

The Rebound of Early Post-operative Plasma dd-cfDNA in Kidney Transplant Recipients: A Single-center Study

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Research article

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Abstract

Background: It has been reported that donor derived cell-free DNA(dd-cfDNA) accounts for less than 1.2% of total cell free DNA in stable kidney allograft recipients, and dd-cfDNA may be a non-invasive biomarker of acute rejection. However, the kinetics of plasma dd-cfDNA level is still unclear, which hinders the further application of dd-cfDNA in kidney transplantation (KTx). The purpose of this study was to explore the correlation between plasma dd-cfDNA and delayed graft function(DGF) and pulmonary infection after KTx, and to explore the diagnostic value of dd-cfDNA in DGF. In addition, we tried to find out the factors related to the rebound of dd-cfDNA level.

Methods: A total of 183 kidney transplant recipients were enrolled in this study. Peripheral blood samples (10ml) were collected on the 1st, 7th, 14th and 21st day after KTx, and 546 plasma samples were collected. Droplet digital PCR (DDPCR) was used to detect the level of dd-cfDNA(%) and Mann Whitney U test was used to analyze the relationship between dd-cfDNA level and DGF and pulmonary infection. Logistic binary regression analysis was used to analyze the clinical factors related to the increase of dd-cfDNA.

Results: There was no significant difference between DGF group and non-DGF group of dd-cfDNA level ($P > 0.05$). The mean value of dd-cfDNA on day 1 (6.97%) was significantly higher than that on day 7 (1.17%), day 14 (1.09%) and day 21 (1.18%). Logistic binary regression analysis was performed for dd-cfDNA level rebound group and non-rebound group. Pulmonary infection (OR = 2.11, $P = 0.028$) and DGF (OR = 1.37, $P = 0.42$) were significantly correlated with rebound of dd-cfDNA. At the same time, on the 1st, 7th and 14th day after KTx, the levels of dd-cfDNA in pulmonary infection group was significantly higher than non-infection group ($P < 0.05$).

Conclusion: Our results indicate that dd-cfDNA (%) can't be used as a biomarker for predicting DGF. The rebound of plasma dd-cfDNA (%) level was significantly correlated with the presence of pulmonary infection. However, further confirmatory studies are necessary.

Introduction

Monitoring the health of kidney allografts is important for the long-term survival of kidney transplant recipients. The proportion of plasma dd-cfDNA of total cell-free DNA(cfDNA) has been proposed as a non-invasive biomarker to assess the status of transplanted kidneys[1].Following organ transplantation, as cells from the donor allograft degrade, donor-derived cell-free DNA (dd-cfDNA) are released into bloodstream and cleared from blood by the liver and kidney with half-life of about 30 min[2]. In this way, the detection of dd-cfDNA can indicate tissue damage and cell turnover, and biochemical changes earlier than cell death can also reflect changes in dd-cfDNA[3].

It has been reported that dd-cfDNA is $< 1\%$ of the total cell-free DNA in kidney transplant recipients with stable allograft[1], and dd-cfDNA% can be a non-invasive marker of acute rejection[4, 5]. In the mean time,the level of dd-cfDNA can be affected by multiple factors(including acute rejection, warm ischemia,

infection), and its change caused by the kind of factor is difficult to be recognized. Therefore, the kinetics of plasma dd-cfDNA levels remains unclear, which poses obstacles to the further application of dd-cfDNA in KTx.

DdPCR is recommended for quantifying dd-cfDNA levels in transplanted recipients due to its rapid detection and cost-effectiveness[6]. In this study, we used ddPCR technology to detect plasma dd-cfDNA levels in kidney transplant recipients, and to explore the diagnostic value of dd-cfDNA for assessment of DGF. Furthermore, we try to find out the factors related to rebound in dd-cfDNA levels.

Patients And Methods

Patients

Between June 2018 and May 2020, we enrolled 188 patients who received deceased donor kidney for their first transplantation at Sichuan Provincial People's Hospital. The kidney came from the organ transplantation center of Sichuan Provincial People's Hospital and no organs / tissues were obtained from prisoners. Written informed consent was obtained from all patients. The study protocol was approved by the ethics committee of Sichuan Provincial People's Hospital [No. (2020)405]. Kidney allografts from deceased organ donors who met the ethical guidelines for kidney donation were used.

