Application of TruGraf v1: A Novel Molecular Biomarker for Managing Kidney Transplant Recipients With Stable Renal Function

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ABSTRACT

TruGraf v1 is a laboratory-developed DNA microarray-based gene expression blood test to enable proactive noninvasive serial assessment of kidney transplant recipients with stable renal function. It has been previously validated in patients identified as Transplant eXcellence (TX: stable serum creatinine, normal biopsy results, indicative of immune quiescence), and not-TX (renal dysfunction and/or rejection on biopsy results). TruGraf v1 is intended for use in subjects with stable renal function to measure the immune status as an alternative to invasive, expensive, and risky surveillance biopsies.

Materials and Methods. In this study, simultaneous blood tests and clinical assessments were performed in 192 patients from 7 transplant centers to evaluate TruGraf v1. The molecular testing laboratory was blinded to renal function and biopsy results.

Results. Overall, TruGraf v1 accuracy (concordance between TruGraf v1 result and clinical and/or histologic assessment) was 74% (142/192), and a result of TX was accurate in 116 of 125 (93%). The negative predictive value for TruGraf v1 was 90%, with a sensitivity 74% and specificity of 73%. Results did not significantly differ in patients with a biopsy-confirmed diagnosis vs those without a biopsy.

Conclusions. TruGraf v1 can potentially support a clinical decision enabling unnecessary surveillance biopsies with high confidence, making it an invaluable addition to the transplant physician's tool kit for managing patients. TruGraf v1 testing can potentially avoid painful and risky invasive biopsies, reduce health care costs, and enable frequent assessment of patients with stable renal function to confirm the presence of immune quiescence in the peripheral blood.

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IN 2017, a record number of 19,850 Americans received kidney transplants [1,2]. At present, there are over 200,000 Americans living with and dependent on a functional kidney transplant [2]. While kidney transplant is the optimal treatment for most patients with chronic renal failure [3], long-term results still remain suboptimal [4]. Only 47% of deceased donor transplants and 63% of living donor transplants are still functioning 10 years after transplant [5].

A key contributor to long-term graft loss is subclinical immune injury that manifests as undetected subclinical acute rejection (subAR), perhaps due to underimmunosuppression, leading to chronic rejection [5–8]. Significant challenges exist to detecting early injury when the transplanted kidney has the greatest chance of regaining normal function. The subAR phenotype is defined as acute rejection characterized by mononuclear cell infiltration of renal tubules that is histologically detected on a biopsy specimen but without clinically defined renal dysfunction (stable serum creatinine level). Therefore, subAR can by definition only be diagnosed on surveillance biopsies taken as per the individual center's protocol at a fixed time after transplant. However, a recent study of surveillance biopsy practices across US transplant centers shows that only 17% of centers perform surveillance biopsies, with another 21% performing surveillance biopsies in select cases [9]. Therefore, ~62% of transplant centers depend solely on an indication biopsy, triggered by a rise in creatinine before a clinical intervention, by which time there is already substantial and perhaps irreversible damage to the allograft. Moreover, it has recently become recognized that donor-specific antibody is generally the product of T-cell dependent immune responses. In calcineurin inhibitor–based regimens, early T-cell mediated rejection episodes correlate with subsequent de novo donor-specific antibody and antibody-mediated rejection (AMR) [10]. This underscores the urgent need for noninvasive testing of patients with stable renal function to confirm adequacy of immunosuppression as a key element to preventing future AMR.

Current monitoring to detect kidney injury includes measuring serum creatinine levels and immunosuppressive drug levels, both of which are insensitive and nonspecific. Routine surveillance (protocol) biopsies can be used to detect subclinical graft injury but are both expensive and invasive, therefore increasing the risk of infection, bleeding, and even graft loss [11] all while having significant intraobserver variation in interpretation of biopsy results [12], thus deeming them unsuitable for frequent monitoring. Recent reviews have highlighted that biomarkers that correlate with and/or predict allograft injury and improve therapeutic decision-making are priorities in transplantation and specifically underscore the need for robust multicenter validation studies [13–15]. For example, data presented over the past year indicate that analysis of levels of cell-free donor-derived DNA (cf-ddDNA) may be helpful in suggesting the likely absence of severe active rejection (84% negative predictive value [NPV], 61% positive predictive value [PPV]) in the small fraction of patients displaying signs of renal dysfunction and thus already suspected to be rejecting [16]. The same technique has also been reported to be ineffective in stratifying patients with stable renal function, as 96% of these patients have been shown to display levels of cf-ddDNA below the analytical cutoff for severe active rejection [17].

