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ORIGINAL ARTICLE

Dynamics of early post-operative plasma ddcfDNA levels in kidney transplantation: a single-center pilot study

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SUMMARY

Donor-derived cell-free DNA (ddcfDNA) is reported to be a promising noninvasive biomarker for acute rejection in organ transplant. However, studies on monitoring ddcfDNA dynamics during the early periods after organ transplantation are scarce. Our study assessed the dynamic variation in ddcfDNA in early period with various types and status of kidney transplantation. Target region capture sequencing used identifies ddcfDNA level in 21 kidney transplant recipients. Median ddcfDNA level was 20.69% at the initial time post-transplant, and decreased to 5.22% on the first day and stayed at the stable level after the second day. The ddcfDNA level in DCD (deceased donors) group (44.99%) was significantly higher than that in LDRT (living donor) group (10.24%) at initial time, $P < 0.01$. DdcfDNA level in DGF (delayed graft function) recipients was lower (23.96%) than that in non-DGF (47.74%) at the initial time, $P = 0.89$ (19.34% in DGF and 4.46% in non-DGF on the first day, $P = 0.17$). DdcfDNA level at initial time significantly correlated with serum creatinine ($r^2 = 0.219$, $P = 0.032$) and warm ischemia time ($r^2 = 0.204$, $P = 0.040$). Plasma ddcfDNA level decreased rapidly follow an L-shaped curve post-transplant, and level in DGF declined slower than non-DGF. The rebound of ddcfDNA level may indicate the occurrence of acute rejection.

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Key words
acute rejection, delayed graft function, donor-derived cell-free DNA, kidney transplant, target region capture sequencing

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Introduction

Cell-free DNA (cfDNA) has been widely applied in the field of non-invasive prenatal test (NIPT) and liquid biopsy for cancer [1,2]. The presence of donor-derived cfDNA (ddcfDNA) in the blood of transplant recipients was first reported in 1998 [3]. Both of pathogenic-derived and ddcfDNA can be detected in plasma [4,5].

Recent research shows that cfDNA has been accepted as a potential biomarker for kidney transplant rejection. One study revealed that the plasma mean ddcfDNA is 1.2% in stable kidney transplant recipients [6]. Bloom *et al.* [7] found that ddcfDNA% would be a diagnostic criteria for antibody mediated rejection (ABMR), and positive and negative predictive values at a cut off of 1.0% were 44% and 96%, respectively.

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Study of urinary ddcfDNA percentage show that value in acute rejection (20.5 ± 13.9%) was significantly greater compared with stable graft (2.4 ± 3.3%; $P < 0.0001$) or those with chronic allograft injury (2.4 ± 2.4%; $P = 0.001$) [8].

There are no reports on the steady state of the ddcfDNA during the early days of post-kidney transplantation. Likewise, only few literatures on dynamic ddcfDNA monitoring during the early days after kidney transplantation exists. A prospective, observational, multicenter cohort study revealed that the plasma ddcfDNA fractions reached 90% of total cfDNA in a few minutes after liver reperfusion, then ddcfDNA levels decreased rapidly to less than 15% at day 10 [9]. In kidney transplantation, ddcfDNA has been found to reach high values (>5% of total cfDNA) immediately post-transplantation, and rapidly decrease to values of <0.5% within 1 week, but this study only investigated the two time points of ddcfDNA level at the initial time (within 3 days postoperation) [10].

There are many factors affecting the content of ddcfDNA such as cold and warm ischemia time, ischemia-reperfusion injury, residual blood cells or loosely associated cells [6,11]. In living donor liver transplantation, total amount of plasma cfDNA has also been found to increase at post-transplant, and ischemia reperfusion insult or infection possibly contributed to the high levels cfDNA [12]. Becka *et al.* [10] compared the contents of ddcfDNA in kidney transplant (KTx) recipients from living donors and deceased donors and found that recipients with deceased donors had higher ddcfDNA than the recipients with living donors. Till now, no study showed the dynamic variation of ddcfDNA between delayed graft function (DGF) and non-DGF recipients.

Besides, the primary post-transplant clinical complication is infection and rejection, they are often present with similar symptoms and current diagnostic tests often fail to distinguish between infection and rejection [4]. This study employed target region capture sequencing and donor independent method to simultaneously monitor the dynamics of ddcfDNA levels and infection in early post-kidney transplantation, and further analyzes ddcfDNA level in living donor and deceased donor, DGF and non-DGF recipients.

