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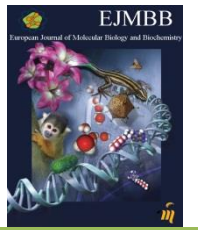

DONOR DERIVED cfDNA IS A NEW INVESTIGATIVE TOOL FOR DETECTION OF GRAFT REJECTION AND ASYMPTOMATIC GRAFT INJURY IN ORGAN TRANSPLANTATION

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ABSTRACT

There is a very important requirement of personalized superior immunosuppression in the field of organ transplantation to minimize premature graft loss. Proficient markers are needed in order to predict rejection and asymptomatic graft injury in organ transplant patients who are under the immunosupression. Prevention of immune activation and exposure to guide tapering are minimal needs for assessment. Allograft integrity is comprehensively monitored with the help of Donor derived cell free DNA [dd-cfDNA]. This review helps to known about prospective benefits of dd-cfDNA to stakeholders [transplant physician, patient, hospital management, laboratory medicine specialist, insurance companies] involved in solid organ transplantation care. There are ninety five articles which contributes strong evidence from forty seven studies shows the role of dd-cfDNA for detection of graft rejection and for monitoring graft integrity. The majority studies are prospective and retrospective cohort studies. There are many techniques used to measure dd-cfDNA in which many of them does not require donor sample. The baseline levels of dd-cfDNA vary by organ type and its normal levels falls rapidly within 2 weeks. The dd-cfDNA levels are elevated in the presence of allograft injury, acute rejections, and infections and returns to normal level after effective treatment. It also have been demonstrated that dd-cfDNA testing is essential for directing potential transplant injury interventions in advance for enhanced long-term result. In contrast with tissue biopsies, dd-cfDNA present in blood offers a rapid and reproducible method to identify graft injuries at early stage and permits more effective personalized immunosuppression. Cell free DNA is novel marker for monitoring health status of solid organ transplant recipients. It can be used in routine clinical practice and have significant clinical outcome with prospective monitoring.

INTRODUCTION

The gold standard method for monitoring the wellbeing state of solid organ transplants has conventionally followed through tissue biopsy of the donor organ. Healthcare system has been redesigned with goal of executing superior value for patients. In suspected clinical conditions, tissue biopsies are mostly prescribed investigations. Tissue biopsies represent an invasive, uncomfortable and inconvenient for patients. The main drawback of tissue biopsy is regular monitoring of transplant organs were not possible and dd-cfDNA helps to show interest in noninvasive strategies to predict graft

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injuries and/or rejections. Existence of cfDNA and its release during apoptosis or necrosis were first recognized by Mandal Metais in 1948[1]. Number of researches have been investigated the use of of cfDNA as marker in of transplant graft injury. The current study demonstrated donor-derived cfDNA is different from recipient cfDNA and proved as promising tool for sensitive prediction of allograft injury. Recent advances in the field of molecular diagnostics are dd-cfDNAs present in transplant recipients blood used clinically as new tool for monitoring for detection of graft rejection and asymptomatic graft injury which leads to irreversible damage[2]. Shotgun or targeted next generation sequencing droplet digital PCR [dd-PCR] used to analyze percentage of dd-cfDNA in solid organ transplantation [3]. Most important problem in solid organ transplantation is scarcity of donor organs. Premature graft loss can be prevented with the help of improved post transplant monitoring which contributes to donor shortages and significant costs associated with retransplantation [4]. In clinical context of patient with unreliable clinical features; biomarkers are needed to provide knowledge on clinical decisions related to exclusion of acute or chronic rejection or early detection of rejection [2]. Early detection of irreversible asymptomatic graft injury is very important for successful solid organ transplantation. Sub-clinical variety of antibody-mediated rejection [ABMR] used for early identification and improve the outcome in renal transplantation [5]. Identification of tissue incompatibility under immunosuppression may reduce the risk of de nova donor specific antibodies [DSA] and transplant organ loss [5]. Overall, accomplishment of more efficient and superior personalized immunosuppression is needed for better- long term outcome in transplantation.