The TruGraf v1 blood test (Transplant Genomics Inc, Mansfield, Mass, United States) is a laboratory-developed test performed as a service available exclusively through the Clinical Laboratory Improvement Amendments–certified laboratory at Transplant Genomics Inc. TruGraf v1 relies on a specific gene expression signature in the peripheral blood (PB) to enable proactive and noninvasive serial assessment of kidney transplant recipients with stable renal function. We have previously described the development of the TruGraf v1 test based on the discovery and validation of signatures derived from the PB in 2 populations of patients: 1. patients following kidney transplant with stable renal function and surveillance biopsy results that revealed no evidence of histologic rejection who were designated as TX, and 2. patients following kidney transplant not meeting the strict criteria for TX who were designated as not-TX. The gene expression profile for TX was first reported by our group in 2014 [18]. A TruGraf v1 blood test reported as TX in a kidney transplant recipient with stable renal function would allow physicians to identify with high probability patients who can be followed routinely, including with serial TruGraf v1 testing, without the need for an invasive surveillance biopsy. This is important because surveillance biopsy results only identify about 15% to 25% of recipients with inflammation [19], indicating that 75% to 85% of surveillance biopsies are unnecessary and avoidable.

Herein we describe a retrospective data analysis of 192 PB samples using the TruGraf v1 classifier that was run on patient samples from 7 transplant centers in the United States that participated in an Early Access Program (EAP) sponsored by Transplant Genomics Inc.

MATERIALS AND METHODS

Patient Population

In this study, the intended use of the TruGraf v1 test (v1.3 classifier) was to allow transplant centers to obtain hands-on experience of the test and for them to form an impression of the value of the test in patient management. In no instances was the result of the TruGraf v1 test used to influence patient management, which was independently decided by the clinicians at the respective EAP centers. Simultaneous blood tests and clinical assessments were performed in 192 patients from 7 transplant centers in the EAP. Of the 192 PB samples, 99 (TX, 75; non-TX, 24) had a biopsy-confirmed diagnosis, and 93 (TX, 83; non-TX, 10) had a clinical diagnosis in the absence of a biopsy assigned by the principal clinical investigator of the respective site and then independently by MRF. The 7 transplant centers were California Pacific Medical Center, Henry Ford Hospital, Houston Methodist, Scripps Clinic, University of Virginia, University of Alabama, and University of Wisconsin. All patients who underwent biopsies and/or clinical assessment had PB samples

(two 2.5-mL PAXgene tubes) drawn at the time of biopsy/clinical assessment. Biopsies were classified using Banff 2007 criteria [20].

Inclusion/Exclusion Criteria

Inclusion criteria were patients >90 days post transplant with stable renal function, defined as a serum creatinine level ≤2.3 mg/dL and an increase in creatinine of <20% compared with the average of a minimum of 2 to 3 preceding values, and/or surveillance biopsy results that revealed no histologic evidence of rejection. Based on these criteria, 158 patients were determined to be TX. An additional 34 patients were determined to be not-TX since they had a serum creatinine level >2.3 mg/dL and an increase in creatinine of >20% compared with the average of a minimum of 2 to 3 preceding values, and/or surveillance biopsy results that revealed no histologic evidence of rejection. A total of 99 patients (52%) had protocol biopsies to confirm immune quiescence by histology. Exclusion criteria were 1. subjects with unstable renal function using the criteria described above, 2. subjects with evidence of BK virus infection, and 3. patients with moderate or severe interstitial fibrosis and/or tubular atrophy (Banff defined interstitial fibrosis and tubular atrophy >25%). None of the centers excluded patients based on age, sex, ethnicity, HLA type, immunosuppression, donor type, or induction since the purpose of the study was to include "all comers" from the participating centers based on their standard of care.

