Urinary T cell subsets and tubular epithelial cells as biomarkers for kidney transplant rejection

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I Background

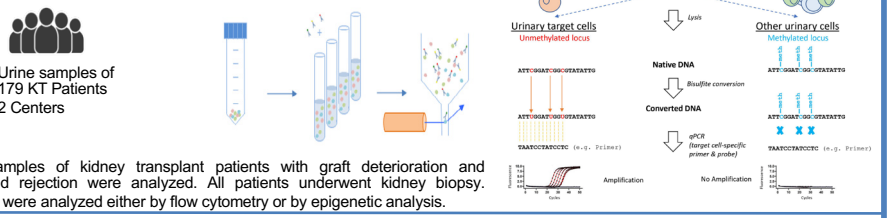
- Detection of kidney transplant rejection remains a challenge
- Non-invasive biomarkers hold high potential to detect rejection
- We developed a biomarker combination to detect rejection by analyzing urine cells using flow cytometry

II Question

Are we able to confirm our previous findings and optimize our biomarkers to detect kidney transplant (KT) rejection?

III Methods

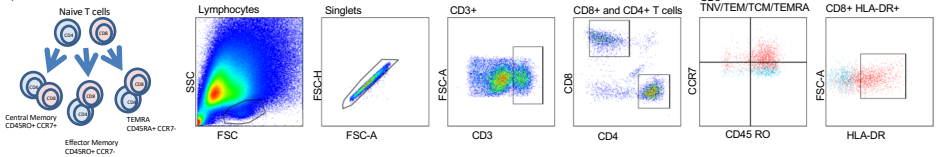
Setup



Urine samples of kidney transplant patients with graft deterioration and suspected rejection were analyzed. All patients underwent kidney biopsy. Samples were analyzed either by flow cytometry or by epigenetic analysis.

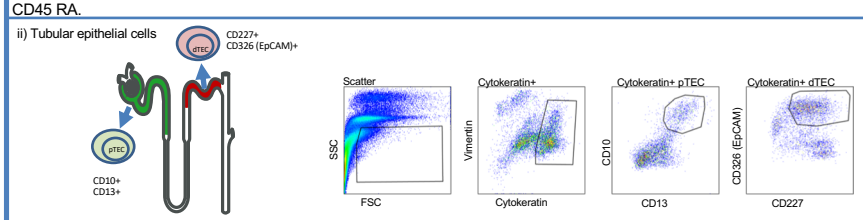
Cell Subtypes & Gating Strategy

i) T cells



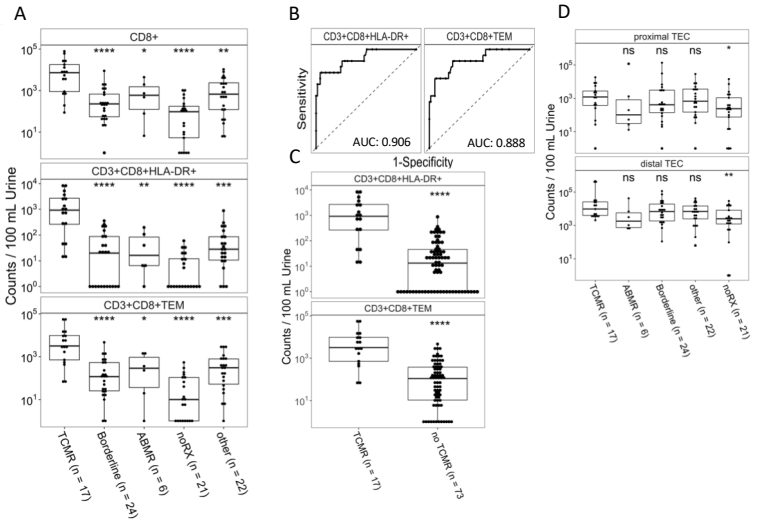
Schematic illustration of T cell subsets and gating strategy. Subsets depicted only for CD8+ T cells. Red: full stained sample, Blue: control. TNV, naive T cells; TEM, effector memory T cells; TCM, central memory T cells; TEMRA, effector memory T cells reexpressing CD45 RA.

ii) Tubular epithelial cells

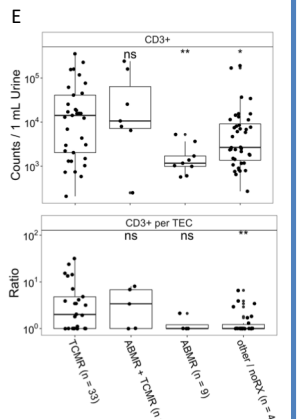


Schematic illustation of a nephron and gating strategy for tubular epithelial cells. Scatter pregate based on previous urine analysis spiked with renal tubular epithelial cells. pTEC, proximal tubular epithelial cells; dTEC, distal tubular epithelial cells.

IVa Flow Cytometry Results



IVb Epigenetics Results



Levels of significance:
ns = p > 0.05
* = p < 0.05
** = p < 0.01
*** = p < 0.001
**** = p < 0.0001

Urinary cell numbers in kidney transplant patients with graft deterioration and renal biopsy. Professional diagnoses from renal biopsies severed to uniquely group graft deterioration. (A) Absolute numbers of urinary CD8+ T cells discriminate patients with TCMR from all other diagnoses. T cell subsets are even more distinctive. (B) ROC curves for CD8+ HLA-DR+ T cells and CD8+ TEM as biomarkers for TCMR. (C) Absolute cell counts for TCMR compared to other groups combined. (D) Urinary tubular epithelial cells separate TCMR from noRX. No further significant differences between TCMR and other analyzed groups. (E) Epigenetic analysis qualitatively confirms T cell quantity in depicted groups as determined by flow cytometry. The ratio of absolute numbers of T cells and TECs discriminates patients with TCMR from patients with other diagnosis than rejection. TCMR, T cell mediated rejection; Borderline, borderline rejection; ABMR, antibody mediated rejection; No RX, no signs of rejection in kidney biopsy; other, other specific pathohistological diagnosis than rejection (e.g. IgA Nephropathy); TEM, effector memory T cells; distal TEC, distal tubular epithelial cells; proximal TEC, proximal tubular epithelial cells; AUC, area under the curve.

CD8+ TEM T cells and HLA-DR+ T cells are most distinctive. Setting a cutoff of 426.7 CD8+TEM T cells per 100ml urine (analyzed with flow cytometry) shows a sensitivity of 88% and a specificity of 77% and setting a cutoff of 44.4 CD8+HLA-DR+ T cells per 100ml urine shows a sensitivity of 88% and specificity of 74%.

V Conclusion and Outlook

We were able to confirm urinary T cells as biomarker for TCMR as previously described. Urinary T cell subsets reflect intrarenal inflammation in TCMR. Urinary tubular epithelial cells mirror intrarenal damage accompanied by rejection and other diagnoses. Epigenetic analysis confirms urine flow cytometry results. T cell subsets yield high potential to monitor kidney transplant patients and to detect rejection. Further prospective trials are required to incorporate the suggested biomarkers into clinical care.

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