Biomarkers of Rejection in Kidney Transplantation

Scott G. Westphal and Roslyn B. Mannon

Alloimmune injury is a major cause of long-term kidney allograft failure whether due to functionally stable (subclinical) or overt clinical rejection. These episodes may be mediated by immune cells (cellular rejection) or alloantibody (antibody-mediated rejection). Early recognition of immune injury is needed for timely appropriate intervention to maintain graft functional viability. However, the conventional measure of kidney function (ie, serum creatinine) is insufficient for immune monitoring due to limited sensitivity and specificity for rejection. As a result, there is need for biomarkers that more sensitively detect the immune response to the kidney allograft. Recently, several biomarkers have been clinically implemented into the care of kidney transplant recipients. These biomarkers attempt to achieve multiple goals including (1) more sensitive detection of clinical and subclinical rejection, (2) predicting impending rejection, (3) monitoring for the adequacy of treatment response, and (4) facilitating personalized immunosuppression. In this review, we summarize the findings to date in commercially available biomarkers, along with biomarkers approaching clinical implementation. While we discuss the analytical and clinical validity of these biomarkers, we identify the challenges and limitations to widespread biomarker use, including the need for biomarker-guided prospective studies to establish evidence of clinical utility of these new assays.

Complete author and article information provided before references.

Correspondence to R.B. Mannon (roslyn.mannon@unmc.edu)

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Introduction

Over the past several decades, there have been substantial improvements in early kidney allograft outcomes, although long-term graft survival remains suboptimal with nearly 50% of deceased-donor kidney transplants failing within 10 years.[1] Alloimmune injury is a key contributor to late graft failure, and early detection and intervention are essential to preserve graft function and optimize long-term graft survival. Conventional strategies for allograft monitoring rely on serial measurements of serum creatinine and estimated glomerular filtration rate (eGFR), along with interval assessments of urine protein and human leukocyte antigen (HLA) donor-specific antibodies (DSA). Despite ubiquitous use, creatinine is neither sensitive nor specific for rejection. When rejection is suspected, "for-cause" biopsies are typically performed, but by the time clinically apparent graft dysfunction becomes evident to prompt the need for biopsy, significant and potentially irreversible graft damage may be present.

To this end, screening for occult alloimmune injury before functional changes using surveillance (protocol) biopsies has been implemented at some transplant centers. Subclinical rejection (SCR), identified in up to 30% of surveillance biopsies,[2] may further negatively impact graft survival.[3] Yet surveillance biopsies are costly, inconvenient, have frequent unactionable histology, have potential for complications (albeit infrequent), and involve logistical concerns about follow-up procedures. As a result, only ~20% of transplant centers perform them.[4] Finally, there are key limitations in biopsy histology, the gold standard for clinical processes. The Banff allograft pathology criteria are utilized by most transplant centers, but biopsy interpretation is subject to sampling error and subjective interpretation, with significant interobserver and intraobserver variability.[5,6]

The limitations of conventional monitoring highlight the need for novel, noninvasive biomarkers to improve timely recognition of alloimmune injury, optimize early interventions, and allow for personalized immunosuppression. This need has long been recognized, but the process from biomarker discovery to validation, refinement, and clinical implementation is long, with many promising biomarkers lost in the developmental process.[7-9] Several new biomarkers have recently been incorporated into clinical practice after receiving coverage approval from the Centers for Medicare & Medicaid Services (CMS).

In this review, we highlight the landscape of novel noninvasive kidney transplant rejection biomarkers. Rather than reviewing all potential biomarkers in the developmental pipeline, the focus of this review is on the noninvasive posttransplant biomarkers recently adopted for clinical use or nearing clinical implementation (Fig 1). We also discuss the areas of uncertainty, the limitations of biomarker-driven care, and future directions regarding the optimal use of emerging kidney transplant biomarkers.

Donor-Derived Cell-Free DNA (dd-cfDNA)

As cells turn over, nonencapsulated fragments of cell-free DNA (cfDNA) are continuously shed into the bloodstream. Within the kidney allograft, cell injury releases donor-derived cfDNA (dd-cfDNA). Measuring this dd-cfDNA or its fraction allows for real-time monitoring of graft injury. Indeed, within a recipient, very low levels of dd-cfDNA are present compared to the total amount (donor + recipient) of circulating cfDNA. Any increase in dd-cfDNA indicates donor cell damage due to rejection or other injury, such as BK virus nephropathy (BKVN).[10]

Early dd-cfDNA assays were impractical for routine use in transplant because they required sex-mismatched donor–recipient pairs or donor genotyping. The newer

