

# The role of dd-cfDNA in kidney transplantation: Is this the future of non-invasive kidney graft assessment?

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## ABSTRACT

Kidney transplantation is a life-saving procedure, which can improve the quality of life of patients with end-stage kidney disease. Although it is the most common transplantation surgery worldwide, it can lead to some serious complications. Nowadays, the most often used procedure to evaluate and diagnose complications like rejection of kidney graft is renal graft biopsy. Although it provides valid results, it remains an invasive procedure. For this reason, various biomarkers have been tested for graft evaluation, with donor derived cell free DNA (dd-cfDNA) being one of the most prominent ones. In this review of the literature, we summarise the different areas of application of this biomarker in kidney transplantation and we highlight its future potential.

**Key Words:** *Kidney transplantation; biomarkers; dd-cfDNA*

## INTRODUCTION

Chronic kidney disease (CKD) is a relatively common condition that affects >10% of the general population worldwide. It can lead to major health complications and lowers the life expectancy of the patients [1]. Apart from the many complications that might occur on the onset of CKD, it may progress to end-stage kidney failure that requires renal replacement therapy. Nowadays, the best renal replacement therapy for end-stage CK is considered to be kidney transplantation (KT) [2]. Kidney transplantation is the most common transplantation surgery worldwide, with more than 25,000 kidney transplants performed in

the United States only in 2022 [3]. Despite the increasing number of kidney transplantation and the amount of research performed around this topic, the long-term survival rates of the kidney graft and kidney recipient remain the same [4]. Some of the complications that affect the long-term survival are antibody mediated rejection, T-Cell mediated rejection, Graft versus host disease and various infections. Non-invasive diagnostic techniques for the evaluation of renal function, monitoring of the KT recipients and diagnosis of complications include serum creatinine and proteinuria, while the “gold standard” in detection of graft injury and rejection remain the invasive technique of biopsy. The above methods create the need for a non-invasive easily accessed biomarker for the detection of complications leading to dysfunction of the graft [5].

This gap in early detection and management of complications has come to fill the donor derived cell free DNA (dd-cfDNA). Dd-cfDNA is non-encapsulated fragmented DNA originating from foreign tissue such as an allograft that is continuously shed into the circulation [6]. This biomarker is measured in serum or urine samples and its

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assessment includes evaluation of its quantity, but also investigation of some quality characteristics, like size of the fragments and methylation pattern [7]. Particularly, for its detection in serum, various types of Polymerase Chain Reaction (PCR) are available, while there is also an alternative approach in female recipients from male donors that is the amplification of Y-chromosome specific genes [8]. Dd-cfDNA is a biomarker with a short half-life (<1,5h) that is released from various types of necrotic or apoptotic cells in the transplanted organ. Absolute and relative increases in blood levels of dd-cfDNA are associated with the amount of graft damage that occurs in events like rejection or subclinical graft injury [9,10]. Apart from quantitative changes, qualitative variations, such as methylation pattern and fragment size, can also indicate graft dysfunction [11]. The samples and the various types of dd-cfDNA quantification are described in Figure 1. Apart from monitoring of transplant recipients, dd-cfDNA appears to be a useful biomarker for the clinical evaluation of renal cancer [12].

## MATERIALS AND METHODS

We conducted a literature review of the medical research databases PubMed and Scopus. We used the following keywords for our search: kidney transplantation, renal transplantation, dd-cfDNA, biomarkers in kidney transplantation, acute rejection, graft injury, nephropathy. Our research was limited to the years 2000 until present and works in the English language. Our exclusion criteria included articles in non-English language, bibliographies that didn't refer to dd-cfDNA as a diagnostic biomarker

