Beyond the Biopsy: Monitoring Immune Status in Kidney Recipients

Roy D. Bloom1 and Joshua J. Augustine2

Abstract
Improved long-term kidney allograft survival is largely related to better outcomes at 12 months, in association with declining acute rejection rates and more efficacious immunosuppression. Finding the right balance between under- and overimmunosuppression or rejection versus immunosuppression toxicity remains one of transplant’s holy grails. In the absence of precise measures of immunosuppression burden, transplant clinicians rely on nonspecific, noninvasive tests and kidney allograft biopsy generally performed for cause. This review appraises recent advances of conventional monitoring strategies and critically examines the plethora of emerging tests utilizing tissue, urine, and blood samples to improve upon the diagnostic precision of allograft surveillance.

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Introduction
Long-term improvement in kidney transplant outcomes is mostly related to better 1-year allograft survival (1–3), concomitant with declining acute rejection under contemporary immunosuppression largely comprising T cell–depleting induction and tacrolimus-mycophenolic acid-corticosteroid maintenance therapy (2). Between years 2 and 10 post-transplant, allograft attrition rates approximate 5%–7% per year (1). Allograft failure occurs from immunologic or nonimmunologic causes, whereas immunosuppression toxicity affects patient morbidity and mortality. Optimizing transplant outcomes is a central recipient care tenet, commencing with pretransplant immunologic assessment. Without a post-transplant measure of immunosuppression burden or risk of rejection versus overimmunosuppression, clinicians rely on nonspecific markers and indication biopsies for guidance, with allograft histology or transplant failure the gold standard against which nonspecific tests are measured. Noninvasive monitoring tools are being developed for use in several contexts, including as diagnostic, prognostic, and/or predictive markers (Table 1). We herein review conventional tools used for these assessments and highlight emerging tissue, urine, and blood biomarkers aimed at improving precision.

Pretransplant Immunologic Risk Assessment

Preexisting Donor-Specific Antibody and Histocompatibility Leukocyte Antigen Epitope Mismatch
Detectable preexisting donor-specific antibody (DSA), ascertained at transplant, is associated with chronic allograft failure (4). Patients with pretransplant DSA and positive crossmatches have high rates of persistent or recurrent DSA post-transplant. Despite desensitization protocols, antibody-mediated rejection occurs in approximately 50% of patients with persistent DSA post-transplant (5,6).

Alloantibodies to HLA engage the HLA molecule’s epitope. Epitope binding affinity is determined by an eplet, a single or small number of polymorphic amino acids near the HLA surface that influence antibody specificity and immunologic risk (7). A seminal 286-patient study demonstrated that locus-specific epitope mismatch correlated more robustly with de novo DSA formation than traditional DR/DQ mismatch (8). Epitope mismatch has since been correlated with rejection and allograft loss when combined with nonadherence (9) and with immunosuppression minimization (10). More recent application of HLA-DR/DQ single-molecular eplet mismatch further improved correlation with de novo DSA and facilitated stratification of recipients into low–, intermediate–, and high–alloimmune risk categories (11). Molecular mismatch risk category was associated with de novo DSA, antibody- and T cell–mediated rejection, and graft loss, findings since validated elsewhere (12,13). Molecular mismatch risk stratification should be easily implementable in HLA laboratories, with potential to positively affect post-transplant outcomes.

Nonhistocompatibility Leukocyte Antigen Mismatching and Nonhistocompatibility Leukocyte Antigen Antibodies

Recently, donor/recipient non-HLA allelic mismatches associating with antibody-mediated rejection were identified through exome sequencing of mononuclear cell DNA (14). Genes for kidney and blood vessel cell surface proteins incurred risk independent of HLA mismatch. In an unrelated study, the presence pretransplant of non-HLA antibodies targeting glomerular endothelium (MHC class 1–related chain A, endothelin 1 type A, and angiotensin type 1 receptor) has been
associated with early post-transplant microvascular injury resembling antibody-mediated rejection (15). These preliminary observations indicate that non-HLA-related biomarkers are promising tools to assess post-transplant immunologic risk of potential candidates.

