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Association of Under Immunosuppression as Defined by Torque Teno Viral Load and Clinical Outcomes in Kidney Transplant Recipients

Ahmed N[1], Speroni L[2], Chen K[3], Guo K[3], Zhao L[3], Rebello C[3], Kleiboeker S[3], Kinsella B[3], Friedewald J[3], Desai A[3]
[1]A.T. Still University School of Osteopathic Medicine, Mesa, AZ, [2]Chapman University, Orange, CA, [3]Northwestern University Feinberg School of Medicine, Chicago, IL

Background

- Identifying markers for over-immunosuppression remains challenging
- Torque teno virus (TTV) is promising due to its high prevalence in healthy individuals and lack of association with human disease
- Previous studies suggest TTV viral load correlates with the net state of immunosuppression, with lower TTV loads associated with subclinical alloreactivity and reduced graft injury[1]

Research objectives

- Validate TTV as a reliable measure of net state of immunosuppression
- Determining if under-immunosuppression differs when assessing a combination of TTV viral load and other biomarkers as determined by reaching a 2-year composite endpoint as opposed to solely relying on a TTV viral load threshold of <10^4

Methods

- 1,980 samples collected from 256 kidney transplant recipients (KTRs) enrolled in the Clinical Trials in Organ Transplantation-08 (CTOT-08; NCT01289717) study[2]
- The primary clinical composite endpoint (CME) included three validated criteria: 24-month biopsy (central read) indicating chronic injury-IFTA (Banff grade ⩾II IFTA [ci ⩾2 or ct ⩾2]), BPAR on any "for-cause biopsy" (central read), or a >10 mL/min/1.73 m² decrease in estimated glomerular filtration rate between 4 and 24 months post-transplant according to the Chronic Kidney Disease Epidemiology Collaboration
- Under-immunosuppression was characterized by a low TTV, high TruGraf score, high TRAC, positive biopsy for rejection, previous higher TTV load by a logarithmic scale, unstable creatinine, weak induction therapy, high Banff score, and decrease in BK/CMV viral load
- TTV copies/mL were calculated by applying the sample's TTV NGS read number to a linear regression equation derived from analysis of a sample subset by an independent TTV qPCR assay
- For the TTV qPCR assay, viral concentrations were derived from a standard curve defined as under immunosuppressed by $<10^4$

Table 1: Frequency Table for Reaching The Delta eGFR Endpoint

Defined by viral load and biomarkers

Ct group	No	Yes
Group 4	37	7
Group 1	30	1
Group 2	49	16
Group 3	45	8

Pearson's Chi-squared test
X-squared = 7.1303, df = 3, p-value = 0.06786

Defined by TTV load $<10^4$

Ct group	No	Yes
Group 4	85	17
Group 1	19	2
Group 2	31	7
Group 3	25	5

Pearson's Chi-squared test
X-squared = 0.84732, df = 3, p-value = 0.8381

There is no statistical significance between the categories of delta eGFR and under immunosuppression count group (**p=0.068**) defined by viral load and biomarkers
There is no significant association between the categories of delta eGFR component and under immunosuppression count group (p=0.8381) when removing the NA observations defined by TTV viral load $<10^4$

Table 2: Logistic Regression for Reaching The Delta eGFR

Defined by viral load and biomarkers

Ct group	Odds ratio	95% CI	P value
Group 1 and group 4	0.176	0.021-1.512	0.113
Group 2 and group 4	1.726	0.644-4.624	0.278
Group 3 and group 4	0.940	0.312-2.833	0.912

Defined by TTV load $<10^4$

Ct group	Odds ratio	95% CI	P value
Group 1 and group 4	0.526	0.112-2.473	4.161e-01
Group 2 and group 4	1.129	0.427-2.983	8.065e-01
Group 3 and group 4	1.000	0.335-2.981	1.000

No statistical significance was noted in graft outcome when logistic regression was adjusted for age and gender.

Table 3: Frequency Tables for Biopsy Chronic Fibrosis Endpoint Analysis

Defined by viral load and biomarkers

Ct group	No	Yes
Group 4	38	6
Group 1	27	4
Group 2	58	7
Group 3	44	9

Pearson's Chi-squared test
X-squared = 7.1303, df = 3, p-value = 0.06786

Defined by TTV load $<10^4$

Ct group	No	Yes
Group 4	89	13
Group 1	18	3
Group 2	31	7
Group 3	28	2

Pearson's Chi-squared test
X-squared = 0.84732, df = 3, p-value = 0.8381

Biopsy component defined as Banff IFTA grade 2 or higher (interstitial fibrosis and tubular atrophy)

There is no significant association between the categories of IFTA2 component and under immunosuppression count group (p=0.807) when removing the missing values of IFTA2 observations.

There is no significant association between the categories of IFTA2 component and under immunosuppression count group (p=0.5571) when removing the NA observations defined by TTV load $< 10^4$

Results

Definitions:

1. Under immunosuppression percentage for a patient:
   number of "Under" defined as $<10^4$/number of total immunosuppression records.
2. Under immunosuppression count group:
   group1: ct=0;
   group2: ct>=1 and ct<3;
   group3: ct>=3 and ct<5;
   group4: ct>=5

   Recipient categorical age group:
   group1: AGE<50;
   group2: AGE>=50 and AGE<=65;
   group3: AGE>65;

- No significant difference was observed when comparing results between viral and biomarkers to a defined TTV viral load of $<10^4$

Conclusions

- Data showed a trend towards a significant association between more frequent episodes of under immunosuppression and the delta eGFR endpoint.
- Limitations of the study include small sample size, missing data points, and short 2-year follow up.

Further studies

- Longer term data collection and follow up is recommended to better asses clinical outcomes in the three categories of immunosuppression. Next steps will be to evaluate graft survival in CTOT8 data out to 10 years.
- The desired goal is to utilize TTV viral load defined by under immunosuppression of <10^4 which will allow real time informed titration of immunosuppression to prevent over/under immunosuppression.