Predictive value of hypothermic machine perfusion parameters combined perfusate biomarkers in deceased donor kidney transplantation

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Abstract

Background: Delayed graft function (DGF) is the main cause of renal function failure after kidney transplantation. This study aims at investigating the value of hypothermic machine perfusion (HMP) parameters combined with perfusate biomarkers on predicting DGF and the time of renal function recovery after deceased donor (DD) kidney transplantation.

Methods: HMP parameters, perfusate biomarkers and baseline characteristics of 113 DD kidney transplantations from January 1, 2019 to August 31, 2019 in the First Affiliated Hospital of Xi'an Jiaotong University were retrospectively analyzed using univariate and multivariate logistic regression analysis.

Results: In this study, the DGF incidence was 17.7% (20/113); The multivariate logistic regression results showed that terminal resistance (OR: 1.879, 95% CI 1.145–3.56) and glutathione S-transferase (GST)(OR = 1.62, 95% CI 1.23–2.46) were risk factors for DGF; The Cox model analysis indicated that terminal resistance was an independent hazard factor for renal function recovery time (HR = 0.823, 95% CI 0.735–0.981). The model combining terminal resistance and GST (AUC = 0.888, 95% CI: 0.842–0.933) significantly improved the DGF predictability compared with the use of terminal resistance (AUC = 0.756, 95% CI 0.693–0.818) or GST alone (AUC = 0.729, 95% CI 0.591–0.806).

Conclusion: According to the factors analyzed in this study, the combination of HMP parameters and perfusate biomarkers displays a potent DGF predictive value.

Keywords: Hypothermic machine perfusion; Perfusate biomarker; Kidney transplantation; Delayed graft function; Prognosic factors

Introduction

Deceased donor (DD) is the main source of organ donation in China. Among multiple factors that affect the renal transplantation outcome, the quality of the donor kidney is the most important one. Accurate donor kidney quality evaluation is the core of effective organ utilization, which is beneficial to the optimal organ distribution and is of great significance for individual management after kidney transplantation.[1,2] Hypothermic machine perfusion (HMP) is the pressure-control device for renal preservation, which could provide objective data for the donor kidney evaluation.[3] Multiple studies found that terminal perfusion resistance is an independent delayed graft function (DGF) risk factor after renal transplantation.[3-5] However, the predictive value of using terminal resistance alone was poor, and the AUC (area under the receiver operating character-istic curve) of DGF was reported in the range of 0.58 to 0.61.[6] Discussions about traditional biomarkers have different conclusions,[7-9] some studies suggested that GST (glutathione S-transferase), IL-8 (interleukin-18), NGAL (neutrophil gelatinase-associated lipocalin), and NAG (N-acetyl-β-D-glucosaminidase) could be used as predictive DGF indicators.[10-12] According to the clinical experience and database, we hypothesized that machine perfusion parameters and perfusate biomarkers could be used as objective donor kidney quality indicators, and predict DGF to a certain extent. However, considering the production mechanism of perfusate biomarkers, their content is not only related to donor kidney quality, but also to the perfusion range and degree.[9] The purpose of this study was to establish a prediction model combing HMP parameters with perfusate biomarkers, as well as to evaluate its efficacy in predicting DGF and renal function recovery after renal transplantation.

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Methods

Ethical approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (The approval number is XJTU1AFCRC2019SJ-008). The study meets the ethics standards set out in the *Helsinki Declaration* and Istanbul guidelines. All donors meet organ donation standards in China. After donors' relatives signed the voluntary documents for organ donation and other relevant documents, the donor heart stopped for 3 to 5 min and was judged to be cardiac death.

Inclusion and exclusion criteria

Donor inclusion criteria: 1) meeting the criteria of Chinese DD organ donation; 2) no history of drug abuse and high-risk activities such as history of intravenous drug use; 3) no metastatic malignant tumor, incurable malignant tumor and some early-stage malignant tumors could also be considered after successful treatment; 4) with no active and untreated systemic bacteria, viral or fungal infections; 5) the identity of the patients was clear.