Sample collection

Peripheral blood sample(10 ml) was separately collected from kidney recipients on day 1, day 7, day 14 and day 21 after KTx, and placed in blood collection tube (Streck, US). The connecting tube from the tube was removed and then immediately gently reversed 8 to 10 times to mix the liquid in the tube. The peripheral blood samples were placed in a special medical vaccine refrigerator and transported to the laboratory by the designated clinical service specialist.

Cell-free DNA extraction

Plasma was separated from peripheral blood sample by centrifugation at 4 °C, 1000 rpm/min for 10 minutes. The NucleoSnap DNA Plasma kit (MN, Germany) was used to separate circulating cfDNA from 4 mL plasma according to the manufacturer's instructions. The cfDNA extracted were stored in the refrigerator at -80°C for further testing.

Digital Droplet PCR (ddPCR) workflow and dd-cfDNA(%)calculation

According to the demand of ddPCR reaction system, the configured reaction system is added to the DG8™ Cartridges, Droplet Generation Oil for Probes is added at the same time, and finally it is sealed with DG8™ Gaskets. Then droplets were prepared on the QX200 droplet generator device (American Bio - Rad) about 2 min, and transferred to a 96-well plate (Eppendorf, Germany) and sealed with an aluminum membrane for PCR reaction. At the end of the PCR reaction, the droplets were read in the QX200 droplet reader and

analyzed using QuantaSoft version 1.7.4 software (US Bio-Rad). In this study, dd-cfDNA (%) is part of the total circulating cfDNA, not measured by quantifying the absolute level of dd-cfDNA.

Definition of DGF, pulmonary infection and the rebound of dd-cfDNA

DGF is defined as recipients who received dialysis within the first week after KTx. Patients with a pulmonary infection was diagnosed by the following clinical manifestations: fever, cough and sputum, decreased oxygen saturation, abnormal laboratory test results, abnormal imaging X-ray, CT. As reported by Beck et al., the mean plasma dd-cfDNA (%) of kidney transplant recipients with stable transplant function is 1.2%[6]. The value of dd-cfDNA (%) higher than 2% is defined as rebound on day 7. The rebound of dd-cfDNA is also defined as the dd-cfDNA% value of one point(day 7, day 14, and day 21),which is significantly higher than the point of the last time .

Statistical analysis

Mann-whitney U tests was used to compare the plasma dd-cfDNA% of kidney recipients between DGF and non-DGF pulmonary infection and non-infection. Clinical factors related to dd-cfDNA% level rebound was analyzed by Logistic binary regression analysis. Two-tailed P-values<0.05 were considered statistically significant. All analyses were performed using Graphpad Prism software version 8.0 (Graphpad Software, INC., USA).

Results

Patients

From June 2018 to May 2020, 188 patients were included, excluding 5 recipients according to the following criteria (Figure 1),and the effective data of 183 patients were finally used to analyze.

As shown in Table 1, 66.12% of these study participants were male (n = 121), and the age range was 17–68 years (mean, 41 years). The patients all accepted the immunosuppressive triple plan, and Corticosteroids was administered as initial immunosuppressive therapy. Basiliximab was used as an immune induction therapy, maintenance therapy drugs are tacrolimus or cyclosporine, mycophenolate mofetil or Mycophenolate Sodium Enteric-coated Tablets and glucocorticoids. There were no significant differences in sex, age, BMI, warm ischemia time,cold ischemia time,HLA mismatches and initial immunosuppression between DGF group and non-DGF group.

Table 1 Demographic characteristics of the kidney allograft recipients. Values are given as mean ± standard deviation or n(%) BMI, body mass index HLA Human leukocyte antigen.

Donor	All (n=99)	DGF (n=34)	non-DGF(n=89)	P
Age, years	45.63±15.00	48.33±14.67	44.88± 15.06	0.20
BMI,kg/m2	23.04±3.74	23.56±3.56	22.89±3.83	0.32
Recipient	All (n=183)	DGF (n=40)	non-DGF(n=143)	P
Age, years	41.59±9.84	42.08±10.32	41.18±10.27	0.63
Male sex	183 (121)	40 (26)	143 (95)	0.50
BMI,kg/m2	21.46±2.96	21.31±3.00	21.50±2.95	0.76
Warm ischemia time, min	16.61±5.95	17.18±6.53	16.45±5.80	0.50
Cold ischemia time, h	10.06±5.53	9.68±5.58	10.17±5.53	0.62
Transplant-related				
HLA mismatches				
1-2	10 (5.46%)	2 (20.00%)	8 (80.00%)	0.61
3-4	86 (47.00%)	17 (19.77%)	69 (80.23%)	
5-6	87 (47.54%)	21 (24.14%)	66 (75.86%)	
Initial immunosuppression				
Corticosteroids	183	40	143	0.5
Tacrolimus	140 (76.50%)	29 (19.23%)	111 (80.77%)	
Cyclosporine	43 (23.50%)	11 (23.08%)	32 (76.92%)	
Mycophenolate mofetil	127 (69.40%)	27 (21.26%)	100 (78.74%)	0.77
Mycophenolate Sodium Enteric-coated Tablets	56 (30.60%)	13 (23.21%)	43 (76.79%)	