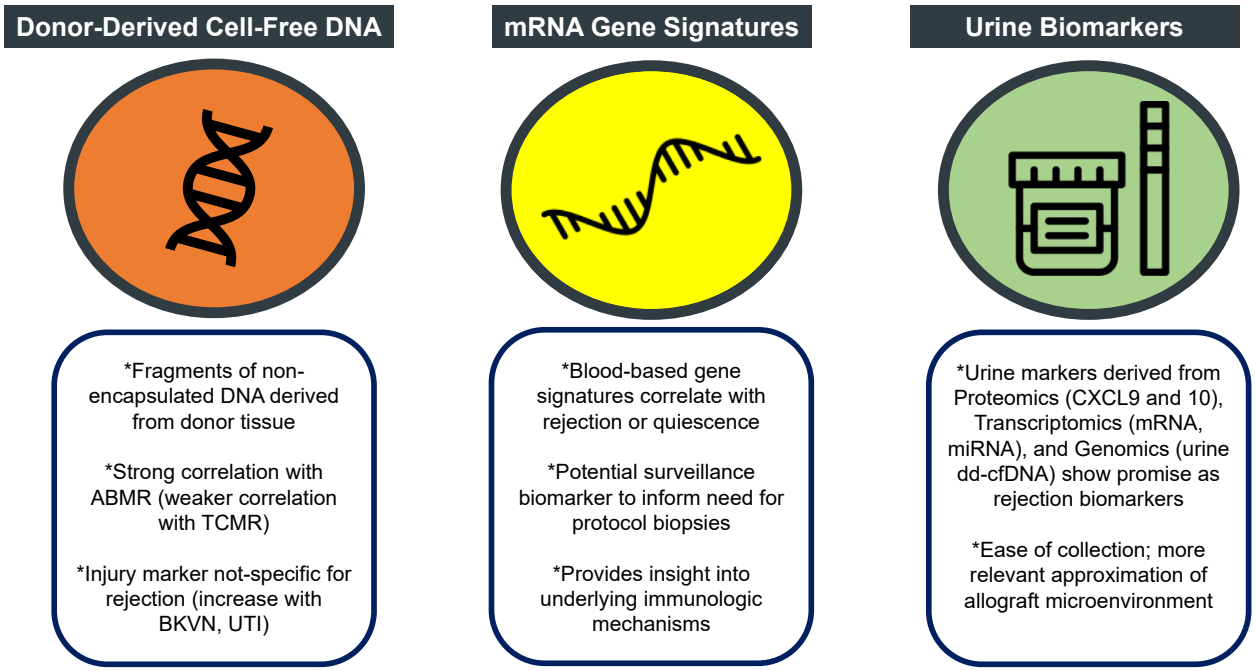

Figure 1. Noninvasive biomarkers of kidney transplant rejection. Abbreviations: ABMR, antibody-mediated rejection; BKVN, BK-polyoma virus nephropathy; dd-cfDNA, donor-derived cell-free DNA; mRNA, messenger RNA; miRNA, microRNA; TCMR, T-cell–mediated rejection; UTI, urinary tract infection.

assays leverage random or targeted single-nucleotide polymorphism (SNP) variants to identify donor and recipient genomic differences by using polymerase chain reaction (PCR), next-generation sequencing (NGS), and bioinformatics to efficiently quantify dd-cfDNA. With the recent availability of several new dd-cfDNA assays, their clinical use has expanded.

Donor-Derived cfDNA as a Diagnostic Rejection Biomarker

Although dd-cfDNA assays are not yet approved by the US Food and Drug Administration (FDA), several clinically available commercial assays have received CMS coverage. These assays, their manufacturers, and methodologies are

- AlloSure (CareDx): targeted NGS (266 SNPs)[11]
- Prospera (Natera): massively multiplexed PCR (>13,000 SNPs)[12]
- Transplant Rejection Allograft Check (TRAC) (Eurofins-Viracor): NGS and genome-wide recipient data[13]
- VitaGraft (Oncocyte): digital droplet PCR[14]

Table 1 reviews the diagnostic performance characteristics from key validation studies for these assays.

AlloSure, the first CMS-approved dd-cfDNA assay, was initially tested in the prospective Diagnosing Active Rejection in Kidney Transplant Recipients (DART) Study, which paired dd-cfDNA with for-cause biopsies.[11] In this study, dd-cfDNA was found to be higher in patients with histologic rejection compared with nonrejection and at a 1.0% threshold identified rejection with 59% sensitivity, 85% specificity, and receiver-operating characteristic (ROC) area under the curve (AUC) 0.74, significantly outperforming serum creatinine (AUC = 0.54). However, dd-cfDNA was elevated primarily in patients with antibody-mediated rejection (ABMR) and T-cell–mediated rejection (TCMR) Banff grade 1B or higher; AlloSure was unable to detect TCMR Banff 1A or borderline rejection (BR).

