Non-Invasive Metabolite-Based Urine Signature Detects Over-Immunosuppression in Renal Transplant Recipients

Managing complications related to over-immunosuppression represents a growing challenge in post-transplant care, with infections accounting for the second leading cause of death with functioning graft (DWFG) in renal transplant recipients (RTRs) within the first year. At present, there are no clinically validated biomarkers to detect over-immunosuppression. Polyomavirus-associated nephropathy (PVAN) is a specific type of opportunistic infection indicative of over-immunosuppression that occurs in 5-10% of RTRs and causes up to 60% of graft failures. Biopsy remains the gold standard for diagnosis, and an increased blood viral copy number is associated with an increased risk for PVAN. Using urine, we leveraged metabolic profiling and machine learning to develop a novel metabolite-based signature to differentiate RTRs with a stable graft (no rejection or infection) from those developing biopsy-confirmed PVAN during follow-up. These results could lead to a non-invasive assay for the early detection of PVAN.

**WHAT IS THE PROBLEM?**

**DIFFERENTIAL METABOLITE RESONANCES IN OVER VS. STABLE RTRS**

Using a Kruskal-Wallis (KW) non-parametric one-way analysis of variance, we identified 24 differential metabolite resonances with fold-change >1.5 and p-value <0.05 between Over and Stable patients. This false discovery rate (FDR) was calculated to correct for multiple hypothesis testing, with many metabolite resonances with an FDR <0.1. Using chemical shift mapping, many differential resonances were mapped to the same metabolite. Resonances requiring experimental validation to distinguish matching-ambiguities are listed as “Unknown” and tracked by chemical shift.

**GENERATING THE CHAMPION MODEL**

Pre-defined feature sets were generated including “Known Metabolic Resonances” (differential metabolite resonances assigned to a unique metabolite), “Unknown Metabolic Resonances,” and “Differential Clinical Features” (available sample-donor information such as gender, leukopenia, HLA matching, drug dose, trough levels) with 10-fold cross-validation of the data (Support Vector Machine [SVM]) and 83% accuracy on a test set.

**PERFORMANCE OF myOLARIS SCORE FOR DETECTION OF OVER-IMMUNOSUPPRESSION & STABLE RTRS**

The champion model was converted into a proprietary myOLARIS Score that ranges from 0 to 1, such that Over patients (orange) score lower than Stable patients (blue). Applying the myOLARIS Score to the full dataset demonstrated excellent separation between Over and Stable RTRs with an overall accuracy of 83%. This suggests that a urine metabolite signature can identify patients who are at risk for developing PVAN over those that are at low risk for either a rejection or infection event, i.e., the stable patients. The high positive predictive value (PPV) of 81% and high negative predictive value (NPV) of 86% offer the exciting possibility that this assay could be used for both rule-in early diagnosis of PVAN and rule-out diagnosis of any event (rejection or infection), potentially eliminating the need for a biopsy.

**CONCLUSIONS AND NEXT STEPS**

Determining the appropriate balance of immunosuppressant remains a clinical challenge for kidney transplant recipients, as well as all solid organ transplant recipients. Underimmunosuppression can lead to life-threatening complications, while over-immunosuppression can lead to chronic graft rejection, while over-immunosuppression can lead to complications such as infection, cardiovascular disease, post-transplant diabetes and malignancy. While numerous tools are available to detect risk of under-immunosuppression, there are few, if any, tools to detect and prevent complications of over-immunosuppression. In this study, we demonstrated that a metabolite-based urine signature was able to classify over-immunosuppressed RTRs with PVAN from those with a stable graft with high accuracy. Validation studies in larger cohorts with longitudinal samples are underway, which could lead to a powerful new biomarker for post-transplant monitoring, empowering earlier diagnosis of PVAN and providing physicians and patients confidence when appropriate immunosuppression has been achieved for stable graft function.

**REFERENCES**


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