Identification of Prognostic Biomarkers for Antibiotic Associated Nephrotoxicity in Cystic Fibrosis

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Abstract

Individuals with cystic fibrosis (CF) suffer chronic lung infections requiring frequent antibiotic treatment, some of which carry risk of acute kidney injury (AKI). Despite the burden of kidney disease in CF, we lack effective methods for early detection of antibiotic associated AKI.

To identify novel biomarkers of antibiotic associated AKI in patients with CF by first identifying candidate markers in an ex-vivo human microphysiological system (MPS) and then translating these findings to a cohort of patients with CF.

Treating the MPS with polymyxin E resulted in a statistically significant increase in the pro-apoptotic Fas gene. Effluent analysis demonstrated an acute rise of soluble Fas (sFas) that correlated with cellular injury. In 16 patients with CF, urinary sFas was significantly elevated during antibiotic treatment. Over 3 years of follow up, 7 cases of incident chronic kidney disease (CKD) were identified. Urinary sFas was associated with subsequent development of CKD.

Introduction

- Chronic lung infections in CF may require patients to undergo repeated therapy with nephrotoxic antibiotics, such as aminoglycosides and polymyxins.
- Patients with CF are at high risk of AKI and chronic kidney disease (CKD), an increasing cause of morbidity in CF.
- Standard metrics for diagnosing AKI (creatinine) are ineffective in identifying renal injury in CF patients.
- Fas/FasL is a key pathway in kidney cell death that characterizes injury and could indicate subclinical AKI in CF.

Methods and Materials

Microphysiological system:
We populated the "kidney on a chip" with primary human proximal tubule epithelial cells from 3 donors and modeled nephrotoxin injury through exposure to 50, 75 and 100 µg/mL polymyxin E for 48 hours. We analyzed gene transcriptional responses by RNAseq and tested MPS effluents to determine if corresponding candidate biomarkers increased with MPS injury. (Fig. 1)

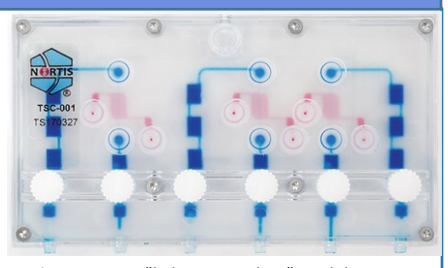

Figure 1. MPS "kidney on a chip," modeling antibiotic associated kidney injury.

Clinical Cohort:
Candidate biomarkers identified in the MPS treatment were translated to urine studies of a cohort of 16 patients with CF undergoing treatment with nephrotoxic antibiotics. Urinary biomarkers were measured prior to antibiotics, during and 2 weeks after antibiotics. Renal function was monitored for a median of 3 years after antibiotic therapy.

Patient Characteristics	N = 16
Age, mean (SD), years	33.8 (± 12.0)
Sex, male, n (%)	7 (44 %)
Prevalent CKD	1 (6%)
Diabetes mellitus	7 (44%)
Hypertension	1 (6%)
Aminoglycoside therapy	9 (56%)
Polymyxin therapy	5 (31%)
Aminoglycoside & polymyxin	1 (6%)
Other	1 (6%)
Days of antibiotic therapy, mean (range), days	13.9 (12-14)

Patient Outcomes	N = 16
Incident CKD during follow-up, n (%)	7 (44%)
Stage 2	3 (19%)
Stage 3a	1 (6%)
Stage 3b	1 (6%)
Stage 4	2 (13%)
Median duration to incident CKD, days (range)	369 (104 – 751)
Mean duration to incident CKD, days (range)	417 (104 – 751)
Death	1 (6%)

Results

- Polymyxin E treatment resulted in a statistically significant increase in the pro-apoptotic Fas gene relative to control: fold-change=1.63, FDR q-value=7.29x10-5. Effluent analysis demonstrated an acute rise of soluble Fas (sFas) concentrations that correlated with cellular injury.
- In all 16 patients with CF, urinary sFas concentrations were significantly elevated during antibiotic treatment, regardless of development of AKI.
- Over a median of 3 years of follow up, we identified 7 cases of incident chronic kidney disease (CKD). Urinary sFas concentrations during antibiotic treatment were significantly associated with subsequent development of incident CKD (unadjusted relative risk=2.02 per doubling of urinary sFas, 95% CI=1.40, 2.90, p<0.001).

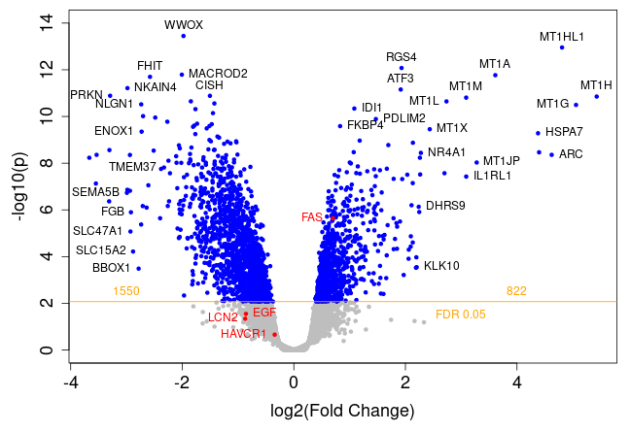

Figure 2. Volcano plot of all differentially regulated genes between polymyxin E and control

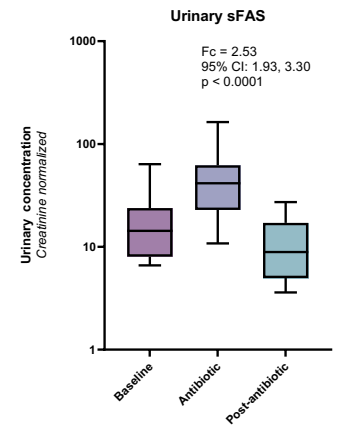

Figure 3. Urinary sFas concentrations among 16 patients with CF at baseline, during antibiotics and 2 weeks after antibiotics.

Discussion

- sFas, a novel urinary biomarker, was upregulated in the MPS after polymyxin E exposure. The Fas/FasL system is a key biologic pathway regulating apoptosis in kidney epithelial cells.
- Despite only 2 patients developing mild AKI (creatinine), urinary sFas concentrations were more than 2-fold greater during antibiotic treatment compared to prior to antibiotics.
- Urinary markers of tubular injury (KIM-1, NGAL) were also elevated, and markers of kidney health (EGF, uromodulin) were decreased in during antibiotic therapy in the CF cohort.
- Urinary sFas could be used as a sensitive and timely biomarker for the development of subclinical AKI in patients with CF receiving nephrotoxic antibiotics. While validation in multiple clinical cohorts is necessary, possible future use of urinary sFas may be as a marker for early detection of kidney injury in patients with CF, assessing CKD risk and guiding antibiotic dosing and duration.

Conclusions

We utilized a human kidney MPS to discover novel biomarkers of epithelial cell injury due to nephrotoxic antibiotics. Fas gene expression and effluent sFas increased with polymyxin E exposure in the MPS. We translated findings that sFas may be an early marker of kidney epithelial cell injury and, in turn, kidney dysfunction to a pilot clinical cohort of patients with CF receiving nephrotoxic antibiotics. The increase in sFas during antibiotics was associated with risk of CKD. Overall, these findings suggest that urinary sFas is a novel biomarker of subclinical kidney injury and may provide early signs prior to a rise in serum creatinine in patients with CF at risk of long-term kidney dysfunction.

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