Definition of the Problem:

The goal of organ transplantation is to save lives and increase quality of life. Critical overarching strategic goals for the transplant community are to increase the number of patients receiving transplants, improve access to transplantation for all, and improve long term transplant outcomes. Improving long term transplant outcomes is central to achieving those strategic imperatives. However, despite enormous success in other phases of the transplant endeavor, dramatic improvements in long term allograft survival have proven elusive. One of the key reasons for this may be that the transplant community continues to monitor allograft function utilizing legacy modalities that often have not changed in decades. For example, in the case of renal allograft function, clinicians still rely primarily on monitoring serum creatinine, urine protein, and donor specific antibody. The emergence of molecular diagnostic techniques has the potential to alter that paradigm and may allow significant improvements in long term allograft function. Our goal in commenting on this topic is to provide clarity for clinicians and advocate for access to these modalities as clinically appropriate for the patients we serve.

The role of donor derived cell-free DNA (dd-cfDNA) in kidney transplant surveillance:

Perhaps the largest unmet for which molecular diagnostics may play a role is in the surveillance of renal transplant recipients for rejection. That population of patients is large, legacy surveillance techniques are limited in efficacy, and improvements in long-term kidney allograft survival have proven elusive over many years. The clinical utility provided by dd-cfDNA is highlighted by the findings of several key studies:

The Circulating Donor-Derived Cell-Free DNA in blood for diagnosing Acute Rejection in Kidney Transplant Recipients (DART) study assessed 1272 samples from 384 unique kidney recipients from 14 clinical sites. In the DART study, Bloom et al reported that dd-cfDNA was a better discriminator of acute rejection than serum creatinine, with median levels of dd-cfDNA in recipients with active rejection significantly higher (1.6%) than in those without active rejection (0.3%). A dd-cfDNA threshold of 1.0% yielded 85% specificity and 59% sensitivity to discriminate active rejection from the absence of rejection. At a 1.0% dd-cfDNA threshold, PPV was 61% and NPV was 84%. Furthermore, at a dd-cfDNA threshold of <0.21%, NPV for acute rejection was 95% (Bloom et al, 2017).

Jordan et al found that elevated dd-cfDNA levels are associated with increased likelihood of de novo DSA development in kidney recipients. They also demonstrated that dd-cfDNA levels above a threshold of 2.9%, in conjunction with a positive DSA, confer an 89% PPV for ABMR (Jordan et al. 2018).

Stites et al evaluated the ability of elevations of dd-cfDNA to predict the clinical course of kidney recipients with the finding of TCMR 1A or Borderline acute rejection on biopsy. They discovered that a dd-cfDNA level over 0.5% predicted longitudinal eGFR decline, while those kidney recipients with the same biopsy findings but a dd-cfDNA level < or = to 0.5% did not have evidence of eGFR decline during the period of study follow-up. Notably, the patients with dd-cfDNA levels > 0.5% and a biopsy finding of TCMR 1A or Borderline TCMAR at study entry had a 40.5% likelihood of denovo DSA development and 21.5% likelihood of development of recurrent AR during the study follow-up. In contrast, those with dd-cfDNA levels less than or equal to 0.5% experienced a 2.7% likelihood of development of de novo DSA and 0% likelihood of recurrent AR.

Bu et al published the findings of the Assessing Donor-derived cell-free DNA Monitoring Insights of kidney Allografts with Longitudinal surveillance (ADMIRAL) study in 2021. This large (1092 kidney transplant recipients), multi-institutional cohort study validated many areas of clinical utility for dd-cfDNA in renal transplant recipients and confirmed the relationship between acute rejection and dd-

cfDNA levels elucidated in the DART study. Data from this study builds on prior work to demonstrate areas of clinical decision utility for dd-cfDNA, some of which are briefly reviewed below.

Relationship of dd-cfDNA and acute rejection:

Bu et confirmed that dd-cfDNA is a significantly better (P<0.001) discriminator of rejection than serum creatinine, with the AUROC for creatinine in prediction of rejection being 0.495 (95% CI; 0.38-0.59), while the AUROC for dd-cfDNA was 0.798 (95% CI; 0.72-0.87). In the 1092 patients studied, dd-cfDNA results also stratified for type of rejection, with the dd-cfDNA result in ABMR having a median of 1.8%, in TCMR a median of 0.7%, and in no rejection a median of 0.23%. The authors reported that a 1% increase in dd-cfDNA level was associated with a 3.3-fold increase in the likelihood of rejection diagnosis (P<0.001), and with a hazard ration for rejection of 1.89 (95% CI; 1.78-2.1).

