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Diagnosis of Early Delayed Graft Function (DGF) Using TIMP-2*IGFPB-7 Product in Transplant Recipients: Preliminary Results

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Results: At time of transplantation, ADV-CD4Tvis were detectable in 30/31 patients (intervention group), CMV-CD4Tvis and HSV-CD4Tvis only in 12/31. No significant ADV- or HSV-DNAemia was found; only two patients showed transient CMV-DNAemia based on CMV-reactivation. Five primary CMV-infections with seroconversion and boost of CMV-CD4Tvis were observed without significant CMV-DNAemia. The mean level of ADV-CD4Tvis was 1.63(SD1.25), 2.03(SD1.8), 2.18(SD2.51) and 1.97 cells/µl(SD1.34) 1,6,12 and 24 months after KTx. In case of CD4Tvis <2cells/µl 125 dose reductions of immunosuppressants (96% based on ADV-CD4Tvis) were performed in 28/31 children with a median of 4 Tvis-based dose reductions (range 0-10) per patient. 48% of these were carried out in the first six months.

Conclusions: Under the intensified immunosuppression during the initial post-KTx period low ADV-CD4Tvis levels were observed with subsequent increase after dose reduction of the immunosuppression. ADV-CD4Tvis are most suitable for immune monitoring because of their high prevalence (even in children) and stability combined with absence of ADV-DNAemia. Routine monitoring of ADV-CD4Tvis is recommendable especially in the first post-KTx year to prematurely identify overimmunosuppression.

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PO2190

Regulatory T Cells, BK Virus Infection, and Long-Term Outcomes in Kidney Transplant Recipients

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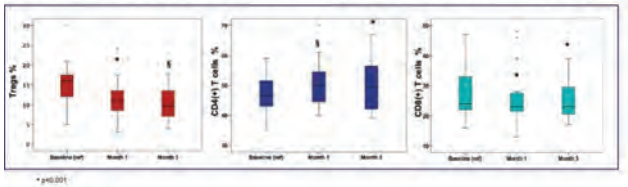
Background: Regulatory T cells (Tregs) may inhibit pathogen-specific immunity in infectious disorders. We monitored Treg levels during BK virus (BKV) viremia/viruria, examined pattern of Tregs that might contribute BKV infection, and assessed their prognostic value for the KTx outcomes.

Methods: We evaluated 20 KTx recipients (male:13, mean age:41±12 years, living donor 15) in whom BKV viremia/viruria was detected at a median 12.6 (IQR, 4.6-31.2) months after KTx. Serum and urine BKV DNA were measured by real-time PCR at baseline, 1 and 3 months after detection of BKV viremia/viruria. Lymphocyte profile and CD4(+)CD25(+)FoxP3(+) Tregs were measured by flow cytometry concurrently at these time points. Graft outcomes over 8 years were examined in relation to BK viremia, viruria levels, and lymphocyte profiles.

Results: At the time of diagnosis of BKV viremia/viruria, 17 (85%) patients were on calcineurin inhibitor (CNI)-based triple immunosuppression. CNI was discontinued in 9 patients, sirolimus was started in 3 of them. Mycophenolic acid was switched to azathioprine or the dose was decreased in all patients. Reduction in overall immunosuppression was associated with a decrease in serum and urine BKV DNA levels. Tregs and CD8(+) T lymphocytes were significantly decreased and CD4(+) T lymphocytes were increased during this period (Figure 1). After a median follow-up of 8.1 years (IQR, 3.3-8.5), 6 (30%) patients lost their allografts. There were no significant differences in mean Tregs levels between patients with and without graft failure (p=0.63). Serum and urine mean BKV DNA levels were similar between patients with and without graft failure (p=0.38 and p=0.20, respectively).

Conclusions: Tregs may play a role in BKV infection, reduction in the overall amount of immunosuppression is associated with improvement of BKV viremia/viruria accompanied by a decrease in Treg levels. Future work is needed to discriminate predictors of allograft failure in patients with BK nephropathy.

Figure 1. Tregs, CD8(+) lymphocytes were significantly decreased and CD4(+) T lymphocytes were increased during follow up.



PO2191

Expansion and Characterization of Regulatory T Cell Populations from Korean Kidney Transplant Recipients

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Background: The development of immunosuppressants has enabled remarkable progress in kidney transplantation (KT). However, current immunosuppressants cannot achieve induction of immune tolerance and their nonspecific immunosuppressive effects result in many adverse effects. Regulatory T cells (Tregs) play crucial roles in controlling allospecific immune responses. This study evaluated the distribution of Tregs and their effects on kidney allograft function in Korean KT recipients.

