Immune monitoring of allograft status in kidney transplant recipients

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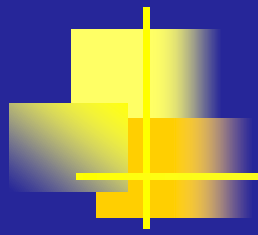


Introduction

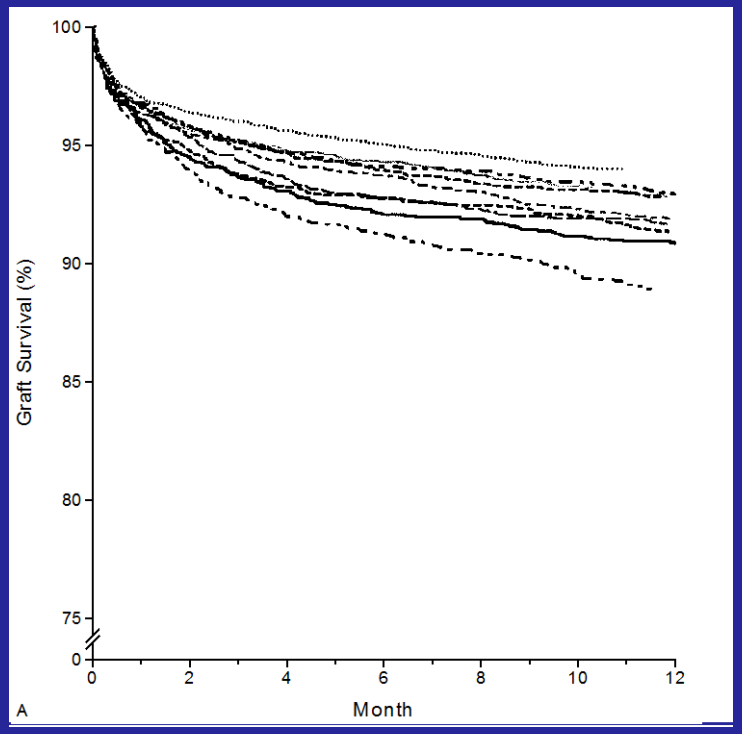
The New England
Journal of Medicine

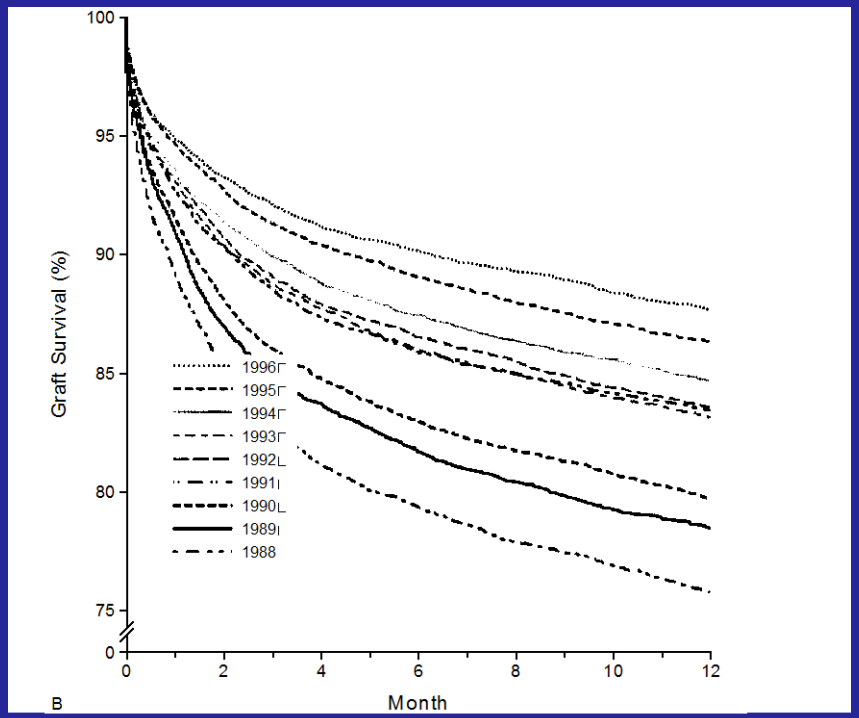
VOLUME 342 MARCH 2, 2000 NUMBER 9

IMPROVED GRAFT SURVIVAL AFTER RENAL TRANSPLANTATION IN THE UNITED STATES, 1988 TO 1996

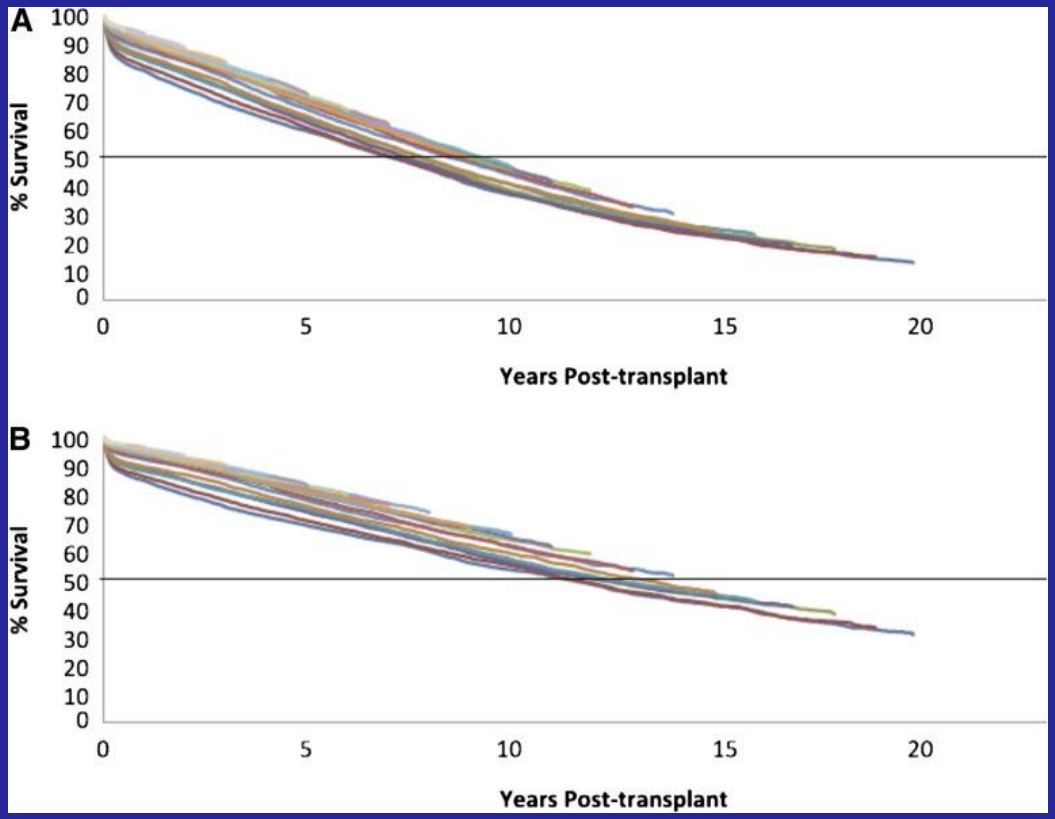
SUNDARAM HARIHARAN, M.D., CHRISTOPHER P. JOHNSON, M.D., BARBARA A. BRESNAHAN, M.D., SARAH E. TARANTO, B.A., MATTHEW J. MCINTOSH, PH.D., AND DONALD STABLEIN, PH.D.

