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## Emerging role of cell-free DNA in kidney transplantation

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### Abstract

Monitoring kidney transplants for rejection conventionally includes serum creatinine, immunosuppressive drug levels, proteinuria, and donor-specific antibody (DSA). Serum creatinine is a late marker of allograft injury, and the predictive ability of DSA regarding risk of rejection is variable. Histological analysis of an allograft biopsy is the standard method for diagnosing rejection but is invasive, inconvenient, and carries risk of complications. There has been a long quest to find a perfect biomarker that noninvasively predicts tissue injury caused by rejection at an early stage, so that diagnosis and treatment could be pursued without delay in order to minimize irreversible damage to the allograft. In this review, we discuss relatively novel research on identifying biomarkers of tissue injury, specifically elaborating on donor-derived cell-free DNA, and its clinical utility.

**Key Words:** Biomarker; Donor-derived cell-free DNA; Kidney allograft outcomes; Kidney transplant; Allograft biopsy

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**Core Tip:** Donor-derived cell-free DNA (dd-cfDNA) is now available as a noninvasive biomarker to evaluate the risk of rejection in kidney allografts and other organ transplants. The technology utilizes next generation sequencing and does not require donor genotyping. In this review we discuss the current literature on the utility of dd-cfDNA in kidney transplantation, the limitations, and future directions.

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## INTRODUCTION

Kidney transplants offer the best survival to patients with end-stage kidney disease [1]. Conventional monitoring of kidney transplant recipients includes serum creatinine, proteinuria, and donor-specific antibodies (DSA), which are neither sensitive nor specific. Surveillance biopsies are performed for allograft monitoring in a few centers, but are invasive and have multiple disadvantages including bleeding risk, inconvenience, sampling error, and poor reproducibility in interpretation. They generally have low yield as the majority reveal normal histology and have not been validated to improve outcomes.

The risk of renal allograft dysfunction from acute rejection (AR) during the first year after transplant is around 10%-15%. AR can be either acute T cell-mediated rejection (TCMR) characterized by lymphocytic infiltration of tubules, interstitium, and in severe cases, vessels causing cytotoxic injury or acute antibody-mediated rejection (ABMR) caused by DSA resulting in complement activation and lysis of target cells. A rise of serum creatinine is a delayed marker of the assessment of AR. By the time the serum creatinine rises, significant histological damage has already occurred, thereby significantly lowering the chance of complete recovery from injury. There have been multiple efforts to develop a noninvasive biomarker that promptly, accurately, and inexpensively predicts immunological allograft injury at an early stage with high sensitivity, specificity, and predictive value [2]. An ideal biomarker should assess the risk of injury, diagnose and monitor the injury and the pharmacological response, have prognostic value, and assess the safety of treatment [3].

## NONINVASIVE BIOMARKERS IN ORGAN TRANSPLANTATION

Over the last decade, the emphasis has been on finding the perfect biomarker, which will help to predict, diagnose, and treat rejection in order to improve short- and long-term transplant outcomes. Various biomarkers in different categories have been studied, including blood mRNA (Granzyme B, Perforin, FasL, HLA-DRA, and multigene signature); blood donor-derived cell-free DNA (dd-cfDNA); blood proteins (DSA, C1Q binding, Pleximune, Immuknow, kSORT, IFN- $\gamma$  Elispot and, TCR repertoire); urinary mRNA (Perforin, Granzyme B, PI-9, CD103, FOXP3, CXCL10, NKG2D, TIM3, Granulysin, and multigene signature); and urinary proteins (CXCL9, CXCL10, and Fractalkine) [4]. The markers have varying degrees of sensitivity and specificity and are summarized in Table 1. dd-cfDNA has recently become as one of the most commonly used biomarkers. The purpose of this review is to outline the discovery and utility of dd-cfDNA and to evaluate the available data regarding its use in kidney transplantation.