RNA Extraction, Amplification, and Hybridization

Briefly, total RNA was extracted from PAXgene Blood RNA (in vitro diagnostic) tubes (Qiagen, Valencia, Calif, United States). PAXgene tubes were processed using PAXgene Blood microRNA reagents on the QIAcube instrument (Qiagen, Valencia, Calif, United States). Samples were processed to remove globin RNA using the Ambion GLOBINclear-Human Kit (Thermo Fisher Scientific, Carlsbad, Calif, United States). The Affymetrix 3' IVT (in vitro transcript) PLUS labeling system was used to perform in vitro transcription and labeling reactions (3' IVT) on globin-reduced RNA. Array hybridization and subsequent washing, staining, and array scanning steps were completed on Affymetrix HG-U133+ arrays using the standard GeneTitan Gene Expression array workflow (Affymetrix, Santa Clara, Calif, United States). Predefined specifications for yield, array data quality, and control sample classifier results were used as acceptance criteria prior to sample data being analyzed on the TruGraf test.

TruGraf v1 Test

We previously reported on the analytical performance of the blood-based TruGraf v1 gene expression assay with classifier version 1.0 (v1.0) using support vector machine algorithms to select genes that discriminate TX (positive result) from not-TX [21] and have described an economic analysis of the cost-effectiveness of this approach [22]. In further analyzing the gene-specific data, we found that highly variable expressing genes were contributing to an increased noise level in the assay's performance. Therefore, the classifier was modified by using Random Forests algorithm to select component genes, which enabled in-depth interrogation of each gene's weighting and contribution to the assay's performance (TruGraf classifier version v1.1). This resulted in performance improvements in both the accuracy and PPV of the TX phenotype (positive result) [23]. The test was further modified using the same classifier as v1.1, but with not-TX as the positive class (classifier version v1.3) using a different threshold of 0.5 to improve the certainty of TX calls by specifically choosing to have a higher NPV.

The TruGraf v1 blood test results were reported dichotomously as TX or not-TX. The clinical phenotypes were determined independently by the principal investigator at each center based on their assessment of the renal function, other laboratory and clinical data, and/or the results of surveillance biopsy. Based on comparing the histologic and/or clinical phenotypes and the TruGraf v1 molecular phenotypes, the principal investigator then made an assessment as to whether the TruGraf v1 results were concordant with clinical phenotype. The molecular testing laboratory was blinded to the renal function and biopsy results, and samples were assigned a deidentified number only. Based on these assessments, the TruGraf v1 blood test result and the clinical phenotype were then analyzed for accuracy (concordance of histologic and molecular phenotypes), the accuracy of a TruGraf v1 test result (number of samples correctly called), the NPV and PPV of the test, and the sensitivity and specificity of the test. For centers not performing surveillance biopsies, the primary objective of the study was to assess whether a TruGraf v1 blood test result of TX corresponded with the physician's evaluation of clinical assessment, indicating a state of immune quiescence. For centers performing surveillance biopsies, the primary objective was to ascertain whether a TruGraf v1 blood test result of TX corresponded with normal histology on the biopsy, thereby indicating that in these cases a surveillance biopsy may have been unnecessary and avoidable.

Statistical Analyses

Statistical analyses for the diagnostic metrics were performed using MedCalc for Windows, version 15.0 and prevalence adjustment was done using VassarStats: Website for Statistical Computation (http://vassarstats.net/).

RESULTS

Of the 192 patients enrolled in the EAP, there were 90 women (47%) and 102 men (53%). Mean age of the subjects was 54.6 years, and the median age was 58 years, with a range of 21 to 81 years.