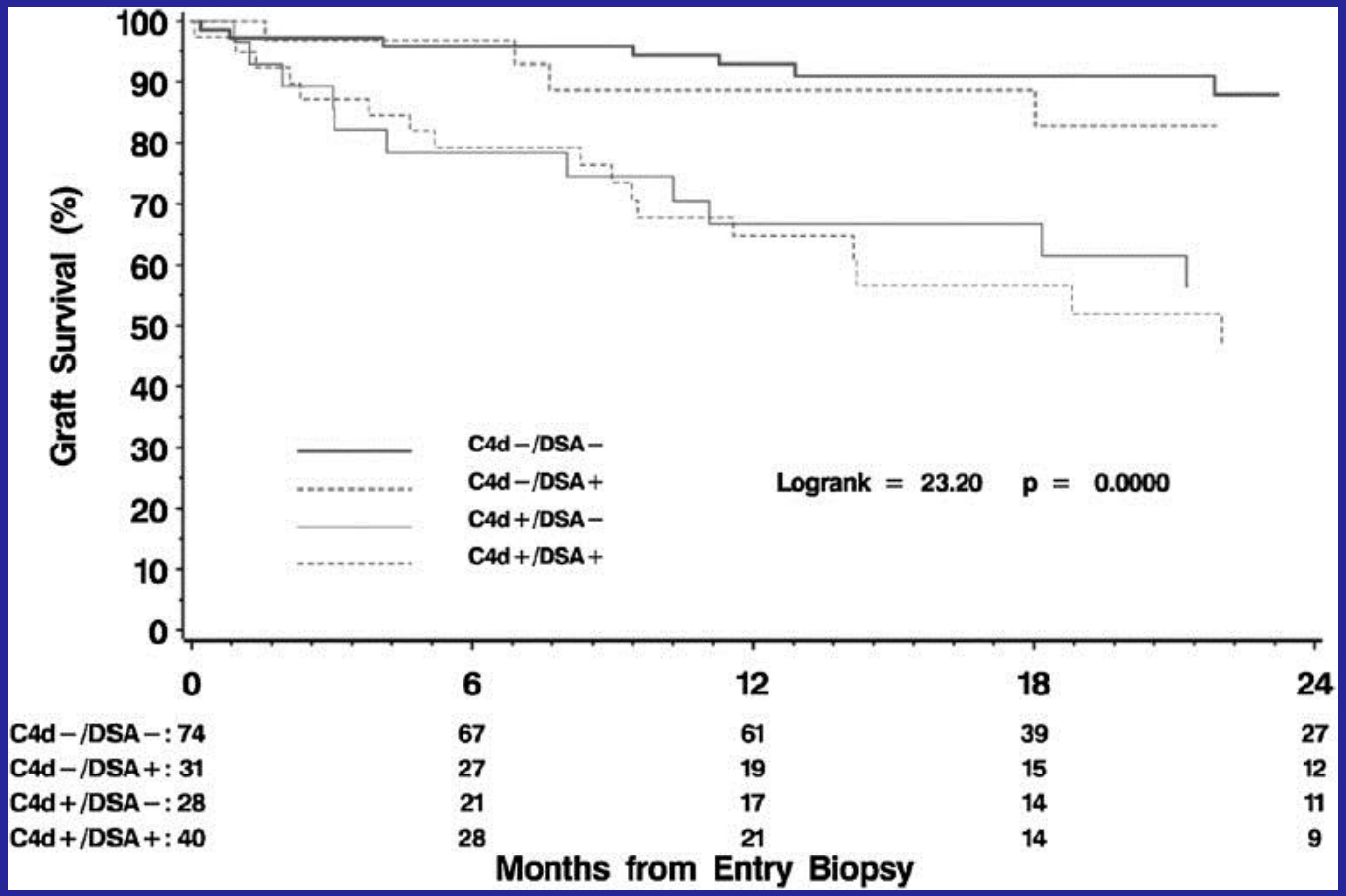
IMPROVED GRAFT SURVIVAL AFTER RENAL TRANSPLANTATION IN THE UNITED STATES, 1988 TO 1996