**Enzyme-Linked Immune Absorbent Spot**

Enzyme-linked immune absorbent spot (ELISPOT) quantitatively measures the cytokine secreting cell frequency. ELISPOT assays for IFN-γ identify effector memory T cells pretransplant when mixed with T cell–depleted donor cells. Pretransplant ELISPOT positivity correlated with rejection in higher-risk populations (16) and during tacrolimus withdrawal in lower-risk patients (17). The association of pretransplant ELISPOT with rejection is tempered by antithymocyte globulin therapy. A recent analysis found a rejection rate of 15% in ELISPOT-positive patients versus 3% in negative patients (18). Among ELISPOT-positive patients who received antithymocyte globulin induction, there was no rejection. In Clinical Trials in Organ Transplantation 01 (CTOT-01), pretransplant ELISPOT similarly predicted allograft function only in the nondepleting induction subgroup (17).

**Peripheral Blood Gene Signature**

Investigators from the Study of the Genomics of Chronic Allograft Rejection (GoCAR) recently performed whole-blood RNA sequencing in patients pretransplant, developing a 23-gene set that predicted early T cell rejection and associated with late graft loss (19). Further validation of this signature is underway.

**Conventional Post-Transplant Monitoring**

**Allograft Function**

Studies demonstrate that 1-year kidney transplant function predicts longer-term failure (20, 21), a risk that is greater with worsening albuminuria (22–24). Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend ongoing serum creatinine and urine protein monitoring, with dysfunction episodes evaluated by allograft ultrasound (25). Allograft biopsy is indicated when diagnosis is uncertain, for evaluating proteinuria, or where histologic findings will affect treatment.

**Drug-Level Monitoring**

Although drug monitoring for tacrolimus, cyclosporin, sirolimus, and everolimus is routine, the utility of mycophenolic acid levels is not established (26). Trough calcineurin inhibitor levels correlate fairly well with total drug exposure. Out of range levels may signify nonadherence; underdosing; formulation change; or unrecognized drug-drug, food-drug, or gut-drug interactions, providing opportunity for patient counseling or regimen adjustment before rejection or toxicity ensues.

Tacrolimus-level variability over time predicts underdos-ing/nonadherence and rejection (27–30). High intrapatient tacrolimus-level variability is associated with interstitial fibrosis/tubular atrophy (IFTA) (27), rejection, and allograft failure (28). In one study, recipients with de novo DSA had higher proportions of tacrolimus levels <5 ng/ml; moreover, levels were significantly lower in the 6 months preceding de novo DSA detection than at earlier time points (29).

Time in therapeutic range, typically used in anticoagulation management, is a newer concept in transplantation. Time in therapeutic range—a calculation of the percentage of time a level is within the predefined target range in individual patients—has been applied to tacrolimus therapy (30). On the basis of target levels 5–10 ng/ml within the first post-transplant year, time in therapeutic range <60% associates with de novo DSA and rejection risk by 12 months

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**Table 1. Context of use of biomarkers in kidney transplantation**