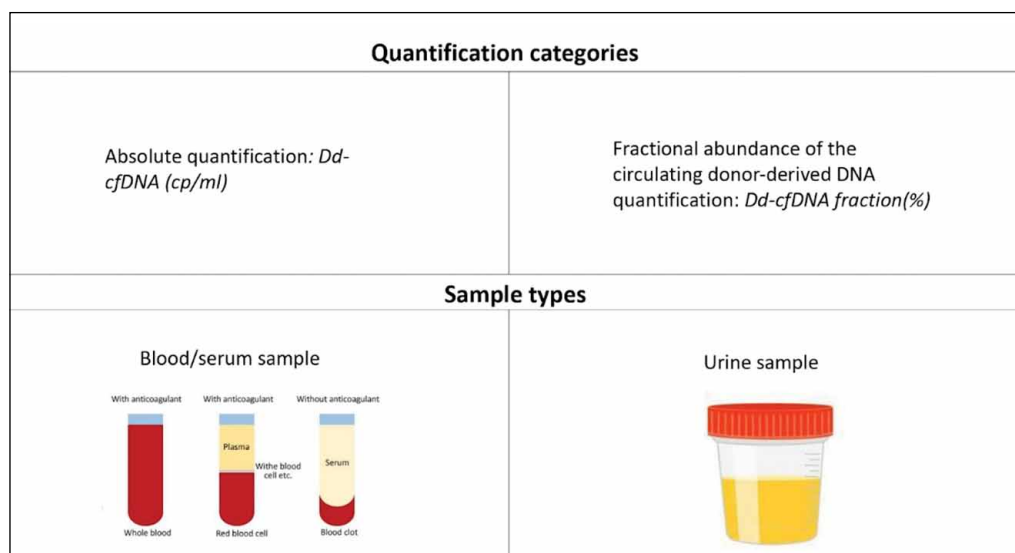
in kidney transplantation and research that wasn't about human recipients.

## RESULTS

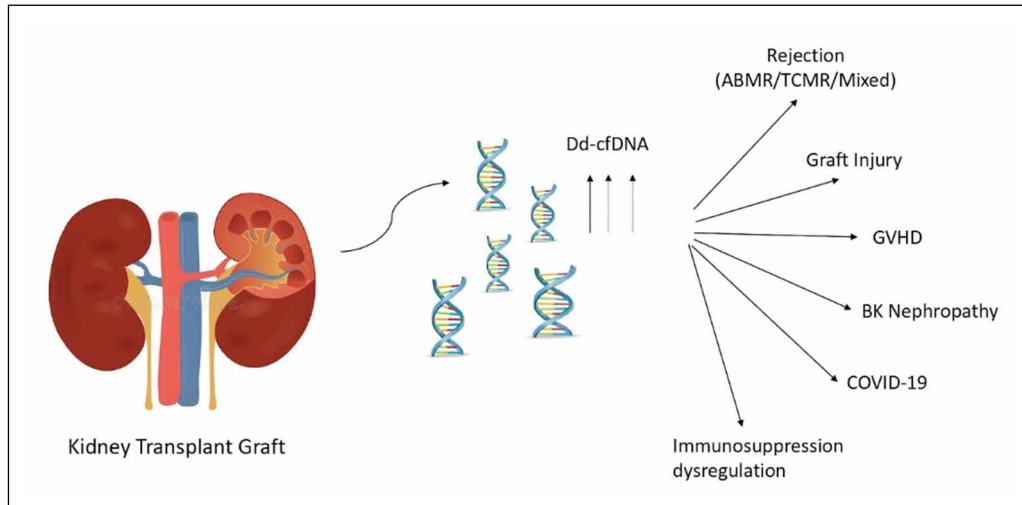
Main areas of application of dd-cfDNA in kidney transplantation are listed below and in Figure 2. The main research studies about dd-cfDNA application in kidney transplantation are listed in Table 1