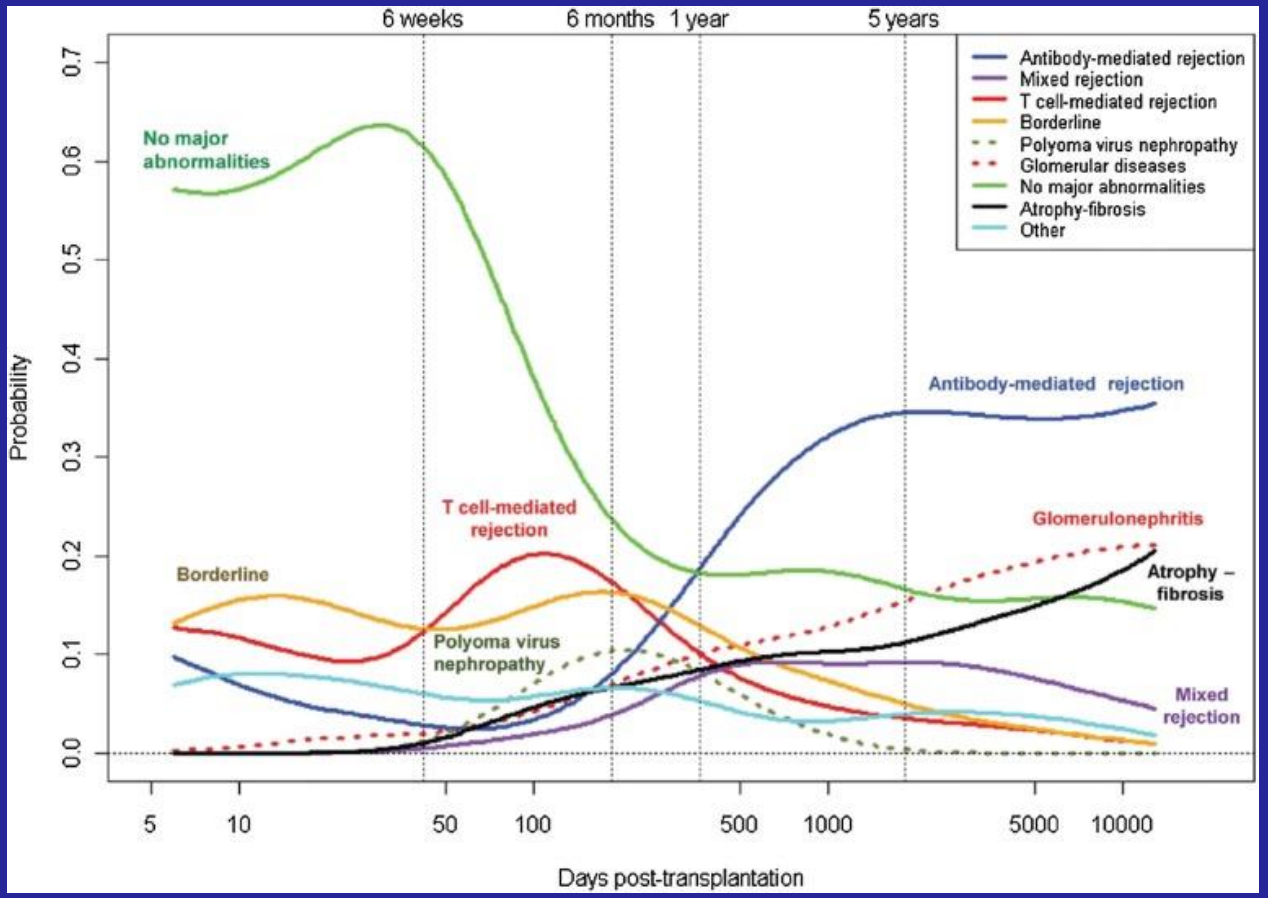




Seeking Clarity in Preventing Late Kidney Transplant Failure




Graft Survival (%)
100
90
80
70
60
50
40
30
20
10
0
C4d−/DSA−
C4d−/DSA+
C4d+/DSA−
C4d+/DSA+
Logrank = 23.20 p = 0.0000
0
6
12
18
24
C4d−/DSA−: 74 67 61 39 27
C4d−/DSA+: 31 27 19 15 12
C4d+/DSA−: 28 21 17 14 11
C4d+/DSA+: 40 28 21 14 9
Months from Entry Biopsy


6 weeks
6 months
1 year
5 years
Antibody-mediated rejection
Mixed rejection
T cell-mediated rejection
Borderline
Polyoma virus nephropathy
Glomerular diseases
No major abnormalities
Atrophy-fibrosis
Other
No major abnormalities
Antibody-mediated rejection
T cell-mediated rejection
Glomerulonephritis
Borderline
Atrophy – fibrosis
Polyoma virus nephropathy
Mixed rejection
Probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
5
10
50
100
500
1000
5000
10000
Days post-transplantation

Introduction

- Improved methods are needed to individualize immunosuppression for each patient to ensure adequate coverage.

- while reducing the risks of chronic allograft changes and infection.

Introduction

- Lag between rise of Cr and tissue damage,
- Protocol biopsy

0041-1337/04/7708-1194/0
TRANSPLANTATION

Vol. 77, 1194–1198, No. 8, April 27, 2004
Printed in U.S.A.

SUBCLINICAL REJECTION AND BORDERLINE CHANGES IN EARLY PROTOCOL BIOPSY SPECIMENS AFTER RENAL TRANSPLANTATION

IAN S. D. ROBERTS,[1,3] SRIKANTH REDDY,[2] CHRISTINE RUSSELL,[2] DAVID R. DAVIES,[1] PETER J. FRIEND,[2] ASHOK I. HANDA,[2] AND PETER J. MORRIS[2]

Background. **To determine the significance of early subclinical rejection, we reviewed protocol biopsies performed on days 7 and 28 during a 4-year period.**

Methods. **The study was confined to patients (n=115) with stable graft function at the time of biopsy; 76 adequate biopsies at day 7 and 79 at day 28 were performed.**

Results. **At day 7, 10 biopsy specimens (13%) showed acute rejection (AR) and 9 (12%) showed borderline changes. Eight of 10 patients with AR received immediate pulsed methylprednisolone (MP) and one untreated patient developed clinical rejection (CR) within 3 days. Four of nine patients whose biopsy specimens showed borderline changes received MP and three untreated patients developed CR within 3 days. At day 28, six biopsy specimens (8%) showed AR and 13 (16%) showed borderline changes. Three of six patients with AR received immediate pulsed MP and one untreated patient developed CR within 6 days. Ten of 13 patients with borderline changes had been treated for AR in the previous 3 weeks. Twelve patients with subclinical rejection or borderline changes at day 28 were never subsequently treated for rejection, and outcome at 6 years did not differ from those patients whose biopsy specimens showed no rejection.**

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Received 25 June 2003.

Revision requested 8 August 2003. Accepted 14 October 2003.