The results of the TruGraf v1 blood test and the comparison with the clinical diagnosis (phenotype) in the 192 kidney transplant recipients with stable renal function are shown in Table 1A. The TruGraf v1 test result called 124 patients (65%) TX and 68 patients (35%) not-TX. The overall accuracy of the TruGraf v1 blood test (concordance between TruGraf v1 result and clinical phenotype) was 74% (142/192), and the accuracy of a TruGraf v1 test result of

Table 1A. Results of TruGraf Blood Test and Comparison With Clinical Phenotype in All 192 Kidney Transplant Recipients with Stable Renal Function

	Clinical Phenotype not-TX	Clinical Phenotype TX
TruGraf Blood Test not-TX	26	42
TruGraf Blood Test TX	8	116

Accuracy = 142/192 (74%).
Accuracy of TruGraf TX result 116/124 (94%).
NPV = 91%.
PPV = 48%.
Sensitivity = 76%.
Specificity = 73%.

Table 1B. Results of TruGraf Blood Test and Comparison With Clinical Phenotype in 99 Kidney Transplant Recipients With Stable Renal Function and Biopsy-Confirmed Phenotypes

	Clinical Phenotype Not-TX	Clinical Phenotype TX
TruGraf Blood Test not-TX	17	19
TruGraf Blood Test TX	7	56

Accuracy = 73/99 (74%).
Accuracy of TruGraf TX result 56/63 (89%).
NPV = 89%.
PPV = 48%.
Sensitivity = 71%.
Specificity = 75%.

TX was 93% (116/123). Additional performance metrics of the TruGraf v1 test were NPV 91%, PPV 48%, sensitivity 76% (95% confidence interval [CI]; CI, 58.83–89.25), and specificity 73% (95% CI, 65.81–80.12). These diagnostic metrics were adjusted for prevalence, and the unadjusted NPV and PPV of the classifier on the EAP samples were 93% and 38%, respectively, showing that adjusting for true prevalence did not change the NPV significantly (90%) but improved the PPV to 48%. We also performed an analysis on the subset of 99 patients that had a biopsy-confirmed diagnosis; results are shown in Table 1B. The TruGraf v1 test result called 63 patients (64%) TX and 36 patients (36%) not-TX. The overall accuracy of the TruGraf v1 blood test (concordance between TruGraf v1 result and clinical phenotype) was 74% (73/99), and the accuracy of a TruGraf v1 test result of TX was 89% (56/63). Additional performance metrics of the TruGraf v1 test were NPV 89%, PPV 48%, sensitivity 71% (95% CI, 48.91–87.38), and specificity 75% (95% CI, 63.30–84.01). Finally we performed an analysis on the subset of 93 patients who did not have a biopsy-confirmed diagnosis (Table 1C). The TruGraf v1 test result called 61 patients (66%) TX and 32 patients (34%) not-TX. The overall accuracy of the TruGraf v1 blood test (concordance between TruGraf v1 result and clinical phenotype) was 74% (69/93), but the accuracy of a TruGraf v1 test result of TX was 98% (60/61). Additional performance metrics of the TruGraf v1 test were NPV 96%, PPV 51%, sensitivity 90% (95% CI, 55.50–99.75), and specificity 72% (95% CI, 61.38–81.55).

Table 1C. Results of TruGraf Blood Test and Comparison With Clinical Phenotype in 93 Kidney Transplant Recipients With Stable Renal Function With Clinical Diagnosis Without Biopsy

	Clinical Phenotype Not-TX	Clinical Phenotype TX
TruGraf Blood Test not-TX	9	23
TruGraf Blood Test TX	1	60

Accuracy = 69/93 (74%).
Accuracy of TruGraf TX result 60/31 (98%).
NPV = 96%.
PPV = 51%.
Sensitivity = 90%.
Specificity = 72%.
Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

Table 2A. Results of TruGraf Blood Test and Comparison With Clinical Phenotypes from 192 EAP Samples by Center. TruGraf Results and Comparison with Clinical Phenotype in 82 Kidney Transplant Recipients with Stable Renal Function from CPMC

Accuracy = 62/82 (76%)
Accuracy = of TruGraf TX result 53/54 (98%)
NPV = 96%
PPV = 53%
Sensitivity =90%
Specificity = 74%

Abbreviations: CPMC, California Pacific Medical Center; HFH, Henry Ford Hospital; HM, Houston Methodist; NPV, negative predictive value; PPV, positive predictive value SC, Scripps Clinic; UAB, University of Alabama; UVa, University of Virginia; UW, University of Wisconsin.

