



ORIGINAL ARTICLE

Hypothermic pulsatile preservation of kidneys from uncontrolled deceased donors after cardiac arrest – a retrospective study

Xavier Matillon¹ , Fabrice Danjou², Palmina Petruzzo^{1,3}, Olivier Thauinat^{4,5,6} , Thomas Rimmele⁷, Claire Delsuc⁷, Alexandre Faure⁷, Maud Rabeyrin⁸, Vannary Meas Yedid⁹, William Hanf¹⁰, Emmanuel Morelon^{4,5,6}, Lionel Badet¹ & Ricardo Codas¹

1 Department of Urology and Transplantation, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France

2 Département d'Information Médicale, Hôpital Saint Camille, Bry-sur-Marne, France

3 Department of Surgery, University of Cagliari, Cagliari, Italy

4 Service de Néphrologie, Transplantation et Immunologie Clinique, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France

5 INSERM U1111, Centre International de Recherche en Infectiologie (CIRI), Ecole Normale Supérieure de Lyon, CNRS, UMR 5308, Lyon, France

6 Université Claude Bernard – Lyon I, Lyon, France

7 Department of Anaesthesiology and Intensive Care, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France

8 Department of Pathology, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France

9 Unité d'Analyse d'Images Biologiques, Institut Pasteur, CNRS URA 2582, Paris, France

10 Department of Nephrology, Alpes-Leman Hospital, Contamine-sur-Arve, France

Correspondence

Palmina Petruzzo, Department of Transplantation, Edouard Herriot Hospital, 5 Place d'Arsonval, 69437 Lyon, France.

Tel.: +33 4 72 11 11 42;

fax: +33 4 72 11 05 82;

e-mails: palmina.petruzzo@chu-lyon.fr and petruzzo@medicina.unica.it

SUMMARY

Kidneys from uncontrolled donors after cardiac arrest (uDCD) suffer from a period of warm ischemia between cardiac arrest and cold flushing. Aim of the study was to evaluate renal outcomes of uDCD kidneys selected on the basis of renal Resistance Index (RI) and its influence on graft function and survival. The study included 44 kidneys procured from 26 uDCD starting 1.1.2006 until 12.31.2013. The donors (Maastricht category II) underwent cardiopulmonary resuscitation by assisted ventilation and chest compression; the organs were preserved with *in situ* cold perfusion or a normothermic regional perfusion. All kidneys were perfused on hypothermic (1–4 °C) pulsatile perfusion machine (RM3; Waters Medical System) and discarded when RI ≥ 0.5 mmHg/ml/min after 6 h of perfusion. There was one (2.2%) primary non function, while 37 recipients (84.1%) experienced delayed graft function. Graft survival was 97.6% at 1 and 3 post-transplantation years. Linear regression models showed that lower values of RI at the end of perfusion were associated with higher values of Modification of Diet in Renal Disease at 3 ($P = 0.049$) and 6 months after transplantation ($P = 0.010$) and with higher values of inulin clearance at 1 year ($P = 0.030$). RI showed to be a useful tool to select uDCD kidneys allowing to achieve good clinical results.

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Key words

donor management, outcomes, perfusion machine, Resistance Index, uncontrolled deceased donors

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Introduction

Kidneys from donors after cardiac death (DCD) offer the possibility to expand the donor pool, answering to the increasing demand for organs. Kidneys from DCD suffer from a period of warm ischemia (WI) between cardiac arrest and cold flushing, particularly the kidneys from uncontrolled donors after cardiac arrest. Indeed, in these cases the period of circulation arrest is relatively unknown and the efficacy of cardiopulmonary resuscitation is difficult to assess [1]. These conditions determine an inevitable unknown ischemic injury which influences graft outcome. The utilization of the hypothermic machine perfusion [2,3] seems not only to improve tissue preservation but also to predict graft function, providing perfusion parameters, such as the resistive index (RI). The assessment of organ viability [4] is particularly important in kidneys from uncontrolled DCD (uDCD) that are exposed to high risk of primary nonfunction (PNF) and delayed graft function (DGF).

This study was performed to evaluate renal function and graft outcomes in perfused uDCD kidneys and also to investigate the influence of RI on graft function and survival.