<table>
<thead>
<tr>
<th>Test</th>
<th>Commercially Available</th>
<th>Susceptibility</th>
<th>Diagnostic</th>
<th>Prognostic</th>
<th>Serial Monitoring</th>
<th>Reference Nos. Cited</th>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>4–6</td>
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<td>Eplet mismatch</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7–13</td>
</tr>
<tr>
<td>Serum creatinine/GFR</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>20, 21</td>
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<td>UP/Cr</td>
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<td>+</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>22–24</td>
</tr>
<tr>
<td>Tacrolimus TTR/CV</td>
<td>+</td>
<td>+</td>
<td>P</td>
<td>P</td>
<td>+</td>
<td>27–31</td>
</tr>
<tr>
<td>EBV NAT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>23, 38, 42</td>
</tr>
<tr>
<td>BKV NAT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>39–41, 43, 46</td>
</tr>
<tr>
<td>De novo DSA</td>
<td>+</td>
<td>+</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>7, 13, 32, 37</td>
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<td>Surveillance biopsy</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>Pretransplant ELISPOT</td>
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<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>16–18</td>
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<tr>
<td>MMDX</td>
<td>+</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>64–67</td>
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<td>GoCAR 13-gene set</td>
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<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>69</td>
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<tr>
<td>Urine chemokines</td>
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<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>25, 70–72</td>
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<td>Blood gene profiling</td>
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<td>P</td>
<td>P</td>
<td>P</td>
<td>54, 75</td>
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<tr>
<td>kSORT</td>
<td>–</td>
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<td>P</td>
<td>P</td>
<td>P</td>
<td>73, 74</td>
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<tr>
<td>dd-cfDNA</td>
<td>+</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>77–83</td>
</tr>
</tbody>
</table>

DSA, donor-specific antibody; +, established use for biomarker test; UP/Cr, urine protein-urine creatinine ratio; TTR/CV, time in therapeutic ratio/coefficient of variation; P, potential use for biomarker pending further validation studies; EBV, Epstein–Barr virus; NAT, nucleic acid testing; BKV, BK virus; ELISPOT, enzyme-linked immune absorbent spot; MMDX, microarray-based molecular diagnostic system; GoCAR, Study of the Genomics of Chronic Allograft Rejection; kSORT, kidney Solid Organ Response Test; dd-cfDNA, donor-derived cell-free DNA.
and allograft loss by 5 years post-transplant. Analysis incorporating both time in therapeutic range and coefficient of variation suggests the immunologic risk associated with high intrapatient tacrolimus-level variation is due to low time in therapeutic range rather than variability in and of itself (31). Use of these simple tracking tools is an actionable strategy to monitor medication nonadherence, optimize dosing, and improve outcomes.

**Donor-Specific Antibody**

De novo DSA develops in 15%–20% of patients within the first few post-transplant years (7,13). One study monitored de novo DSA over a mean of 6.2 years in 315 sensitized patients, demonstrating that its presence associated with HLA-DR mismatch and patient nonadherence and adversely affected 10-year allograft survival (7). Other DSA risk factors include immunosuppression minimization, DQ mismatching, and early T cell-mediated rejection (32), with mostly class 2 DSA identified in this latter setting (33,34).

In patients with antibody-mediated rejection, de novo DSA appears to have greater negative effect than preexisting DSA. In a 205-patient antibody-mediated rejection cohort evenly divided between recipients with pretransplant DSA and de novo DSA, antibody-mediated rejection occurred earlier in the latter group (median 85 versus 1437 days). Patients with de novo DSA demonstrated more proteinuria, greater class 2 antibody, higher antibody titers, more frequent transplant glomerulopathy, and worse allograft survival at 8 years (35).

Transplantation Society guidelines recommend DSA monitoring in the setting of recipients with pretransplant DSA (36), immunosuppression reduction, patient nonadherence, or a rejection episode occurrence, with close allograft function surveillance when detected (37). Transplant biopsy, similarly recommended upon DSA detection, notably has no evidence grade. In this setting, we believe biopsy may have diagnostic utility, although acknowledge absence of data demonstrating the procedure results in improved outcomes.

**Viral Screening**

Through the interplay between antiviral and alloimmune responses, viruses result from overimmunosuppression but may also trigger rejection. Virus-specific T cells crossreact to alloantigen have been demonstrated in the circulation of Epstein–Barr virus (EBV)– and/or cytomegalovirus-infected patients (38). BK virus (BKV) impairs allograft function through cytopathic injury yet is associated with de novo DSA and rejection (39,40). Mechanisms linking BKV to alloimmunity are unclear but include immunosuppression reduction or heterologous immunity. Supporting the latter, BKV nephropathy allograft biopsies have been shown to simultaneously contain both BKV-reactive and alloreactive T cell clones (41).