Tables 2A–G show the performance of the TruGraf v1 classifier at each EAP center defined by the same diagnostic metrics (sensitivity, specificity, NPV, PPV, and accuracy) adjusted for true prevalence. The overall accuracy for TruGraf v1 at the 7 centers ranged from 56% to 79%, with prevalence-adjusted NPV ranging from 74% to 100% and the prevalence-adjusted PPV ranging from 0% to 56%. In participating transplant centers performing surveillance biopsies, the overall concordance between the histology and the TruGraf v1 result was 73/99 (74%). Perhaps most importantly, the accuracy of a TruGraf v1 TX classification ranged from 83% to 100% across all 7 transplant centers.

DISCUSSION

The first objective of the current study was to further confirm the previously published clinical validity of the TruGraf v1 test in a blinded fashion based on results from a group of 7 transplant centers that participated in an EAP. A PB signature of TX for a patient with stable renal function indicates that there is a high degree of confidence that if the patient had undergone a biopsy at the same time as the PB was drawn, the results would have shown no evidence of either rejection or possibly of any other histologic abnormality. This confidence is based on a large reference dataset of patients used to discover and validate the TX PB signature, with stable renal function, whose biopsy results demonstrated no histologic abnormalities. The finding in the current study in a cohort of 192 patients from 7 centers who represent a range of patient populations that differ in ethnicities, immunosuppression regimens, and standard of care further confirmed the results from our earlier validation studies [21,23].

Table 2B. TruGraf Results and Comparison with Clinical Phenotype in 9 Kidney Transplant Recipients with Stable Renal Function from HFH

Accuracy = 7/9 (78%)
Accuracy = of TruGraf TX result 6/6 (100%)
NPV = 100%
PPV = 56%
Sensitivity = 100%
Specificity = 75%

Table 2C. TruGraf Results and Comparison with Clinical Phenotype in 27 Kidney Transplant Recipients with Stable Renal Function from HM

Accuracy = 22/27 (81%)
Accuracy = of TruGraf TX result 13/14 (93%)
NPV = 96%
PPV = 55%
Sensitivity =90%
Specificity = 76%

Table 2E. TruGraf Results and Comparison with Clinical Phenotype in 25 Kidney Transplant Recipients with Stable Renal Function from UAB

Accuracy = 14/25 (56%)
Accuracy = of TruGraf TX result 12/14 (86%)
NPV = 78%
PPV = 27%
Sensitivity = 50%
Specificity = 57%

Furthermore, we would like to orient the reader to the dichotomous results for TruGraf v1, where the positive test is not-TX and the negative test is TX. Therefore, PPV is the measure of confidence for not-TX, and NPV is the measure of confidence for TX. A result of TX in a kidney transplant recipient would allow physicians to identify with high probability patients with stable renal function who are likely to be immune quiescent and can be followed routinely, including with serial TruGraf v1 testing, without the need for a surveillance biopsy. In addition, when reducing immunosuppression post transplant, a signature of TX in a patient with stable renal function may reassure the clinician that there is no impending rejection as a result of reduction in immunosuppression, and a follow-up TruGraf v1 test at a time decided at the clinician's discretion can either confirm the adequacy of immunosuppression, or a change in result could mandate an appropriate intervention.

Conversely, a signature of not-TX, whether obtained in the process of routinely testing a patient with stable renal function or following reduction in immunosuppression, might prompt the clinician to monitor the patient more closely, perhaps to reverse the reduction in immunosuppression, and to repeat the TruGraf v1 test. If the second test result is also not-TX, a biopsy should be considered.

The TruGraf v1 test is designed primarily to provide noninvasive information that can be used to support decisions to perform fewer surveillance biopsies while having high confidence that the vast majority of those patients spared a surveillance biopsy will have an immune quiescent phenotype (TX). In patients whose test result is not-TX, clinicians must use all other clinical information and medical judgement to decide whether a TruGraf v1–prompted biopsy is needed, whether the patient should be tested further, or whether immunosuppression should be increased. Therefore, the TruGraf v1 result should not be interpreted as a stand-alone definitive diagnostic but rather a guide to strengthen and support physician decisions regarding the need for a clinical intervention such as a biopsy or a change in immunosuppression.