Patients and methods

Study design and definitions

Consecutive kidneys from uDCD, procured from 1.1.2006 to 12.31.2013, were included in this retrospective study. Recipients were transplanted in a single center (Dept. of Transplantation, Hopital Edouard Herriot, Lyon, France).

According to the French law on uDCD program, this study includes only Maastricht category II donors with cardiac arrest without any cardiopulmonary resuscitation (asystolic warm ischemia) less than 30 min; age ranging between 18 and 55, without traumatic cardiac arrest as cause of death or history of chronic renal disease, arterial hypertension, diabetes, cancer, sepsis, and drug addiction.

In addition, the WI time had to be less than 120 min (it can be 150 min when the chest compression was performed using a device).

Warm ischemia time was considered the time from the cardiac arrest and the start of organ perfusion, including the no-touch period of 5 min and the period of manual or mechanical cardiopulmonary resuscitation (Fig. 1).

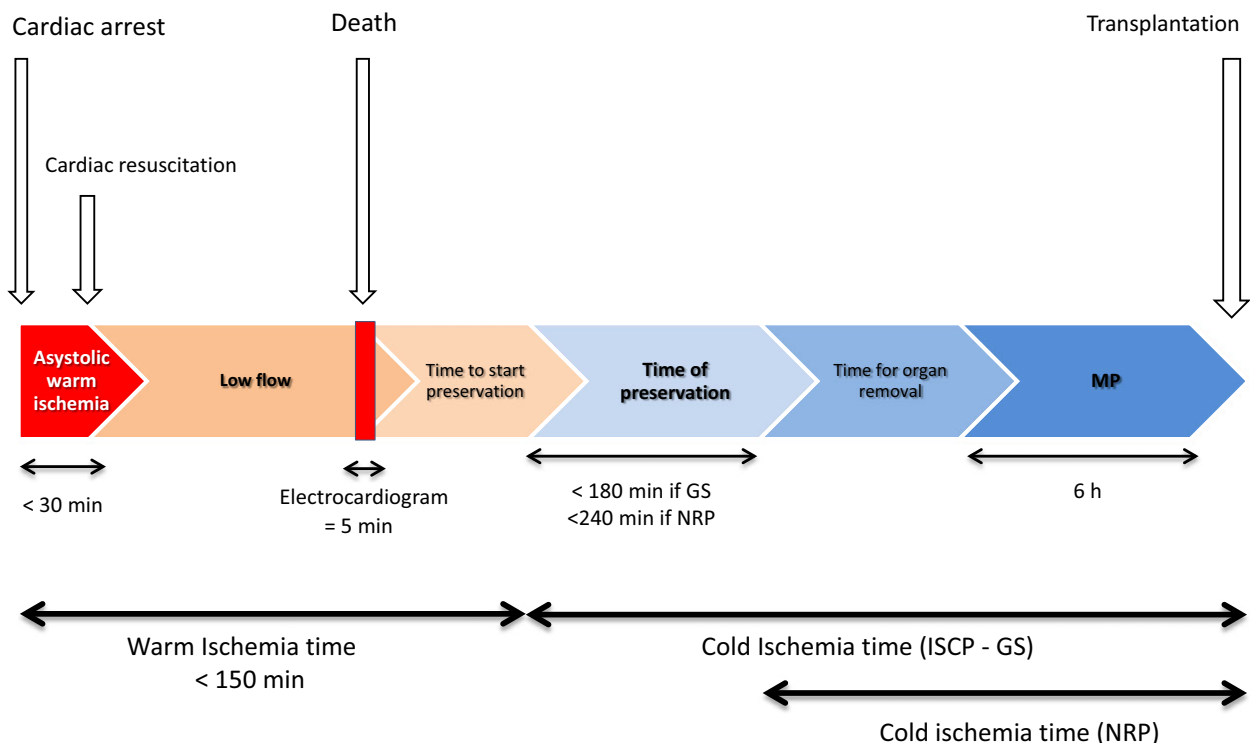


Figure 1 Timelines and clinical pathway in the process of uncontrolled kidney donation. GS, *in situ* cold preservation using “Gillot sonde”; NRP, normothermic regional perfusion using subdiaphragmatic extracorporeal membrane oxygenation; MP, machine of perfusion.