Patients and methods

Between January 3, 2018 and February 15, 2018, 21 adult patients were enrolled to receive a kidney graft. Seven received living donor kidney transplantation (LDRT), three with ABO incompatible (ABOi) and four with ABO compatible (ABOc); 14 received kidney transplantation with donation after cardiac death (DCD). The study was approved by the ethics committee of the first affiliated hospital of Zhejiang University and a written, and informed consent was obtained from each recipient.

Induction and immunosuppressant treatment

Induction therapy was adopted with simulect or anti-thymocyte globulin (ATG) or rituximab and immunosuppressant with tacrolimus combined with myfortic or mycophenolate mofetil and prednisone. Tacrolimus dosing was adapted to maintain target concentrations at 10–15 ng/ml in the first week.

Definition of DGF and acute rejection, treatment of acute rejection

DGF is defined as recipients who received dialysis within the first week. The acute rejection was diagnosed by biopsy according to Banff's 2015 [13]. Treatment of biopsy-proven acute rejection was by steroids (500 mg/day up to 3 days) and/or ATG 50 mg/day for 5–7 days.

Blood collection

Repeated ddcfDNA determinations were scheduled to be performed on the study patients at specific per-protocol post-operative times including initial time (3-h post-operative recovery kidney blood), 1–7 days as well as another two times (10 days; 14 days) when DGF was diagnosed.

Plasma and reference standard

Peripheral blood sample (8 ml) was collected from kidney transplantation patients with cfDNA blood collection tubes (Streck, Omaha, NE, USA). Plasma was separated by centrifugation at 1600 *g* for 10 min, 600 μl of supernatant was used to extract cfDNA for pathogen detection, and remaining supernatant was subjected to a second centrifugation at 16 000 *g* for 10 min, the cfDNA was extracted from the supernatant using the Circulating Nucleic Acid kit (Qiagen, Cat. No 55114, Shanghai, China). To inspect the accuracy of our method, peripheral blood from two healthy specimens were assembled to simulate a "donor" and "recipient" DNA. The "donor" DNA was mixed with "recipient" DNA at specific ratios: 0.6%, 1%, 3%, 6%, 9%, 12%, 15%, 18%, and 21%.

Library construction and target region capture sequencing

The purified cfDNA was then quantified by Qubit fluorometer (Qubit3.0, Life Technologies, Shanghai, China). DNA sequence library was constructed by applying KAPA LTP library preparation kit (KAPA, KK8235). A total of 6200 human SNP loci as well as 13 Kb species-specific regions of 20 pathogens are enriched with a custom TruGrade® DNA Oligos pool (IDT, San Diego, CA, USA). Capture hybridization was carried out according to the manufacturer's protocol. Captured libraries were characterized using the Agilent 2100 Bioanalyzer (High Sensitivity DNA Kit, Beijing, China), then pooled and sequenced (illumina X-ten, 10 ± 5 million, PE 150 bp, San Diego, CA, USA).

Bioinformatics and ddcfDNA quantification

Sequencing raw data were trimmed by removing low quality reads, adapter contamination reads and PCR duplications. Then reads were aligned against the human genome reference (GRCh38; https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.38) using BWA (http://bio-bwa.sourceforge.net). All polymorphism alleles were output when SNP calling by Samtools (-A -uv -t DP, AD). For each of the 6200 SNPs, the reads number were counted for each allele, and minor allele ratio (MAR) value for the informative SNP locus (recipients' homozygous SNP loci with at least one alternative allele read) was then calculated for the ddcfDNA quantification. Bayes approach was applied to quantify the ddcfDNA level. For each informative SNP, Binomial model was employed to estimate the donor derived allele frequency, and donor genotype is estimated based on donor specific allele frequency in the population.

Pathogens detection

We built a custom database which included 984 bacteria species, 248 fungus species, 657 viruses, 34 parasites and human genomes GRCh38. Sequencing reads which met the following conditions were counted: (i) less than 5 bp mismatches in an alignment; (ii) occurrence of less than 2 indel in an alignment; (iii) at most 2 soft-clipping appeared in CIGAR string of bwa output; (iv), insert size less than 500 bp or estimated insert size is equal to mapped length of a read when soft-clipping occurrence simultaneously in both end of paired-end reads; (v), mapped length longer than 100 bp. Samtools was employed to calculate the depth of each pathogens genome sequence. Regions that overlapped with probe sequence were treated as on-target area, and those outside the on-target area as well as the flanking 500 bp regions were considered as off-target area.