Relationship of dd-cfDNA and longitudinal eGFR decline:

Bu et al analyzed dd-cfDNA levels and renal function from year 1 to year 3 after kidney transplantation, and found that a dd-cfDNA level > 0.5% was associated with significant eGFR decline at 3-years post-transplant. Furthermore, persistently (> 1 value) elevated (>0.5%) dd-cfDNA levels almost doubled (HR 1.97) the risk of a 25% decline in eGFR (95% CI; 1.39-2.68, P = 0.041).

Relationship of dd-cfDNA and de novo DSA development:

In the ADMIRAL study, Bu et al evaluated 961 patients with paired dd-cfDNA and DSA results, none of whom had preexisting DSA at study entry. The authors found that dd-cfDNA levels >0.5% almost tripled (HR 2.71, P = 0.001) the likelihood of future de novo DSA development. In multivariable analysis, each 1% increase in dd-cfDNA levels was associated with a 20% higher likelihood of subsequent de novo DSA development (HR 1.19; P = 0.004). Interestingly, the increases in dd-cfDNA occurred a median of 91 days prior to the detection of de novo DSA, suggesting that, much as it is for serum creatinine, dd-cfDNA is a leading, rather than a lagging indicator of DSA development.

Relationship of dd-cfDNA and renal allograft injury:

The ADMIRAL study utilized a composite allograft injury metric to assess dd-cfDNA levels in injured and what were termed quiescent allografts. The composite score included those recipients with BK viremia, urinary tract infection, rejection, de novo DSA, proteinuria and recurrent FSGS. Recipients without any of these criteria were termed quiescent. The median dd-cfDNA level in those meeting injury criteria was 0.51% (95% CI; 0.48-1..2%), and the median dd-cfDNA level in those without criteria for allograft injury (those deemed quiescent) was 0.21% (95% CI; 0.19-0.34). The median values were highly statistically different (P <0.0001). The dd-cfDNA scores in the quiescent group mirrors that of the stable kidney recipients from the multicenter DART study and reaffirms the typical low dd-cfDNA levels seen in stable allografts. In ADMIRAL, the AUROC for dd-cfDNA to predict injury versus quiescence was 0.727 (95% CI; 0.71-0.88), while the AUROC for creatinine was only 0.575 (95% CI; 0.52-0.62). The median creatinine in quiescent patients was 1.32 mg/dl (95% CI; 1.16-1.65), while in those with allograft injury, the median creatinine was 1.48 (95% CI; 1.1-2.7) (P = 0.08). Thus, creatinine was not able to effectively discriminate the allograft injury recipients from the quiescent allograft recipients, while dd-cfDNA assessment was.

Summary of role of dd-cfDNA in kidney recipient surveillance:

Collectively, these and other data point to significant clinical utility of dd-cfDNA in clinical decision formation in kidney transplant recipients, with low dd-cfDNA levels providing evidence of immune quiescence and providing the ability to forego unnecessary biopsies, moderately elevated or rising levels signaling a markedly elevated risk of rejection, and higher levels providing a high degree of confidence in

the presence of underlying antibody meditated rejection (ABMR). The demonstrated clinical utility of dd-cfDNA includes a significant improvement relative to serum creatinine measurement in the detection of both TCMR and ABMR. As a marker of allograft injury, dd-cfDNA is an earlier indicator of allograft injury than de novo DSA and of serum creatinine.

SOCIETY RECOMMENDATIONS REGARDING CLINICAL UTILITY AND DECISION ANALYSIS:

The most data have been accumulated in adult transplant recipients, and these recommendations are therefore most applicable to adult patient populations.

We suggest that clinicians consider measuring serial dd-cfDNA levels in kidney transplant recipients with stable renal allograft function to exclude the presence of subclinical antibody mediated rejection.