Cold ischemia time was considered the time from preservation to the vascular anastomosis time, including the perfusion machine time.

Vascular anastomosis time was the second period of warm ischemia required to perform them.

Graft function in the early postoperative period was defined as immediate function without the need for dialysis treatment. DGF was defined as temporary dialysis treatment initiated in the first week of transplantation while PNF as inadequate renal function necessitating continuation of dialysis treatment or re-transplantation.

Graft failure, censored for death, was defined as permanent return to dialysis.

Renal function was assessed using serum creatinine, glomerular filtrate rate calculated using Modification of Diet in Renal Disease (MDRD) formula and inulin clearance at 12 months after transplantation.

Recipients' criteria of inclusion were age <65 years, first renal transplantation, ABO compatibility and no previous HLA sensitization.

Management of uncontrolled donors after cardiac death

The potential donors (Maastricht category II) underwent cardiopulmonary resuscitation by assisted ventilation and chest compression. The period of cardiac arrest was known for all donors. After the arrival at the hospital and the no-touch period of 5 min, the organs were preserved with in situ cold perfusion or using normothermic regional perfusion (NRP), a normothermic and regional extracorporeal membrane oxygenation.

The intra-aortic double-balloon catheter (Gillot catheter with three lumens) and a venous vent were surgically inserted into femoral arterial and vein respectively after an injection of 25 000 UI of heparin. The intra-aortic catheter was perfused with a heparinized (5000 UI/l) preservation solution (IGL-1) at a rate of 250–500 ml/min until blood washout, and maintained at 100 ml/min. Finally, peritoneal refrigeration with 4 l isotonic saline at 4 °C was performed. The duration of this procedure has to be less than 180 min according to the French uDCD protocol.

The in situ cold perfusion was the only available technique from September 2006 to January 2010, and then, it was replaced by NRP.

Pulsatile perfusion machine

All the recovered kidneys were machine perfused using hypothermic (1–4 °C) pulsatile perfusion (RM3; Waters Medical System, Rochester, MN, USA). The preservation

solution used was the Machine Perfusion Solution-Belzer MPS. The system is pressure controlled, and the pressure was set at 40 mmHg. RI was calculated by the quotient of mean perfusion pressure (mmHg) divided by perfusate flow rate (ml/min). Kidneys with RI >0.5 mmHg/ml/min after 6 h of perfusion were discarded.

Mean RI value at the end of the perfusion was 0.264 ± 0.073 (Table 2).

The perfusion was not preceded by static cold preservation, according to the French protocol for uDCD, while there was a short period of static cold preservation between the end of the perfusion and the vascular anastomoses.

Immunosuppressive treatment

The immunosuppressive treatment included an induction therapy based on antithymocyte globulins (ATG; Genzyme, Cambridge, MA, USA) and a maintenance therapy including low dose of steroids, mycophenolate mofetil, and anticalcineurin inhibitors (27 patients were treated with tacrolimus, 17 with cyclosporine A, and only one patient was switched to tacrolimus and another one to azathioprine).

Histological assessment

All the acute rejection episodes were proven by biopsies. Moreover, patients underwent systematic 18-G needle-core biopsies at M3 and M12 after transplantation. Paraffin sections were stained with Masson's trichrome and periodic acid–Schiff. Acute and chronic lesions were evaluated by a single pathologist according to Banff 2007 classification. The borderline changes were also analyzed. Masson-stained interstitial fibrosis was quantified by computerized color image analysis as described [5].

Statistical analysis

Donor and recipient characteristics are reported in Tables 1 and 2, respectively, as proportion or mean \pm standard deviation upon data type. For descriptive Tables 1 and 2, we report *P*-values of differences between NRP and in situ cold perfusion for each variable, using Mann–Whitney *U*-test or Fisher's exact test as required.

Using linear regression models with stepwise procedure, we tested which parameters were associated with RI at the beginning and at the end of perfusion, and with MDRD and inulin clearance values at different time points of the follow-up. For RI prediction, we used inverse-normal transformation of RI and considered only donor

Table 1. Donors' characteristics and ischemia times.