## DD-CFDNA

Plasma cell-free DNA has been used as a biomarker in prenatal testing, cancer diagnosis, and organ transplantation [5-8]. Multiple studies have shown that allograft-derived cell-free DNA can be detected and quantified as a fraction of total cell-free DNA in the plasma or serum of various solid organ transplant recipients [8-10] such as kidney [11], heart [12], lung [13], pancreas [14], and liver [15]. Similar studies were also done looking at cell-free DNA excretion in urine [16]. This noninvasive marker was extensively studied in heart transplant recipients by Snyder *et al* [9], where significantly increased levels of dd-cfDNA were noted with biopsy-proven AR. Severity of rejection worsened with increasing levels of dd-cfDNA. In addition to being a marker of rejection, dd-cfDNA can also be used as an individualized tool to assess the efficacy of immunosuppressive treatment. In a study of liver transplant recipients, higher tacrolimus levels were associated with lower amounts of dd-cfDNA, suggesting that the relationship could be used as a tool for optimizing immunosuppressant drug dosing [17]. The presence of dd-cfDNA in the plasma of solid organ transplant patients was first described in 1990's, and involved measurement of Y chromosome DNA

**Table 1 Biomarkers studied in the field of transplantation**

| Ref.                           | Biomarker name                      | Biomarker assay method       | Sample size            | Rejection type                            | Sensitivity/specificity | PPV/NPV   | Comments         |
|--------------------------------|-------------------------------------|------------------------------|------------------------|---|-------------------------|-----------|------------------|
| Patel <i>et al</i> [56]        | CDC crossmatch                      | Micro-cytotoxicity assay     | 225                    | Hyper acute rejection/ early graft loss   | 0.75/0.97               | 0.80/0.97 | FDA approved     |
| Mahoney <i>et al</i> [57]      | Flow crossmatch                     | Flow cytometry               | 90                     | Early graft loss                          | 0.71/0.74               | 0.33/0.93 | FDA approved     |
| Pei <i>et al</i> [58]          | Luminex                             | HLA beads; flow cytometry    | 10                     | Anti HLA Ab                               | -                       | -         | FDA approved     |
| Ashokkumar <i>et al</i> [59]   | Pleximmune                          | T cytotoxicmemory cell       | 32                     | Acute rejection                           | 0.88/0.94               | 0.93/0.88 | FDA approved     |
| He <i>et al</i> [60]           | Cylex-Immuknow                      | Lymphocyte ATPgeneration     | 42                     | CD4 T cell function                       | -                       | -         | FDA approved     |
| Loupy <i>et al</i> [61]        | C1q bindingassay                    | Flow cytometric C1q binding  | 1016                   | TCMR/ ABMR/graft loss                     | -                       | -         | FDA approved     |
| Hricik <i>et al</i> [62]       | IFN- $\gamma$ ELISPOT               | Donor-reactive memory T cell | 21                     | De novo DSA/ rejection                    | 1.0/0.67                | 0.67/1.0  | Not FDA approved |
| Roedder <i>et al</i> [63]      | KSORT                               | PBLRNA by qPCR               | 143                    | AR  | 0.83/0.91               | 0.81/0.91 | Not FDA approved |
| Bloom <i>et al</i> [33]        | dd-cfDNA                            | PBL single gene sequencing   | 102 (107 samples)      | ABMR and I b or higher TCMR               | 0.59/0.85               | 0.61/0.84 | FDA approved     |
| Acquino-Dias <i>et al</i> [64] | FOXP3                               | PBL, urine (PCR)             | 65 (78 sample)         | AR vs DGF                                 | 0.94/0.95               | 0.94/0.95 |                  |
| Li <i>et al</i> [65]           | Granzyme B, perforin                | Urine mRNA (PCR)             | 85 (151 samples)       | AR  | 0.79-0.83 /0.77-0.83    | -         |                  |
| Hricik <i>et al</i> [66]       | Urine CXCL9                         | Urine ELISA                  | 258                    | TCMR                                      | 0.85/0.81               | 0.68/0.92 | Not FDA approved |
| Suthanthiran <i>et al</i> [67] | Urine 3 gene; CD3E, CXCL10, 18SrRna | Urine RNA by PCR             | 485 pts (4300 samples) | Diagnosis AR 20 d early                   | 0.79/0.78               | -         | Not FDA approved |
| Renesto <i>et al</i> [68]      | TIM-3                               | PBL, urine mRNA PCR          | 115 (160 samples)      | Diagnose AR, values normal post treatment | 0.87/0.95               | 0.87/0.93 |                  |
| Valujskikh <i>et al</i> [69]   | miRNA-210                           | Urine miR-210 PCR            | 81 (88 samples)        | Diagnose AR, values normal post treatment | 0.52/0.74               | -         |                  |