DOI: 10.1097/01.TP.0000118905.98469.91

Invasive methods of allograft monitoring

- The gold standard for diagnosis of allograft dysfunction is the kidney biopsy.
- The procedure is invasive and not without risk of complications.

American Journal of Transplantation 2016; 16: 1298–1305
Wiley Periodicals Inc.

doi: 10.1111/ajt.13622

Brief Communication

Complications of Ultrasound-Guided Renal Transplant Biopsies

T. A. Morgan[1,*], S. Chandran[2], I. M. Burger[3], C. A. Zhang[1] and R. B. Goldstein[1]

or as part of posttransplant management (at 6 and 12 mo postoperatively in adults and at 3 and 12 mo postoperatively in pediatric patients at our institution) to screen

kidney biopsy

- Single-center 5-year retrospective cohort analysis of 2514 biopsies.
- Major complications occurred in 47 of 2514 patients (1.9%) and included hospitalization, transfusion of blood products, operative exploration and interventional radiology procedures.
- The complication rate among “cause” biopsies was significantly higher than in “protocol” biopsies (2.7% vs. 0.33%, $p < 0.001$

kidney biopsy

- Specific patient characteristics associated with increased risk of a complication were
- increased age
- blood urea nitrogen,
- decreased platelet count,
- history of prior renal transplant,
- deceased donor transplant type
- use of anticoagulant medications but not aspirin.

kidney biopsy

- Additionally, there is great variability when it comes to interpretation of biopsy results.

- Histological assessment has its limitations and pathology readings have been noted to be subjective and inconsistent.

kidney biopsy

- To improve histologic assessment and inter-reader variability, novel techniques have been developed that utilize gene expression profiles of kidney biopsy tissue.

Review

OPEN

The Molecular Phenotype of Kidney Transplants: Insights From the MMDx Project

Philip F. Halloran, MD, PhD,[1] Katelynn S. Madill-Thomsen, PhD,[1] and Jeff Reeve, PhD[1]

Abstract. This review outlines the molecular disease states in kidney transplant biopsies as documented in the development of the Molecular Microscope Diagnostic System (MMDx). These states include T cell–mediated rejection (TCMR), antibody-mediated rejection (AMR), recent parenchymal injury, and irreversible atrophy-fibrosis. The MMDx project, initiated through a Genome Canada grant, is a collaboration involving many centers. MMDx uses genome-wide microarrays to measure transcript expression, interprets the results using ensembles of machine learning algorithms, and generates a report. Experimental studies in mouse models and cell lines were extensively used to annotate molecular features and interpret the biopsy results. Over time, MMDx revealed unexpected aspects of the disease states: for example, AMR is usually C4d-negative and often DSA-negative, and subtle "Minor" AMR-like states are frequent. Parenchymal injury correlates with both reduced glomerular filtration rate and increased risk of graft loss. In kidneys with rejection, injury features, not rejection activity, are the strongest predictors of graft survival. Both TCMR and AMR produce injury, but TCMR induces immediate nephron injury and accelerates atrophy-fibrosis, whereas AMR induces microcirculation and glomerular damage that slowly leads to nephron failure and atrophy-fibrosis. Plasma donor-derived cell-free DNA levels correlate strongly with AMR activity, acute kidney injury, and in a complex way with TCMR activity. Thus, the MMDx project has documented the molecular processes that underlie the clinical and histologic states in kidney transplants, and provides a diagnostic tool that can be used to calibrate biomarkers, optimize histology interpretation, and guide clinical trials.

(*Transplantation* 2023;00: 00–00).

gene expression profiles of kidney biopsy tissue

- The MMDx® (molecular microscope diagnostic system, One Lambda, West Hills, CA)
- is a microarray-based test that uses machine learning to assess the risk of kidney transplant rejection.
- The test analyzes messenger RNA (mRNA) expression from a biopsy sample to identify patterns associated with rejection.

gene expression profiles of kidney biopsy tissue

- have good correlation with histological findings, and it may be useful when histological results are borderline or inconclusive

- MMDx has the potential to not only add additional information to the biopsy in question, but also lessen the need for repeat biopsies.

Non-invasive tests of allograft function

- Graft damage and rejection can occur in the absence of an acute rise in serum creatinine.
- Historically, the only way to do determine changes in allograft status before graft dysfunction would be to perform protocol biopsies.
- less than half (46%) of the high-volume transplant centers (defined by annual kidney transplants greater than 50) in the United States perform protocol biopsies.

Non-invasive tests of allograft function

- Measurements of gene transcripts in the blood,
- Tests of lymphocyte function,
- Donor derived cell free DNA analysis, alloantibodies,
- Monitoring for post-transplant infections.
- Urinary biomarkers,
- Artificial intelligence.

Immune monitoring

- Gene expression

- The most widely used is TruGraf® (Eurofins Transplant Genomics, Framingham, MA), which utilizes DNA microarray technology to determine whether a patient's gene expression is more similar to a reference population with adequate immunosuppression than that with inadequate immunosuppression
-

Gene expression

Investigator Assessment of the Utility of the TruGraf Molecular Diagnostic Test in Clinical Practice

M.R. First[a,b,*], V.R. Peddi[c], R. Mannon[d], R. Knight[e], C.L. Marsh[f], S.M. Kurian[f], J.C. Rice[f], D. Maluf[g], D. Mandelbrot[h], A. Patel[i], J. David[a], C. Schieve[a], D. Lee[a], P. Lewis[a], J.J. Friedewald[b], M.M. Abecassis[b], and S. Rose[a]

[a]Transplant Genomics Inc, Mansfield, MA; [b]Comprehensive Transplant Center, Northwestern University, Chicago, IL; [c]California Pacific Medical Center, San Francisco, CA; [d]University of Alabama at Birmingham, Birmingham, AL; [e]Houston Methodist Hospital, Houston, TX; [f]Scripps Center for Organ Transplantation, La Jolla, CA; [g]University of Virginia, Charlottesville, VA; [h]University of Wisconsin, Madison, WI; and [i]Henry Ford Hospital, Detroit, MI

Gene expression

- TruGraf v1 is a well-validated DNA microarray-based test that analyzes blood gene expression profiles as an indicator of immune status in kidney transplant recipients with stable renal function

Gene expression

- Methods. In this study, investigators assessed clinical utility of the TruGraf test in patient management
- In a retrospective study, simultaneous blood tests and clinical assessments were performed in 192 patients at 7 transplant centers, and in a prospective observational study they were performed in 45 subjects at 5 transplant centers
- Results. When queried regarding whether or not the TruGraf test result impacted their decision regarding patient management, in 168 of 192 (87.5%) cases the investigator responded affirmatively.