Recipient inclusion criteria: 1) single organ (kidney) transplantation; 2) kidney transplantation for the first time; 3) no pregnancy or lactating, pregnancy test negative; no pregnancy during the follow-up period; 4) understanding and signing of the informed consent voluntarily before the trial process begun; 5) body mass index $<26\,kg/m^2$.

Donor exclusion criteria: 1) aged < 18 years or > 70 years; 2) serum hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV) positive.

Recipient exclusion criteria: 1) ABO blood group incompatibility; 2) renal function failure within 24 h after renal transplantation; 3) preemptive kidney transplantation: For patients with matched donor kidneys, renal transplantation was performed directly without dialysis; 4) participation in other studies.

Definitions

DGF: One of the following conditions should be met in this study:[13,14] 1) dialysis requirement within 1 week after kidney transplantation; 2) serum creatinine is higher than 400 μmol/L 10 days after renal transplantation; 3) serum creatinine is higher than 300 μmol/L 2 weeks after renal transplantation. Based on the above-described criteria, the transplanted kidney function could be restored eventually.

Primary non-function:[15] Within 3 months after kidney transplantation, in the absence of rejection evidence or surgical factors that lead to transplant failure, the graft function is insufficient and required dialysis, and did not resume normal function ultimately.

Renal function recovery time: the time by which the transplanted kidney function returned to normal (serum creatinine ≤ 140 μmol/L) for the first time.

Clinical and laboratory data

Data of 113 DD kidney perfused with HMP was collected and baseline characteristics of donors and recipients in the First Affiliated Hospital of Xi'an Jiaotong University were analyzed from January 1, 2019 to August 31, 2019 [Table 1]. The terminal perfusion parameters, the perfusate (perfused for 4 h, stored at $-80°C$), as well as the operation and post-operative complications were also recorded. Collection of the recipients' laboratory biochemical indexes within 3 months after transplantation; 113 DD kidney transplant recipients were divided into DGF and non-DGF group according to the DGF occurrence. The patients were followed up for 3 months.

Hypothermic machine perfusion

Perfusate was added into the HMP machine (Organ Recovery Systems, Zaventem, Belgium) under aseptic condition and started the initial perfusate cycle. According to the known high-risk factors of the donor kidney and the condition of the *in situ* perfusion, the initial perfusion pressure was adjusted smoothly according to the perfusion flow after running steadily at 30 to 35 mmHg, maintaining the perfusion flux at the level of 100 to 140 mL/min. The perfusion parameters were recorded after the readings were stabilized.

Table 1: Baseline characteristics of donors and recipients of kidney transplantation included in this study.

Characteristics	DGF group	No-DGF group	P
Donors			
n	20	40	–
Age (years)	32.7 ± 11.4	53.4 ± 9.7	<0.01
Gender			
Male	13 (65.0)	25 (62.5)	>0.01
Female	7 (35.0)	15 (37.5)	>0.01
Cause of death *n* (%)			
Cerebral trauma	11 (55.0)	23 (57.5)	>0.01
Stroke	6 (30.0)	15 (37.5)	>0.01
Hypoxic-ischemic	3 (15.0)	1 (2.5)	<0.01
Encephalopathy		1 (2.5)	
Others			
BMI (kg/m²)	22.4 ± 3.5	23.1 ± 2.9	>0.01
Hypertension	17 (85.0)	29 (72.5)	>0.01
Donor kidney			
Warm ischemia time (min)	9.4 ± 2.6	9.1 ± 1.8	>0.01
Cold ischemia time (min)	384 ± 181	354 ± 186	>0.01
Recipients			
n	20	93	–
Age (years)	35.4 ± 7.3	32.6 ± 5.2	>0.01
HLA mismatch (number)	1.94 ± 1.08	2.01 ± 0.95	>0.01
Dialysis (years)	1.7 ± 1.1	1.9 ± 1.5	>0.01
Basic diseases			
Hypertension	20 (100.0)	90 (96.8)	>0.01
Cardiovascular/ Cerebrovascular disease	8 (40.0)	32 (34.4)	>0.01
Diabetes	2 (10.0)	13 (14.0)	>0.01

Data was presented by *n* (%) or Mean ± SD; DGF: Delayed graft function; HLA: human leukocyte antigen.