Statistical analysis

Serum creatinine and ddcfDNA were compared between LDRT and DCD kidney transplantation, DGF and non-DGF in DCD recipients. The significance of the change in ddcfDNA percentages in all patients, DCD, LDRT group between every observed day and its previous observed day were compared using a non-parameter test of null hypothesis method (Wilcoxon Rank Sum Tests). Pearson correlation coefficients were calculated between ddcfDNA percentages and serum creatinine, warm ischemic time and cold ischemic time at H0 and H1 time point of all LDRT, DCD and DGF patients. *P*-Values <0.05 were considered statistically significant (Taiyun Wei and Viliam Simko, 2017. R package "corrplot": Visualization of a Correlation Matrix. Version 0.84).

Results

Validation of assay precision

To determine the accuracy of our method, validation assay was performed using reference materials. We mixed "donor" genomic DNA sample with another specimen at specific ratios: 0.6%, 1%, 3%, 6%, 9%, 12%, 15%, 18%, and 21%. The level of each concentration was quantified by our method with three replicates for each mixture (Figure. 1). The analyzed levels were found to significantly correlate with the theoretical level ($R^2 = 0.998$).

Demographics of enrolled patients

The demographics of the 21 recipients are listed in Table S1. There were seven recipients who received living donor kidney transplantation. Among the LDRT group, three recipients with ABOi transplant received one dose of 200 mg rituximab and three sessions of plasmapheresis as induction treatment, while the other four recipients with ABOc transplant received no induction treatment. One recipient (p19) experienced acute rejection (Banff type IIA) on the post-operative fourth day, while another recipient (p20) was diagnosed with B19 infection on the post-operative 14th day. Fourteen recipients received DCD kidney transplantation; six recipients received ATG induction and eight recipients received simulect induction. Six of 14 DCD kidney

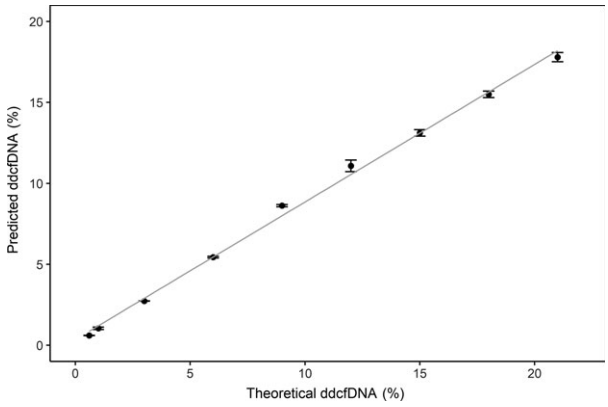

Figure 1 Shows the ddcfDNA assay which identifies 'donor' cfDNA with high linearity and accuracy. Targeted SNP capture sequencing was used to determine the percentage of 'donor' genome present (*y*-axis). The X axis represents the concentration of the theoretical mixture. A linear fit to these points has a slope of 0.84, R^2 value of 0.998.

transplant recipients experienced DGF (p5, 6, 11, 12, 13, 14). Three of six recipients with DGF received allograft biopsy on the post-operative 14th day, p13 and p14 were diagnosed with acute tubule necrosis (ATN), and patient 12 showed acute rejection (Banff type IIA).

Warm ischemia time in LDRT group was significantly lower than that in DCD group, (3.9±2.5 min vs. 15.8±4.0 min, $P < 0.001$); cold ischemia time demonstrated the same result, (156±72 min vs. 328±124 min, $P = 0.003$). Recipients in LDRT group were younger than DCD group; but no significant difference was observed between DGF subgroup and non-DGF subgroup (Table S2). Recipients in LDRT group had lower serum creatinine at each post-operative time when compared with recipients in DCD group (Figure 2a). Serum creatinine value in the DGF subgroup was higher than that in non-DGF subgroup from the fourth day to the seventh day (Figure 2b).