In transplant centers not performing surveillance biopsies, TruGraf v1 offers a noninvasive test with a high NPV for a TX result in patients with stable renal function, which supports the diagnosis of immune quiescence. Patients with not-TX could be followed more closely and retested over time. If a not-TX result persists, then an indication biopsy can be considered. Therefore, the TruGraf v1 test provides a valuable indicator of adequacy of immunosuppression for the majority of transplant centers in the United States that do not perform surveillance biopsies. The results of TruGraf testing in terms of the diagnostic metrics reported varies among the centers. This is typically expected since each transplant center has its own patient management standard of care, and the discrepancies can be attributed to changes in immunosuppression, induction, and maintenance therapies that have been tailored by the transplant team at each center to optimize graft and patient outcomes.

In selecting the threshold in the discovery cohort, priority was given to the balance of the percent of patients who might be spared a biopsy and the confidence in a TX result. The threshold was also set to provide high NPV (high certainty of TX calls), resulting in improved sensitivity for not-TX, and also improved specificity for TX. Approximately 62% of transplant centers in the United States do not perform surveillance biopsies [19]. These centers assume that patients are stable until the serum creatinine is elevated and then do a for-cause biopsy (ie, they wait until they see an indicator of possible substantial damage, depending on the rejection grade, before taking action). Their baseline standard of care thus involves missing opportunities for

Table 2D. TruGraf Results and Comparison with Clinical Phenotype in 19 Kidney Transplant Recipients with Stable Renal Function from SC

Accuracy = 15/19 (79%)
Accuracy = of TruGraf TX result 15/18 (83%)
NPV = 74%
PPV = 0%
Sensitivity = 0%
Specificity = 94%

Table 2F. TruGraf Results and Comparison with Clinical Phenotype in 15 Kidney Transplant Recipients with Stable Renal Function from UVa

Accuracy = 11/15 (73%)
Accuracy = of TruGraf TX result 10/10 (100%)
NPV = 100%
PPV = 53%
Sensitivity = 100%
Specificity = 71%

Table 2G. TruGraf Results and Comparison with Clinical Phenotype in 15 Kidney Transplant Recipients with Stable Renal Function from UW

Accuracy = 11/15 (73%)
Accuracy = of TruGraf TX result 7/8 (88%)
NPV = 92%
PPV = 46%
Sensitivity = 80%
Specificity = 70%

early intervention in 20% to 25% of patients with stable renal function who may harbor subAR and be heading down a path to developing de novo donor-specific antibody and ultimately AMR. Surveillance (protocol) biopsies are used in ~38% of centers to try to identify patients who are not adequately immunosuppressed in advance of seeing a rise in the serum creatinine. We also adjusted the diagnostic metrics prevalence, which many studies reporting diagnostic performance metrics fail to address. Increasing the disease prevalence will lead to an increasing PPV but a decreasing NPV of a diagnostic test. This is of consequence because in most study designs researchers tend to have an equal number of cases and controls in a roughly 50:50 split, thus leading to an overinflated presumption of the true prevalence. The TruGraf v1 performance metrics were adjusted based on true prevalence of subAR (rejection with a stable creatinine) from the multicenter Clinical Trials in Organ Transplantation–08 study of >300 patients enrolled and followed for 2 years post transplant [24].