Subsequent studies assessed the performance of AlloSure using a lower test threshold. In a high immunologic risk cohort, a dd-cfDNA threshold of 0.74% detected ABMR with 100% sensitivity but again was unable to discriminate TCMR from no rejection.[15] The multicenter prospective Assessing Donor-derived Cell-free DNA Monitoring Insights of Kidney Allografts With Longitudinal Surveillance (ADMIRAL) Study (n = 1,092) utilized serial dd-cfDNA monitoring and, by further lowering the test positivity threshold to 0.5% from 1.0%, improved the sensitivity for TCMR (excluding BR) from 45% to 75%.[16]

The Prospera assay was tested using a biorepository of for-cause and surveillance biopsies and discriminated ABMR, TCMR, and mixed rejection from no rejection.[17] The prospective Trifecta Study (n = 300) paired Prospera with biopsy samples for both histologic diagnosis and tissue molecular gene expression diagnosis using the microarray-based Molecular Microscope Diagnostic System (MMDx).[18] Median dd-cfDNA was highest in MMDx-diagnosed ABMR (2.11%), followed by TCMR (1.61%) and mixed-rejection (1.56%), and lowest in the no-rejection group (0.33%). For histologic diagnosis,

Table 1. Diagnostic Performance of Commercially Available Blood-based Biomarker Assays From Key Discovery/Validation Studies

Study	Design	Biopsy Classification	Borderline Rejection Classification	Rejection Rate	Biopsy Indication	Positive Test Threshold	Rejection Type	Sensitivity	Specificity	NPV	PPV	ROC-AUC
Donor-Derived Cell-Free DNA												
Allosure												
Bloom[11]	• Prospective (n = 107) • Multicenter	Banff 2013	No rejection	25%	For cause	1.0%	Any rejection	59%	85%	84%	61%	0.74
							ABMR	81%	83%	96%	44%	0.87
Huang[15]	• Prospective (n = 63) • Single center	Banff 2013	No rejection	54%	For cause	0.74%	Any rejection	79%	72%	75%	77%	0.71
							ABMR	100%	72%	100%	69%	0.82
							TCMR	—	—	—	—	0.42
Bu[16]	• Prospective (n = 1,092) • Multicenter • Serial dd-cfDNA	Banff 2019	No rejection	9%	For cause, surveillance	0.5%	Any rejection	78%	71%	90%	50%	0.80
							ABMR	79%	59%	88%	42%	0.80
							TCMR	75%	50%	84%	36%	0.70
Gupta[36]	Prospective (n = 208)	Banff 2017 + MMDx	Rejection	38%	For cause, surveillance	0.82%	Any rejection	58%-61%[a]	83%-90%[a]	—	—	0.75-0.80[a]
Prospera												
Sigdel[17]	Biorepository (n = 277)	Banff 2017	No rejection	14%	For cause, surveillance	1.0%	Any rejection	89%	73%	95%[b]	52%[b]	0.87
Halloran[18,25]	Prospective (n = 300)	Banff 2019 + MMDx	Rejection	40%	For cause	1% +78 cp/mL	Any rejection	73%-82%[a]	79%-80%[a]	82%-90%	68%-70%	0.82-0.86[a]
TRAC												
Bixler[19]	Biorepository (n = 77)	Not listed	Not defined	20%[c]	For cause	0.7%	Any rejection	58%	85%	86%[d]	55%[d]	0.85
Park[20]	CTOT-08 post hoc analysis (n = 428)	Banff 2019	Rejection	24%	Surveillance	0.7%	Any rejection	47%	88%	84%	56%	0.72
							ABMR	68%	88%	93%	54%	0.84
							TCMR	28%	88%	87%	31%	0.62
VitaGraft												
Oellerich[21]	• Prospective (n = 189) • Serial dd-cfDNA	Banff 2017	Rejection	8%	For cause, surveillance	52 cp/mL	Any rejection	73%	73%	98%	13%	0.83
mRNA Gene Expression Profile												
TruGraf												
Friedewald[46]	Prospective (n = 307)	Banff 2007	Rejection	28%[e]	Surveillance	0.375 (Not-TX)	Any rejection	47%-66%	80%-87%	78%-88%	47%-61%	0.85
							ABMR[f]	38%	85%	87%	33%	0.71
							TCMR[f]	48%	85%	90%	37%	0.80

(Continued)

Table 1 (Cont'd). Diagnostic Performance of Commercially Available Blood-based Biomarker Assays From Key Discovery/Validation Studies

Study	Design	Biopsy Classification	Borderline Rejection Classification	Rejection Rate	Biopsy Indication	Positive Test Threshold	Rejection Type	Sensitivity	Specificity	NPV	PPV	ROC-AUC
Tutivia												
Bestard[51]	Prospective (n = 151)	Banff 2019	Rejection	31%	For cause, surveillance	Score >50	Any rejection	51%	85%	79%	60%	0.69
Combined dd-cfDNA+Gene Expression Profile												
TRAC + TruGraf												
Park[20]	CTOT-08 post hoc analysis (n = 428)	Banff 2019	Rejection	24%	Surveillance	0.7%; 0.375 (Not-TX)[g]	Any rejection	69%	74%	88%	46%	0.81

Abbreviations: ABMR, antibody-mediated rejection; CTOT-08, Clinical Trials in Transplantation-08; cp/mL, copies per milliliter; eGFR, estimated glomerular filtration rate; dd-cfDNA, donor-derived cell-free DNA; GEP, gene expression profile; MMDx, Molecular Microscope Diagnostic System; mRNA, messenger RNA; NPV, negative predictive value; PPV, positive predictive value; ROC-AUC, receiver operating characteristic area under the curve; TCMR, T-cell mediated rejection; TRAC, Transplant Rejection Allograft Check.