Methods: We enrolled 144 KT recipients with stable graft function between 1989 and 2018. Differentiation and expansion of Tregs were studied by flow cytometry to compare the Tregs subpopulations. Tregs were defined as $CD4^{+}CD25^{high}CD127^{low/-}FoxP3^{+}$ cells.

Results: Among the 144 patients, 75 patients (65.8%) were males and mean follow-up period was 144.3 ± 111.5 months. All patients received calcineurin inhibitors as maintenance immunosuppressants. Patients with follow-up period more than 144.3 months tended to have more gating Tregs numbers than that in shorter follow-up period (92.3 ± 142.4 vs. 50.1 ± 76.4, p = 0.061, respectively). There were no significant differences in Tregs subpopulations between patients with serum creatinine more than 1.5 md/dL and patients with serum creatinine less than 1.5 mg/dL. In terms of the number of Tregs, when the trough level of tacrolimus was at an appropriate level, the number of Tregs tended to be higher than that of Tregs when the trough level of tacrolimus was low or high, and the organ function of the transplant was also stable.

Conclusions: Tregs counts may be associated with transplant outcomes considering that there is a relationship between these cells and kidney graft function.

Table 2-1. Regulatory T cell subpopulation according to the patient's characteristics.

	Gating cell number	P value	Gate
Male (n=73)	76.7 ± 129.5	0.295	29.1
vs. Female (n=40)	vs. 56.6 ± 73.5		vs. 3
LDKT (n=85)	75.6 ± 121.5	0.113	32.0
vs. DDKT (n=23)	vs. 44.5 ± 72.1		vs. 3
Follow-up duration ≤ 147.5 months	50.6 ± 76.9	0.073	36.4
(n=87)	vs. 89.0 ± 138.5		vs. 2
vs. Follow-up duration > 147.5 months (n=56)			
Tacrolimus (n=70)	49.3 ± 69.4	0.095	34.3
vs. Cyclosporine (n=39)	vs. 94.7 ± 158.1		vs. 2
MMF (n=73)	65.0 ± 121.7	0.558	33.2
vs. No MMF (n=40)	vs. 78.1 ± 95.8		vs. 3
PDN (n=79)	58.1 ± 89.6	0.181	34.1
vs. No PDN (n=34)	vs. 96.3 ± 152.4		vs. 2
Tacrolimus/MMF/PDN (n=49)	44.0 ± 66.4	0.427	36.8
vs. Cyclosporine/MMF/PDN (n=9)	vs. 88.9 ± 158.8		vs. 2
Median tacrolimus level ≤ 5.7 ng/ml	56.9 ± 72.7	0.363	31.7
(n=38)	vs. 41.7 ± 66.1		vs. 3
vs. Median Tacrolimus level > 5.7 ng/ml (n=35)			
Mean tacrolimus level ≤ 5.8 ng/ml	57.6 ± 73.3	0.266	30.9
(n=39)	vs. 38.9 ± 63.8		vs. 3
vs. Mean Tacrolimus level > 5.8 ng/ml (n=31)			
Median tacrolimus dose ≤ 2.5 mg	46.6 ± 62.5	0.963	37.6
(n=36)	vs. 45.7 ± 80.2		vs. 3
vs. Median tacrolimus dose > 2.5 mg (n=22)			
Mean tacrolimus dose ≤ 2.6 mg (n=36)	46.6 ± 62.5	0.963	37.6
vs. Mean tacrolimus dose > 2.6 mg (n=22)	vs. 45.7 ± 80.2		vs. 3
Median cyclosporine level ≤ 87.7	53.5 ± 111.1	0.095	32.0
ng/ml (n=20)	vs. 138.1 ± 189.3		vs. 2
vs. Median cyclosporine level > 87.7 ng/ml (n=19)			
Mean cyclosporine level ≤ 98.1 ng/ml	52.9 ± 105.5	0.078	30.7
(n=23)	vs. 154.8 ± 201.1		vs. 2
vs. Mean cyclosporine level > 98.1 ng/ml (n=16)			

Regulatory T cell subpopulation according to the patient's characteristics.