Since only a little over half of the patients in this study had a biopsy-confirmed diagnosis, it was imperative to test whether there was any skewing of the results, especially in the patients who were assigned a diagnosis based on only clinical results. Though Trugraf had a slightly higher sensitivity, PPV, and NPV in the patients without a biopsy, the specificity as well as the accuracy was similar to the patient's with biopsy-confirmed results. Therefore, we can safely conclude that there is no implicit bias toward samples that were specifically confirmed by histology. This is important in the context that most clinical centers do not perform routine biopsies, and, therefore, most blood samples analyzed using Trugraf are not confirmed histologically with a biopsy. A simple minimally invasive (PB), or noninvasive (urine) test to help guide immunosuppression and optimize patient management is widely viewed as desirable, especially since such a test would reduce the number of unnecessary biopsies performed given the disadvantages of biopsies (painful, costly, risky; and 75%-80% are negative for rejection). Similarly, a diagnostic test is desired that could be used in patients with stable renal function in place of or that informs of the need for a surveillance and/or protocol biopsy and detects the majority of patients who are truly stable without adding significant risk (understanding that these patients are stable and are tested routinely). As described above, use of the TruGraf v1 blood test in patients with stable renal function would represent a reasonable and necessary advance in health management of kidney transplant recipients, providing a noninvasive anytime indicator of adequacy of immunosuppression, and eliminating the need for surveillance biopsies in more than 50% of cases.

Finally, we would like to give the correct context for a molecular test for rejection and the history of similar tests developed to date. Others have also reported gene expression profiles in the PB diagnostic of acute kidney transplant rejection but only in the context of renal dysfunction [18]. A recent study reported that cf-ddDNA in the PB of kidney transplant recipients was able to detect severe graft injury; however, it was not specific to rejection in patients who underwent a biopsy to investigate renal dysfunction [16]. Gene expression based molecular detection of subAR in PB has been explored in recent studies. The Kidney Solid Organ Response Test assay used in both the Assessment of Acute Rejection in Renal Transplantation [25] and Evaluation of Sub-Clinical Acute rejection PrEdiction [26] studies was designed to primarily predict acute rejection; however, only a small proportion of PB samples were paired with surveillance biopsies. Other drawbacks include the lack of blinded central histology reads to assign clinical phenotypes when discovering and training the models, use of best-fitting models, indeterminate values, and nonprevalent cohorts potentially skewing performance metrics [27,28].

CONCLUSIONS

Peripheral blood gene expression profiling may prove to be a highly effective and minimally invasive approach, allowing for more frequent, less expensive, and minimally risky testing of kidney transplant recipients in order to assess adequacy of immunosuppression. Such a test could provide valuable information that can be used to support a physician's decision regarding the timing of a biopsy in a patient with normal renal function, thereby avoiding the complications of a biopsy unless absolutely clinically indicated. Similarly, changes in therapy, and monitoring the impact thereof, during immunosuppressive drug minimization or withdrawal can be safely done using a minimally invasive test. We propose that a patient management strategy that uses TruGraf v1 serial PB testing has the potential to decrease the number of surveillance (protocol) biopsies for a proportion of patients in transplant centers that currently perform protocol biopsies. Additionally, based on the data from this study, we also reason that the TruGraf v1 test has added value in transplant centers that do not routinely perform protocol biopsies, allowing clinicians to focus their attention on the management of patients that may be at highest risk of demonstrating immune activation based on TruGraf v1 results.

REFERENCES

[1] Houchens RL, Ross DN, Elixhauser A, Jiang J. Nationwide Inpatient Sample Redesign Final Report. 2014. HCUP NIS Related

Reports ONLINE. April 4 2014. U.S. Agency for Healthcare Research and Quality. Available at: http://www.hcupus.ahrq.gov/db/nation/nis/nisrelatedreports.jsp.

[2] UNOS. Annual Report 2014. Available at: https://unos.org/about/annual-report/2014-annual-report/.

[3] Tonelli M, Wiebe N, Knoll G, et al. Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes. Am J Transplant 2011;11:2093–109.

[4] Hart A, Smith JM, Skeans MA, et al. Kidney. Am J Transplant 2016;16(Suppl. 2):11–46.

[5] Rush D, Nickerson P, Gough J, et al. Beneficial effects of treatment of early subclinical rejection: a randomized study. J Am Soc Nephrol 1998;9:2129–34.

[6] El Ters M, Grande JP, Keddis MT, et al. Kidney allograft survival after acute rejection, the value of follow-up biopsies. Am J Transplant 2013;13:2334–41.