These viral infections typically occur within 6 months post-transplant. Except for preemptive treatment strategies or guiding therapy in infected individuals, no cytomegalovirus screening recommendations exist (23). KDIGO guidelines suggest EBV nucleic acid testing in EBV-seronegative recipients of EBV-seropositive kidneys once in week 1, monthly for 3–6 months, then quarterly until 12 months post-transplant, and when treating rejection (23). Although American Society of Transplantation (AST) Infectious Diseases Community of Practice (IDCOP) guidelines suggest more frequent testing (42), both guidelines advocate immunosuppression reduction for worsening EBV viremia.

BKV viremia and BKV nephropathy prevalence rates are 10%–30% and 2%, respectively (43). Routine screening enables BKV detection before nephropathy affects kidney function (44). KDIGO suggests monthly monitoring through 6 months and then quarterly for 6 months. Because BK viral loads >10,000 copies per milliliter predict BKV nephropathy (45), KDIGO recommends this level as a threshold above which immunosuppression should be reduced. Recent AST IDCOP guidelines recommend monthly testing until 9 months and then quarterly until 24 months because 30% of BKV infections occur beyond 6 months post-transplant (46). Because intragraft BKV replication may be focal, up to one third of BKV-infected biopsy samples may test negative; this false-negative rate declines as viral loads exceed six log10 copies per milliliter (46). We recommend allograft biopsy in recipients with both BK viremia and either new allograft dysfunction or another abnormal diagnostic biomarker.

**Torque Teno Virus—An Emerging Immunosstat?**

Torque teno virus (TTV) is an apathogenic virus with no known therapies (47). In the transplant context, a recent study reported that around 43 days prebiopsy, patients with rejection had lower blood TTV levels than nonrejecting patients (48). A subsequent prospective, observational study incorporated TTV viral load measurements weekly initially and then quarterly until 12 months post-transplant (49). Torque Teno viral load peaked 3 months post-transplant; thereafter, each TTV viral load log increase associated with a 22% lower rejection odds and an 11% greater odds for another infection. Viral loads between 1 × 106–107 copies per milliliter were identified as the “sweet spot” for optimally minimized risk for rejection and infection. Although further validation is required, TTV represents another potential immune status monitoring tool.

**Surveillance Biopsies**

Historically performed in “high-risk” patients, the rationale for surveillance kidney transplant biopsy is determination of “subclinical rejection” described in cyclosporintreated patients, where 30% of recipients biopsied by protocol early post-transplant displayed histologic tubulitis despite stable laboratory values (50,51). A similar study subsequently conducted in tacrolimus-MMF-prednisone–treated patients observed subclinical rejection rates <5%, with no differences in patient/allograft survival or IFTA at 2 years post-transplant (52,53). The investigators concluded there was no benefit to surveillance biopsies in patients receiving this immunosuppression regimen.

Recent surveillance biopsy studies have focused on subclinical borderline T cell–mediated rejection, detected in approximately 25%–40% of participants (54–56). Follow-up biopsies have demonstrated histologic progression despite treatment (54,55). Although these data may support surveillance biopsy (and potentially, rebiopsy when
subclinical injury is detected), it should be borne in mind that consensus around the histologic definition of borderline T cell rejection (57) and effectiveness of treatment in this setting is not established.

Surveillance biopsies may have value in patients undergoing major immunosuppression modification. Heilman et al. (58) correlated 1- or 4-month post-transplant biopsy findings with a 12-month biopsy in 256 recipients who underwent rapid corticosteroid withdrawal. Although 6% developed overt rejection by 12 months, early surveillance biopsy revealed subclinical rejection or inflammation in 27%. Both subclinical rejection and inflammation predicted greater IFTA at 12 months. Another study randomized low-immunologic risk recipients to continued tacrolimus versus conversion to sirolimus at 3 months post-transplant (59). Despite similar kidney function, 24-month surveillance biopsies showed more subclinical inflammation and IFTA in the sirolimus arm.