PO2192

Diagnosis of Early Delayed Graft Function (DGF) Using TIMP-2*IGFPB-7 Product in Transplant Recipients: Preliminary Results

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Background: DGF is acute kidney injury (AKI) defined as need for dialysis within one week of renal transplant. AKI is defined by a change in serum creatinine (SCr), however early recognition is limited by delay in creatinine rise. Accurate early biomarkers may lead to prevention or treatment of established AKI. The product of two novel biomarkers of cell cycle arrest, tissue Inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor binding protein (IGFBP-7) have shown promise in predicting AKI. In prior studies, TIMP-2*IGFPB-7 ≤0.3 had high negative predictive value, and ≥2 high positive predictive value for AKI. **Aims** - 1) Investigate the early diagnostic value of TIMP-2*IGFPB-7 for DGF; 2) Correlate TIMP-2*IGFPB-7 with long term graft function.

Methods: This is a prospective, double-blinded single center observational study with goal enrollment of 150 transplant recipients. Urine TIMP-2*IGFBP-7 was measured in $(ng/mL)^2/1000$ with a commercial kit, Nephrocheck (Astute Medical, San Diego, CA) at 4-12 hours, 48-72 hours and 72-96 hours post-transplant. SCr was measured just prior to transplant, 1 week post-transplant, and at 1, 3, 6, 9 and 12 months post-transplant.

Results: Thus far, 64 patient samples have been collected, 11 with DGF. Mean TIMP-2*IGFPB-7 were 3.08 ± 0.63 vs 0.54 ± 0.23 (p-value <0.001) at 4-12 hours, 3.39 ± 0.93 vs 0.38 ± 0.13 at 24-48 hours (p-value <0.001), and 1.73 ± 0.76 vs 0.62 ± 0.27 (p-value =0.09) at 72-96 hours in DGF vs non DGF patients respectively. Mean SCr at 1 week were 6.14 ± 0.71 mg/dL in DGF vs 2.13 ± 0.26 mg/dL (p-value <0.001) in non-DGF. Correlation between peak TIMP-2*IGFPB-7 at 24-48 hours and sCr at 1, 3, 6, 9, and 12 months, was nonsignificant.

Conclusions: These preliminary results confirm the use of TIMP-2*IGFBP product measured by Nephrocheck in the diagnosis and prediction of DGF in the post-kidney transplant period as early as 4-12 hours, and peaking at 24-48 hours. The non-DGF TIMP-2*IGFBP-7 means were higher than prior reports, suggesting mild renal injury in the peritransplant period in those patients without DGF. The current sample size is too small and underpowered as of yet to draw conclusions on prediction of long-term renal dysfunction.

Funding: Commercial Support - Astute Medical Inc

PO2193

Kidney Injury in Hematopoietic Stem Cell Transplant (HCT) Recipients: Transcriptome Profiling and Development of Urinary Biomarkers

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Background: In kidney biopsies of HCT recipients, thrombotic microangiopathy with/without tubulointerstitial/microvascular inflammation suggest the possibility of kidney being a target of graft versus host disease. We tested the hypothesis: (i) kidney inflammation/injury in HCT recipients is immune mediated, (ii) urinary mRNA profile may be used as a noninvasive biomarker.

Methods: (i) RNA-sequencing of native kidney from 6 HCT recipients with kidney injury was performed. We compared the transcriptome profile to that of allograft kidney. (ii) Urine samples from 9 HCT recipients were collected. We calculated the CTOT-04 signature score for each recipient. We compared the score to that of kidney allograft recipients.

Results: Of the 4188 genes (26% of 16375) that were different (FDR-P<0.05) between HCT and Normal, 2152 were shared among HCT, ACR, and AMR; 1442 were unique to HCT **(Figure 1)**. Shared genes revealed enrichment of innate and adaptive immune system pathways. Urinary cell CTOT-04 signature score was higher in AKI/tubulitis and interstitial inflammation in the native kidney and resembled ACR of kidney allograft recipients **(Figure 2)**.

Conclusions: In recipients of HCT: (i) kidney inflammation/injury is immune mediated, (ii) urinary cell mRNA profiling is useful for diagnosing kidney injury

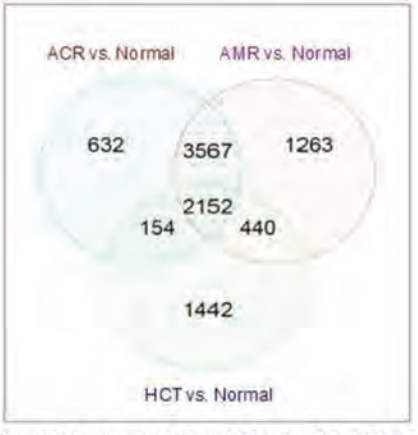

Figure 1. Differential expression of mRNAs. We did mRNA transcriptome profiling of kidney tissue by RNA sequencing (6HCT recipients with kidney injury and 51 allograft recipients [16 ACR, 17 AMR and 18 Normal]). Venn diagram depicts the number of mRNAs that were statistically significant (FDR-P <0.05) between HCT versus Normal, ACR versus Normal and, AMR versus Normal. Probability values were adjusted for false discovery rate using the Benjamini-Hochberg method and is the expected proportion of false positives among all the statistically significant P-values (<0.05)

