159.7], p=0.040, Figure 1A.
- uOLFM4 was higher in patients with septic shock, median 375.2 [IQR 67.9-943.3] than those without septic shock, median 78.3 ng/mL [IQR 25.7-172.4], p=0.034, Figure 1B.

Results (continued)

- Of 18 patients with septic shock, 8 with AKI had higher uOLFM4 than 10 without AKI, median 969.3 [IQR 178.6-1087.0] vs 143.2ng/mL [IQR 61.4-424.4], p=0.048, Figure 1C.

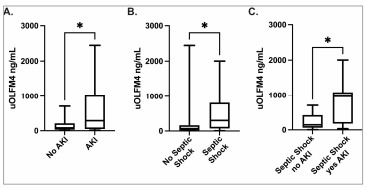

Figure 1. Urine Olfactomedin 4 levels based on A. AKI status (n=18/group), B. Septic shock status (18 vs 15/group), C. Septic shock+ AKI vs septic shock no AKI. *Denotes p<0.05. uOLFM4- urine olfactomedin 4

- There is correlation between urine NGAL and uOLFM4 (r2 0.59, p=0.002), but some patients had high NGAL and low uOLFM4, and vice versa (Figure 2).

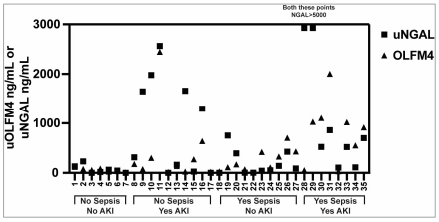

Figure 2. Correlation of urine NGAL and uOLFM4. Individual patients, grouped by AKI and sepsis status. OLFM4 level is marked by a triangle and NGAL level marked by a box

- Immunofluorescence showed nearly all OLFM4 co-expressed with LOH cells, in all 5 cases of acute tubular necrosis. 2 of 4 controls had rare OLFM4 staining, which co-expressed with LOH cells (Figure 3).

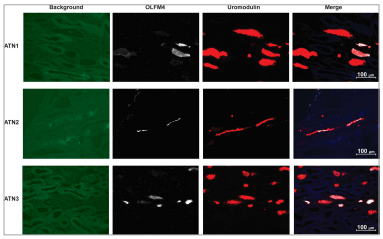

Figure 3. Immunofluorescence of 3 human kidney biopsies with acute tubular necrosis (Columns left to right). Background shows distorted tubular architecture from AKI. OLFM4 staining appears in white. Uromodulin, staining loop of Henle cells, appears in red. Merged images of the white OLFM4 overlaying the red loop of Henle cells. ATN-acute tubular necrosis. OLFM4- olfactomedin4.

Conclusion

- Urine OLFM4 is elevated in patients with AKI and septic shock and is increased in patients with septic shock and AKI above those with septic shock and no AKI.
- There was a strong correlation between OLFM4 and NGAL. In the patients where correlation is poor, further investigation is warranted to explore the possible clinical significance of this discrepancy.
- Given OLFM4 colocalization to human LOH, we propose OLFM4 may be a LOH-specific AKI biomarker.
- Future studies will look to corroborate these findings prospectively, focusing on septic AKI.