ABMR: Antibody-mediated rejection; AR: Acute rejection; CDC: Complement dependent cell cytotoxicity; dd-cfDNA: Donor-derived cell-free DNA; ELISA: Enzyme-linked immunosorbent assay; FDA: Food and Drug Administration; HLA: Human leukocyte antigen; IFN: Interferon; PBL: Peripheral blood lymphocyte; PCR: Polymerase chain reaction; TCMR: T cell-mediated rejection.

particles in a female recipient from a male donor[18].

## BIOLOGY AND KINETICS OF CELL-FREE DNA

Levels of cell-free DNA fluctuate randomly during the day[19,20] and vary with multiple factors including age[21], exercise, obesity[22], malignancy, transplant[20], acute coronary syndrome[23], stroke[24], and other pathological conditions. The concentration of cell-free DNA may vary from 3.5-100 ng/mL[20,25]. Cell-free DNA is rapidly cleared from the plasma. Its The mean clearance half-life of fetal Y chromosome DNA particles from maternal plasma was reported by Lo *et al*[26] to be 16 min. Cell-free DNA is primarily cleared by apoptosis, necrosis, and active secretion. Less than 20% is secreted in urine[27] and partly degraded by the liver[28] and endonucleases in the plasma and other tissues. Yu *et al*[29] found clearance of fetal cell-free DNA to occur in two phases, one with a half-life of about 1 h and a slower phase with a half-life of about 13 h. Nearly complete disappearance of fetal cell-free DNA occurs by 2 d postpartum.

In kidney transplant recipients, the kinetics of dd-cfDNA were described in detail by Shen *et al*[30], where the authors compared the dynamics of degradation of dd-cfDNA in the immediate post transplant period in kidney transplant recipients from

living donors (LD) compared with deceased donors (DD) where donors had cardiac death, and some experienced delayed graft function. Based on their analysis, the mean dd-cfDNA concentration was 20.69% at 3 h, 5.22% by about 16 h, and 0.85% by day 7. The concentrations were significantly higher in recipients of kidneys from DDs than LDs initially (45% *vs* 10%) and on day 7 d (1.11% *vs* 0.59%) probably because of higher levels of ischemia reperfusion injury in the former group. Other large solid organs, such as livers may have more cell turnover and larger proportions of dd-cfDNA released in the recipient. Beck *et al*[10] found dd-cfDNA fractions of 90% immediately after transplant with steady state levels below 15% by day 10.

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## MEASURING DD-CFDNA

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The technology of measuring dd-cfDNA initially had some limitations as the assays required prior recipient and donor genotyping and were time consuming and expensive[8,9]. Newer technologies that have been validated as clinical-grade assays measure dd-cfDNA in transplant recipients by polymerase chain reaction (PCR) such as real-time quantitative PCR, droplet digital PCR, or next generation sequencing (NGS) as described by Grskovic *et al*[19]. Droplet digital PCR and NGS have been clinically investigated and validated over a wide range for detecting rejection in transplant recipients[31,32]. The basic principle of measuring dd-cfDNA is by measuring single nucleotide polymorphisms (SNPs) that are homozygous in the recipient and differ from those of the donor. That can be accomplished in the absence of donor genotyping[33]. There are no standardized assays to be used for transplantation, in terms of the number of SNPs. The commercially available assays using NGS technology so far are Allosure, (CareDx, Brisbane, CA, United States), which targets 266 SNPs[34]; Prospera, (Natera Inc, San Carlos, CA, United States), which targets 13392 SNPs[35], Viracor Transplant Rejection Allograft Check (TRAC) combined with TruGraf, and (Eurofins Viracor, (Lee's Summit, MO, United States), which targets 70000 SNPs[36]. There is one study that compared the results of two commonly available commercial assays in United States; Allosure and Natera, involving 76 kidney transplant recipients. It found no significant differences in the test results for predicting rejection or other test characteristics, but found some differences in the test result turnaround time[36,37]. The recipient genotype is determined at each SNP and the relative fraction of dd-cfDNA is computed using custom bioinformatics tools. The performance of the assay was validated in 1117 samples from related and unrelated transplant recipients with reliability and precision. The turnaround time of the test was 3 d, which was considered as a practical time frame for transplant recipients.