Dd-cfDNA level and DGF

There was no significant difference of dd-cfDNA(%) between DGF and non-DGF on day 1, day 7 and day 14 respectively (Fig. 2). Due to the small number of plasma samples on day 21, it was not included for analysis. Therefore, dd-cfDNA% may not be suitable to be used to distinguish DGF from non-DGF.

Kinetics of the dd-cfDNA% in the entire cohort of patients

The level of complete dd-cfDNA(%) in 183 patients are shown in Fig. 3. We collected all 500 plasma samples included in the patients. On the whole, the level of dd-cfDNA(%) on day 1 is relatively higher than the latter, and the overall trend is declining. It is worth noting that some patients have a significant rebound in dd-cfDNA(%) levels in this study. Among the dd-cfDNA(%) rebound, the maximum difference between the dd-cfDNA (%) levels at two adjacent time points (day 21 minus day 14, day 14 minus day 7, day 7 minus day 1) is 16.3%.

Dd-cfDNA level and pulmonary infection

To explain the phenomenon of dd-cfDNA(%) rebound, we retrospectively analyzed the clinical data and reports of these patients in an attempt to find out the cause. Notably, The level of dd-cfDNA (%) is significantly higher in pulmonary infection group on day 1, day 7 and day 14 ($P < 0.05$, Figure 4). 26 patients in the dd-cfDNA(%) level rebound group exposed to pulmonary infection. 42 patients in the dd-cfDNA(%) level non-rebound group exposed to pulmonary infection. A multivariate logistic regression analysis showed that pulmonary infection was significantly associated with dd-cfDNA(%) rebound (OR = 2.11, 95% CI, 1.08-4.13, $P < 0.05$, Figure 5). Logistic binary regression analysis was performed on the dd-cfDNA% rebound group and non-rebound group, and pulmonary infection, DGF, warm ischemic time (WIT), and cold ischemic time (CIT) were included as control variables, and the relative risk of pulmonary infection increased. Furthermore, DGF (OR=1.37), WIT (OR=1.02), and CIT (OR=1.03) were also associated with dd-cfDNA(%) rebound.

Discussion

In recipients with stable grafts, the plasma dd-cfDNA kinetics seem to follow an L-shaped curve with high percentages in the immediate postengraftment phase followed by a swift decrease to a stable baseline level[1]. However, dd-cfDNA levels were early increased during acute rejection, graft infection episodes [7] (including sepsis, urinary tract infections, respiratory tract infections and cytomegalovirus infection), ischemia-reperfusion injury[8] and warm ischemia[9]after KTx. Obesity is another factor contributing to cfDNA quantity, which is twice as high as that of thin people [10]. Our study revealed that the rebound of plasma dd-cfDNA levels was correlated with DGF and pulmonary infection in the early post-kidney transplantation period. But warm ischemia time and cold ischemia time were not correlated with the rebound of plasma dd-cfDNA levels, which is inconsistent with their results.

The release of dd-cfDNA in the recipient's blood secondary to cell damage in the graft can be a promising biomarker to assess allograft health. It has been proposed that mean plasma dd-cfDNA% in kidney transplant recipients with stable graft function is about 1.2%[6]. Within the first 7 days after KTx, dd-cfDNA level in DGF declined slower than non-DGF, however, dd-cfDNA% has not yet been demonstrated the potential ability to distinguish DGF from non-DGF [9], and similar results were obtained in our study. Studies have shown that there is no association between an abnormal dd-cfDNA% dynamics and the occurrence of DGF or predictive biopsy (early) performance ($p = 0.696$)[11]. However, the release of dd-cfDNA% is related to graft damage (including urinary tract infection, prerenal acute kidney injury, surgical complications or the occurrence of hydronephrosis). However, the occurrence of one or more other early adverse events was closely related to the abnormal decline of dd-cfDNA% after transplantation ($p = 0.001$)[11].