Analytical test validation of dd-cfDNA fractions as Biomarker

The main underlying principle for using dd-cfDNA as a biomarker in organ transplantation is based on the genome of transplant tissue reveals information about organ transplant rejection or compatible[6]. In kidney and liver transplant recipients, presence of donor specific DNA in plasma was illustrated by Dennis Lo in 1998[7]. In this study, dd-cfDNA measured in female recipient's organs by using Y-chromosome specific PCR method from male donors. In conclusion, plasma dd-cfDNA is considered as graft cell death marker, discharged from necrotic or apoptotic cells in transplanted organ recipients and may therefore used as identification marker in graft rejection. This investigation with Y chromosome PCR method have limitation is that it need sex mismatch repairing. In condition of graft cell death, chromosomal DNA is set free in the form of nucleosomes into the blood stream. The process of cfDNA liberated either from necrosis gives rise to large fragments [~ 10000 bp] or apoptosis gives rise to small fragments [180-200 bps]. In blood circulation the median half life of these fragments is ~30 minutes to 2 hrs [8]. There are many validated methods for measurement of dd-cfDNA in blood circulation has been developed. Droplet PCR with set of preselected SNPs [single nucleotide polymorphism] is one of the best available methods. This method has important benefit, which does not require prior genotyping of the donor [9]. Detection of SNPs and heterologous [non recipient allele] in plasma can be done with help of informative assays. To establish dd-cfDNA fraction [%] in plasma, there are 4 independent informative SNPs assays used. Absolute quantification of dd-cfDNA fractions in plasma can be done by multiplying total cfDNA[cp/ml X dd-cfDNA fractions [%]; to determine high accuracy, modification for cfDNA extraction and dd-cfDNA amplification efficiency are needed [2]. Other methods include next generation shotgun [6], or next generation sequencing [NGS] [10, 11]. Deletion/insertions polymorphism are used in PHABRE-PCR [12], Hybrid captured NGS [13], and INDEL qPCR [14, 15]. The analytical performance of dd-PCR and NGS methods are compared as depicted in figure 1 and table 1. The performance of dd-PCR and NGS methods are similarly attained for limit of blank [LoB], Limit of Detection [LoD], Limit of quantification [LoQ], Lower limit of quantification [LLoQ], and imprecision, but the linear quantifiable range was found to be wide for dd-PCR in contrast with targeted NGS assay[9, 10, 16]. Recent findings in liver transplant patients demonstrates monitoring dd-cfDNA with current NGS method may not be possible, due to the elevated values[above 10%] noticed with graft injury in these patients[17]. Considering pre-analytical errors are significant for all types of analysis can be reduced by implementing standard approach and training technical staff by encouraging them to report errors [18]. Pre-analytical errors can be prevented in reporting result of molecular diagnostic investigations by introducing new techniques such as failure effect mode analysis [FEMA] which prevents maximum errors of analysis in routine clinical biochemistry lab [19]. Special cfDNA collection tubes [e.g Streck cfDNA BCTR]for shipping of samples to an external laboratory, which stabilizes white blood cells. These samples in collection tubes are stable at room temperature about 1 week at room temperature. Otherwise, separation of EDTA plasma within 4hrs is vital. The use of relative fractions of dd-cfDNA can be caluculated as dd-cfDNA% and influenced by amount of cfDNA present in recipients blood. The dd-cfDNA% lowered in certain conditions for example infections, exercise, non-graft-associated vascular compromise and medications which cause discharge of host DNA into the blood stream. Most significant, majority of cfDNA [90%] in plasma sample comes from white blood cells i.e from neutrophils and lymphocytes] which undergoes natural cell death called apoptosis [20].

Table 1: analytical test performance of ddPCR and NGS methods. Data from [9,10,16]

	ddPCR	NGS	Targeted NGS assay
LoB	0.10 %	0.11%	0.10%
LLoQ	0.15%	0.15%	0.20%
LoD	0.15%	0.15%	0.16%
Linear quantifiable range	0.15-99.9%	0.15-15%	0.20-16%
Imprecision	3-12%	4.3%	6.8%

Figure 1 shows dd-PCR and NGS method contrasted with targeted NGS assay. LoB: Limit of Blank; LoD: Limit of Detection; LLoQ: Lower limit of quantification. [Data from 9,10,16].