Gene expression

- The prospective study indicated that TruGraf results supported physicians' decisions on patient management 87% (39/45) of the time, and in 93% of cases physicians indicated that they would use serial TruGraf testing in future patient management.

- A total of 21 of 39 (54%) reported results confirmed their decision that no intervention was needed, and 17 of 39 (44%) reported that results specifically informed them that a decision not to perform a surveillance biopsy was correct.

Gene expression

- Conclusions. TruGraf is the first and only noninvasive test to be evaluated for clinical utility in determining rejection status of patients with stable renal function and shows promise of providing support for clinical decisions to avoid unnecessary surveillance biopsies with a high degree of confidence.

Gene expression

- AlloMap® Kidney (CareDx, Brisbane, CA) uses next-generation sequencing and targeted RNA sequencing technology for gene expression profiling to assess immune quiescence.

- The AlloMap Kidney test is a gene expression profile utilizing the RNA-seq platform to measure immune quiescence in kidney transplant patients.

Gene expression

-
- **Results/Conclusions:** Analytical validation showed robust performance characteristics with an accuracy correlation coefficient of 0.997 and a precision coefficient of variation of 0.049 across testing.

- Clinical validation from the prospective, multi-center studies of 235 samples (66 rejection and 169 quiescence specimens) demonstrated the sensitivity of 70% and specificity of 66% for allograft rejection, while the negative predictive value was 95% to discriminate rejection from quiescence at 10% prevalence of rejection.

gene expression

- Another gene expression test available for assessing rejection is the kSORT® (kidney Solid Organ Response Test, Immucore, Norcross, GA).

- The kSORT looks at relative mRNA expression levels to detect patients who are at higher risk of rejection .

- Despite promising results in early studies, a large retrospective multicenter study of 1,763 samples from 1,134 patients found that kSORT could not be validated for acute rejection in the first year after transplantation ($p = 0.46$)

urinary biomarkers

urinary biomarkers

TABLE 1.
Summary of relevant urine biomarkers for renal allograft injury

Reference	Biomarkers	Test design	Sensitivity	Specificity	PPV	NPV	AUC
Transcriptomics							
Suthanthiran et al[11]	*CD3ε* mRNA + *IP-10* mRNA + *18S rRNA*	TCMR vs non-TCMR	79%	78%	–	–	0.850
Nissaisorakarn et al[12]	*CD3ε* + *CD105* + *CD14* + *CD46* + *18S rRNA*	TCMR vs AMR	–	–	–	–	0.810
Sigdel et al[15]	*BASP1, CD6, CXCL10, CXCL9, INPP5D, ISG20, LCK, NKG7, PSMB9, RUNX3, TAP1* (**uCRM Score**)	AR vs HC	95.35%	97.78%	–	–	0.9886
		AR vs bAR + HC	87.10%		–	–	0.9677
		AR vs BKVN + bAR + HC	76.92%		–	–	0.9111
Kaminski et al[16]	*CXCL9* mRNA Unnecessary	AR vs HC	93%	76%	–	–	0.91
Lorenzen et al[17]	*miR-210*	AR vs HC	74%	52%			0.70
Millán et al[18]	*miR-155*	AR vs non-AR	85%	86%	88%	100%	0.875
	CXCL10 mRNA		84%	80%	90%	85%	0.865
		Metabolomics					
Nissaisorakarn et al[12]	3-sialyllactose, xanthosine + quinolinate + X-16397 + *CD3ε* mRNA + *IP-10* mRNA + 18S rRNA	TCMR vs non-TCMR	90%	84%	–	–	0.930
Sigdel et al[26]	Glycine, adipic acid, glutaric acid, *N*-methylalanine, inulobiose, threose, sulfuric acid, taurine, asparagine, 5-aminovaleric acid, myoinositol	AR vs HC	92.9%	96.3%	96.3%	92.9%	0.985
	arabinose, 2-hydroxy-2-methylbutanoic acid, octadecanol, and phosphate	BKVN vs non-BKVN	88.9%	94.%	72.7%	98.2%	0.940
Blydt-Hansen et al[27]	Proline, PC:aa:C34:4, kynurenine, sarcosine, methionine sulfoxide, PC:ae:C38:6, threonine, glutamine, phenylalanine, alanine	TCMR vs non-TCMR	83%	83%	97%	45%	0.880
		TCMR + borderline tubulitis vs non-TCMR	95% (training) 74% (validation)	75% (training) 65% (validation)	–	–	0.900 (training)
		Proteomics					
Park et al[33]	$CD3^+$ extracellular vesicles (**iKEA**)	TCMR vs non-TCMR	92.8% (discovery) 63.6% (validation)	87.5% (discovery) 100% (validation)	–	–	0.911 (discovery) 0.837 (validation)
Sigdel et al[34]	Tamm-Horsfall protein (UMOD)	AR vs non-AR	–	–	–	–	0.973
	Pigment Epithelium-Derived factor (PEDF) or SERPINF1		–	–	–	–	0.932
	CD44		–	–	–	–	0.846
Sigdel et al[35]	11-peptide panel	AR vs HC	–	–	–	–	0.939
	12-peptide panel	BKVN vs HC	–	–	–	–	0.832
	12-peptide panel	CAI vs HC	–	–	–	–	0.995
Lim et al[36]	Tetraspanin-1 and hemopexin	TCMR vs HC	64%	72.%	–	–	0.744
Kanzelmeyer et al[37]	79-peptide panel	cAMR vs non-cAMR	100%	75%	–	–	0.92
	79-peptide panel + CKD273		88%	92%	–	–	0.92
Mertens et al[38]	Alpha-1-B glycoprotein, afamin, apolipoprotein A1, apolipoprotein A4, Ig heavy constant α1, Ig heavy constant γ4, leucine-rich α2-glycoprotein 1, alpha-1 antitrypsin, antithrombin, and transferrin	AMR vs non-AMR	95% (training) 95% (validation)	96% (training) 76% (validation)	–	–	0.98 (training) 0.88 (validation)

urinary biomarkers

TABLE 1. (Continued)

