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

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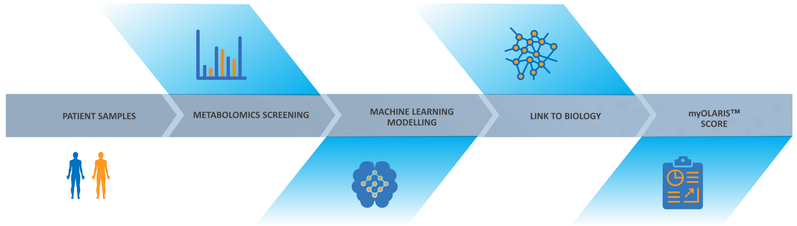
WHAT IS THE PROBLEM?

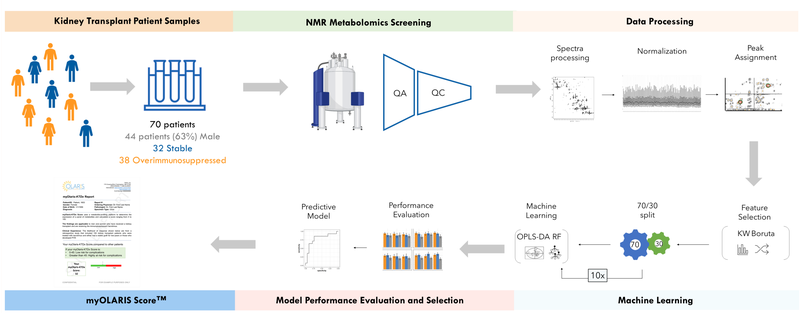
Managing complications related to over-immunosuppression represents a growing challenge in post-transplant care, with **infections accounting for the second leading cause of death with functioning graft (DWFG)** in renal transplant recipients (RTRs) within the first year[1]. At present, there are no clinically validated biomarkers to detect over-immunosuppression[2]. **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[3]. Biopsy remains the gold standard for PVAN diagnosis, and only strongly 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.

THE OLARIS CEREBRO PLATFORM COMBINES METABOLOMICS & MACHINE LEARNING

Altered metabolism has been linked to kidney function and kidney transplant tolerance. By measuring the complete set of metabolites in an individual (their metabolome), it is possible to identify biomarkers that correlate with disease status, prognosis, and therapeutic response. We detect and quantify **metabolites** from hundreds of patient biofluid samples (blood and/or urine). Using **NMR-** and **MS-** based metabolomics and **machine learning,** we identify a biomarker-based score that can monitor patient response to treatment with a high degree of accuracy.

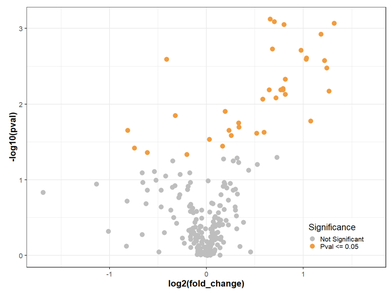


STUDY DESIGN



Urine metabolites were extracted and analyzed via 1D ^{1}H and 2D ^{1}H-^{13}C Heteronuclear Spectrum Quantum Coherence (HSQC) NMR spectroscopy of samples from **70 RTRs collected 3 months post-transplant, including N=38 with subsequent biopsy-proven PVAN (labeled "Over") and N=32 with stable graft function and no histological signs of rejection or indications of over-immunosuppression for 2 years (labeled "Stable").** Spectra were processed using in-house processing and normalization tools and metabolite resonance peaks were assigned to metabolites based on chemical shift mapping to a library of known metabolites. Differential metabolite resonances between Over and Stable samples were then used to generate cross-validated machine learning models, including orthogonal partial least square discriminate analysis (OPLS-DA) and random forest (RF). The model with the highest stability and overall performance was selected as the champion model. Based on the champion model, we then developed a scoring method (**myOLARIS-*KTdx*™ Score**) with high accuracy to differentiate Stable and Over RTRs.

DIFFERENTIAL METABOLITE RESONANCES IN OVER VS. STABLE RTRS



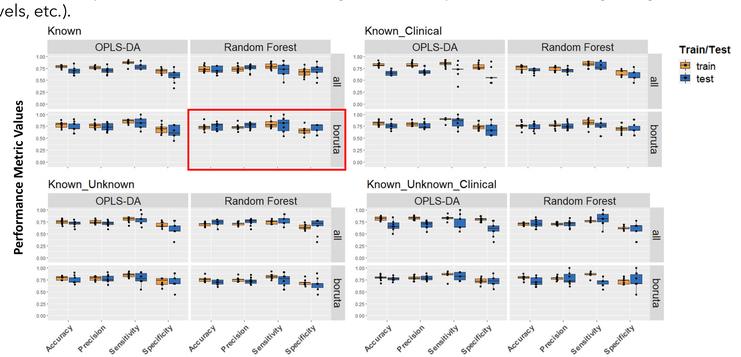
Annotation	1H ppm	13C ppm	Fold change (Stable/Over)	P-value	FDR
Trigonelline	9.117	148.323	1.74	0.001	0.052
Trigonelline	8.841	147.243	1.578	0.001	0.052
Trigonelline	8.08	130.247	1.567	0.006	0.087
4-Ethylbenzoic acid	7.817	131.399	2.405	0.007	0.087
Unknown_7.531_129.748	7.53	129.739	2.268	0.001	0.056
Unknown_7.532_134.804	7.531	134.794	2.366	0.003	0.065
3-Hydroxymandelic_acid,L-Phenylalanine,4-Methoxyphenylacetic acid	7.292	132.713	1.758	0.007	0.092
Unknown_7.348_121.584	7.347	121.584	1.73	0.006	0.087
Unknown_7.085_121.7	7.084	121.706	2.047	0.002	0.056
Salicyluric_acid	6.974	120.638	1.729	0.006	0.087
Unknown_7.274_116.531	7.273	116.5	1.967	0.002	0.056
Homoveratric_acid	6.914	115.412	1.758	0.005	0.084
Quinic acid	1.994	40.118	1.602	0.002	0.056
Hippuric acid	7.818	129.759	1.701	0.006	0.087
Trigonelline	4.441	50.943	1.627	0.001	0.052
Unknown_3.813_62.593	3.81	62.602	1.509	0.024	0.19
Unknown_2.712_47.752	2.712	47.748	0.598	0.038	0.261
Unknown_2.566_47.724	2.566	47.755	0.569	0.022	0.185
Unknown__3.787_73.579	3.785	73.587	0.655	0.044	0.293
4-Aminohippuric acid, Pteroyltriglutamic acid	7.624	131.127	1.646	0.008	0.095
Hippuric acid	7.618	134.806	2.494	0.001	0.052
Hippuric acid	7.531	131.362	2.33	0.003	0.056
Hippuric_acid	3.969	46.485	2.04	0.003	0.056
Unknown_3.876_35.932	3.874	35.924	2.107	0.017	0.163