[a]Range based on histology or MMDx comparator.
[b]Adjusted to 25% rejection prevalence.
[c]BK nephropathy grouped with rejection.
[d]Adjusted to 15% rejection prevalence.
[e]Sample level prevalence.
[f]ABMR and TCMR data reported in post hoc analysis.[20]
[g]Requires only 1 of dd-cfDNA or GEP to be positive to classify as rejection.

dd-cfDNA was also strongly correlated with mixed rejection (3.18%) and ABMR (1.57%), was only modestly elevated in TCMR (0.88%) followed by no rejection at <6 weeks after transplant (0.68%), and was lowest in the no-rejection group at >6 weeks (0.16%).

TRAC assay characteristics were derived at a dd-cfDNA 0.7% threshold[19] and validated in a post hoc analysis of the Clinical Trials in Transplantation–08 (CTOT-08) Study that paired dd-cfDNA and a gene expression profile with surveillance biopsies in a multicenter cohort of kidney transplant recipients.[20] The dd-cfDNA discrimination was better for ABMR than TCMR. In cases without SCR, 88% of participants had subthreshold (<0.7%) dd-cfDNA levels. Notably, in the 103 SCR cases, only 47% had elevated dd-cfDNA, indicating that dd-cfDNA missed over half the cases of SCR in this cohort.

VitaGraft was studied in a prospective cohort (n = 189) with relatively low rejection rate (8%). Optimal discrimination for rejection occurred at a quantitative threshold of 52 copies/mL (AUC = 0.83) versus dd-cfDNA fraction of 0.43% (AUC = 0.73), with high negative predictive value (NPV) for rejection (98%) but low positive predictive value (PPV) because dd-cfDNA did not differentiate rejection from acute tubular necrosis.[21] Correlation was modestly improved using absolute quantitative dd-cfDNA levels compared to dd-cfDNA fraction. This observation has also been reported elsewhere[22-25] and reflects the potential impact of fluctuations in background recipient cfDNA levels due to inflammation, infection, and posttransplant time, which could falsely raise or lower the dd-cfDNA fraction.[26,27]

Notably, these key dd-cfDNA studies employed different study designs and context of use (clinical rejection versus SCR), different iterations of Banff classification of allograft pathology, and inconsistent categorization of BR. Specificity and sensitivity of each assay depend on the threshold used for defining a positive test, and a varied rejection prevalence impacts PPV and NPV. As a result, superiority or inferiority of one assay to another cannot be ascertained from these studies. Attempts to compare assays head to head have not shown meaningful performance differences.[28,29]

Donor-Derived cfDNA as a Prognostic or Predictive Biomarker

Donor-derived cfDNA may add value as a predictive or prognostic biomarker. In a post hoc analysis of the DART Study, dd-cfDNA > 1% and dd-cfDNA variability (>0.34% from baseline) were prognostic of a future decline in graft function (eGFR ≥ 25%) and de novo DSA (dnDSA) formation.[30] In another cohort receiving serial dd-cfDNA testing, patients with a >1 result above a 0.5% dd-cfDNA threshold were similarly found to be at higher risk of eGFR decline, and patients who developed dnDSA experienced a 125% median dd-cfDNA increase 3-months prior to DSA development.[16]

Also, dd-cfDNA may risk stratify histologic findings. TCMR 1A and BR represent heterogeneous diagnoses,

ranging from trivial inflammation that may resolve without treatment to clinically significant rejection that impacts graft function and survival,[31] and dd-cfDNA may provide insight into the significance of these diagnoses. A multicenter study included 79 biopsies with histologic diagnosis of BR/TCMR 1A and found that paired dd-cfDNA > 0.5% indicated a higher risk of subsequent rejection, DSA formation, and eGFR decline, potentially identifying patients at risk of progression.[32] Finally, in another cohort (n = 180) with paired biopsy and dd-cfDNA, a model incorporating dd-cfDNA with Banff biopsy scores better prognosticated future eGFR decline than histology alone.[33]

Donor-Derived cfDNA to Monitor Rejection Treatment Response

Conventional strategies to assess rejection treatment response are lacking, relying on serum creatinine improvement (a lagging indicator), change in DSA quantity, or histologic improvement by repeat biopsy.[34] Given this situation, dd-cfDNA may provide a more dynamic gauge of treatment response to guide subsequent management.[35] In a cohort treated for rejection (n = 26), follow-up biopsies were obtained 6-8 weeks after rejection treatment and paired with posttreatment dd-cfDNA.[36] Patients with histologic treatment response showed a significant decrease in dd-cfDNA (0.95% to 0.20%), whereas dd-cfDNA was unchanged in those without histologic improvement (0.76% to 0.82%). Conversely, eGFR did not differ in either group before or after rejection treatment. Similarly, Shen et al[37] evaluated patients (n = 28) treated for TCMR or ABMR and demonstrated that dd-cfDNA improved immediately after rejection treatment while eGFR remained unchanged. The magnitude of the dd-cfDNA decrease from pretreatment values correlated with 1- and 6-month eGFR values.