Dynamics of ddcfDNA concentration in kidney transplantation recipients

The ddcfDNA kinetics seemed to follow an L-shaped curve with high concentration in the immediate post-transplantation phase followed by a swift decrease to a stable baseline level (Figure 3a). At the initial time (3 h after recovery of kidney blood flow), the median value of the concentration of ddcfDNA was 20.69%, then it decreased to 5.22% on the first day (exact time about 16.4 h post-transplantation), the ddcfDNA concentration from the second to the seventh day was 1.98%, 2.09%, 0.98%, 1.95%, 1.5%, 0.85%, respectively. Further statistical results showed that the concentration of ddcfDNA was significantly lower on the second day than on the first day ($P = 0.039$), but no significant difference was observed between the second day and the third day ($P = 0.89$), and P value was 0.17 when the third day versus the fourth day performed, which means that the concentration of ddcfDNA was on high level at initial time, and then decreased to a stable level on the second day in kidney transplantation.

Figure 3b shows the decrease curve of ddcfDNA concentration between DCD kidney transplant and living donor kidney transplant. The ddcfDNA level of recipients in DCD group (44.99%) was significantly higher than that in LDRT group (10.24%) at initial time, $P < 0.01$, which means DCD group have been affected by IRI greater. Although the ddcfDNA level of two groups decreased over time, ddcfDNA level was still significantly higher (1.11%) in DCD group than that in LDRT group (0.59%) on the seventh day ($P < 0.05$). In

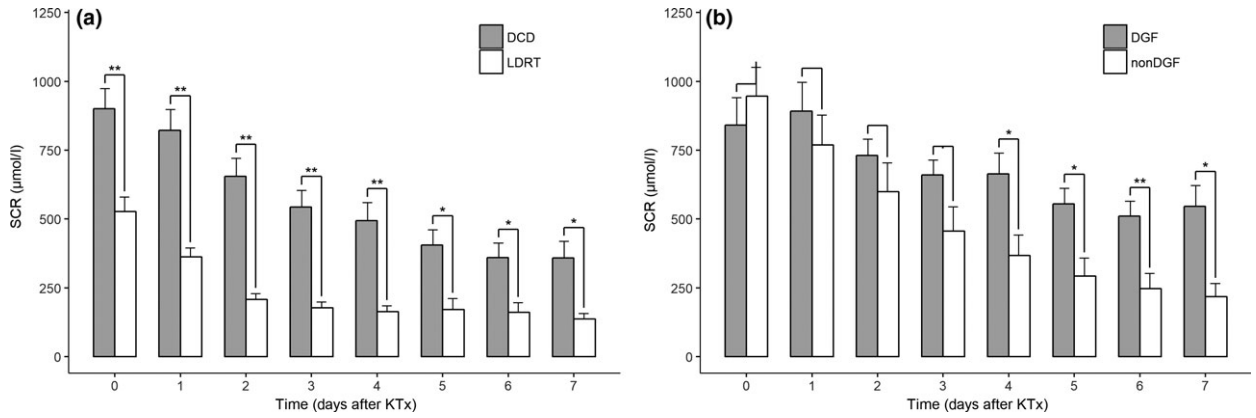

Figure 2 Serum creatinine level at different time points. (a) serum creatinine level between DCD group and LDRT group; (b) serum creatinine level between DGF subgroup and non-DGF subgroup. *$P < 0.05$, **$P < 0.01$.

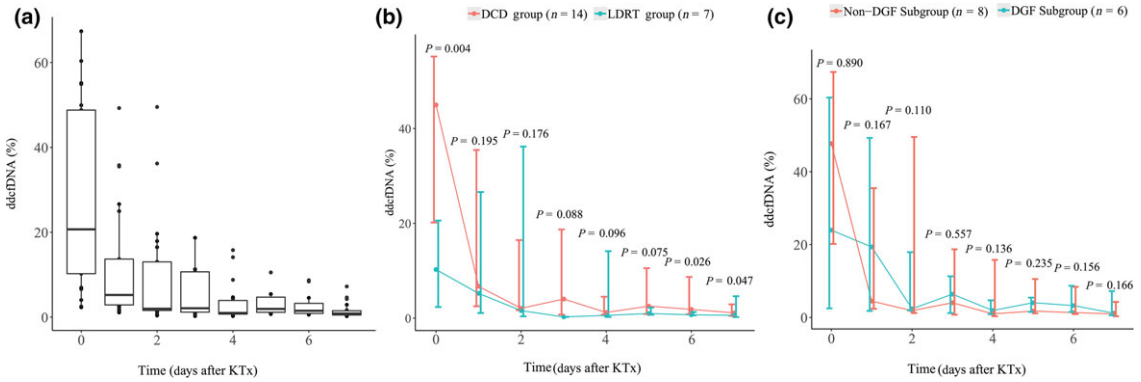

Figure 3 The dynamics of plasma ddcfDNA in kidney transplant recipient. (a) The ddcfDNA concentration at different time points; (b) The ddcfDNA concentration at different time points between DCD group and LDRT group; (c) The ddcfDNA concentration at different time points between DGF subgroup and non-DGF subgroup.