In this study, we firstly found that pulmonary infection is significantly associated with the rebound of plasma dd-cfDNA level, which may be caused by the following reasons: 1.inflammation directly leads to the destruction of donor kidney cells; 2.anti-infective drugs have nephrotoxicity, which may indirectly lead to kidney damage; 3. immunosuppressive agents cause damage to donor kidney cells. But the specific reasons need to be further explored.

Overall, elevated dd-cfDNA indicates allograft injury as may occur with AR, infection, or acute tubular injury, and it is related to ischemia-reperfusion injury[8] warm ischemia[9], but may also be found in clinically stable patients with normal histology. In general, no matter which organ is affected, the amount of cfDNA in plasma seems to correlate with the volume of damaged tissue and in some cases with the prognosis.[12]. Recent data suggest that increased cfDNA is associated with acute kidney injury[13]. On the other hand, a multivariate analysis in patients on haemodialysis showed that an important determinant of cfDNA is the blood pressure suggesting that vascular injury might contribute to the pool of plasma DNA in some patients[14].

However, our study has several limitations. The detection time points are not continuous, which brings obstacles to accurately assessing the dynamic changes of dd-cfDNA levels. Protocol biopsy was lack, especially when considering subset comparisons, makes detect acute rejection as cause of DGF

inadequate statistical power. Nevertheless, our results clearly showed that the rebound of plasma dd-cfDNA level is correlated with DGF and pulmonary infection early after transplantation, and seems to be predictive of adverse events, although further confirmatory studies are warranted.

Conclusions

In conclusion, the rebound of plasma dd-cfDNA levels during the early post-transplant period are significantly associated with the presence of DGF and pulmonary infection.

Abbreviations

donor derived cell-free DNA dd-cfDNA

delayed graft function DGF

Droplet digital PCR ddPCR

warm ischemic time. WIT

Declarations

Acknowledgments

Not Applicable

Authors' contributions

QR, JYW and SZ were responsible for the concept and design of the study. JYW and XMQ are responsible for the data analysis and drafting of this paper. XXW and PS are responsible for data interpretation and manuscript revision. QR and SZ are responsible for revising and finalizing the manuscript. XMQ and JYH are responsible for data acquisition and interpretation. All authors participated in the final approval of this paper and agreed to be responsible for all aspects of the work.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional ethics committees of Sichuan Provincial People's Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all patients. Patients' identification information was replaced with codes before data extraction for privacy concerns. This research upheld the principles of the Declaration of Istanbul. This article does not contain or report any studies with human participants or animals performed by any of the authors.

Consent for publication

Not Applicable

Competing interests

The authors declare that they have no competing interests.