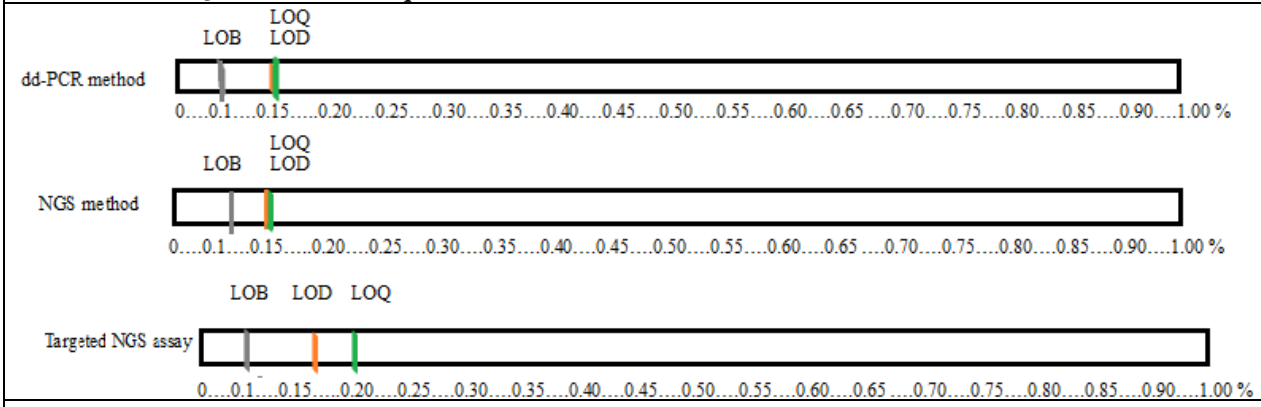
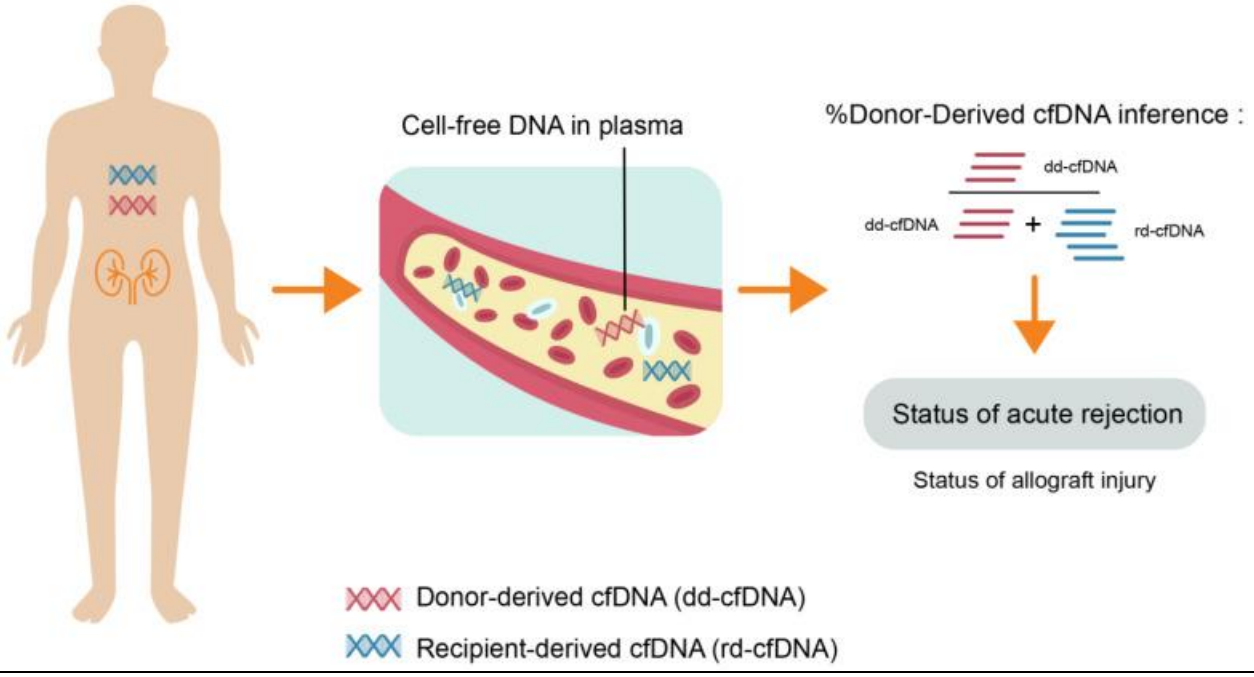


Figure 2. Adapted from Charat Thongprayoon etal study shows donor derived cell free DNA used to identify allograft injury and acute rejections



Patient selection for dd-cfDNA analysis

Monitoring of dd-cfDNA is done in patients of kidney, liver, heart and lung transplant recipients. Sequential analyses of dd-cfDNA are performed in patient recipients to find out acute, chronic rejections and other graft injuries [e.g. acute tubular necrosis], but also used to keep away from unnecessary biopsies. Application of dd-cfDNA done in combined kidney/pancreas transplant recipients. [21] This is not functional to transplants of identical twins.