A recent US transplant center survey found that surveillance biopsies were performed by 38 of 83 (46%) responding centers; 20 centers biopsied all patients, whereas 18 were more selective (60). This increased biopsy rate compared with a prior survey (61) may reflect currently perceived need for histology in lieu of reliable immune monitoring tools. An analysis that examined complications after 2514 kidney allograft biopsies observed fewer major complications in surveillance than for-cause procedures (0.3% versus 3%), with surgical intervention undertaken in 0.6% and no attributable graft losses or death (62). These findings support that surveillance kidney transplant biopsies are safe.

Limitations of Conventional Monitoring and Biopsy

Serum creatinine, widely available, inexpensive, and with rapid turnaround, is neither specific nor sensitive, and often, it is a late injury indicator. Kidney biopsy is costly, sampling error prone, limited by subjective interpretation, and inconvenient, and it carries some risk. Surveillance biopsies are lower yield than indication biopsies because many patients with unremarkable histology will be biopsied. Moreover, optimal timing and surveillance biopsy frequency are unknown. Finally, although identifying morphologic changes that predict outcomes, surveillance biopsies have yet to result in interventions established to improve outcomes.

These collective limitations, coupled with technological advancement, have spawned interest in finding more specific, noninvasive tissue, urine, and blood biomarkers. An important premise for seeking status quo alternatives is that allograft injury and damage are driven by subclinical, often repetitive events (63), underscoring the need for scalable monitoring strategies.

Novel Tissue Diagnostics

Halloran et al. (64) analyzed molecular allograft rejection phenotypes by measuring mRNA transcripts in biopsy tissue. Comparing T cell-mediated and antibody-mediated rejection biopsies with normal histology revealed that prominent transcripts for both rejection types were induced by IFN-γ. Effector T cell and myeloid cell expressions were T cell–mediated rejection specific, whereas transcripts for natural killer cell localization and endothelial injury were unique to antibody-mediated rejection. There was some transcript overlap between rejecting and nonrejecting allografts (65), but generally strong associations of transcripts with rejection and rejection subtypes were preserved in validation testing.

Application of this “microarray-based molecular diagnostic system” (MMDX) was demonstrated in a multicenter study (66). MMDX defined antibody-mediated rejection in 41% of biopsies where it was not reported originally and revealed antibody-mediated rejection transcript signaling in C4d-positive and -negative biopsies. The MMDX system has been used to analyze biopsies with inflammation in areas of IFTA, revealing predominant antibody-mediated rather than T cell–mediated rejection (67). Recently, the Banff Working Group described a 770 biopsy tissue–derived gene panel using a Nano-String platform. Genes were categorized by host organ transplant responses, including rejection, tolerance, drug toxicity, and viral infection, with plans for future multicenter validation using a commercially available assay (68).

The GoCAR study utilized mRNA microarray analysis to predict fibrosis progression at 1 year from biopsy tissue collected at 3 months post-transplant (69). Using the Chronic Allograft Damage Index score, 12-month biopsies with scores of > 2 were analyzed, identifying a gene set that correlated with fibrosis. After application to 3-month biopsies in cases where fibrosis worsened by month 12, a 13–gene set panel was derived that predicted subsequent fibrosis, independent of clinicopathologic variables.

Novel Noninvasive Biomarkers

Ideal diagnostic biomarkers should identify patients with high disease probability (high positive predictive value). Prognostic and predictive biomarkers can then be applied to determine both patients at high risk for a bad prognosis and predicted treatment benefit. Assays should provide greater lead time for detecting and monitoring allograft injury, be reproducible, have a low coefficient of variation, have rapid turnaround, and permit cost-effective surveillance. Moreover, they could provide dynamic analyses of allograft and immune status, enabling precise risk stratification, with potential to guide need for biopsy or immunosuppression modification.

