Next-level approach to AMR and TCMR diagnosis in kidney transplantation; the dynamic duo of urine CXCL10 and donor-derived cell free DNA.

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- **Background:**

•Alloimmune injury, including TCMR and AMR, remains the primary cause of kidney allograft failure today
•Kidney transplant biopsies remain the gold- standard for diagnosis of rejection but these are invasive, resource-intensive, subject to sampling error, interpretation bias and difficult to use as a tool to monitor alloimmune activity over time (including rejection resolution)
•In contrast, serum creatinine is non specific for rejection
•Donor-specific antibody (DSA) has been shown to correlate with AMR and allograft loss but there are no recommendations on surveillance frequency and both TCMR and AMR can occur in the absence of DSA

- **Hypothesis**

While elevated dd-cfDNA reflects glomerulitis and peritubular capillaritis, as seen in AMR, elevated urine CXCL10 reflects more tubulitis, characteristic of TCMR and mixed rejection
Due to these complementary properties, we hypothesized that use of these 2 biomarkers **together** would improve the diagnosis of mixed rejection phenotypes

- **Methodology**

•Single Center, Case Control Study (CHUM Transplant Biobank)
•Inclusion Criteria: adult renal transplant recipients over 18 years of age who underwent an adequate allograft biopsy (for-cause or protocol) associated with both paired urine and plasma samples
•Exclusion criteria: simultaneous dual organ transplant recipients
•Rejection diagnoses were classified by adhering to the Banff 2022 criteria
•Urine CXCL10 was measured at the Chemokine laboratory at the University of Manitoba using the Meso Scale V-Plex assay.
•Cell free DNA was extracted blindly from EDTA plasma samples (Brisbane, California)
• Percent donor derived cell free DNA calculated using the CareDx AlloSeq cfDNA kit (Brisbane, California).

Results

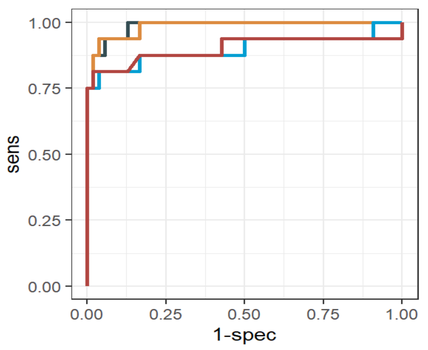
Demographics & history, Main analysis set (later biopsies).

Characteristic	N = 96
recipient age at biopsy, Median (IQR)	52 (40 – 60)
recipient sex, n (%)	
F	30 (31)
M	66 (69)
donor age, Median (IQR)	51 (37 – 57)
donor sex, n (%)	
F	46 (48)
M	50 (52)
living donor, n (%)	25 (26)
cause of renal disease, n (%)	
Glomerular Disease	21 (26)
Polycystic Kidney Disease	26 (32)
Diabetes	15 (18)
Vascular	8 (9.8)
Genetic	4 (4.9)
Other	8 (9.8)
Unknown	14
Delayed graft function, n (%)	11 (11)
Induction, n (%)	
Anti-thymocyte Globulin	12 (13)
Basiliximab	73 (77)
other	10 (11)
Unknown	1
clinical vs subclinical biopsy, n (%)	
clinical	42 (44)
subclinical	54 (56)

Multiple-Regression Analysis: AMR vs no rejection

Biomarker	OR (95% CI)	P value
Serum Creatinine	0.55 (0.03-11.9)	0.69
Urine CXCL10	1.12 (0.43-2.76)	0.8
dd-cfDNA	3.74 (1.54-13)	0.011

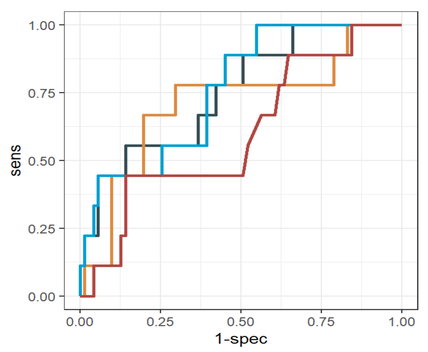
Addition of dd-cfDNA



Black: Creatinine+DSA+CXCL10+dd-cfDNA
Orange:Creatininie+DSA+dd-cfDNA
Red: Creatinine+DSA
Blue: Creatinine+DSA+CXCL10

Multiple-Regression Analysis: TCMR vs no rejection

Biomarker	OR (95% CI)	P-value
Serum Creatinine	2.18 (0.38-14.1)	0.38
Urine CXCL10	1.61 (1.09-2.60)	0.03
dd-cfDNA	1.26 (0.70-2.34)	0.44



Black: Creatinine+CXCL10+dd-cfDNA
Orange:Creatininie+dd-cfDNA
Red: Creatinine
Blue: Creatinine+CXCL10

- **Conclusion**

•dd-cfDNA alone outperforms urine CXCL10 alone as a diagnostic biomarker of clinical AMR

•Addition of dd-cfDNA improves the non-invasive diagnosis of clinical AMR when added to SOC biomarkers (serum creatinine and DSA)

•Urine CXCL10 alone outperforms serum creatinine as a diagnostic biomarkers of TCMR

•Addition of urine CXCL10 improves the non-invasive diagnosis of TCMR when added to SOC serum creatinine

•The benefit of combining dd-cfDNA, urine CXCL10 and serum creatinine to improve the non-invasive diagnosis of TCMR and mixed rejection requires further study