Footnotes

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Figures

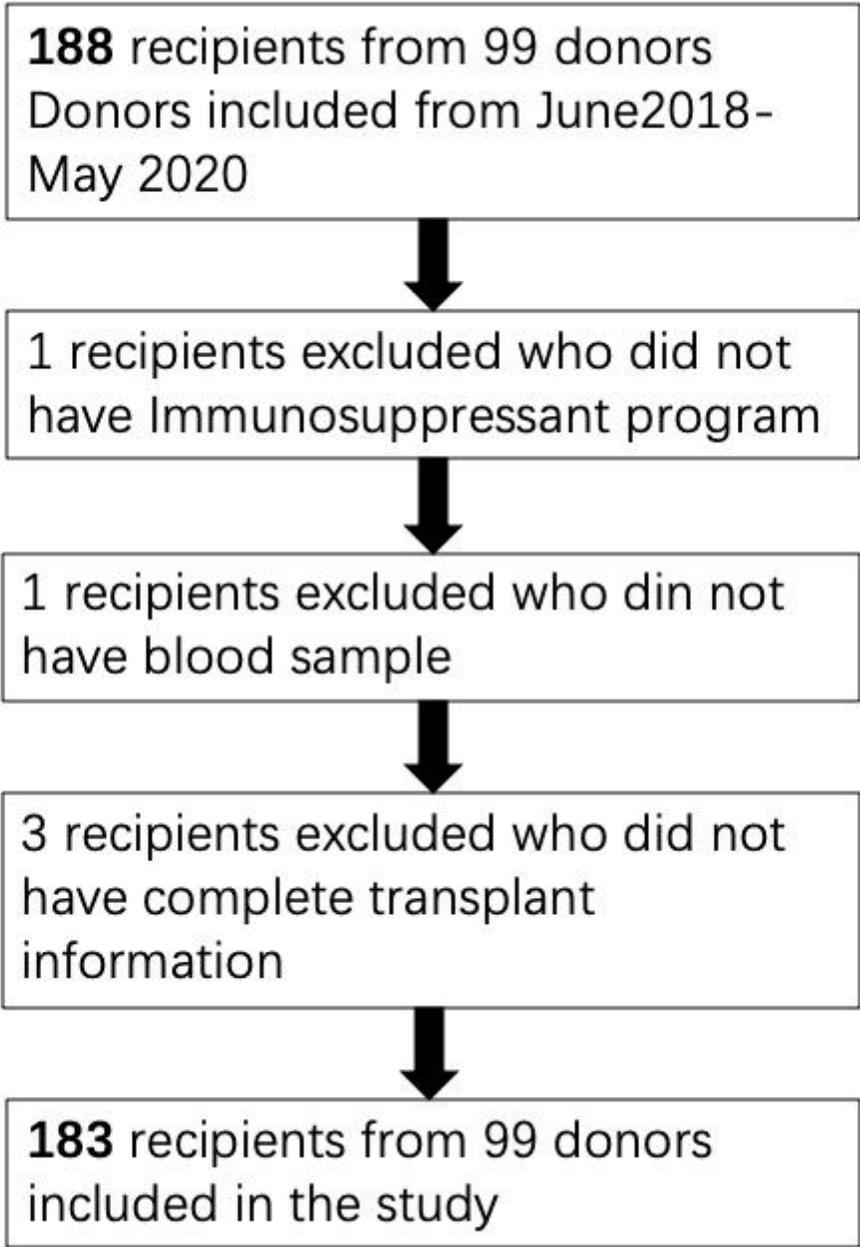


Figure 1

Flowchart of patients

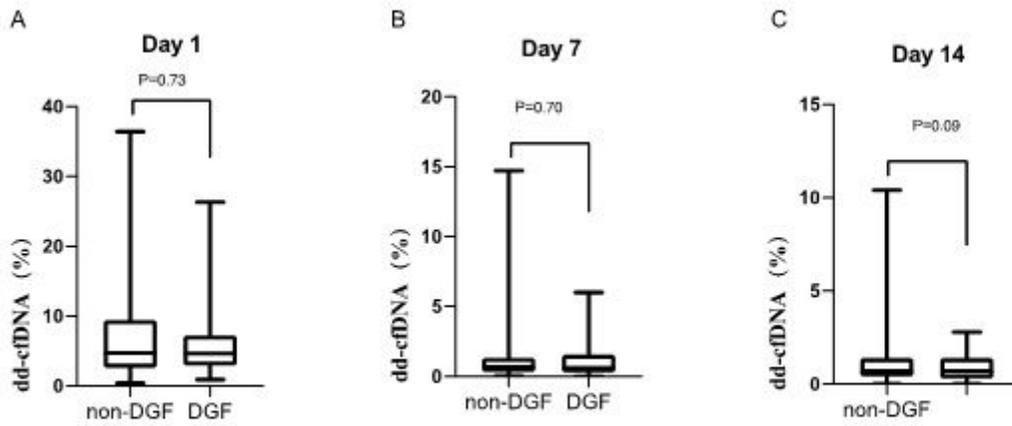


Figure 2

Dd-cfDNA level in the DGF group and the non-DGF group at different time points. (A) The dd-cfDNA on day 1 (B) The dd-cfDNA on day 7 (C) The dd-cfDNA on day 14 . Box and whisker plots; horizontal line represents the median; bottom and top of each box represents Min and Max.

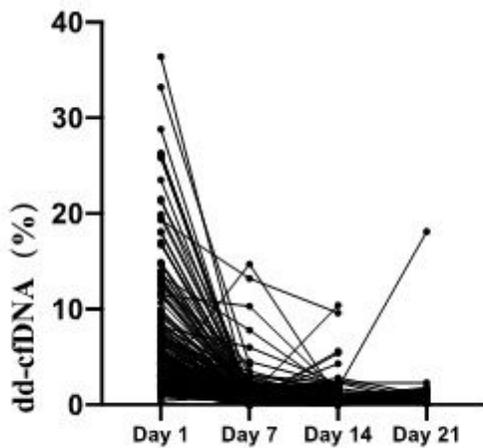


Figure 3

Dynamic changes of dd-cfDNA levels in all patients (n=183)