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## REPORTING DD-CFDNA AS A FRACTION VS ABSOLUTE VALUE

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In clinical application, the dd-cfDNA value is expressed as a fraction of background circulating cell-free DNA fragments. This assumes that the recipient's DNA fragments remain constant. However the host's cell-free DNA fragment levels can vary in different scenarios such as exercise, inflammatory state, and body size[22,38,39]. In a recent report involving 121 stable kidney transplant recipients, there was a significant negative correlation of the average baseline dd-cfDNA fractions between 4-12 wk post-transplantation and increasing recipient BMI[22]. That indicates that dd-cfDNA fractions are influenced by recipient body size.

Previous studies have compared absolute dd-cfDNA values to fractional values[40-42]. The analysis by Whitlam *et al*[40] included 61 samples and reported similar areas under the curve (AUC) for diagnosing ABMR, with an absolute dd-cfDNA value of 0.91 [95% confidence interval (CI): (0.82-0.98)] and a dd-cfDNA fraction of 0.89 (95% CI: 0.79-0.98). Neither measure was very useful in diagnosing 1A and borderline TCMR rejection. In a prospective observational study, Oellerich *et al*[42] compared dd-cfDNA quantification of copies/mL plasma to dd-cfDNA fraction at prespecified visits in 189 patients over 1 yr post kidney transplant. Median dd-cfDNA (copies/mL) was 3.3-fold and the median dd-cfDNA fraction was 2.0 fold higher in patients with biopsy-proven rejection ( $n = 15$  with 22 samples) compared with the median in stable patients ( $n = 83$  with 408 samples). Measuring dd-cfDNA (copies/mL) showed superior performance ( $P = 0.02$ ) with an AUC of 0.83 compared with the dd-cfDNA fraction, which had an AUC of 0.73. A subset analysis found a significant inverse correlation between tacrolimus levels and dd-cfDNA (copies/mL), implying that dd-cfDNA may be useful

in evaluating adequacy of immunosuppression. A subsequent study from the same group evaluated the longitudinal time-dependent changes in total cfDNA (copies/mL), dd-cfDNA (copies/mL) and dd-cfDNA fraction in 303 clinically stable kidney transplant recipients 12-60 mo post-transplantation[41]. Total cfDNA showed a significant decline over time, resulting in increasing dd-cfDNA fractions, with doubling of the 85th percentile value by 5 yr. In contrast, dd-cfDNA (copies/mL) values remained stable during the same period. The authors concluded that measurement of absolute dd-cfDNA concentrations minimize false positive results compared with dd-cfDNA fractions and were hence superior for long-term allograft monitoring. Further large scale studies are still needed to define the ideal method of dd-cfDNA monitoring.

## DD-CFDNA IN DIAGNOSING AR IN KIDNEY TRANSPLANTATION

The Diagnosing AR in Kidney Transplant Recipients (DART) study by Bloom *et al*[33] focused more on dd-cfDNA (Allosure, CareDx, Brisbane, CA) as a novel biomarker in discriminating subclinical rejection from no rejection at an early stage, which could allow early intervention and hopefully better outcomes. It was a prospective multicenter study of renal allograft recipients ( $n = 102$ ) that used targeted amplification of dd-cfDNA by sequencing of SNPs to quantify donor and recipient DNA contributions in the plasma without the need of donor genotyping. A dd-cfDNA level of  $< 1\%$  had an AUC of 0.87 (95%CI: 0.75-0.97) for discriminating ABMR from no rejection. The positive predictive value (PPV) and negative predictive value (NPV) with a cutoff of  $< 1\%$  were 44% and 96% respectively, which was quite significant, suggesting a dd-cfDNA value of  $> 1\%$  may indicate active rejection (TCMR type  $\geq 1$  b or ABMR) where the sensitivity and specificity were 59% and 85% respectively. The hope is that this noninvasive biomarker could replace the need of surveillance biopsies done at some centers to monitor for rejection. A limitation of the study was that the test failed to pick up borderline TCMR type Ia rejection. Measurement of dd-cfDNA as a steady state fraction of recipient cfDNA in kidney transplants was first described by Bromberg *et al*[32], using the Allosure test. The study established that in steady state, a dd-cfDNA fraction above 1.2% could be abnormal and potentially predict AR. The results of the Prospera test were reported by Sigdel *et al*[35] in a single center retrospective study from a curated biobank. Along the same lines, a study by Jordan *et al*[34] combining the use of elevated DSA with dd-cfDNA  $> 1\%$  increased the probability of diagnosis of ABMR. That study involved 87 kidney transplant recipients, 16 had ABMR, and the PPV of a 1% threshold level of dd-cfDNA to detect active ABMR in DSA positive patients was 81%, whereas the NPV was 83%. The PPV for DSA positivity alone was 48%.