### Subclinical graft injury

According to the definition of dd-cfDNA, it is a biomarker primarily used for detection of injury. Graft injury is the result of many complications that are mentioned below, but according to many, there exist a "separate" category of graft injury that it is called "Subclinical graft injury". Nowadays, the only way to diagnose subclinical graft injury or subclinical rejection is through biopsy, an invasive method that is not applicable in all KT centers [13]. Dd-cfDNA might complement or enhance diagnostic accuracy of early injury detection [14]. Butiu M and Halloran PF are highlighting the importance of dd-cfDNA detection in the diagnosis of various types of injury that remain at a subclinical level. Dd-cfDNA levels in these cases remain significantly lower compared to other types of complications, but still detectable and capable to separate normal graft from injured [15,16]. In addition, Erik L. Lum emphasises the clinical utility of surveillance dd-cfDNA evaluation for early diagnosis and better treatment of rejection, especially in centers that a surveillance biopsy is not part of the KT recipient management protocol [17].



**FIGURE 1.** Different methods of dd-cfDNA (donor derived cell free DNA) quantification in possible samples.



**FIGURE 2.** Main areas of diagnostic application of dd-cfDNA (donor-derived cell free DNA), ABMR: Antibody-mediated rejection, TCMR: T-Cell-mediated rejection, GVHD: Graft-versus-Host disease.

## Rejection

Dd-cfDNA has proved to be an effective biomarker in all types of rejection (Antibody mediated, T-cell mediated and mixed rejection) according to Sigdel TK et al, Xu-Tao Chen et al and Bloom et al [18-20]. Particularly, Bloom et al, was the first to report the use of dd-cfDNA in KT recipients in a multicenter study which results have shown a positive correlation between dd-cfDNA levels, antibody-mediated rejection (ABMR) and T cell-mediated rejection (TCMR) with a cutoff level of 1% [20]. Although dd-cfDNA has proved to be a biomarker with high sensitivity for rejection, its effectiveness varies through the different types of rejection. Specifically, Bloom et al, Erik L. Lum et al, Huang E et al and Obrișcă et al highlight that dd-cfDNA is more effective and appears to have higher sensitivity in ABMR than other types of rejection [17,20-22]. Moreover, Halloran PF et al mention in their results that there is a positive correlation between active rejection status and higher dd-cfDNA, proposing that the sensitivity of dd-cfDNA is higher for acute rejection [16,23]. Additionally, Bu's L et al, Halloran's PF et al, Martuszewski's A et al and Huang et al results prove that dd-cfDNA is a more effective biomarker for earlier diagnosis of rejection than donor specific antibodies (DSA) and creatinine serum levels [12,16,21,24]. Lastly, Martuszewski A mentions that high dd-cfDNA serum levels may indicate reduced estimated glomerular filtration rate (eGFR) in the onset of rejection [12].

## Graft-versus-host disease (GVHD)

Graft-versus-host disease (GVHD) is a major life-threatening complication after allograft transplantation. It is

very rare after kidney transplantation and there are no existing guidelines to the diagnosis and treatment of this life-threatening complication. Nowadays, GVHD is mainly diagnosed based on the symptoms, clinical examinations and some general laboratory results. Williams MD et al proposed dd-cfDNA levels as a possible method for early detection and more efficient management of this complication [25].

## BK Nephropathy

BK virus is widely found in the general population with seroprevalence rates of over 90%. In KT recipients BK virus can lead to BK nephropathy (BKPyVAN) due to reactivation of latent infection or transmission of new infection from the donor kidney. According to the existing guidelines, due to high prevalence of BK nephropathy during the first year after kidney transplantation, plasma BK detection through PCR is done in all recipients [26]. Even though an effective, non-invasive screening method exists for the detection of BK virus, it is not capable of distinguishing BK detection from BK related nephropathy that may result in major complications. In this "diagnostic dilemma" the evaluation of dd-cfDNA plays a decisive role. According to Xu-Tao Chen et al evaluation of dd-cfDNA concentration level in urine of KT recipients with positive biopsies for BKPyVAN was higher than in recipients without BKPyVAN, while differences in urine dd-cfDNA concentration were also found between the different stages of nephropathy [19].