Perfusate biomarkers detection

Pretreatment of the specimens: We took out the samples of the perfusate stored at −80°C, thawed at room temperature, centrifuged using a desktop high-speed low-temperature centrifuge (10,000 × g, 10 min, 4°C), distributed the supernatant into 1.5 mL eppendorf (EP) tubes and stored at −80°C for further use. We used enzyme-linked immunosorbent assay to detect interleukin (IL-18) and colorimetry to quantify NAG and GST.

Recipient immune preparation plan

(1) Immune induction: Rabbit anti-human thymocyte immunoglobulin 1.5 to 2.0 mg·kg^{-1}·d^{-1} and methylpred-nisolone 250 mg/d, intravenous drip for 3 to 4 days; (2) Immunosuppressant maintenance: take mycophenolate mofetil from the post-operative day: Cellcept 2.0 g/d, or Myfortic 1.44 g/d, oral administration at 12-hour-interval; Tacrolimus 0.06 to 0.08 mg·kg^{-1}·d^{-1} was taken orally at interval of 12-hour-interval from two days after operation; oral prednisone 10 mg/d was given and maintained from the final day of the immune induction therapy. After that, the drug dose was adjusted according to plasma concentration, serum creatinine level, and body weight.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation, and the differences between the two groups were compared using Student t test or Mann-Whitney U test according to the characteristics of the data. Univariate and multivariate logistic regression were used to screen the independent risk factors for DGF. The Cox proportional hazards model was used to analyze the relationship between the HMP parameters, perfusate biomarkers and the recovery time of the renal allograft function. The evaluation of these DGF risk factors predictive values was performed by drawing the ROC curve and calculating the AUC. We used the SPSS22.0 software (SPSS Inc, Chicago, IL, USA) for statistical analysis. Bilateral $\alpha = 0.05$ was regarded as the test level ($P < 0.05$, the difference was considered as statistically significant).

Results

DGF and renal function recovery after transplantation

We included 60 donors and 113 DD renal transplant recipients in the study (two cases of donor kidney were abandoned as their pathological biopsy score indicated non-suitability for transplantation; four renal transplantation recipients were less than 18 years old, and one was a secondary renal transplantation) [Table 1]. The DGF incidence was 17.7% (20/113), and 93 recipients were included in the non-DGF group. The mean recovery time of the renal function was 8.1 (5.4, 10.1) days. The recovery time in the DGF group was 23.2 (11.4, 37.6) days, which was significantly longer than the 5.3 (3.7, 6.2) days value in the non-DGF group ($P < 0.001$).

Relationship between the HMP parameters and DGF

We compared the differences of HMP parameters between the DGF and non-DGF groups [Table 2]. The analysis showed that there were significant differences in initial flux, terminal flux and terminal resistance between the two groups.

Our univariate logistic regression analysis showed that higher flux, lower perfusion pressure, and lower terminal resistance were protective factors for DGF, while lower flux, higher perfusion pressure and terminal resistance donor kidney represented a higher risk for DGF occurrence [Table 3].

We used the forward stepwise (likelihood ratio) multivari-ate logistic regression approch to analyze initial flux, terminal flux and terminal resistance. The results showed that terminal resistance was an independent risk factor for DGF ($P = 0.021$, OR = 1.879, 95% CI 1.145–3.56). The AUC of predicting DGF using terminal resistance alone was 0.756 (95% CI 0.693–0.818). When the Youden index reaches its maximum, the best cut-off value of terminal resistance for predicting DGF was 0.30.