Based on pivotal validation studies, dd-cfDNA became Medicare reimbursable in October 2017 for noninvasive monitoring of rejection in transplant recipients. A subsequent external validation study by Huang *et al*[43] in 63 kidney transplant recipients with suspicion of rejection, revealed that the dd-cfDNA test did not discriminate TCMR from no rejection. The AUC for TCMR was 0.42 (CI: 0.17-0.66), although performance for diagnosing ABMR was much better, with an AUC of 0.82 (CI: 0.71-0.93). To better understand the long-term outcomes based on dd-cfDNA, a large prospective multicenter observational cohort study, the Kidney Allograft Outcomes AlloSure Registry (KOAR, ClinicalTrials.gov Identifier NCT03326076) is underway and plans to enroll 4000 kidney transplant recipients. KOAR is sponsored by CareDx, and will complete enrollment in December 2021. The ProActive study utilizing the Prosepra test and sponsored by Natera, Inc. (NCT04091984) is also underway and is targeting to enroll 3000 kidney transplant recipients prospectively from the time of transplant surgery. It will assess changes in the utilization of allograft biopsy and clinical outcomes based on physician-directed use of the Prospera test to rule in and rule out active rejection. The planned follow up for the study is 3 yr for most patients and 5 yr for a subset of patients at high immunologic risk.

The utility of dd-cfDNA in first time single kidney transplant recipients (SKTR) was clearly shown in the above mentioned studies, but the validity of the test in repeat kidney transplant recipients (RKTR) was unclear until Mehta *et al*[38] reported a median dd-cfDNA of RKTR ( $n = 12$ ) in the surveillance group that was higher than in the SKTR group (0.29% vs 0.19%,  $P < 0.001$ ). However, both were significantly lower than the established 1% dd-cfDNA rejection threshold[44]. Another study by Sureshkumar *et al*[45], showed that there were no significant differences in dd-cfDNA values for either deceased vs living donor ( $0.39\% \pm 0.42\%$  vs  $0.37\% \pm 0.20\%$ ,  $P = 0.35$ ) or

repeat *vs* first time ( $0.34\% \pm 0.07\%$  *vs*  $0.39\% \pm 0.43\%$ ,  $P = 0.36$ ) kidney transplant recipients. One possible reason for the latter observation could be that the limited number of viable cells in a failed allograft is insufficient to generate enough cell-free DNA fragments.

Using a slightly different platform from Natera to detect dd-cfDNA, Sigdel *et al*[35] have shown promising results. They measured plasma dd-cfDNA with a single SNP-based cell-free assay targeting 13392 SNPs using a massively multiplexed PCR method to detect allograft injury or rejection without knowing the donor genotype. Altuğ *et al* [31] further validated the performance of this method to detect the dd-cfDNA fraction with improved precision over other currently available tests, regardless of donor-recipient relationships. A major limitation was that the study was a retrospective analysis of archived samples from a single center comparing outcomes of patients who underwent for-cause biopsies, with an increased risk of rejection. The superiority in the technique of measuring dd-cfDNA and methodology of those studies was questioned in an editorial by Grskovic *et al*[46]. More studies are needed to prove the superiority of this technique over the other available techniques used to measure dd-cfDNA.

