Performance of Tutivia™ RNA Signature in Kidney Transplant Patients with Multi-organ and Previous Failed Kidney Transplants

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Background

Verici Dx has validated Tutivia, an RNA Signature comprised of a machine learning derived select set of gene expression features + algorithm to produce a prognostic, post-transplant peripheral blood-based risk score (0 – 100) for acute rejection in kidney transplant patients.[1,2] The Tutivia risk assessment results are intended to inform clinicians in support of medical management decision making.

Acute rejection (AR) in the first year is associated with kidney function decline and graft loss, supporting the need for early detection and intervention.[3,4] Graft loss results in patients receiving a 2nd, 3rd or more kidney allograft. These patients are considered to be at higher risk for acute rejection post-transplant.[3,4] Multi-kidney transplantation is prevalent, and approximately 15% of the current kidney transplant waiting list in the US has had at least one previous failed kidney transplant. In addition to multiple kidney transplants, another important subset of transplant patients receive multiple organs, such as kidney/pancreas. Recent publications have reported that utility of dd cfDNA biomarker testing is limited in multi-organ transplant because multiple sources of donor derived cfDNA result in chronically elevated and highly variable cfDNA in these patients.[5] Moeller et al reported prevalence of positive cfDNA tests in 42.2% of patients studied with kidney-heart transplants.[5] Pettersson et al studied cfDNA test performance in patients with previous failed kidney transplant and reported that cfDNA from the failed kidney was detectable in 20% of their clinical patients.[6] The authors further reported that the amount of detected dd-cfDNA from the previous donor is in a range where it could influence clinical decision-making. Huang and colleagues studied performance of dd cfDNA in kidney transplant including 15 patients who had a previous failed kidney transplant. In this group, cfDNA overall test accuracy was poor at 53% (26.6-78.7).[7]

No biomarker has currently been shown to provide sensitive and specific results in support of clinical evaluation in the setting of previous failed transplant / multi-organ transplant. Herein we present the performance characteristics of Tutivia in patients in this population.

Methods and Study Cohort

Tutivia is an RNA Signature using next-gen sequencing to quantify gene expression in the peripheral blood of kidney transplant recipients to inform the algorithm and does not rely on methods requiring differentiating donor from recipient. In the Tutivia all-comers clinical validation study, 21 of 151 (14%) patients had a previous failed kidney transplant, and 17 (11%) of 151 had both kidney and pancreas transplants. Three patients were in both of these groups. Study population is described in Table 1.

We evaluated overall test accuracy in this subset of patients and compared this to the accuracy of Tutivia in the first-time, kidney-only transplant recipients to assess comparability of performance in patients with multiple transplanted organs. All Tutivia test results were derived from blood collected anytime within 6-7 months following transplant and assessed for accuracy in comparison to kidney biopsy, protocol or for-cause, read centrally according to BANFF 2019 criteria. No exclusions were made as long as the patient had a blood sample that passed laboratory QC and an acceptable biopsy; borderline AR was classified as an AR outcome.

Clinical performance for test accuracy was performed using Medcalc (MedCalc® Statistical Software version 20.210 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2022). Tutivia risk score distributions were compared using two way analysis of variance (ANOVA) using R package version 0.4.0 (https://CRAN.R-project.org/package=ggpubr).

Deconvolution was performed on RNA in patient samples from the 35 transplant recipients in the multi-organ/previous failed kidney transplant cohorts to determine the percent composition of various immune cells, Figure 2, in patients with biopsy confirmed rejection vs non-rejection using iSort™ Fractions, an AI-powered digital cytometry platform that deconvolutes bulk RNA-seq data to estimate the relative proportions of distinct immune cell types in a mixed sample. Differences in cell populations were compared using two-sided unpaired t test.

Results

The full validation set included 31% (47/151) AR event rate including ABMR, borderline, TCMR IA or higher, or mixed rejection. Test accuracy in this group was 74.17% (66.43-80.95) with a hazard ratio (HR) of 5.74. The multi-organ subset had a 29% event rate (10/35), Table 2, with a test accuracy of 74.3% (56.74-87.51) and an HR of 5.25 while the remaining validation set after removal of the multi-organ group had an event rate of 32%, test accuracy of 74.14% (65.18-81.82) and HR of 5.89. Negative and positive predictive values were comparable across the comparator groups with NPV = 79% in all, and PPV between 58-60% in all groups, Figure 1A.The patient risk score distributions within the three comparator groups were not different, violin plots, Figure 1B, p=0.95. Additional findings in this clinical set included BKN in 3 patients, one with previous failed transplant, one with kidney/pancreas transplant and one with both, each of whom had no AR on biopsy and low risk Tutivia results. iSort™ Fractions deconvolution data quantified 10 immune cell types from their gene expression profiles, Figure 2. Analysis of composition differences in patients with biopsy confirmed rejection vs no rejection were found in monocytes, P<0.00005, macrophages, P<0.0005, and mast cells, P<0.05.