Summary of relevant urine biomarkers for renal allograft injury

Reference	Biomarkers	Test design	Sensitivity	Specificity	PPV	NPV	AUC
Hirt-Minkowski et al[39]	CXCL10 protein	Inflammation vs no inflammation	61% (surveillance biopsy) 63% (indication biopsy)	72% (surveillance biopsy) 80% (indication biopsy)	–	–	0.69 (surveillance biopsy) 0.74 (indication biopsy)
Raza et al[40]	CXCL10 protein	AR vs non-AR	72%	71%	–	–	0.74
		TCMR vs non-AR	79%	71%	–	–	0.79
Jackson et al[43]	CXCL9 protein	AR or BKV vs CNI toxicity + IFTA + HC + nontransplant control	86%	80%	–	–	–
	CXCL10 protein	AR or BKV vs CNI toxicity + IFTA + HC + nontransplant control Combined omics	80%	76%	–	–	–
Yang et al[45]	Multiple biomarker types: cfDNA, m-cfDNA, CXCL10, creatinine, clusterin, total protein (**Q Score/QSant**)	AR vs HC	94.9% (training) 95.8% (validation)	100% (training) 99.3% (validation)	–	–	0.99 (training) 0.998 (validation)

The table displays the ROC analysis results of various methods to identify allograft injury in urine samples. The bold text in parentheses indicate assay names.

AMR, antibody-mediated rejection; cAMR, chronic AMR; AR, acute rejection; AUC, area under the curve; bAR, borderline acute rejection; BKV, BK virus; BKVN, BKV nephropathy; cfDNA, cell-free DNA; CNI, calcineurin inhibitor; HC, healthy control; HUDSON, heating unextracted diagnostic samples to obliterate nucleases; IFTA, interstitial fibrosis and tubular atrophy; iKEA, integrated kidney exosome analysis; NPV, negative predictive value; m-cfDNA, microbial cell-free DNA; PPV, positive predictive value; ROC, receiver operating characteristic; TCMR, T cell–mediated rejection.

- Unfortunately, there are currently no commercially available urinary biomarker tests.

lymphocyte function

- ImmuKnow® (immune cell function assay, Eurofins Viracor, Lenexa, KS) measures the concentration of adenosine triphosphate (ATP) from CD4+ T-cells after stimulation to monitor the immune response of transplant patients The test assigns patients into three categories based on their intracellular ATP levels:

lymphocyte function

- low (<225 ng/ml), moderate (226-524 ng/ml), and strong (>524 ng/ml).

- Lower ATP levels were correlated with “over immunosuppressed state” and increased risk of infection while the higher ATP levels were correlated with rejection, suggesting that patients should be aimed towards the moderate zone

lymphocyte function

- However, there is significant overlap between stable and infected patients in the moderate range, which limits the test's generalizability

lymphocyte function

- The Pleximark® (Plexision, Pittsburgh, PA) looks at allo-antigen-specific T- cytotoxic memory cells but has only shown to measure likelihood of TCMR .

- A test looking at alloantigen-specific B-cells (PlexABMR®, Plexision, Pittsburgh, PA) is being developed to be able to measure the risk of antibody-mediated rejections (ABMR).

Research Article

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Biomarkers in Medicine

Validation of a gene expression signature to measure immune quiescence in kidney transplant recipients in the CLIA setting

Rocky Cheung[‡,1], Hua Xu[‡,1], Xia Jin[1], Wenlan Tian[1], Kevin Pinney[1], Lihong Bu[2], Steven Stone[1], Robert N Woodward[1], Nikhil Agrawal[1], Shamik Dholakia[1] & Ryan T Phan*[,1]

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[‡]Authors contributed equally

- The AlloMap Kidney test is a gene expression profile utilizing the RNA-seq platform to measure immune quiescence in kidney transplant patients.

- Analytical validation showed robust performance characteristics with an accuracy correlation coefficient of 0.997 and a precision coefficient of variation of 0.049 across testing.

- Clinical validation from the prospective, multi- center studies of 235 samples (66 rejection and 169 quiescence specimens) demonstrated the sensitivity of 70% and specificity of 66% for allograft rejection, while the negative predictive value was 95% to discriminate rejection from quiescence at 10% prevalence of rejection.

(dd-cfDNA) testing.

- One of the more promising technologies that is clinically available is the use of
- donor derived cell-free DNAs (dd-cfDNA) testing.

- cfDNA is non-encapsulated DNA that can be released after cells have been injured.

- In solid organ transplantation, dd-cfDNA has been investigated as a potential biomarker for allograft rejection

American Journal of Transplantation 2015; 15: 2541–2551
Wiley Periodicals Inc.

doi: 10.1111/ajt.13387

Minireview

Cell-Free DNA: An Upcoming Biomarker in Transplantation

E. M. Gielis[1,2], K. J. Ledeganck[1], B. Y. De Winter[1], J. Del Favero[3], J.-L. Bosmans[1,4], F. H. J. Claas[2], D. Abramowicz[1,4] and M. Eikmans[2,*]

SRY, sex-determining region of chromosome Y; TMA, thrombotic microangiopathy; Tr-DNA, transrenal DNA; Tx, transplantation; UTI, urinary tract infection

Received 21 January 2015, revised 01 May 2015 and accepted for publication 13 May 2015

(dd-cfDNA) testing.

- Donor-derived cell-free DNA (ddcfDNA) can be detected in the recipient's blood and urine.
- Different ddcfDNA quantification techniques have been investigated but a major breakthrough was made with the introduction of digital droplet PCR and massive parallel sequencing creating the opportunity to increase the understanding of ddcfDNA kinetics after transplantation.
- The observations of increased levels of ddcfDNA during acute rejection and even weeks to months before histologic features of graft rejection point to a possible role of ddcfDNA as an early, noninvasive rejection marker.

(dd-cfDNA) testing.