DCD group, the data showed that the concentration of ddcfDNA was significantly lower on the second day than that on the first day ($P = 0.02$), but no significant difference was observed between the second day and the third day ($P = 0.54$), which means in DCD group, the concentration of ddcfDNA was at high level at initial time, and then decreased to the stable level on the second day. There was no significant difference between two adjacent time points in LDRT group although the ddcfDNA concentration was 10.24% at initial time and 5.22%, 1.58%, 0.26%, 0.58%, 0.98%, 0.70%, 0.59%, thereafter, respectively. The ddcfDNA concentration decreased to <1% on the third day in LDRT group.

When we further classified the recipients with DCD kidney transplantation into DGF subgroup and non-DGF subgroup, although the ddcfDNA concentration in DGF subgroup was 23.96% lower than 47.74% in non-DGF subgroup at the initial time, no significant difference was observed, $P = 0.89$. The concentration of ddcfDNA in DGF subgroup was 19.34% while it was 4.46% in non-DGF subgroup on the first day ($P = 0.17$). The ddcfDNA reduced 90.6% in non-DGF subgroup and 19.3% in DGF subgroup on the first day, respectively. The ddcfDNA in DGF recipient was higher than that in non-DGF subgroup at the each operative time from the first day to the seventh day, and the reduction in ddcfDNA was slower in DGF recipient when compared with non-DGF subgroup, (Figure 3c).

DdcfDNA level and delayed graft function, acute rejection, and infection

Three recipients (p12, p13, p14) with DGF were maintained with regular hemodialysis and received allograft biopsies at 2 weeks post-operation. DdcfDNA level of p12 declined rapidly but on the fourth day ddcfDNA concentration increased to 2.47%, and 7.23% on the seventh day. This recipient received 10 g/day IVIG treatment from the fifth day to ninth day for 5 days, after which the level of ddcfDNA decreased to 1.09% on the 10th day, increased to 1.47% on the 14th day and received allograft biopsy which resulted in Banff type IIA acute rejection. The ddcfDNA level of p13 decreased gradually, but on the 14th day the value was 2.13%. Recipient p13 received allograft biopsy on the 14th day, and the result showed ATN without a sign of acute rejection. DdcfDNA value of p14 was lower than 1% on the 14th day, and the allograft pathology showed ATN. The results of these recipients suggest that monitoring the dynamic change in the ddcfDNA concentration in DGF can be valuable. The situation that the ddcfDNA decreased initially but then increased later (levels more than 1%) might suggest acute rejection. Doctors should therefore adopt kidney biopsy or intensify the immunosuppression. If the recipient initially had high levels of ddcfDNA (more than 1%) but decreased gradually over time, it may be without problem, because it can be maintained with available treatment options and wait for kidney function recovery (Figure 4).

Recipient p19's ddcfDNA on the first post-operative day was higher than the initial time. The serum creatinine level decreased slowly from initial time to the third day, but increased on the fourth day. The recipient was maintained with 500 mg methylprednisolone pulse from the first to second post-operative day. On the fourth day, the recipient initially received ATG (50 mg/day) treatment for the consideration of acute rejection and received kidney biopsy simultaneously resulting in Banff type IIA acute rejection. The increase