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. The 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

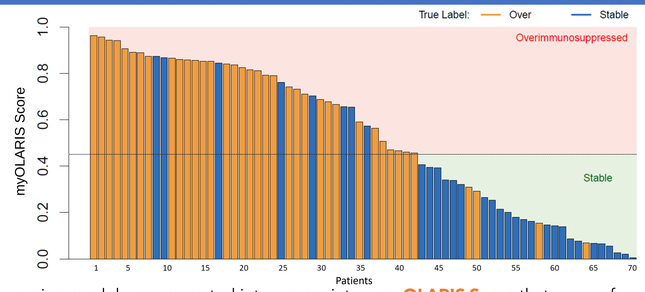
Known Metabolic Resonances	Unknown Metabolic Resonances	Clinical Features	
3-Hydroxymandelic_acid,L-Phenylalanine, 4-Methoxyphenylacetic acid_7.292_132.713	Unknown__3.787_73.579	gender	median_dose_mmf
4-Aminohippuric acid, Pteroyltriglutamic acid_7.624_131.127	Unknown_2.566_47.724	leukopenia_episodes	mean_dose_mmf
4-Ethylbenzoic acid_7.817_131.399	Unknown_2.712_47.752	num_HLA_A_locus	iqr75_BW
Hippuric acid_7.531_131.362	Unknown_3.813_62.593	num_HLA_B_locus	iqr25_BW
Hippuric acid_7.618_134.806	Unknown_3.876_35.932	num_HLA_DR_locus	median_BW
Hippuric acid_7.818_129.759	Unknown_7.085_121.7	acute_rejection_episodes	mean_BW
Hippuric_acid_3.969_46.485	Unknown_7.274_116.531	iqr75_dose_steroids	starting_dose_tac
Homoveratric_acid_6.914_115.412	Unknown_7.346_121.584	iqr25_dose_steroids	iqr75_dose_tac
Quinic acid_1.994_40.118	Unknown_7.531_129.748	median_dose_steroids	iqr25_dose_tac
Salicyluric_acid_6.974_120.638	Unknown_7.532_134.804	mean_dose_steroids	median_dose_tac
Trigonelline_4.441_50.943		dose_MMF	mean_dose_tac
Trigonelline_8.08_130.247		iqr75_dose_mmf	iqr75_level_tac
Trigonelline_8.841_147.243		iqr25_dose_mmf	iqr25_level_tac
Trigonelline_9.117_148.323			median_level_tac
			mean_level_tac

Pre-defined feature sets were generated including "**Known** Metabolic Resonances" (differential metabolite resonances assigned to a unique metabolite), "**Unknown** Metabolic Resonances" (differential metabolite resonances requiring experimental clarification), and "**Clinical** Features" (available sample-donor information such as gender, leukopenia, HLA matching, drug dose, trough levels, etc.).



Data were split 70/30 into training and test data, 10x cross-validated, and assessed based on accuracy, precision, sensitivity, and specificity. **A total of 16 ML models (8 OPLS-DA and 8 RF) were generated using different combinations of "Known", "Unknown" and "Clinical" features** with and without Boruta feature selection. Boruta selects features based on the RF importance of the unchanged vs. shuffled feature and can be used as a feature reduction method[4]. The RF Model with "Known" metabolites and optimized with Boruta feature selection was selected as the champion model (red box) based on optimal performance in training/test cohorts across all metrics.

PERFORMANCE OF myOLARIS SCORE FOR DETECTION OF OVER-IMMUNOSUPPRESSED & STABLE RTRS



The champion model was converted into a proprietary **myOLARIS Score** that ranges from 0 to 1, such that Over patients (orange) have higher scores 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 from 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.**

myOLARIS-*KTdx* Score
84% AUC
83% Accuracy
89% Sensitivity
75% Specificity
81% PPV
86% NPV

CONCLUSIONS AND NEXT STEPS

Determining the appropriate balance of immunosuppressant remains a clinical challenge for kidney transplant, as well as all solid organ transplant. Underimmunosuppression can lead to acute and/or chronic graft rejection, while overimmunosuppression 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 early diagnosis of PVAN and providing physicians and patients confidence when appropriate immunosuppression has been achieved for stable graft function.**

REFERENCES ~ ACKNOWLEDGMENTS

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