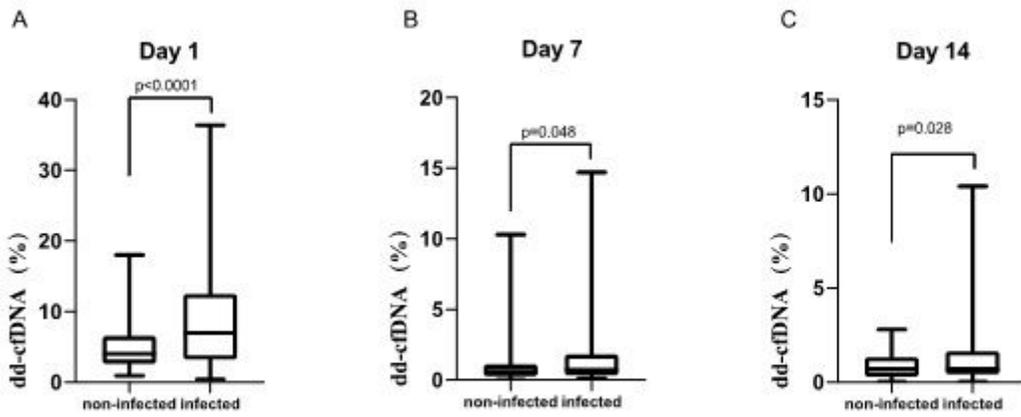


Figure 4

Dd-cfDNA % level in pulmonary infection group and the non- infection group at different time points.(A) The dd-cfDNA % on day 1 . (B) The dd-cfDNA % on day 7.(C) The dd-cfDNA % on day 14.

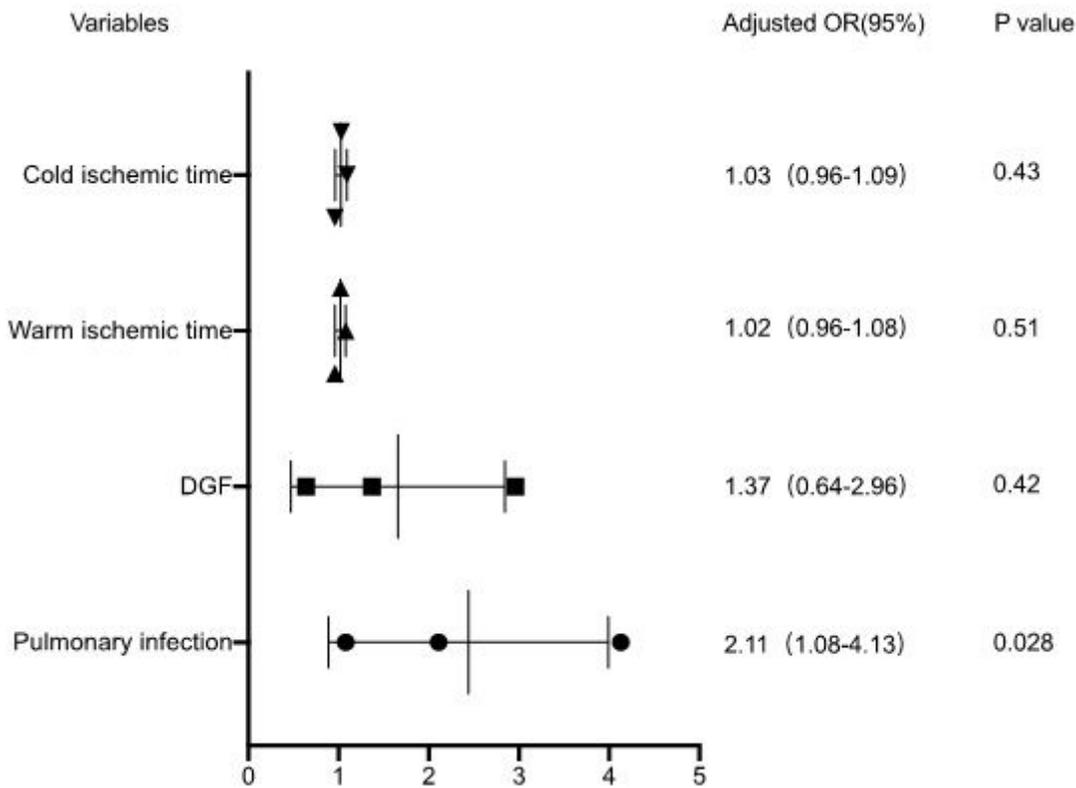


Figure 5

The OR value and P value of cold ischemia time, warm ischemia time, DGF and pulmonary infection were calculated by chi square test.