Table 3: Overview of studies published on quantification of plasma, serum or urinary cfDNA levels or fractions in patients suffering from different posttransplant complications compared to a stable graft function

				Stable graft		Posttransplant complication episode	
Ref.	Study design	Patients	Samples	n	Mean/median total cfDNA or ddcfDNA	n	Mean/median total cfDNA or ddcfDNA
Garcia Moreira et al (37)	**Kidney:** Observational Prospective Longitudinal (0–3 months post-Tx)	**100 KTP:** plasma total cfDNA **17 KTP:** plasma ddcfDNA **30 KTP:** urine cfDNA	Mean 24 plasma samples/patient Mean 7 urine samples/patient	31 KTP	**total cfDNA:** 1280 (1035–1514) GE/mL[1]	69 KTP	**Total cfDNA:** • AR (n = 19): 55 303 (23 557–93 809) GE/mL[1] • ATN (n = 34): 2944 (2265–3338) GE/mL[1] • NTX (n = 13): 6130 (4668–9793) GE/mL[1] • Infection (n = 21): 13 123 (8068–38 522) GE/mL[1]
Sigdel et al (38)	**Kidney:** Observational Retrospective Longitudinal (0–24 months post-Tx)	**21 KTP**	63 biopsy matched urine samples	41 urine samples	**ddcfDNA:** 2.4 (± 3.3) ChrY copies/μg urine creatinine[3]	22 urine samples	**ddcfDNA:** • AR (n = 8): 20.5 (± 13.9) ChrY copies/μg urine creatinine[3] • CAI (n = 10): 2.4 (± 2.4) ChrY copies/μg urine creatinine [3] • BKVN (n = 4): 20.3 (± 15.7) ChrY copies/μg urine creatinine[3]
Gadi et al (40)	**Kidney–pancreas:** Observational Retrospective Longitudinal (before Tx to 5 years post-Tx)	**42 KPTP**	158 serum samples with 65 biopsy matched samples 2–7 serum samples/patient	34 serum samples	**ddcfDNA:** 0.9 (0–35.9) GE/ml[2] or 58.9 (0–28 537) GE/10[6] host cell-free genomes[2]	32 serum samples	**ddcfDNA:** • AR (n = 31): 10.4 (0–57.8) GE/mL[2] or 2613 (0–28 066) GE/10[6] host cell-free genomes[2] • TMA (n = 1): 4.1 GE/mL or 1934 GE/10[6] host cell-free genomes
Beck et al (42)	**Liver, Kidney, Heart:** Observational Prospective Cross-sectional + Longitudinal	**10 LTP** *Cross-sectional:* stable maintenance phase >6 months post-Tx **7 LTP** *Longitudinal:* immediately after Tx **9 KTP** **8 HTP**	NM	10 LTP 9 KTP 8 HTP	**ddcfDNA:** LTP: 3.5% (1.0–8.5%)[4] KTP: 1.2% (0.2–3.5%)[4] HTP: 0.9% (0.1–3.4%)[4]	2 LTP	**ddcfDNA** • AR (n = 2) Patient 1: >60%; Patient 2: 55%

(*Continued*)

(dd-cfDNA) testing.

Table 3: Continued

Ref.	Study design	Patients	Samples	Stable graft n	Stable graft Mean/median total cfDNA or ddcfDNA	Posttransplant complication episode n	Posttransplant complication episode Mean/median total cfDNA or ddcfDNA
Macher et al (39)	**Liver:** Observational Prospective Longitudinal (before Tx to 6–30 days post-Tx)	**10 LTP**	NM	6 LTP	**ddcfDNA:** 133.2 ± 49.6 ng/mL[3]	4 LTP	**Total cfDNA** • AR (n = 1): 7570 ng/mL • hepatic vein thrombosis (n = 1): 17 100 ng/mL hepatic artery thrombosis (n = 2): 11 800 ng/mL, 15 100 ng/mL • Cholestasis and MOF (n = 1): 8010 ng/mL • Biliary peritonitis (n = 1): 8590 ng/mL • Biliary conduit complication (n = 1): 3930 ng/mL **ddcfDNA** • hepatic vein thrombosis (n = 1): 307 ng/mL • hepatic artery thrombosis (n = 1): 14 800 ng/mL • Cholestasis and MOF (n = 1): 1414 ng/mL • Biliary peritonitis (n = 1): 0 ng/mL • Biliary conduit complication (n = 1): 711 ng/mL
Snyder et al (34)	**Heart:** Observational Retrospective Longitudinal (0.5–22 months post-Tx)	**7 HTP**	44 plasma samples 3–8 samples/patient	38 plasma samples	**ddcfDNA:** 0.92% (± 1.16%)[3]	6 plasma samples	**ddcfDNA** • AR: 2.75% (± 1.81%)[3]
De Vlaminck et al (45)	**Heart:** Observational Prospective Longitudinal (1 day to 24 months post-Tx)	**65 HTP**	565 plasma samples with 356 biopsy matched samples	NM	**ddcfDNA:** 0.06% (± 0.11%)[3]	NM	**ddcfDNA:** • AR (mild n = 147, moderate to severe n = 24) Patient1: 5.75% Patient 2: >10% Patient 3: 2.0%, 9.0%, and 4.9%

(*Continued*)

Monitoring for alloantibodies

- Around 13% to 30% of kidney transplant recipients develop dnDSA, and presence of dnDSA is associated with poorer outcomes

- For example, one study following 508 renal transplant patients (64 with dnDSA) reported that recipients without dnDSA had eGFR decline of 0.65 mL/min/1.73m2 per year and presence of dnDSA led to eGFR decline of 3.63 mL/min/1.73m2 per year ($p < 0.001$)

dnDSA

- The risk factors for developing dnDSAs include
- inadequate immunosuppression
- infections such as BK virus and cytomegalovirus.

dnDSA

- Current guidelines from the Transplantation Society recommend monitoring for DSA in patients with DSA pre-transplant when:

- immunosuppression is being reduced (in the setting of infection or for other reasons),
- when there is concern for non-adherence, and in patients with a rejection episode .

dnDSA

- Some evidence suggests that the presence of DSAs alone, without biopsy-proven rejection or acute inflammation, may not be associated with graft failure,

- highlighting our continued reliance on kidney biopsy. That said, the frequency of monitoring DSAs is at the discretion of the transplant center.

Monitoring for viral infections

- Torque teno virus (TTV)

- is a non-pathogenic virus that is almost ubiquitous worldwide, with 90% of healthy individuals and up to 100% of transplant recipients infected.