Table 1. Study Population

Study Population	Overall (N=35)		
Recipient age		**NO_TRANSPLANTS**	
Mean (SD)	51.5 (13.50)	0	14 (40.0%)
Median [Min, Max]	51 [24,79]	1	18 (51.4%)
		2	3 (8.6%)
Sex		**CIT**	
Male	25 (71.4%)	Mean (SD)	10.1 (7.40)
Female	10 (28.6%)	Median [Min, Max]	10.31 [0.46, 25.5]
Race		**HLA mm**	
Black	10 (28.6%)	0 - 4	14 (40%)
White	23 (65.7%)	5 - 8	21 (60%)
Missing	2 (5.7%)		
Ethnicity		**DE_NOVO_DSA**	
Hispanic/Latino	2 (5.7 %)	No	35 (100.0%)
Not Hispanic/Latino	32 (91.4%)	Yes	0 (0.0%)
Missing	1 (2.9%)		
Donor Status		**Induction Medication**	
LRD	7 (20.0%)	ATG/Thymo	20 (57.1%)
LURD	5 (14.3%)	ATG/Thymo & IL2RA	1 (2.9%)
SCD	14 (40.0%)	IL2RA	2 (5.7%)
ECD	4 (11.4%)	Campath	12 (34.3%)
DCD	5 (14.3%)	Methylprednisolone	35 (100.0%)
Kidney Pancreas Transplant		**PRA**	
No	18 (51.4%)	Class I >30%	10 (28.6%)
Yes	17 (48.6%)	Class II >30%	15 (42.9%)

Table 2. Biopsy

HP Findings	Number (%)
ABMR	5 (14%)
Mixed	2 (6%)
TCMR IA+	1 (3%)
Borderline	2 (6%)
Death	3 (7%)
Graft Loss	2 (6%)
No Rejection/normal	25 (71%)
AKI/ATN	4 (11%)
BKN	3 (9%)
TMA	3(9%)
Other	2 (6%)

Figure 1. Tutivia Clinical Performance Comparisons

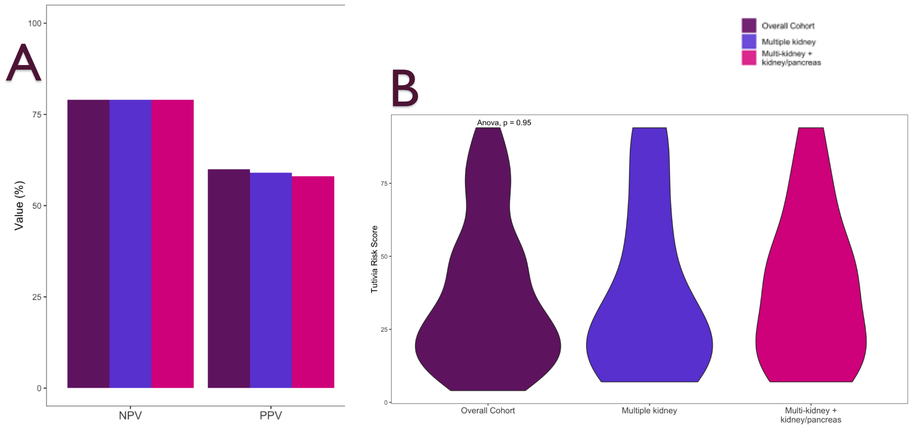
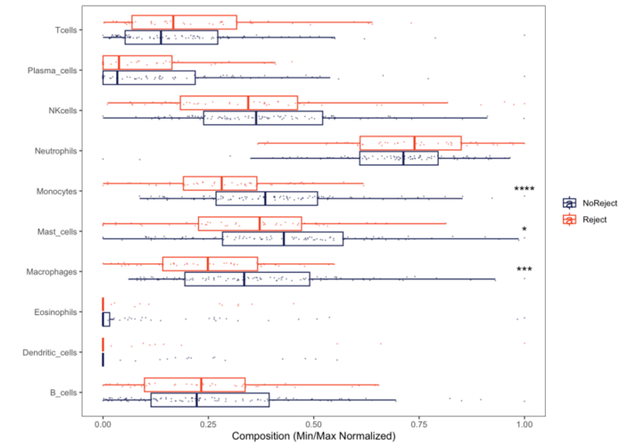


Figure 2. Immune Cell Composition in Tutivia



*<0.05, **P<0.005, ***P<0.0005, ****P<0.00005

Conclusions

- Biomarker dd cfDNA testing in patients with previous failed transplant or in multi-organ recipients can be prone to elevated and highly variable results with poor test accuracy. The accuracy of 74% across the Tutivia validation set, and in first time kidney transplant recipients, patients with previous failed kidney transplant and those with multi-organ transplant demonstrates that Tutivia is able to achieve comparable accuracy in the multi-organ recipient patients providing an advancement in biomarker testing with significant improvement in test accuracy in higher-risk kidney transplant patients, such as those with previous failed transplant.
- One limitation is this study includes a limited size study cohort, n = 35. This may have particularly impacted the immune cell deconvolution as comparisons were made between 10 acute rejection patients and 25 non-rejection patients. Moreover, the types of AR were different, and may well have had different immune cell profiles, but the size of the cohort was too small to evaluate different types of rejection separately.
- A second limitation is that each patient was evaluated at a single timepoint that correlated to biopsy. More studies with a larger population are needed to evaluate the performance of Tutivia™ longitudinally and to evaluate risk results with correlation to long-term outcomes.
- Tutivia represents an advancement to support clinicians that has not been previously offered in biomarker transplant biology. The favorable hazard ratio, PPV and NPV in Tutivia™ clinical performance in this particularly challenging population demonstrates that RNA Signature Tutivia™ informs prognostic interpretations supporting clinical management.

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