Urinary Biomarkers

Chemokines CXCL9 and CXCL10 recruit effector T cells in response to IFN-γ and have been identified as urinary markers of acute rejection in separate clinical trials. In CTOT-04, Suthanthiran et al. (70) analyzed urinary mRNA collected serially from 485 kidney recipients within the first post-transplant year. A three-gene signature was derived using CD3ε, CXCL10, and 18S ribosomal RNA that discriminated T cell–mediated rejection from no rejection. Analysis of urine samples from rejectors showed increased chemokine gene expression as early as 120 days before biopsy.
<table>
<thead>
<tr>
<th>Test</th>
<th>Suggested Testing Frequency</th>
<th>Potential Benefit</th>
<th>Limitation(s)</th>
<th>Comment</th>
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<tbody>
<tr>
<td><strong>Conventional</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UP/Cr</td>
<td>1, 3 mo, then quarterly</td>
<td>Low cost, actionable surveillance test</td>
<td>• Lacks sensitivity to detect some rejection, inflammation</td>
<td>Opportunity for antiproteinuric therapy/targeted therapy</td>
</tr>
<tr>
<td>Tacrolimus TTR/CV</td>
<td>Ongoing</td>
<td>Actionable, low cost, monitor nonadherence</td>
<td>• Targeted levels not specific for immune response of individual patients</td>
<td>Can incorporate into electronic medical record</td>
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<tr>
<td>EBV NAT</td>
<td>Monthly for 6–12 mo</td>
<td>IS management guide in EBV+/- recipients</td>
<td>• Not standardized</td>
<td>Reduce IS for rising viral load</td>
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<tr>
<td>BKV NAT</td>
<td>1, 3, 6, 12 mo</td>
<td>Guide IS management</td>
<td>• Not standardized</td>
<td>Reduce IS for rising viral load</td>
</tr>
<tr>
<td>DSA</td>
<td>1, 3, 6, 12 mo</td>
<td>Risk stratification/guide IS management</td>
<td>• Lacks specificity</td>
<td>Opportunity to enroll in contemporary clinical trials</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Not standardized</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• No proven surveillance benefit</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• $$</td>
<td></td>
</tr>
<tr>
<td><strong>Emerging</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surveillance biopsy</td>
<td>Once in first 3 mo?</td>
<td>Risk stratification, relatively safe</td>
<td>• Risk of complications</td>
<td>Possible use in conversion/minimization regimens, patients with DSA, clinical trials</td>
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<td>• Sampling error</td>
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<td></td>
<td></td>
<td></td>
<td>• No proven outcome benefit</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>• $$$</td>
<td></td>
</tr>
<tr>
<td>MMDX</td>
<td>TBD</td>
<td>Discriminates rejection types, with improved consistency</td>
<td>• Some diagnostic overlap</td>
<td>Consider for patients being biopsied where histology findings are unclear to guide therapy</td>
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<td></td>
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<td>• No prospective intervention or outcome data</td>
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<td></td>
<td>• $$$</td>
<td></td>
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<td>Urine chemokines</td>
<td>TBD</td>
<td>Predicts rejection, reproducible</td>
<td>• Not specific, standardized</td>
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<td></td>
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<td></td>
<td>• Not commercially available</td>
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<td></td>
<td></td>
<td></td>
<td>• No proven outcome benefit</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• $$</td>
<td></td>
</tr>
<tr>
<td>ELISPOT</td>
<td>Prior to transplant</td>
<td>Risk stratification</td>
<td>• Limited utility in depleting induction setting</td>
<td>May be more practical in live donor recipients</td>
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<td></td>
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<td>• $$</td>
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<tr>
<td>Blood gene profiling</td>
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<td>• No proven outcome benefit</td>
<td>Consider where 2- to 3-d result delay will not affect management plan</td>
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<td></td>
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<td></td>
<td>• Multiday turnaround</td>
<td></td>
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<td></td>
<td></td>
<td>• $$$</td>
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<tr>
<td>kSORT</td>
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<td>May risk stratify rejection</td>
<td>• No proven outcome benefit</td>
<td>Lack of benefit in recent large, multicenter, retrospective study</td>
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<tr>
<td></td>
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<td></td>
<td>• Multiday turnaround</td>
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<td>dd-cfDNA</td>
<td>TBD</td>
<td>High NPV for rejection</td>
<td>• No proven outcome benefit</td>
<td>Consider for ruling out AMR, where 2- to 3-d result delay will not affect management plan</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Multiday turnaround</td>
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<td></td>
<td>• Not proven for surveillance</td>
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**Table 2. Conventional and emerging biomarkers used in kidney transplant**