Relationship of dd-cfDNA, HLA-DSA, and ABMR

Development of dnDSA correlates with graft rejection and failure,[38] yet not all DSA-positive patients develop ABMR.[39,40] Given its ability to detect endothelial injury associated with ABMR, dd-cfDNA might clarify decision making in DSA-positive patients with diagnostic uncertainty.[41] Detection of dnDSA often prompts a biopsy, even if graft function is stable. In 1 study of DSA-positive patients undergoing biopsy (n = 87), combining DSA and dd-cfDNA for the diagnosis of ABMR (NPV 83%, PPV 81%) was more accurate than either alone.[42]

Not all cases of ABMR have detectable HLA-DSA. Whether these rejections are caused by non-HLA antibodies, HLA-DSA not detected by standard platforms, or incomplete donor typing, DSA-negative ABMR has a comparable risk of graft failure to DSA-positive ABMR.[43] In a post hoc analysis of the Trifecta Study,[40] >50% of ABMR cases diagnosed by histology or MMDx lacked detectable DSA, yet dd-cfDNA was elevated in both DSA-negative and DSA-positive ABMR. Of the patients with DSA-negative ABMR, 77% had dd-cfDNA > 1.0%. Conversely, in DSA-positive patients without rejection, the median dd-cfDNA was not elevated. For biopsies with ABMR, dd-cfDNA > 1.0% (75%) was more common than DSA (44%), and a combination of dd-cfDNA quantitative level, dd-cfDNA percent, DSA, and posttransplant time was highly accurate for the diagnosis of ABMR (AUC = 0.88).

Emerging Evidence for Value of Adding dd-cfDNA to Clinical Management

Despite the promise of dd-cfDNA as a transplant biomarker, many early studies used small sample sizes and lacked external validation, which potentially hampered their generalizability and limited its widespread implementation. However, recent larger, unselected cohort studies have provided further support for the clinical role of dd-cfDNA. In an 18-month interim analysis of patients (n = 1,631) receiving serial Prospera testing in the Pro-Active registry, dd-cfDNA increased 5 months before ABMR detection and 2 months before TCMR diagnosis,[44] highlighting its potential in surveillance. Another recent multicenter study (n = 2,882) paired Allosure with for-cause and surveillance biopsies; a strong correlation was demonstrated between dd-cfDNA and ABMR, TCMR, and mixed rejection, as well as a correlation with disease severity and activity.[45] Importantly, improved discrimination for rejection was shown when dd-cfDNA was added to a standard-of-care monitoring model (AUC = 0.82) compared with standard of care alone (AUC = 0.78), which included not only creatinine and eGFR but also additional donor/recipient factors, proteinuria, and DSA. Although additional studies are needed to establish its clinical relevance and utility, these recent studies highlight the potential value added from dd-cfDNA.

Blood-Based Gene Expression Signatures

Peripheral messenger RNA (mRNA) gene expression profiles (GEP) provide another blood-based biomarker for rejection or immune quiescence. Unlike dd-cfDNA, an injury marker not specific for rejection, GEPs provide insight into the molecular mechanisms of immunologic activity, potentially upstream of significant injury.

TruGraf (Eurofins-Transplant Genomics) was the first CMS-approved GEP assay. This 57-gene microarray-based GEP was validated in the CTOT-08 Study,[46] which paired TruGraf measurements with surveillance biopsies in patients (n = 307) with stable graft function. In this context, TruGraf had a strong NPV (88%) for SCR, and the PPV was 61%. The high NPV highlights the potential utility of TruGraf as an alternative to surveillance biopsies in stable patients or as part of a targeted surveillance biopsy approach that may provide a higher yield. TruGraf also provides prognostic information; the number of positive ("Not-TX") test results correlated with worse 24-month allograft outcomes and dnDSA development compared with patients who had persistent negative results ("TX"). A

subsequent multicenter study performed serial testing during the first year after transplant (n = 240) and found that patients with >1 Not-TX results were associated with interstitial fibrosis, reduced eGFR, and inferior death-censored graft survival.[47]

Another GEP, developed in the Genomics of Chronic Allograft Rejection (GoCAR) Study,[48] is commercially available as Tutivia (Verici Dx). This 17-gene signature profile differs from TruGraf[49] in that it discriminates early SCR and predicts risk of future rejection and graft loss.[50] In a recent multicenter validation study, the assay was paired with either surveillance or for-cause biopsies in a cohort of transplant recipients (n = 151), unlike the CTOT-08 Study.[51] Tutivia discriminated rejection (AUC = 0.69) significantly better than serum creatinine (AUC = 0.51), and the addition of creatinine had almost no effect on its diagnostic performance. Applying a cutoff score of 50 (range, 0-100) to group the patients into high-risk or low-risk groups yielded a sensitivity 51%, specificity 85%, NPV 79%, PPV 60% for rejection. The PPV was higher in for-cause biopsies whereas the NPV was higher in surveillance biopsies. The assay classified patients with BKVN as low rejection risk, differing from other assays that do not discriminate BKVN from rejection.