## Correlation with other pathologies

Dd-cfDNA is a biomarker of all types of injury. Because

**TABLE 1.** Main research articles referred to dd-cfDNA applications. Type of study, area of application, detection method, sensitivity and specificity of the method are mentioned in this table.

Author	Year	Type of study	Number of patients	dd-cfDNA application	dd-cf DNA detection method	Sensitivity	Specificity
Obrîșcă B et al	2022	Cross-sectional cohort study	171	Monitoring of recipients and rejection detection	Targeted next-generation sequencing assay in serum samples	94.4% for ABMR	NA
Butiu M et al	2022	Cross-sectional cohort study	171	Subclinical allograft injury	Quantification of serum levels by targeted next-generation sequencing assay	NA	NA
Halloran PF et al	2022	Cohort study	289	Rejection	dd-cfDNA (%) measurement in plasma sample	NA	NA
Erik LL et al	2022	Case report	1	subclinical ABMR	Dd-cfDNA quantification in serum sample	NA	NA
Xu-Tao Chen et al	2022	Single-center prospective observational study	113	rejection and BKPyVAN	Quantification of dd-cfDNA in plasma and urine samples	NA	NA
Williams MD et al	2022	Case report	1	GVHD	Quantification of dd-cfDNA in plasma samples	NA	NA
Reusing JO Jr et al	2022	Retrospective analysis	29	COVID-19 disease severity	dd-cfDNA was processed by PCR targeting in blood samples	NA	NA
Lihong Bu	2021	Prospective multicenter cohort study	1092	Rejection	Quantification of dd-cfDNA in blood samples using targeted next-gene ratio 85% for active rejection and 83% for all types of rejection sequencing assay	NA	NA
Peabody J et al	2020	Randomized clinical trial	924	Monitoring	Quantification of dd-cfDNA in blood samples	NA	NA
Sigdel TK et al	2019	Retrospective analysis	193	Rejection and graft injury	dd-cfDNA was processed by PCR targeting in blood samples	88.7%	72.6%
Oellerich M et al	2019	Prospective observational study	189	Rejection and graft injury	Measurement of dd-cfDNA fraction (%) and d-cfDNA (cp/mL) quantification in blood samples	73%	73% for dd-cfDNA (cp/mL) vs 69% for dd-cf %
Roy D Bloom et al	2017	Prospective cohort study	384	Rejection	Quantification of fraction of dd-cfDNA in blood plasma	59% for active rejection and 81% for all types of rejection	85% for active rejection and 83% for all types of rejection

NA: Non applicable, ABMR: Antibody-mediated rejection, dd-cfDNA: Donor derived cell free DNA, BKPyVAN: BK Polyomavirus Nephropathy, GVHD: Graft versus host disease, PCR: Polymerase chain reaction.

of this general ability to detect graft injury, it has a role in the investigation of various pathological situations that may occur in KT patients. Reusing JO Jr et al found a positive correlation relation between COVID-19 severity and median total cfDNA level, while they also mentioned that total cfDNA levels were elevated in every diagnosis of COVID-19 [27]. This observation may indicate that serious COVID-19 disease may result to some level of graft injury. Moreover, it is known that malignancies and particularly

renal cancer propose one of the major causes of death in transplanted patients [28]. Hongbiao Lu et al mention the possible role of cf-DNA in early detection of renal cancer (metastatic and non-metastatic) [29].

### Monitoring of recipients

The clinical utility of dd-cfDNA evaluation is based on its non-invasive character and its fast evaluation. The above parameters result to the proposal of dd-cfDNA in

surveillance level in all KT recipients. Particularly, Oellerich M et al have mentioned the utility of correlation dd-cfDNA concentration with tacrolimus levels and their results suggest negative relation between higher dd-cfDNA and tacrolimus levels [30]. This study implies the possible role of dd-cfDNA as a method for personalisation of immunosuppression [31]. Moreover, Thongprayoon C et al have proved that dd-cfDNA can detect various types of graft injury in the subgroup of repeat transplant patients [14]. Lastly, dd-cfDNA levels also reflect the extend of early ischaemia-reperfusion injury that can affect the graft function if not monitored properly [10].