[7] Loupy A, Vernerey D, Tinel C, et al. Subclinical rejection phenotypes at 1 year post-transplant and outcome of kidney allografts. Am J Soc Nephrol 2015;26:1721–31.

[8] Nankivell BJ, Chapman JR. The significance of subclinical rejection and the value of protocol biopsies. Am J Transplant 2006;6:2006–12.

[9] Mehta R, Cherikh W, Sood P, Hariharan S. Kidney allograft surveillance biopsy practices across US transplant centers: a UNOS survey. Clin Transplant 2017;31.

[10] Velidedeoglu E, Cavaille-Coll MW, Bala S, Belen OA, Wang Y, Albrecht R. Summary of 2017 FDA Public Workshop: antibody-mediated rejection in kidney transplantation. Transplantation 2018;102:e257–64.

[11] Morgan TA, Chandran S, Burger IM, Zhang CA, Goldstein RB. Complications of ultrasound-guided renal transplant biopsies. Am J Transplant 2016;16:1298–305.

[12] Mengel M, Sis B, Halloran PF. SWOT analysis of Banff: strengths, weaknesses, opportunities and threats of the international Banff consensus process and classification system for renal allograft pathology. Am J Transplant 2007;7:2221–6.

[13] Lo DJ, Kaplan B, Kirk AD. Biomarkers for kidney transplant rejection. Nat Rev Nephrol 2014;10:215–25.

[14] Menon MC, Murphy B, Heeger PS. Moving biomarkers toward clinical implementation in kidney transplantation. J Am Soc Nephrol 2017;28:735–47.

[15] Willis JC, Lord GM. Immune biomarkers: the promises and pitfalls of personalized medicine. Nat Rev Immunol 2015;15:323–9.

[16] Bloom RD, Bromberg JS, Poggio ED, et al. Cell-Free DNA and active rejection in kidney allografts. J Am Soc Nephrol 2017;28:2221–32.

[17] Bromberg JS, Brennan DC, Poggio E, et al. Biological variation of donor-derived cell-free DNA in renal transplant recipients: clinical implications. J Appl Lab Med 2017;2.

[18] Kurian SM, Williams AN, Gelbart T, et al. Molecular classifiers for acute kidney transplant rejection in peripheral blood by whole genome gene expression profiling. Am J Transplant 2014;14:1164–72.

[19] Mehta R, Sood P, Hariharan S. Subclinical rejection in renal transplantation: reappraised. Transplantation 2016;100:1610–8.

[20] Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant 2008;8:753–60.

[21] First MR, Pierry D, McNulty M, et al. Analytical performance validation of a molecular diagnostic signature in kidney transplant recipients. J Transplant Technol Res 2017;7.

[22] First MR, Lee D, Lewis P, Rose S. An economic analysis of the cost effectiveness of blood gene expression profiling in kidney transplant recipients. J Health Med Econ 2017;3.

[23] First MR, Whisenant T, Friedewald JJ, et al. Clinical utility of peripheral blood gene expression profiling of kidney transplant recipients to assess the need for surveillance biopsies in subjects with stable renal function. J Transplant Technol Res 2017;7.

[24] Friedewald JJ, Kurian SM, Heilman RL, et al. Development and clinical validity of a novel blood-based molecular biomarker for subclinical acute rejection following kidney transplant. Am J Transplant 2019;19:98–109.

[25] Roedder S, Sigdel T, Salomonis N, et al. The kSORT assay to detect renal transplant patients at high risk for acute rejection: results of the multicenter AART study. PLoS Med 2014;11:e1001759.

[26] Crespo E, Roedder S, Sigdel T, et al. Molecular and functional noninvasive immune monitoring in the ESCAPE study for prediction of subclinical renal allograft rejection. Transplantation 2017;101:1400–9.

[27] Abecassis M, Kaplan B. Transplantation: biomarkers in transplantation—the devil is in the detail. Nat Rev Nephrol 2015;11:204–5.

[28] Kurian SM, Whisenant T, Mas V, et al. Biomarker guidelines for high-dimensional genomic studies in transplantation: adding method to the madness. Transplantation 2017;101:457–63.