Investigating dd-cfDNA in intervention efficacy

Determination of dd-cfDNA utility is to find out multiple types of graft injuries, including acute, chronic rejection and helpful in avoiding graft rejection [22]. It is also useful to avoid inconvenient and invasive biopsies, shows response to rejection treatment, identified under

immunosupression and facilitates immunosuppression. Recent study shows that cell free DNA gives more decisive information in comparison with tissue biopsies [23]. Research conducted in kidney and heart transplant patients shows pooled negative predictive values [NPV] ranges from 75-98%, pooled positive predictive values ranges from 12-77%, pooled sensitivity ranges from 59-89% and specificity from 62-93%[24-27]. However this test is not specific for rejection, as increased values are also seen in other graft injuries [e.g., acute tubular necrosis, BK-virus nephropathy, ischemia/reperfusion injury. Research findings from nineteen studies reveals importance of dd-cfDNA levels were able to predict acute rejections with moderate to good performance [ROC AUC ranging from 0.59 to 0.97]. Liver threshold values of dd-cfDNA are higher in comparison with other organ types.

Plasma dd-cfDNA levels as tool for detection of transplant organ injury

More number of studies demonstrated a quick fall of plasma dd-cfDNA to steady state base line levels in uncomplicated patients by around day's 7-10 post transplants. Research findings shows decline in levels of dd-cfDNA are slower rate in heart transplant recipients in compared with the liver transplant recipients [28]. In steady state plasma dd-cfDNA levels vary by organ type. Study shows higher levels of circulating dd-cfDNA in liver transplant recipients in contrast to cardiac or renal transplant recipients [22]. Research proves hypothesis of the higher levels of dd-cfDNA relates to a more amount of transplanted cell mass of double lung and liver recipients in contrast to single lung transplants [29]. The amount of dd-cfDNA fractions are up to 8.5 to 55 % higher found in urine of kidney transplant recipients in comparison to plasma levels [30]. Recent research shows elevated levels of dd-cfDNA in heart transplant recipients with a left ventricular assist device [LVAD] in comparison without device [31]. Additionally, research findings demonstrates dd-cfDNA levels increases slowly in lung transplant recipients after 3 months period of transplantation, which eventually progress to chronic stage and leads to the functional loss of lungs[32]. In prospective cohort studies, the gold standard test [Protocol biopsies] frequently applied in patients with clinical evidence of graft dysfunction, which mean that utility of dd-cfDNA in identification sub clinical graft injury is uncertain. Prospective studies in the present review shows minimum interval required is one month between tests with shorter duration in post transplant period when the risk of infections and AR are highest.

Plasma dd-cfDNA levels in association with acute rejections

Most of the studies show strong association between dd-cfDNA levels and biopsy proven acute rejections [BPAR] out of this one study demonstrates significant increased levels of dd cfDNA at a stage of biopsy proven acute rejections. Research conducted by Bloom etal shows elevated levels of antibody mediated rejection [AMR] than T cell mediated rejection [TCMR] [11]. Additionally to this view, high levels of dd-cfDNA in recipients noticed when de nova donor specific antibodies [DSA] are developed [33]. Study conducted by stoltz etal shows increased levels of dd-cfDNA were reported up to 31 weeks before clinical diagnosis of acute rejections in pediatric recipients [34]. A study conducted in simultaneous kidney-pancreas transplant recipients and liver transplant recipients demonstrates elevations of dd-cfDNA during episodes of rejections and biopsy proven acute rejections. The levels of dd-cfDNA increased in acute rejections before clinical manifestations, two studies shows levels of dd-cfDNA elevated up to 4-6 days before serum amino transferases rise and 8-15 days before confirmation of rejection [9, 35]. There is significant association between levels of dd-cfDNA and acute rejections of cardiac transplant recipients [recognized on endomyocardial biopsy]. Studies confirmed there is strong association for antibody mediated rejections [AMR] and more in intensified T-cell mediated rejections [TCMR] in comparison with mild TCMR [36-39].