**Legend:**
- UP/Cr, urine protein-urate creatinine ratio
- TTR/CV, time in therapeutic ratio/coeficient of variation
- NAT, nucleic acid testing
- IS, immunosuppression
- NK, BK virus
- DSA, donor-specific antibody
- MMDX, microarray-based molecular diagnostic system
- TBD, to be determined
- ELISPOT, enzyme-linked immune absorbent spot
- NPV, negative predictive value
- AMR, antibody-mediated rejection

UP/Cr, urine protein-urate creatinine ratio; TTR/CV, time in therapeutic ratio/coeficient of variation; EBV, Epstein–Barr virus; NAT, nucleic acid testing; IS, immunosuppression; BKV, BK virus; DSA, donor-specific antibody; MMDX, microarray-based molecular diagnostic system; TBD, to be determined; ELISPOT, enzyme-linked immune absorbent spot; NPV, negative predictive value; kSORT, kidney Solid Organ Response Test; dd-cfDNA, donor-derived cellfree DNA; AMR, antibody-mediated rejection.
In CTOT-01, Hricik et al. (71) analyzed protocol and for-cause biopsies in 255 first kidney recipients. Rejection was present in 33% of indication biopsies, and urinary CXCL9 protein could detect rejection. CXCL9 also correlated with inflammation and was elevated up to 30 days before rejection. For the entire cohort, CXCL9 at 6 months correlated with rejection and functional deterioration by 24 months post-transplant.

In CTOT-09, tacrolimus withdrawal resulted in urinary CXCL9 elevation in six of 14 patients; four had rejection, five had de novo DSA, and two had BKV (25). Urinary CXCL9 alone could not differentiate BKV from rejection. Prospective trials investigating urinary chemokine monitoring and outcomes are planned (72).

**Blood Biomarkers**

To date, three blood biomarker assays have been evaluated in the post-transplant setting: kidney Solid Organ Response Test (kSORT), whole-genome peripheral blood gene expression profiling, and donor-derived cellfree DNA (cfDNA). One caveat with these assays in their quest to supplant allograft biopsy is that histology continues to serve as their rejection “gold standard.”

**Kidney Solid Organ Response Test.** This whole blood–derived molecular assay uses quantitative PCR to detect a 17-gene panel. The Assessment of Acute Rejection Trial study, involving 436 patients, collected aggregated blood samples in a cross-sectional manner and matched them with contemporaneous kidney allograft biopsy. The kSORT analysis suite, an algorithm that used varying numbers of smaller subsets of the gene panel, differentiated rejection from no rejection on the basis of patterns consistent with increased inflammation or immune quiescence (73). Study limitations included lack of serial blood samples, cohort
heterogeneity, and inability to distinguish T cell-mediated rejection. A real-world, retrospective, multicenter study comprising 1763 samples from 1134 patients could not validate kSORT for detecting rejection in the first post-transplant year (74). Observational and interventional studies are ongoing.

**Peripheral Blood Gene Expression Profiling.** Most established is a DNA microarray-based gene expression test that identifies 57 classifier genes that distinguish subclinical acute rejection (with stable allograft function) from histologic quiescence. In the pivotal study, where subclinical acute rejection was the primary end point, patients were followed for 24 months with gene profiling paired with surveillance biopsies between 2 and 6 months, at 12 and 24 months, and with for-cause biopsies (54). The subclinical acute rejection incidence was 42%, although it was mostly “borderline” T cell rejection. At the optimal diagnostic threshold, the sensitivity, specificity, positive predictive value, and negative predictive value of the biomarker for subclinical acute rejection were 64%, 87%, 61%, and 88%, respectively. Secondarily, investigators observed that the biomarker profile that correlated with subclinical acute rejection also associated with worse transplant outcomes after 24 months. Real-world experience in centers not using surveillance biopsies reflects similar performance characteristics in confirming immune quiescence (75).

