Validation of Urinary Cell Biomolecular Markers of Acute Cellular Rejection: A Report from CTOT-4 Trial Investigators.

M. Suthanthiran, R. Ding, V. Sharma, M. Abecassis, D. Dadhania, B. Samstein, S. Knechtle, W. Hancock, J. Schwartz, J. Liu, N. Bridges, A. Shaked CTOT-04 Investigators

Background: Development of validated urinary cell biomarkers of acute rejection could minimize biopsy associated complications and costs as well as improve allograft surveillance. **Methods:** The NIAID-CTOT-4 Trial prospectively enrolled 497 adult renal allograft recipients (patients) and investigated the accuracy of urinary cell mRNA levels for diagnosing acute rejection. Urine specimens collected at any time of for cause kidney biopsy, were centrifuged and cell pellets were shipped to the PCR Core blinded to clinical information and biopsy diagnosis. A pre-amplification enhanced kinetic quantitative PCR assay was used to measure mRNA levels, and the absolute copy numbers were normalized/controlled using 18s rRNA copy number. A NIH-sponsored Scientific and Clinical Coordinating Center (SACCC) performed data collection, coordination and independent statistical analyses. **Results:** Among the 2076 urine specimens with mRNA profiling, 1843 were from patients who did not require biopsy during the post-transplantation follow-up; 155 from patients with normal biopsies; 43 from patients with Banff grade IA or higher (ACR) biopsies, 16 patients with borderline changes; 11 from patients classified as Other/CAN; and 8 from patients with biopsies showing antibody mediated rejection. Data analysis restricted to those who underwent biopsies demonstrated that urinary cell levels of perforin ($P<0.0001$, Mann-Whitney test), granzyme B (<0.0001), CD3 (<0.0001), IP-10 (<0.0001), CD103 (0.002), and Foxp3 (0.002) were higher in the 43 samples from patients with ACR compared to the 155 samples from patients with normal biopsies. Data analysis that included all 2076 urine cell mRNA profiles demonstrated that urinary cell levels of perforin ($P<0.0001$, Mann-Whitney test), granzyme B (<0.0001), CD3 (<0.0001), IP-10 (<0.0001), CD103 (0.004), and Foxp3 (0.02) were higher in the 43 samples from patients with ACR compared to the 2033 samples from patients without ACR. Receiver Operator Character Curve analysis of a prediction model comprised of levels of perforin, IP-10, Foxp3 and TGF beta1 demonstrated that ACR is predicted with a sensitivity of 86% and a specificity of 70% (AUC: 0.85, 95% CI: 0.79-0.90). **Conclusions:** Validated urinary cell biomarkers can replace kidney biopsy for the acute cellular rejection, and therefore reduce biopsy associated complications and costs and improve the care of renal allograft recipients.