Correlation of dd-cfDNA levels in other clinical events

In kidney transplant recipient's high levels of dd-cfDNA found with BK virus nephropathy and urinary tract infections/pyelonephritis[11,39]. In liver transplant recipients elevated levels correlates with active hepatitis B and C infections [17] but not in cholestasis [40]. In lung transplant recipients, increased levels noticed with infections and chronic allograft lung dysfunctions [41]. Study conducted Zhang etal demonstrates strong association between early dd-cfDNA levels and organ dysfunctions at twelve months period which predicts declining long term organ survival [42]. Specifically, a highly variable "peak spiked" pattern of dd-cfDNA levels in the early post transplant period was positively correlated with long-term organ dysfunction which indicates repeated acute graft injury may deteriorate long-term functions [43]. In lung transplant recipients the elevated mean levels of dd-cfDNA in the first 6 months after transplantation correlates with higher incidence of bronchiolitis obliterans syndrome and inferior survival [36]. Elevated median levels associated with a combined endpoint of death in heart transplant recipients, retransplantation, hemodynamic negotiation or organ dysfunction at 3 years [44]. Donor derived cell free DNA is the segment of total cell free DNA which is derived from donor and recipient blood used to asses status of allograft injury and acute rejections [45] as shown in figure 1. The newer techniques help to find out accurate measurement of dd-cfDNA in HLA matched individuals [46]. Recent research shows dd-cfDNA as diagnostic investigation in renal transplants recipients and demonstrates its performance similar to the use of troponin I in identification of myocardial infarction

[47] though difference exists in intra-individual and inter-individual.

Perspectives

Major benefit is the prediction of injury before onset of clinical manifestation, which permits accurate treatment of acute rejection and other causes of organ injury that have the prospective to improve outcome. Monitoring dd-cfDNA levels in blood are most reproducible and rapid method of investigative tool to predict graft injuries at early actionable stages without protocol biopsies. Patients who are under immunosupression and at risk of DSA formation, dd-blood dd-cfDNA levels help to reduce immunosupression. On a wider range, monitoring of dd-cfDNA levels has potential in clinical practice which saves cost in patient diagnosis and improves management of transplant patients. This review study illustrates best and accurate technique by comparing the performance of dd-PCR, NGS and targeted NGS method. There are number of techiniques to detect dd-cfDNA reliably and reaches to baseline within 2 weeks of transplantation when initial ischemia-reperfusion injury subsided. Baseline levels of dd-cfDNA varies based up on organ types which narrates more cellular mass in transplanted organ and elevated levels in liver and lung transplant recipients. The major research findings show a strong association with AR and other causes of allograft injury. High levels found in AR and AMR which returns to baseline after successful treatment. The newer techniques permit accurate detection of dd-cfDNA in HLA compatible transplants. In retrospective cohorts, patient selection is based up on clinical manifestation of transplant organ injury. Cell free DNA have pivotal role in assessment of pre transplant injury and graft viability in deceased-donor transplantation. To determine the origin of circulating cfDNA specific to organ, methylation patterns are used [48]. In the context of islet cell transplantation application of beta cell specific cfDNA detection can be used to predict early graft injury [49]. A latest abstract provides information about donor plasma mitochondrial levels separately detect slow, delayed and primary non graft functions after renal transplantation [50]. Earlier detection of graft injury before clinical manifestation is window for accurate treatment of AR and other causes of graft injury. Furthermore researches are needed to validate the threshold levels of dd-cfDNA in intervention and in improvement transplant outcomes in current clinical practice.

Abbreviations

dd-cfDNA: Donor derived cell free DNA; dd-PCR: Droplet digital PCR; DSA: Donor specific antibodies; ABMR: Antibody mediated rejection; SNP: Single nucleotide polymorphism; NGS: Next generation sequencing; FMEA: Failure mode Effect analysis; LVAD: Left ventricular assist device; BPAR; Biopsy proven acute rejections; AR: Acute rejections; AMR: Antibody mediated rejections; TCMR: T-cell mediated rejections.

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Competing interests

The author declares that they have no competing interest.

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