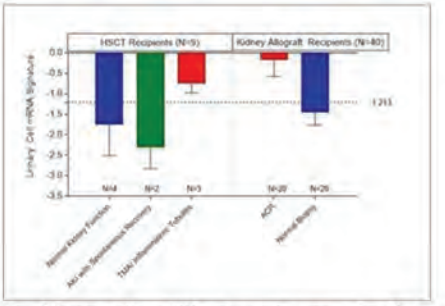

Figure 2. Urinary cell mRNA signature score in HCT recipients and kidney transplant recipients. CTOT-04 signature (Suthanthiran et al. N Eng J Med 2013) is a score derived from urinary cell mRNA profiling of kidney transplant recipients for the noninvasive diagnosis of acute rejection of kidney allograft. The score is a liner combination of urinary cell levels of CXCL10 mRNA, CD3e mRNA, and 18S rRNA, quantified by RT-PCR assay and expressed as copies/µg of total RNA. The dotted line in the figure is the cut point value (-1.213) for the diagnosis of ACR in kidney transplant recipients. We collected urine from HCT recipients and kidney allograft recipients, isolated total RNA from urinary cells, reverse transcribed to cDNA, measured the absolute quantity of urinary cell transcripts, and calculated the CTOT-4 signature score for each patient. Figure depicts the mean ± SE of the CTOT-04 signature score.

PO2194

The Role of Hyperleptinaemia and Low Values of Interleukin 10 in De Novo Donor-Specific Antibody Production After Kidney Transplantation

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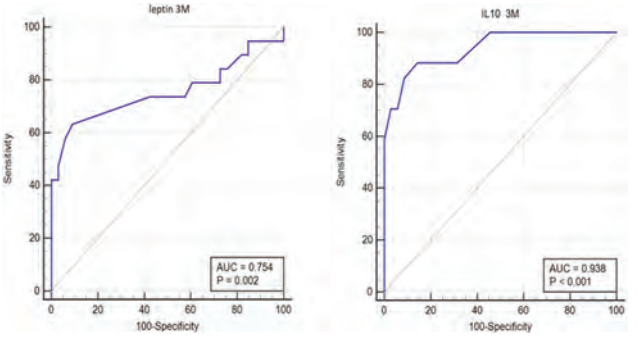
Background: White adipose tissue secretes a number of peptide hormones, including leptin, adiponectin, and several cytokines. The aim of this paper was to determine the role of selected adipocytokines (leptin and adiponectin) and interleukins (IL-10 and IL-6) on the development of graft rejection in protocol biopsy after kidney transplantation.

Methods: In a prospective analysis (n=104), we monitored the values of leptin, adiponectin, IL-6, and IL-10 prior to the transplantation and in the 3rd month after the transplantation. The protocol biopsy of the graft was performed in the 3rd month after the transplantation. The group was divided into the following according to the biopsy result: negative result, IFTA 1, borderline, and DSA positive.

Results: After adjusting for the differences in the baseline recipient and donor characteristics, we identified the hyperleptinaemia baseline (HR=2.0444, P=0.0341) and month 3 (HR=49.8043, P<0.0001) as independent risk factors for borderline changes in the protocol biopsy. The hyperleptinaemia baseline (HR=7.4979, P=0.0071) and month 3 (HR=9.7432, P=0.0057) are independent risk factors for de novo DSA positivity. A low value of IL-10 month 3 is a risk factor for de novo DSA positivity (HR=3.0746, P=0.0388).

Conclusions: Higher leptin levels might play a role in rejection and de novo DSA production. We also confirmed the influence of low values of IL-10 on the development of de novo DSA. We assume that values of adipocytokines in context of other risk factors can predict the immunological risk of patients after kidney transplantation.

Funding: Government Support - Non-U.S.



ROC curve. Leptin 3M and IL-10 3M for the endpoint of de novo DSA and borderline

PO2195

Diagnostic Performance of Donor-Derived Cell-Free DNA Assay (AlloSure®) in Kidney Transplant Recipients with Graft Dysfunction: A Single-Center Study

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Background: Circulating donor-derived cell-free DNA (dd-cf-DNA) is a non-invasive biomarker of kidney allograft injury with a high negative predictive value for ruling out active rejection in patients with evidence of graft dysfunction. At our center, we