Additional gene signatures show promise in various contexts. Allomap Kidney (CareDx) is a 5-gene classifier modified from a similar assay used in heart transplantation.[52] This assay discriminated immune quiescence from rejection (TCMR and ABMR) with AUC = 0.78, highlighting its potential as a surveillance biomarker of immune activity.[53] The European multicohort BIOMARGIN Study analyzed an 8-gene assay that displayed diagnostic accuracy for ABMR in patients with and without graft dysfunction.[54] Lastly, the 17-gene Kidney Solid Organ Response Test (kSORT) initially showed promise in detecting rejection (AUC = 0.94),[55] but subsequent validation was less robust (AUC = 0.71),[56] and it had even worse performance (AUC = 0.51) in a prospective multicenter study (n = 1,763), with "real-world" rejection prevalence.[57]

Although several GEPs have shown promise for detecting subclinical rejection, further independent validation is necessary in larger, real-world populations to demonstrate reproducibility and reliability at different time periods after transplant,[58] and their clinical utility will require demonstrating improved outcomes when utilized.

Combining GEP and dd-cfDNA Biomarkers

The diagnostic performance of combining GEP and dd-cfDNA has been evaluated. A post hoc analysis of the CTOT-08 Study examined the ability of paired GEP (TruGraf) and dd-cfDNA (TRAC) to detect SCR in patients undergoing surveillance biopsies.[20] This study considered BR and "suspicious for ABMR" as rejection. The GEP better detected subclinical TCMR (AUC = 0.80) than dd-cfDNA (AUC = 0.62), whereas dd-cfDNA preferentially detected subclinical ABMR (AUC = 0.84) compared with GEP (AUC = 0.71). Combining GEP with dd-cfDNA detected all rejection types with NPV 88% and PPV 81% (AUC = 0.81), performing significantly better than either assay alone.

Similar findings were noted in a post hoc analysis (n = 99) of the DART Study.[53] Unlike the CTOT-08 Study, the DART Study included only for-cause biopsies, paired with dd-cfDNA (Allosure) and GEP (AlloMap Kidney). Both assays correlated with rejection, but there was non-overlap and only weak correlation between the assays, suggesting a potential role for complementary testing because the combined assays discriminated rejection from nonrejection better than either alone. Larger, multicenter registry studies are ongoing to further assess the role of combining these GEP with dd-cfDNA.

Urine Biomarkers

Urine may be the ideal marker given its direct proximity to the transplant microenvironment, along with its relative ease of collection and potential for point-of-care testing. Promising transcriptomic, proteomic, genomic, and metabolic urine biomarkers have emerged but have not yet been incorporated into routine clinical practice.

Urine Gene Expression Signatures

Several potential urine transcriptomic gene signature biomarkers incorporating mRNA, microRNA, and exosomal RNA have been evaluated to detect or predict acute rejection, but they have not been further validated.[59,60] An obstacle to urine gene expression is the relative instability and rapid degradation of urinary cell pellets. An alternative approach analyzes mRNA expression in urine exosomes, which are more stable and less prone to degradation.[61] An exosomal mRNA signature was developed and validated from a cohort of for-cause biopsies with paired urine samples.[62] The exosome signatures discriminated rejection from nonrejection (sensitivity = 85%, specificity = 94%, NPV = 93%, PPV = 86%, AUC = 0.93), as well as TCMR from ABMR. Subsequent studies evaluating various urine exosomal mRNA and microRNA signatures show promise[63] but have not been validated nor clinically implemented.

Urine Chemokines

Urine chemokines, particularly C-X-C motif ligand 9 and 10 (CXCL9 and CXCL10) have emerged as potential proteomic rejection biomarkers. The CTOT-01 Study evaluated urine CXCL9 mRNA and protein and found that low CXCL9 protein levels identified the patients least likely to develop rejection and eGFR decline.[64] CXCL10, which is expressed in allograft infiltrating leukocytes as well as renal tubular epithelial cells, appears to be sensitive for detecting subclinical rejection.[65,66] However, these chemokines are not specific for rejection and are elevated in other urinary inflammatory conditions (eg, BKVN, infection).[67] To account for these confounders, studies incorporating clinical variables (ie, age, eGFR, BK viral load) with urine