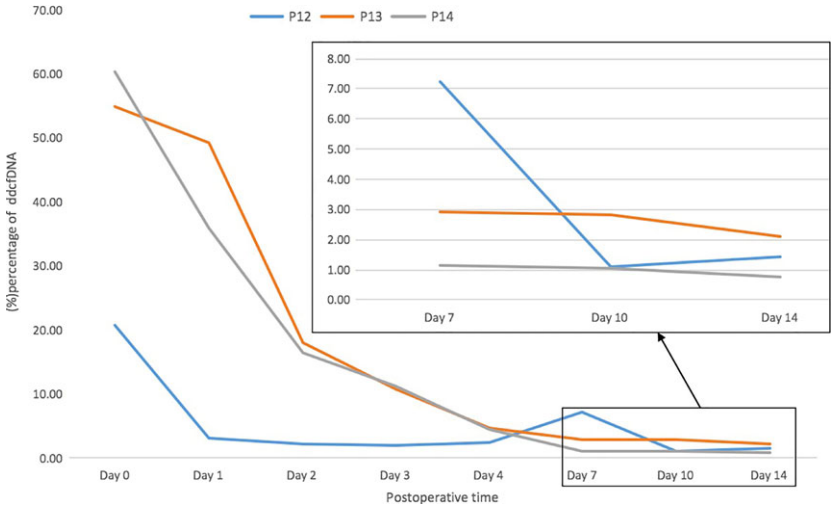

Figure 4 dynamic ddcfDNA levels among three recipients with DGF received kidney biopsy at two weeks.

in ddcfDNA on the first post-operative day, which is earlier than the increase in serum creatinine, may suggest acute rejection (Figure 5).

More interesting, we identified 55 reads of human parvovirus B19 on the second day, 2363 reads on the fourth day and 29 407 reads on the seventh day in recipient p20. In clinical, this recipient was identified with 3.4 × 10E8 copies of human parvovirus B19 by qPCR method at 14th day post-transplantation without anemia. This recipient experienced anemia with low levels of reticular cells (0.1%) and received 20 g/day IVIG treatment and switched tacrolimus to cyclosporine on the 24th day. At this time point, 4 323 853 reads of human parvovirus was identified, ddcfDNA increased from 0.28% on 7th day to 0.74% on the 24th day (Figure 6).

Analysis of ddcfDNA correlated factors

DdcfDNA level at initial time was significantly correlated with serum creatinine ($r^2 = 0.219$, $P = 0.032$), and significantly correlated with WIT ($r^2 = 0.204$, $P = 0.040$). At the same time, serum creatinine was significantly correlated with WIT ($r^2 = 0.456$, $P < 0.001$). The result means that long WIT caused kidney ischemia insult, and ischemia insult was the reason of high level

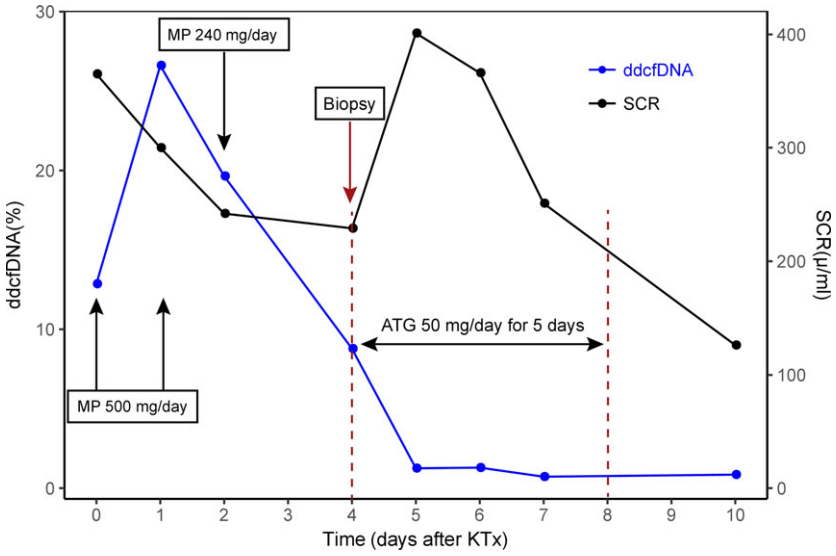

Figure 5 The ddcfDNA level and serum creatinine levels at different time. ATG: anti-thymocyte globulin; MP: methylprednisolone; SCR: serum creatinine.

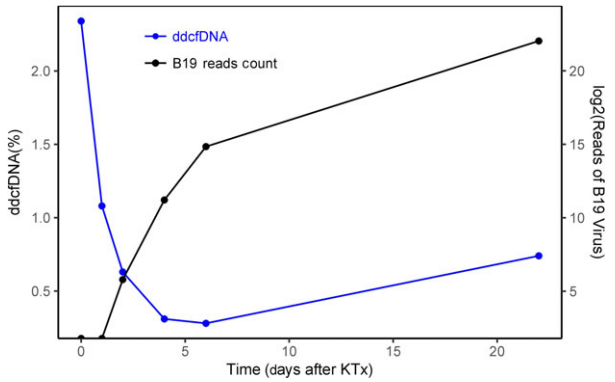

Figure 6 The dynamics of ddcfDNA level and number of reads on human parvovirus B19 virus.

of initial post-operative ddcfDNA (Figure 7a). DdcfDNA level on the 1st day after post-operative recovery kidney blood was significantly correlated with serum creatinine ($r^2 = 0.198$, $P = 0.043$), serum creatinine was significantly correlated with WIT ($r^2 = 0.333$, $P = 0.006$) and significantly correlated with CIT ($r^2 = 0.191$, $P = 0.047$) (Figure 7b).