By drawing the Kaplan-Meier survival curves of the DGF and non-DGF groups [Figure 1], we revealed that the renal function recovery rate in the DGF group was lower than that in the non-DGF group at the same time. The difference between the two groups was confirmed by Log-Rank test ($P < 0.001$). Based on this, we defined 'renal function recovery' as the endpoint of the observation and analyzed the correlation between the HMP parameters and the renal function recovery time using the backward stepwise (likelihood ratio) Cox proportional hazard regression model. Finally, we revealed that terminal resistance was an independent risk factor affecting the recovery of renal allograft function ($P = 0.036$, HR = 0.823, 95% CI 0.735–0.981).

Table 2: HMP parameters between DGF and non-DGF groups.			
Parameters	**DGF group**	**Non-DGF group**	**P value**[*]
Initial flux (mL/min)	60.33 ± 20.14	77.12 ± 20.3	0.036
Initial pressure (mmHg)	36.25 ± 3.87	34.45 ± 3.61	0.045
Initial resistance (mmHg·mL^{-2}·min^{-1})	0.59 ± 0.21	0.47 ± 0.19	0.055
Terminal flux (mL/min)	97.31 ± 20.78	108.16 ± 12.74	0.021
Terminal pressure (mmHg)	30.69 ± 2.76	29.08 ± 3.84	0.083
Terminal resistance (mmHg·mL^{-2}·min^{-1})	0.41 ± 0.12	0.23 ± 0.15	0.018
Perfusion time (h)	4.97 ± 2.03	4.86 ± 1.93	0.176

Data was represented by Mean ± SD. [*]$P < 0.05$ is considered to be statistically significant; SD: Standard deviation DGF: Delayed graft function.

Table 3: Univariate logistic regression analysis of perfusion parameters.

Perfusion parameters	P	OR (95%CI)
Initial flux (mL/min)	0.036	0.663 (0.452–0.975)
Initial pressure (mmHg)	0.045	9.620 (1.051–18.083)
Initial resistance (mmHg·mL^{-2}·min^{-1})	0.055	1.874 (0.932–3.021)
Terminal flux (mL/min)	0.021	0.625 (0.419–0.932)
Terminal pressure (mmHg)	0.083	6.221 (0.476–8.923)
Terminal resistance (mmHg· mL^{-2}·min^{-1})	0.018	1.979 (1.125–3.45)
Perfusion time (h)	0.176	2.542 (0.642–5.863)

Figure 1: Kaplan–Meier survival curves of the DGF and non-DGF groups. Log-Rank test was used to test the statistical significance between the two groups. DGF: Delayed graft function.

Correlation between the perfusate biomarkers and DGF

The mean concentration of NAG ($P = 0.026$) and GST ($P = 0.016$) in the DGF group was significantly higher than that in the non-DGF group. However, the IL-18 concentration in the DGF group was slightly higher than in the non-DGF group, and there was no significant difference between the two groups ($P = 0.212$)

The univariate logistic regression analysis of NAG ($P = 0.043$) and GST ($P = 0.012$) indicated that both of them were risk factors for DGF. Furthermore, the multivariate logistic regression analysis revealed that GST was an independent risk factor for DGF ($P = 0.029$, OR = 1.62, 95% CI 1.23–2.46), AUC = 0.729, 95% CI 0.591–0.806. According to the maximum Youden index, 9.04 µg/ml was considered to be the best cut-off value in predicting DGF. We used the Cox regression hazard model to analyze the correlation between the perfusate biomarkers and the renal function recovery time. The results showed that neither GST ($P = 0.088$) nor NAG ($P = 0.103$) were significant risk factors affecting the renal function recovery time.

The prognosis of the HMP parameters combined perfusate biomarkers in predicting DGF

The AUC of predicting DGF using terminal resistance alone was 0.756 (95% CI 0.693–0.818). GST was also used to predict DGF occurrence, and the AUC was 0.729 (95% CI 0.591–0.806) [Figure 2A and 2B].

