Urinary Cell Messenger RNA Expression Signatures Anticipate Acute Cellular Rejection: A Report from CTOT-04

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Background: NIAID-CTOT4 tested the following primary hypotheses: (i) urinary cell mRNA profiles measured at the time of allograft biopsy are diagnostic of acute rejection of renal allografts; and (ii) sequential measurements of urinary cell mRNA profiles are predictive of acute rejection in the near future. We previously reported that urinary cell mRNA profiles at the time of allograft biopsy are diagnostic of acute cellular rejection (ACR) of renal allografts. We now report that sequential measurements of urinary cell mRNA profiles accurately predict future ACR. Methods: The CTOT4 prospectively enrolled 497 adult renal allograft recipients. Urine specimens collected at scheduled visits were centrifuged and cell pellets were shipped to the PCR Core blinded to clinical information and biopsy diagnosis. A quantitative PCR assay was used to measure copy numbers of 10 pre-specified mRNAs. An independent Statistical and Clinical Coordinating Center performed data management and 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 biopsies with no rejection; 43 from patients with Banff grade IA or higher (ACR) biopsies; 16 from patients with borderline changes; 8 from patients with AMR; 11 other. The mRNA levels were screened using univariate t-tests and the candidate genes were then included in a stepwise logistic regression model predicting presence/absence of ACR from the mRNA predictors. Figure 1 lists the mRNA combinations predictive of ACR 90 to 60 days (area under the curve [AUC]: 0.88, P<.001), 59 to 30 days (AUC: 0.88, P<.001); and 29 to 16 days (AUC: 0.93, P<.001) prior to the biopsy identification of ACR. Conclusions: mRNA profiles of longitudinally collected urine specimens predict the development of future ACR with a high degree of accuracy. The dynamics in mRNA expression and the anticipatory molecular signatures could be invaluable for individualizing immunosuppression and preventing imminent rejection prior to tissue injury.

Table 1. Urinary Cell mRNA Expression Signatures Predictive of Future ACR

90 to 60 Days Prior to Biopsy DX of ACR	**59 to 30 Days Prior to Biopsy Dx of ACR**	**29 to 16 days Prior to Biopsy DX of ACR**
Perforin	Perforin	Perforin
PI-9	IP10	PI-9
	CXCR3	IP10
	Foxp3	CXCR3
		CD3
		Granzyme B
AUC=0.88	**AUC=0.88**	**AUC=0.93**