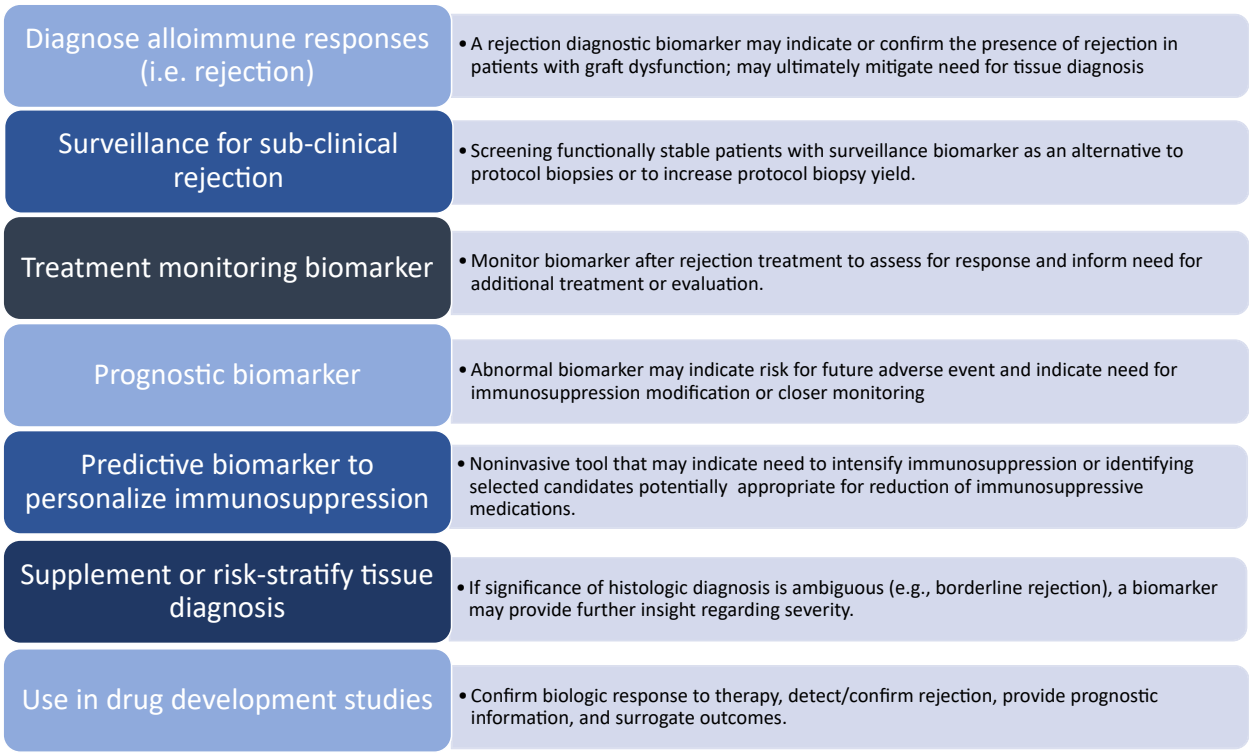

Figure 2. Potential clinical purposes of noninvasive post–kidney transplant biomarkers. Current studies identify the potential for these purposes, but further evidence is needed to determine the clinical utility for these purposes and for practical and consistent implementation.

chemokines have had high diagnostic accuracy for acute rejection.[68,69]

Clinical translation of CXCL10 monitoring has been limited in part by the accessibility of a practical, quantitative, reliable monoclonal-based antibody assay. A prospective, multicenter study recently validated a CXCL10 bead-based immunoassay (Luminex) readily available to HLA laboratories.[70] Using this platform, which is now commercially available, CXCL10 measurements were found to be precise and reproducible. Its relative ease of use and lower cost makes this a potentially attractive monitoring approach.

Based on its promise as a diagnostic and prognostic biomarker, a randomized controlled trial assessed the impact of CXCL10-driven posttransplant care. This study confirmed that low levels were associated with low risk for rejection, and elevated levels were associated with inflammation (eg, rejection, BKVN). However, the study did not demonstrate benefit on clinical 1-year outcomes.[71] Additional CXCL10-driven management trials that assess for clinical utility are ongoing.[72]

The Challenges of Biomarker Driven Clinical Management

The CMS coverage approval of new biomarkers led to relatively quick integration into clinical practice. New biomarkers have potential to transform kidney transplant management (Fig 2), but their impact on long-term graft outcomes remains unknown, and current use is not consistent across transplant programs. The limitations of these assays must be understood and defined to ensure appropriate interpretation (Box 1).

Many biomarkers are being used in clinical contexts that differ from those in which they were validated. Providers should be cautious when they apply assays that were validated to detect SCR in functionally stable patients to assess for acute rejection in patients with graft dysfunction. Similarly, biomarkers validated in patients

Box 1. Key Challenges in the Clinical Implantation of Post–Kidney Transplant Biomarkers of Rejection

- Defining and using biomarkers in the appropriate context of use.
- Understanding which biomarkers are best suited for patients with differing pretest rejection probability.
- Evaluating larger sample sizes to validate role of biomarkers to predict rejection or monitor treatment responses.
- Determining test cost-effectiveness given high price and insurance coverage challenges.
- Improving processing and shipping logistics, with more rapid turnaround time.
- Establishing the clinical utility using evidence derived from biomarker-driven clinical trials.

receiving for-cause biopsies may perform differently for surveillance screening, given differences in pretest probability. Pretest characteristics and risk, which impact NPV and PPV, must be considered because a biomarker result may require different interpretation in a high-alloimmune risk patient early after transplant versus a lower-risk patient farther out from transplant. The analytic factors of a given assay must be considered, such as defining biomarker thresholds which directly impact test sensitivity and specificity. Technical/logistical factors remain a limitation to widespread implementation because most assays require sending samples to specialized laboratories; the resulting delays in turnaround times by several days can limit their use where a result is required urgently.