Discussion

In this study, we demonstrated the dynamics of ddcfDNA and the time to reach stable state in kidney transplantation. The ddcfDNA level is significantly correlated with warm ischemia time and serum creatinine. Our data highlight ddcfDNA may be a new indication of rejection when dynamic ddcfDNA increase over time. This method not only effectively measured ddcfDNA but also identified the presence of pathogens.

Our results showed ddcfDNA kinetics follow an L-shaped curve with high percentages in the immediate post-engraftment phase followed by a swift decrease to a stable baseline level. DdcfDNA levels, immediately post-transplantation, were higher in DCD recipients than in living donor recipients and were significantly related with warm ischemia time. That means the high ddcfDNA level was caused by ischemia-reperfusion injury. Ischemia-reperfusion injury, residual blood cells or cells loosely associated with the graft might be the major source of early high ddcfDNA levels [6, 11, 14].

The ddcfDNA concentration in DGF subgroup was 23.96% lower than 47.74% in non-DGF subgroup at the initial time. The concentration of ddcfDNA in DGF subgroup was 19.34% and 4.46% in non-DGF subgroup on the first day ($P = 0.17$), whiles the reduction rate was 90.6% in non-DGF subgroup and 19.3% in DGF subgroup on the first day. These recipients with DGF needed longer time to reach the stability although had the lower initial ddcfDNA levels. As we known, cfDNA was cleared mainly by deoxyribonuclease. Deoxyribonuclease activity is inversely proportional to the amount of cfDNA. Low deoxyribonuclease activity in plasma could be the cause of increased amount of cfDNA in some diseases [15]. The highest deoxyribonuclease activity among all tested tissues and body fluids was found in urine. The enzyme activity in the kidney is higher than in most analyzed organs [16]. The physiological or pathological role of deoxyribonuclease activity in the kidney is not clear. So, we can consider that recipients with DGF may have low deoxyribonuclease activity in the transplant kidney, which lead to lower clearance of cfDNA. Even though the activity of this enzyme has not been identified, we hope to carry out further study on the relationship between ddcfDNA level and deoxyribonuclease activity. Some studies showed that cfDNA in plasma could be filtered by glomerulonephritis, termed trans-renal cfDNA. This may be another reason for DGF recipients with high level of ddcfDNA [17,18].

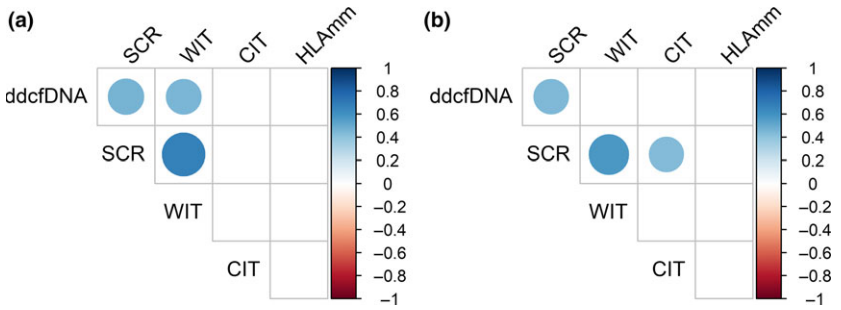

Figure 7 Heatmap of parameter correlations. (a) ddcfDNA at the 3 hours post-operative recovery kidney blood; (b) ddcfDNA on the first day post-operative recovery kidney blood. CIT: cold ischemia time; HLA-MM: human lymphocyte antigen mis-match; SCR: serum creatinine; WIT: warm ischemia time.