- TTV is insensitive to conventional antiviral drugs used in transplantation, but it has the potential to be used as a marker of immune status in transplant recipients.

- TTV actively replicates and over 90% of the viruses are cleared by the immune system daily. T-cell function is thought to be crucial for viral control.

- Observational studies have shown that low TTV load is associated with a higher risk of rejection, while high TTV load is associated with a risk of infection .

OPEN

Torque Teno Virus Load Is Associated With Subclinical Alloreactivity in Kidney Transplant Recipients: A Prospective Observational Trial

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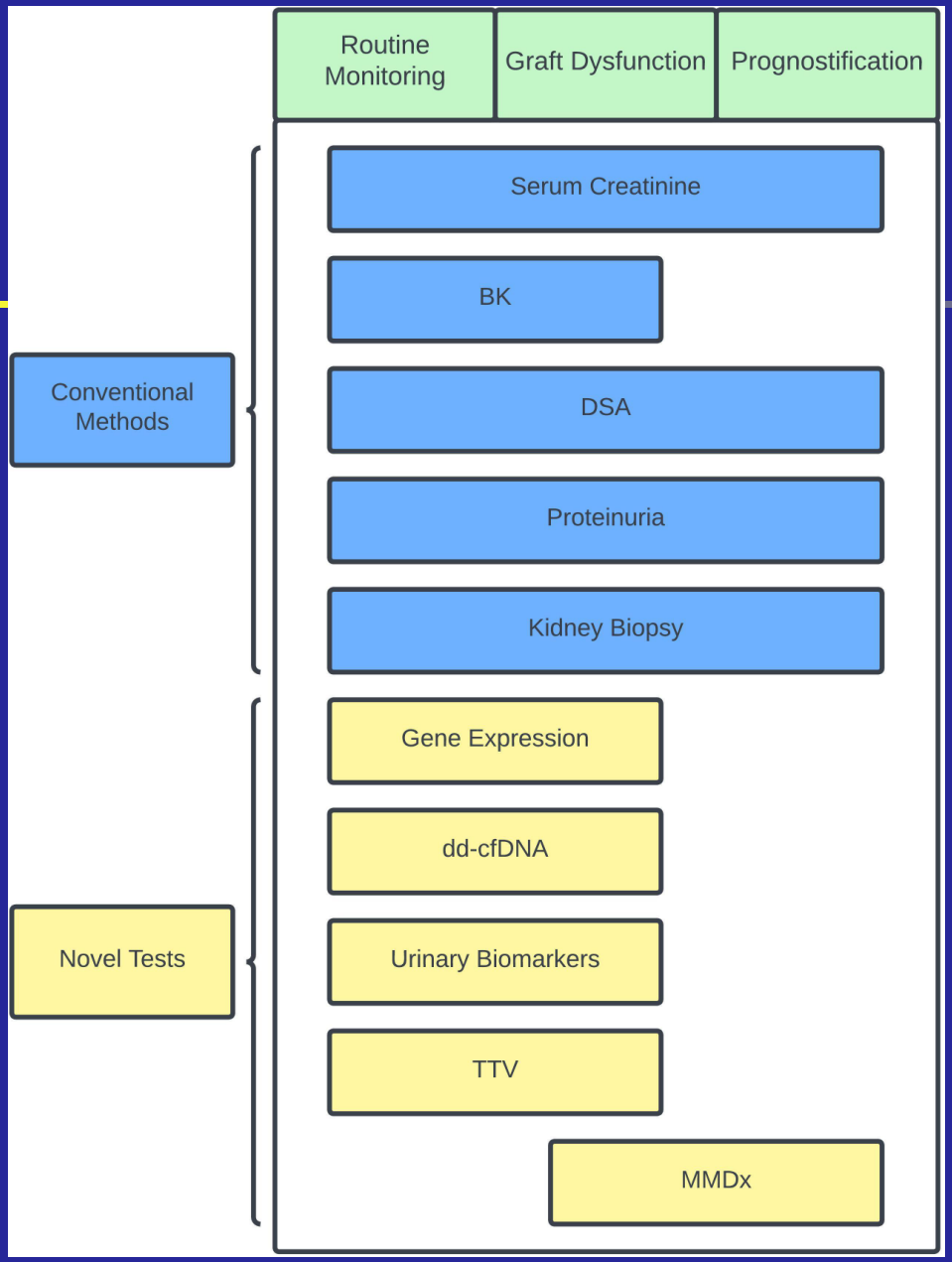
Text

Background. Nonpathogenic torque teno viruses (TTVs) are highly prevalent in transplant recipients and associated with immunosuppression. Studies in kidney transplant patients have proposed assessment of TTV load for risk stratification of clinically overt graft rejection. The value of TTV quantification in the context of subclinical rejection has not been evaluated. **Methods.** In this prospective trial, 307 consecutive kidney transplant recipients were subjected to per-protocol monitoring of plasma TTV. TTV was analyzed in the context of protocol biopsies (n = 82), scheduled 1 year posttransplantation. **Results.** TTV load at the time of biopsy was lower in recipients with rejection (n = 19; according to Banff, including borderline changes suspicious for acute T cell-mediated rejection) than those without rejection (n = 63) whereby each log increase in TTV copies/mL decreased the risk for rejection by 9% (risk ratio 0.91, 95% confidence interval, 0.85-0.97; $P = 0.004$). Development of chronic lesions (cg, cv, ci, ct, ah, ptcml) was associated with the number of days with a TTV load $<1 \times 10^6$ copies/mL between months 3 and 12 posttransplant (β 0.07, 95% confidence interval, 0.01-0.14; $P = 0.02$). **Conclusions.** This trial demonstrates an association between TTV and subclinical graft rejection in kidney transplant recipients. A TTV load $<1 \times 10^6$ copies/mL suggests suboptimal immunosuppression.

(*Transplantation* 2021;105: 2112–2118).


Routine Monitoring
Graft Dysfunction
Prognostification
Conventional Methods
Serum Creatinine
BK
DSA
Proteinuria
Kidney Biopsy
Novel Tests
Gene Expression
dd-cfDNA
Urinary Biomarkers
TTV
MMDx