Considering that the perfusate biomarkers concentration were not only related to the degree of renal damage, but also to the range of perfusion, we combined terminal resistance and GST, which were proved to be independent risk factors in previous studies and established a combined interaction model. This model showed efficacy for DGF predictability, and its AUC was 0.888 (95% CI 0.842–0.933). The optimal cut-off value of this model was 0.344, according to the Youden index. The diagnostic sensitivity and specificity were 83.3% and 79.5% separately. Tmodel that combined terminal resistance and GST significantly
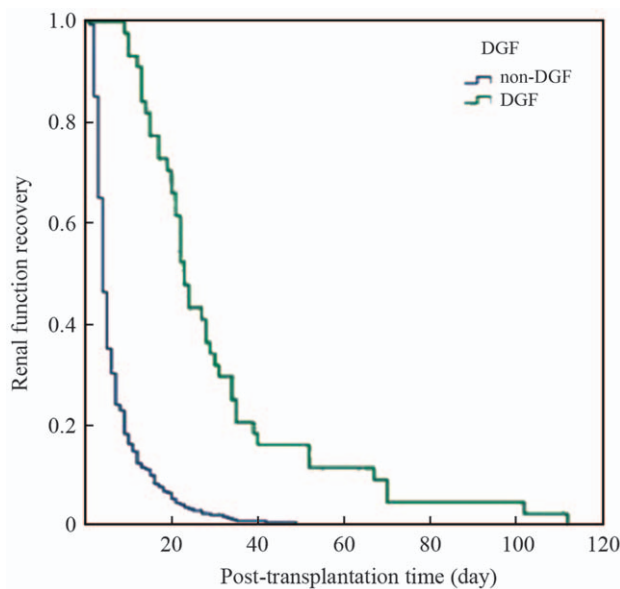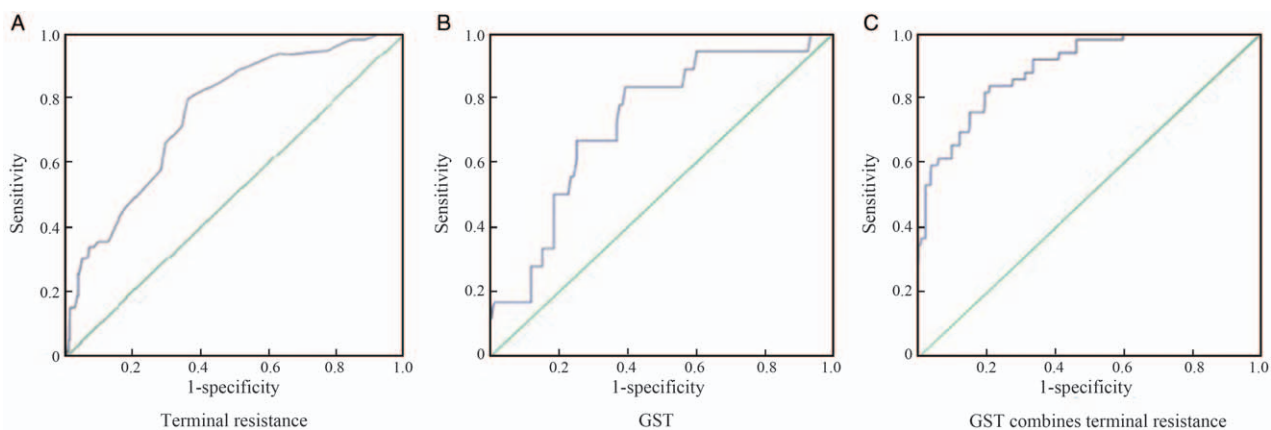
Figure 2: ROC of terminal resistance (A), GST (B) and the combined model (C) predicts DGF occurrence were shown respectively. The AUC of these three models implied that the combined model significantly improved prognostic efficacy. GST: Glutathione thiotransferase; DGF: Delayed graft function; AUC: Area under curve.

Table 4: The prognostic model including terminal resistance and GST interactive item.