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Delayed graft function had a higher risk of acute rejection in the modern era of kidney transplantation [19]. It is important to differentiate acute rejection from recipients with DGF. There were six recipients with DGF in this study, and three of them received allograft kidney biopsy. One recipient (p12) with decreased ddcfDNA initially and increased thereafter showed acute rejection. Another recipient (p13) with a gradual decrease in ddcfDNA, with high level not more than 2% on the 14th day, had ATN without sign of acute rejection. The third recipient (p14) with rapidly decreased ddcfDNA to less than 1% on 14th day also had ATN without sign of acute rejection. It may suggest that recipient may experience acute rejection if recipient's ddcfDNA increased thereafter during early days of transplant, regardless of whether there is a high level of ddcfDNA, as long as its level is decreasing gradually, there may be no coexistence with rejection in recipients with DGF. The recipient (p20) showed low initial ddcfDNA (12.9%) but ddcfDNA increased at 1st day although the recipient still received methyprednisone pulse, while the serum creatinine level decreased slowly from initial time to the third day, but increased on the fourth day. Biopsy-proved acute rejection was confirmed on the fourth day eventually, which also showed that the increase in ddcfDNA can be used to predict acute rejection earlier. The level of ddcfDNA is associated with rejection, however, it does not guarantee rejection in cases where the ddcfDNA is more than 1% which is reported in humoral and Banff IB type rejection [12]. Therefore, early monitoring of the dynamics of ddcfDNA can better identify and predict an acute rejection. Dynamic surveillance of ddcfDNA may provide a new strategy to identify acute rejection.

Aside from acute rejection, infection is another critical complication that impacts the survival of allograft patients after organ transplantation. Based on the current data, plasma ddcfDNA levels have shown marked increases both during acute rejection and graft infection [4], pointing to the necessity of a combined pathogen monitoring strategy. This phenomenon was also observed in our case (p19) with human parvovirus B19 infection. In the liver transplantation, hematoma, CMV infection, and acute rejection led to increased ddcfDNA fractions up to 90% compared to 10% during stable graft function [6]. Infection can also lead to an increase in level of ddcfDNA, and one study reported that a combination of ddcfDNA with PCT can distinguished the infection leading to the ddcfDNA increase [20].We detected human parvovirus B19 reads in recipient p19 at the 2nd post-operative day, and the dynamic increase in B19 reads over time. At last, the recipient was confirmed with human parvovirus B19 infection with low hemoglobin and recovered after treatment with IVIG. Our un-published data shows that this technique can also identify the polyomavirus viremia in patients with high level of ddcfDNA and confirmed with biopsy-proved acute rejection.

QPCR is the conventional methods to detecting BKV and parvovirus clinical, however, QPCR only allows the identification of known pathogens with limited throughput. Here, we employed liquid hybridization technology and NGS to simultaneously monitor acute rejection and infection in allograft patients using peripheral blood sample. Targeted 2000 pathogenic microorganisms can be detected by sequencing the species-specific genome region except for BKV and parvovirus. So the method has the merit of high-throughput. This technique is noninvasive and simultaneously provided the information of rejection and infection, therefore it provides an good health surveillance tool for allograft patients.

It should be noted that there are several limitations in our paper. First, the limited sample size, especially when considering subset comparisons, does not have adequate statistical power to detect AR vs ATN as cause of DGF. Second, there is the heterogeneity of the population and heterogeneity of the induction treatment compounded with the small sample size. Third, protocol biopsy was lack. However, our data mainly revealed the dynamic variation in ddcfDNA in early period with various types and status of kidney transplantation.

In conclusion, we established target region capture sequencing method which not only identify ddcfDNA but also monitor pathogens. Our data demonstrated that plasma ddcfDNA at initial time after kidney transplant was high and achieve stable levels on the second day, whiles the ddcfDNA level in DGF recipients declined slowly. The ddcfDNA level in early period was correlated with warm ischemia time and serum creatinine. The phenomenon of ddcfDNA rising again after decreasing may be a cue for acute rejection.

Authorship

JS and YZ: performed experiments and wrote the article. YC, WL, WP, and JW: performed blood collection, cfDNA library construction and target region enrichment. JG, GL performed pathogen identification. XL, GY, and HS: analyzed the data. JC: edited the article. TJ and RW: designed experiments, interpreted data, and reviewed all data and article.

Conflict of Interest

The authors have declared no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. The detail parameters in 21 kidney transplant recipients.

Table S2. Clinical parameters comparison in LDRT group and in DCD group, and in DGF subgroup and non-DGF subgroup.

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