There have been multiple recent meta-analyses compiling the data from studies of the potential of dd-cfDNA as a biomarker to distinguish between different types of allograft rejection in kidney transplant recipients. A meta-analysis by Wijtvliet *et al*[47] included seven studies and one by Xiao *et al*[48] included nine studies. Both revealed significantly higher levels of dd-cfDNA in patients with ABMR compared with those with no rejection. The diagnostic accuracy was less for early TCMR, particularly Banff 1A and borderline. The meta-analysis by Xiao *et al*[48] revealed that the incidence of ABMR was 12%-37% in patients with elevated dd-cfDNA, with a pretest probability of 25%, positive likelihood of 58%, and negative likelihood of 6%, suggesting it may be a good test to rule out rejection. The presence of DSA can enhance the ability of dd-cfDNA to diagnose ABMR[34]. Zhang *et al*[49] showed that patients with positive DSA but without ABMR on biopsy had a higher baseline dd-cfDNA value compared with transplant recipients with neither DSA nor ABMR. The study suggests that the dd-cfDNA level may help in differentiating possibly “benign” DSA from the more damaging DSA that can cause ABMR. The majority of stable kidney transplant recipients have a median dd-cfDNA value of 0.21% with an NPV of 95%; suggesting that dd-cfDNA could be a reasonably accurate marker to rule out active rejection<sup>33</sup>A recent meta-analysis reported similar results[48].

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## DD-CFDNA IN SUBCLINICAL REJECTION

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Subclinical rejections are usually diagnosed in protocol biopsies, and there has been some data to suggest that subclinical rejections portend worse long-term graft outcomes; yet there is no data to suggest that treatment of this improves outcomes [50]. A study by Gielis *et al*[51] using dd-cfDNA measured by NGS in 43 patients who had 107 protocol biopsy specimens did not differentiate subclinical rejection from pyelonephritis or acute tubular injury. Bloom *et al*[33] reported that in the DART study, dd-cfDNA did not predict early TCMR, the majority of which were subclinical rejections. Even though the efficacy of diagnosing subclinical rejection is low, use of dd-cfDNA in combination with other markers of graft dysfunction such a DSA, chemokines, gene transcripts, and other novel biomarkers, might be able to predict rejection in immunologically high risk recipients[50]. In a recently published multicenter study involving 79 patients with steroid-treated borderline/1A TCMR, those with dd-cfDNA  $\geq 0.5\%$  had a steeper decline in glomerular filtration rate (median 8.5% *vs* 0%), more frequent development of DSA (40.5% *vs* 2.7%) and recurrent rejection rates (21.4% *vs* 0%) at 3-6 mo post-initial diagnosis than patients with a value  $< 0.5\%$ [52].

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## DD-CFDNA FOR SURVEILLANCE AND MONITORING

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The ideal frequency of monitoring dd-cfDNA has not been established, but studies have shown that, depending on the type of donor organ (*i.e.* living or deceased with or without DGF), the dd-cfDNA value nadirs at 2 wk post transplant, from the ischemia reperfusion injury. Hence, the monitoring should begin at 2 wk post transplant[30]. Some studies, like as the DART study[33], measured dd-cfDNA monthly for 3 mo and quarterly thereafter for a year, which might be a good frequency to follow. Various

other studies to look at the outcomes of using this biomarker as a tool for surveillance to monitor rejection in all transplant recipients or a subset of those with high immunological risk are ongoing and are described in [Table 2](#). Interestingly, dd-cfDNA was elevated in pathologies other than rejection, such as BK nephritis[53] and infection[54].

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## LIMITATIONS OF DD-CFDNA AS A BIOMARKER

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The use of the dd-cfDNA assay has limitations that need to be kept in mind. The test may give inaccurate results if performed within first 2 wk of transplant, in pregnant women, within 24 h of kidney biopsy, in patients who received whole blood or WBC components within a month of testing, in those with history of allogeneic bone marrow transplantation, kidney transplant from monozygotic twin and in multiorgan transplant recipients. In dual organ transplants from a single donor, a cutoff value above which one could anticipate an increased risk of rejection has not been defined, and an increased value will not distinguish which organ is experiencing the injury. A positive result in single organ recipients does increase the risk of rejection, but cannot distinguish the grade and type of rejection. Confirmatory diagnosis of type and intensity of rejection is still based on biopsy findings. Occasionally increased levels of dd-cfDNA were seen in BK nephritis or other causes of allograft injury other than rejection.