Risk factors	Multivariate logistic regression	
	β	*P*
Terminal resistance	1.776	0.027
GST	3.823	0.039
Terminal resistance GST	0.079	0.048
Constant term	−7.603	0.160

GST: Glutathione thiotransferase.

improved the DGF predictability compared with using either of the approaches alone [Table 4].

Discussion

HMP not only protects isolated allografts but provides a window for observation and intervention. The real-time parameters and extractable/added perfusate have become a new focus of transplant physicians.[16-18] Several studies revealed that low perfusion flux and high perfusion resistance often indicated poor donor kidney quality, which was also closely related to bad prognosis after transplantation. However, there is no unified conclusion about this question and the critical value of specific perfusion parameters. There are more than ten kinds of biomarkers reported internationally in the study of perfusate biomarkers.[8,19] GST is considered to be one of the most promising biomarkers.[19,20] Some studies reported that after 4 h of HMP, the concentration of GST in perfusate no longer increased, indicating that GST mainly comes from cell damage caused by previous ischemia injury[11] In contrast, NGAL was found gradually up-regulated after ischemia.[10] IL-18 is a kind of cytokines produced by activation of the inflammatory and immune process during ischemia-reperfusion injury, which can cause cellular damage through various mechanisms[11] Several studies have shown that IL-18 is associated with DGF.[9] Therefore, based on the existing results of these researches, we selected the above several perfusion parameters and biomarkers for detection, and to analyze their connection with the outcome of kidney transplantation.

In this study, by the method of logistic regression, terminal resistance was considered as a significant risk factor for DGF (OR = 1.879), and then we also screened GST as the risk factor for DGF (OR = 1.62). In the study with renal function recovery time as the end point, although the result of terminal resistance showed statistical significance ($P <$ 0.05), HR < 1 means that it is not a risk factor for recovery time, which was not consistent with previous results. And GST also didn't show effective predictability in COX analysis ($P >$ 0.05), the reason for this may be that HMP parameters can only show renal perfusion and initial injury, both NAG and GST are early injury biomarkers, and the recovery of renal function is also related to later immune and inflammatory activation, so these factors do not show significant predictability at the end of this study. Finally, we constructed a predictive model containing the interaction terms included GST and terminal resistance (AUC = 0.888, 95% CI 0.842–0.933), and uncovered its predictive efficacy of DGF improved significantly when

compared with using terminal resistance (AUC = 0.756, 95% CI 0.693–0.818) or GST alone (AUC = 0.729, 95% CI 0.591–0.804).

The innovation of this article is that we not only screened risk factors for DGF occurrence, but established the prediction model of combining parameters and biomarkers. Compared with other similar studies, we firstly described the predictive value of the combination of these two risk factors in predicting kidney transplantation outcome. Most foreign studies have not reported about combining the two factors to analyze the prognosis of renal transplantation. Although there was a discussion in Hall's research, this study didn't analyze the interaction between the two indicators.[11] In Hoogland *et al* study,[9] the mean duration of cold ischemia (27 ± 6 h) and perfusate time (21 ± 6 h), mean warm ischemia time (26 ± 11 min) were much higher than those in China, and the proportion of controllable DD was lower, which conditions were not suitable for the situation of China. There is a lack of effective combination predictive model based on HMP in China, and the result of this study make up for the gap in this area. However, this study also has some limitations: on account of the limitation of the sample number, even though the correlation between HMP parameters and biomarkers is obvious, these data are not enough to show the data characteristics of perfusate biomarkers among the groups with large differences in perfusate parameters. In the case of larger sample sizes, a stratified analysis could be used to compare differences of perfusate biomarkers between layers, which can improve the efficacy of the predictive model.

In summary, we analyzed the characteristics of several representative HMP parameters and perfusate biomarkers in DD donor kidney in detail and their association with the prognosis of renal transplantation, and proposed the model combined terminal resistance and GST can improve the predictive efficacy of DGF. This study not only provides a scientific support for more efficient application of HMP and reasonable usage of the prognostic model, but also offers a more adequate theoretical basis for renal quality assessment and guidance for clinical interventions.

Conflicts of interest

None.

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