A peripheral blood 17-gene signature using targeted RNA expression has been reported from GoCAR that associates with rejection on 3-month surveillance biopsy, although it requires further validation (76).

**Donor-Derived Cellfree DNA.** Cellfree DNA (cfDNA) is nonencapsulated circulating DNA. Degrading into approximately 166-base nucleosomal units, cfDNA has a half-life around 30 minutes. Donor-derived cfDNA are DNA fragments in recipient circulation originating from donor tissue injury. The most common detection approach is on the basis of detecting differences in highly homoygous single-nucleotide polymorphisms with high allelic frequency between donor and recipient. Normally a miniscule fraction of total cfDNA, donor-derived cfDNA increases with increasing allograft injury. Levels are elevated very early post-transplant after ischemia-reperfusion injury, declining to baseline steady state more rapidly in living than deceased donor recipients (77).

The prospective, observational Diagnosing Active Rejection in Kidney Transplant Recipients (DART) trial investigated donor-derived cfDNA as a rejection marker (78). At a 1% diagnostic cutoff, donor-derived cfDNA levels measured concomitantly with for-cause biopsy differentiated active rejection from no rejection, outperforming serum creatinine. Donor-derived cfDNA performed most robustly for discriminating antibody-mediated rejection from no antibody-mediated rejection, with sensitivity, specificity, positive predictive, and negative predictive values of 81%, 83%, 44%, and 96%, respectively. Data, frequently single center, with different donor-derived cfDNA assays from the United States, Europe, and Australia have since been reported. Most (77,79–81), although not all (82), studies have confirmed DART’s observation. Some assays have additionally shown promise for T cell-mediated rejection (79,83) and ATN (77). Reported differences in performance characteristics between assays, and between studies with the same assay, are likely related to differences in diagnostic thresholds used or different histologic classifications (Banff 2013 versus 2017). Although attention has focused on donor-derived cfDNA’s ability to discriminate rejection from nonrejection, it most plausibly reflects overall injury burden regardless of triggering cause. Better-quality data are required to evaluate donor-derived cfDNA’s potential for diagnosing and predicting rejection and other allograft injury, as well as postintervention surveillance.

**Where to from Here?**

At present, the published quality of evidence regarding emerging biomarkers is variable, the clinical framework for their use is not clearly established (Table 2), and they have not been independently validated. Despite these limitations, costly commercially available biomarkers are now widely used in various unproven contexts. All demonstrate a strong negative predictive value, indicating potential to avoid unnecessary biopsies; however, they perform less well in identifying patients at risk for a poor outcome (lower positive predictive value). Furthermore, studies have not compared these biomarkers against most conventional approaches or against one another. Table 3 highlights knowledge gaps related to optimal use of novel biomarkers, including testing frequency and timely resulting as well as other logistic and technical considerations. Finally, it is necessary to determine whether they are cost effective in low- and high-immunologic risk recipients alike. Figure 1 depicts hypothetical time frames for using these biomarkers in practice assuming their clinical utility was established.

Kidney transplantation’s successful evolution has created a high bar for biomarkers to overcome. Outcomes have improved with conventional monitoring, and death accounts for most allograft loss. Moreover, acute rejection rates are <10% (2), while subclinical acute rejection is relatively infrequent and its treatment benefit unproven (43,44). Moving beyond measuring serum creatinine, immunosuppression drug levels and indication biopsy will require large, prospective, observational and interventional randomized controlled studies to demonstrate that these promising biomarkers result in improved outcomes in order to justify their incorporation into routine post-transplant care.

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