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## FUTURE DIRECTIONS

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The availability of dd-cfDNA for clinical use in recent years is a step in the right direction toward noninvasive monitoring of allograft health, especially following kidney transplantation. A number of recent publications have described the utility of dd-cfDNA in kidney transplant recipients. In general, studies have found that dd-cfDNA was more useful in diagnosing ABMR, with less clear impact toward diagnosing milder forms of TCMR. One possible reason for the early rise in dd-cfDNA levels in ABMR is the associated microvascular injury in the allograft, with earlier release of cell-free DNA fragments into the circulation. Emerging reports suggest that dd-cfDNA is predictive of short-term adverse graft outcomes in TCMR1A at a lower threshold dd-cfDNA level. Despite being clinically available as an attractive option for noninvasive allograft evaluation, there are still many unanswered questions on the optimal utilization of these biomarkers. More large studies and experience are needed. Some of these questions are: (1) Should we use absolute dd-cfDNA levels or dd-cfDNA fractions? (2) What is the role of surveillance using dd-cfDNA in stable kidney transplant recipients, and would there be a favorable impact on long-term transplant outcomes? And (3) Is it cost effective to perform serial dd-cfDNA measurements? Puttarajappa *et al*[55] used a Markov model to perform an economic analysis comparing noninvasive biomarker monitoring to protocol biopsy during the first 12 mo following kidney transplantation. Assuming an incidence of 12% subclinical TCMR and 3% subclinical ABMR, protocol biopsy yielded more quality-adjusted life years at a lower cost compared with biomarkers. Hopefully many of these questions will be answered once the results of large database studies such as KOAR and ProActive become available.

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## CONCLUSION

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Noninvasive monitoring of early diagnosis of kidney allograft injury is a need of the hour. Among the various biomarkers that have been studied, dd-cfDNA captured the most attention and data is emerging. The available literature finds dd-cfDNA to be valuable for the early diagnosis of ABMR, but its role in milder forms of TCMR is less clear. Similarly, the favorable impact of dd-cfDNA in allograft surveillance on long-term outcomes is also not clear. Results from ongoing large outcome studies could shed further light onto this.

**Table 2** Trials of donor-derived cell-free DNA in kidney transplantation

|             |  |                    |
|-------------|--|--------------------|
| NCT02424227 | Noninvasive blood test to diagnose acute rejection after kidney transplantation (DART)   | Completed          |
| NCT03765203 | Utility of a novel dd-cfDNA test to detect injury in renal posttransplant patients (QIDNEY)  | Completed          |
| NCT03326076 | Evaluation of patient outcomes from the kidney allograft outcomes allosure registry (KOAR)   | Recruiting         |
| NCT04091984 | The Prospera kidney transplant active rejection assessment registry (ProActive)  | Recruiting         |
| NCT04057742 | Allosure for the monitoring of antibody-mediated processes after kidney transplantation (All-MAP)                                      | Recruiting         |
| NCT03759535 | Study in detection cfDNA for the early stage diagnosis of acute rejection post-renal transplantation                                   | Not yet recruiting |
| NCT03984747 | Study for the prediction of active rejection in organs using donor-derived cell-free DNA detection (SPARO)                             | Recruiting         |
| NCT04130685 | Donor-derived cell-free DNA for surveillance in simultaneous pancreas and kidney transplant recipients                                 | Recruiting         |
| NCT04166149 | Eliminating the need for pancreas biopsy using peripheral blood cell-free DNA (PancDX)   | Recruiting         |
| NCT03859388 | Longitudinal changes in donor-derived cell-free DNA with tocilizumab treatment for chronic antibody-mediated rejection                 | Enrolling          |
| NCT04225988 | Comparison of tacrolimus extended-release (envarsus xr) to tacrolimus immediate-release in HLA sensitized kidney transplant recipients | Recruiting         |
| NCT04177095 | Immune monitoring to facilitate belatacept monotherapy   | Recruiting         |
| NCT04239703 | Intercomex donor-derived cell-free DNA study   | Recruiting         |

dd-cfDNA: Donor-derived cell-free DNA; HLA: Human leukocyte antigen.

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