The search for novel biomarkers has led to an unprecedented increase in biomarker publications over the past two decades. However, a recent systematic review raised concerns about the quality and clinical applicability of biomarker research.[73] This study found that most biomarker studies lack rigorous design: >80% were retrospective, 74% were single center, <5% reported external validation, the sample sizes were generally small, and there was a general lack of transparency with potential for inaccurate interpretation. As noted previously, diagnostic characteristics derived from a small-sample study population with stringent enrollment conditions may not be applicable to real-world patients. Several ongoing registry studies (eg, NCT03326076, NCT04491552, and NCT04091984) are evaluating biomarkers in large cohorts of unselected patients, and these results may demonstrate independent association of these biomarkers with activity and severity of rejection, and value added to the standard of care.

Beyond analytical and clinical validity, demonstrable clinical utility is critical.[74] As such, additional prospective, interventional studies are needed to show beneficial outcomes of biomarker-driven care in current standard-of-care posttransplant management including creatinine/eGFR monitoring, assessment of proteinuria, monitoring for HLA-DSA, and incorporating both donor and recipient characteristics. The first trial attempting to evaluate biomarker-driven management (CXCL10) compared with the standard of care did not demonstrate a benefit.[71]

The economic impact of biomarker use also remains unknown. Initial biomarkers receiving CMS local coverage determination (LCD) were approved at a reimbursement rate of >$2,800/test, set by the CMS contractor Palmetto GBA Molecular Diagnostics Services (MolDX) Program. Given the cost and frequency that these tests are obtained, cost-effectiveness is a concern.[75] More widespread implementation was challenged in March 2023, when the MolDx program reimbursement guidance was updated with more restrictive requirements for biomarker coverage.[76] This guidance continues to be updated (Table 2), resulting in more stringent approaches to biomarker use.

Table 2. Updated Local Coverage Determination for Transplant Biomarker Usage

Requirement	Expectation	Comments
Overall requirement	The 4 intended uses that are established require that the physician is concerned about at least 1 of the following: • Rejection (and would otherwise obtain a biopsy to evaluate the allograft). • Adequacy of immunosuppression (and would otherwise obtain a biopsy to evaluate the allograft or is considering doing so). • Probability of rejection with concerning clinical information (clinical pretests/information that inform whether a subsequent biopsy would likely be avoided). • Results of an inconclusive biopsy (wherein the test may subsequently preclude another biopsy).	A biopsy must be considered or have been performed and is equivocal.
Frequency of biomarker testing	Surveillance testing would be covered only in lieu of a surveillance biopsy. Requires a transplant center to have a clinical protocol in place stating a surveillance biopsy would otherwise be performed	• Excludes the ~65% of transplant centers that do not utilize routine surveillance for all recipients. • Complicates current treatment standards where immune high-risk populations may be treated with more frequent monitoring.
Concurrent molecular testing and biopsy	Limits use of testing when a biopsy is either planned or already performed.	Presumes intent for allograft biopsy already made by clinician and hence suggests that biomarker results have no use in the context of an allograft biopsy histologic result.
Multimodality molecular testing	Only 1 molecular biomarker would be approved for a given encounter, removing coverage for combined simultaneous biomarkers.	Treats all molecular biomarkers identically when they have been derived by different intents with different context of use.

Information derived from CMS, L398956 (March 2023).[76]

Conclusion

Noninvasive blood and urine biomarkers have great potential to transform the management of kidney transplant patients. As additional biomarkers become clinically available, understanding the context of use and the strengths and limitations of each assay will be imperative to ensure appropriate utilization and interpretation. Despite widespread incorporation of new biomarkers, prospective studies are still required to demonstrate the clinical benefit and utility of these new assays.

Article Information

Authors' Full Names and Academic Degrees: Scott G. Westphal, MD, and Roslyn B. Mannon, MD.

Authors' Affiliations: Division of Nephrology, Department of Internal Medicine, University of Nebraska Medical Center (SGW), and Medical Service, Nebraska Western Iowa Veterans Affairs Health Care System (SGW, RBM), Omaha, Nebraska.

Address for Correspondence: Roslyn B. Mannon, MD, 983040 Nebraska Medical Center, MSB 5569, Omaha, NE 68198-3040. Email: roslyn.mannon@unmc.edu

Additional Information: ORCiDs are 0000-0003-1776-3680 (Dr Mannon) and 0000-0001-5843-0677 (Dr Westphal).

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