## DISCUSSION

Dd-cfDNA appears to be an easily accessible biomarker, with various diagnostic applications in kidney transplantation. Its non-invasive character in combination with its reliability and its fast measurement can make it the 'gold standard' for graft monitoring in the future. Particularly, it can be proved to be really useful for the monitoring of patients during COVID-19 era and other future similar situations, when the access of patients in hospitals is not easy [32].

Dd-cfDNA is not a biomarker exclusively used in KT, but with the same mechanisms can also detect various complications in all organ transplants [33,34]. Apart from the diagnosis of various transplant related complications, cfDNA can be proved to be useful in the differential diagnosis of other kidney related disorders [35]. Firstly, cfDNA appears increased in plasma or urine samples in the incident of renal tumour, an augmentation in which tumour-related cell necrosis and apoptosis may result [36,37]. In cancer cases, not only the quantification of cfDNA, but also the detection of particular qualitative characteristics in cfDNA analysis can be useful. Moreover, cfDNA as mentioned above, is a biomarker of necrosis and due to that characteristic, it is found elevated in the onset of haemolytic uremic syndrome and glomerulonephritis [38]. Additionally, it has been previously mentioned in bibliography that cfDNA can be a prognostic factor for patients with sepsis-induced acute kidney injury or other sepsis related inflammatory complications [39,40].

Dd-cfDNA is usually calculated as fraction percentage, but there are certain situations where the host's cfDNA levels increase resulting in total cfDNA concentration increase and false negative results [30]. Based on this possible problematic diagnostic situation, Halloran et al propose the combination of dd-cfDNA fraction and quantity as the examination with the best diagnostic value [16]. Generally, a combination of non-invasive biomarkers, like eGFR and creatinine levels, with dd-cfDNA quantification can prove

to be the 'gold standard' in KT recipients monitoring [41]. When taking into consideration dd-cfDNA levels into the differential diagnosis of possible graft injury or dysfunction, there are various non pathological situations that should be considered. These situations include transplantations where the graft is derived from deceased donors, a situation most likely explained by ischaemia-reperfusion injury, when higher base-line levels of dd-cfDNA occur [42].

Despite the benefits and potential diagnostic usages that dd-cfDNA offer, there are certainly limitations. Firstly, KT recipients due to immunosuppression or other pathological situations are prone to leukopenia, leukocytosis and inflammatory illness that may influence fractional dd-cfDNA determination. Additionally, medical treatments for other diseases can lead to increase in dd-cfDNA due to graft injury [43]. Moreover, dd-cfDNA is a potential biomarker for many KT complications having to do with graft injury, and not only those related to rejection. There are also certain cases of KT where dd-cfDNA cannot be a potential biomarker, these include identical twin donor/recipient pairs and donor/recipient siblings from consanguineous marriages. Lastly, dual organ transplants from a single donor and multiple organ transplants from different donors also pose some limitations in the application of dd-cfDNA as a diagnostic method [44]. Increase and expansion of the possible SNPs used for dd-cfDNA detection, measurement or usage of epigenetic pattern differences as a method of detection and combination of quantification of various non-invasive biomarkers could be potential solutions to the limitations mentioned above [45].

## CONCLUSIONS

Our review highlights the importance of dd-cfDNA as a potential biomarker for monitoring of KT recipients and the detection of the most commonly found complications. It provides up-to-date information that can benefit research conducted for dd-cfDNA diagnostic usage in kidney transplantation. As far as we know, this is the only review of the literature that highlights all the possible diagnostic applications of dd-cfDNA. There are certain limitations in our review of the literature, including a lack of variability of the research papers due to the novelty of the technique as well as language limitations. These difficulties only enhance the need for more detailed research in this new and exciting field.

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manuscript; K.-E.K. critically reviewed the manuscript; and G.T. supervised, assisted with the data curation, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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