We recommend that clinicians measure dd-cfDNA levels in kidney transplant recipients with acute allograft dysfunction to exclude the presence of rejection, particularly antibody-mediated rejection (ABMR).

We do not recommend the use of blood gene expression profiling (GEP) in kidney transplant recipients for the purpose of diagnosing or excluding sub-clinical rejection, as adequate evidence supporting such use is still lacking.

We do not recommend the use of blood GEP to diagnose or exclude the presence of acute graft rejection in kidney transplant recipients with acute allograft dysfunction given the paucity of data to support this practice.

We recommend that dd-cfDNA may be utilized to rule out subclinical rejection in heart transplant recipients.

We recommend that clinicians utilize peripheral blood GEP as a non-invasive diagnostic tool to rule out acute cellular rejection in stable, low-risk, adult heart transplant recipients who are over 55 days status post heart transplantation.

We recommend that there is still insufficient evidence to recommend dd-cfDNA or GEP testing in liver transplant recipients.

CAVEATS AND RECOMMENDATIONS FOR FUTURE STUDIES

None of these recommendations should be construed as recommending one biomarker over another in the same diagnostic niche.

We strongly recommend ongoing further clinical studies to clarify the scenarios in which molecular diagnostic studies should be utilized.

We specifically recommend that studies be carried out to evaluate the potential role of dd-cfDNA surveillance in kidney transplant recipients to improve long term allograft survival.

PAYER REIMBURSEMENT

We strongly encourage commercial payers to align reimbursement practices for molecular diagnostic testing with clinical guidelines and emerging practice patterns, as well as with Medicare beneficiary coverage patterns.

Summary:

The accumulated evidence supporting the use of dd-cfDNA argues that its use should no longer be considered investigational. The use of dd-cfDNA is evidence-based and validated, and a prime example of much needed innovation in transplant organ surveillance. It has demonstrated utility in the early detection of allograft injury and assistance with clinical decision-making regarding allograft biopsy and treatment initiation. Salient factors that argue for the utilization of dd-cfDNA as a surveillance and for-cause diagnostic modality include:

- Medicare beneficiaries with renal transplants have dd-cfDNA as a covered benefit. This technology is widely used in the MC patient population yet not covered by most commercial payers. This disparity in access is concerning.

- Long term outcomes for kidney recipients are suboptimal. Approximately 20% of allografts fail by 5 years after transplantation. Those allograft losses are bad for patients, bad for payers, and worsen the supply-demand mismatch for kidney transplants because many of those patients ultimately undergo repeat transplantation. Better long-term renal allograft survival is a key that will unlock global cost savings and facilitate transplanting more patients.

- Transplant providers have been managing post-kidney transplant surveillance in much the same way for decades, and innovation and adoption of new tools are needed to improve long-term outcomes. Creatinine is an insensitive marker of allograft injury, and is essentially a lagging indicator of allograft dysfunction, particularly when initial renal function is good. The dependence on creatinine measurement and renal allograft biopsy in the detection of renal allograft injury one of reluctant necessity born of a paucity of better options. The emergence of dd-cfDNA provides a better, and clinically useful, marker of allograft injury that can directly impact patient care and outcomes.

- The rapidly emerging technology of dd-cfDNA provides a validated, evidence-based means to improve the surveillance of renal allografts. Taken as a whole, this technology is being widely adopted by the transplant community and appears useful in both detection of allograft injury and, via a high negative predictive value of low dd-cfDNA levels for underlying rejection, may also prevent unnecessary renal allograft biopsies with their attendant cost and potential morbidity.

- Renal allograft failure and return to dialysis is a disaster for individual patients, and enormously expensive for payers.

  - Allograft failure is associated with approximately $78,079 in additional costs in the first year of return to dialysis.

  - Long-term patient survival is much better with allograft function relative to return to dialysis.

Renal transplantation is a life-saving intervention that also saves money relative to maintenance dialysis. Utilizing biomarkers, particularly dd-cfDNA, for detection of renal allograft injury is an emerging standard of care that can directly aid in clinical decision making that may improve patient and allograft survival. We support access of transplant recipients to these promising diagnostic technologies and advocate for ongoing study of these tools to clarify their utility and cost effectiveness.

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