	All donors (n = 26)	NRP (n = 9)	In situ cold perfusion (n = 17)	P value
Gender (M/F)	24/2	7/2	17/0	0.111
Age (years)	43.0 ± 10.5	42.2 ± 12.2	43.4 ± 9.8	0.958
BMI (kg/m ²)	26.2 ± 4.2	27.9 ± 2.6	25.3 ± 4.6	0.061
Serum creatinine (μmol/l)	135.6 ± 38.8	116.5 ± 27.5	144.6 ± 40.7	0.043
MDRD (ml/min/1.73 m ²)	52.3 ± 11.7	56.4 ± 15.4	51.1 ± 10.6	0.595
Asystolic time (no flow, min)	16 ± 6.5	17.4 ± 8.4	15.3 ± 5.3	0.874
Time (min) of in situ cold perfusion (Gillot sonde)			164.7 ± 39.3	
Time (min) of NRP		190.3 ± 38.8		
Flow (l/min)		1.8 ± 0.5		

NRP, normothermic regional perfusion.

P-values of differences between NRP and *in situ* cold perfusion donors for each variable using Mann–Whitney U-test or Fisher's exact test.

Table 2. Recipient characteristics and results.

	All recipients n = 44	ECMO recipients n = 16	<i>In situ</i> cold perfusion recipients n = 28	P value
Gender (M/F)	33/11	12/4	21/7	1.000
Age (years)	45.4 ± 10.5	44.1 ± 9.4	46.2 ± 11.2	0.339
BMI (kg/m ²)	24.4 ± 3.3	24.8 ± 3.1	24.1 ± 3.4	0.292
Patient undergoing dialysis before transplantation	39	16	23	0.141
Awaiting list time (days)	507 ± 472	666 ± 535	419 ± 418	0.190
Total warm ischemia time (min)	130.8 ± 16.2	135.9 ± 17.5	128.1 ± 15.3	0.148
Total cold ischemia time (min)	903.8 ± 227.6	758.3 ± 136.1	987 ± 229	0.001
RI at the beginning of perfusion	0.599 ± 0.470	0.326 ± 0.120	0.774 ± 0.527	0.001
RI at 6 h of perfusion	0.264 ± 0.073	0.230 ± 0.075	0.285 ± 0.064	0.026
Vascular anastomosis time (min)	31 ± 11	30 ± 9	31 ± 11	0.833
PNF	1	1	0	0.364
DGF	37	13	24	0.692
Number of post transplant dialysis	7 ± 12	9 ± 19	5 ± 5	0.766
3 months MDRD	40 ± 16	41 ± 21	39 ± 13	0.700
1 year MDRD	44 ± 15	43 ± 18	45 ± 14	0.785
1 year Inuline clearance	48 ± 14.5	54.6 ± 9.1	45.5 ± 15.5	0.109
Length of hospital stay (days)	22 ± 10	18 ± 6	25 ± 10	0.015
Kidney graft loss	4	2	2	0.615

P-values of differences between NRP and *in situ* cold perfusion recipients for each variable using Mann–Whitney U-test or Fisher's exact test.

parameters as independent variables in the model (age, gender, BMI, serum creatinine, number of external electric shocks, adrenaline dose, type of cold perfusion procedure, cold ischemia time, warm ischemia time). For MDRD and inulin clearance models, we considered the same donor parameters plus recipient ones (age, gender, BMI, awaiting list time, dialysis before transplantation, number of HLA incompatibility, episodes of acute rejection) as well as RI at the beginning and at the end of the perfusion.

Finally, to test the association between DGF and RI, we used a binary logistic regression model, considering the same above reported confounders.

All statistical procedures were performed using SPSS version 20.0 software package (IBM Corp., Armonk, NY, USA) and considering a critical alpha of 0.05.

Results

Donors' and recipients' characteristics

From 1.1.2006 to 12.31.2013, kidneys from 38 uncontrolled donors after circulatory death (uDCD) were machine perfused (RM3 Machine; Waters Medical System).

Eighteen kidneys were discarded on the basis of the RI; moreover, secondary parameters were organ discoloration, complexity of vascular anatomy, and, in some cases, histological assessment.

In addition, two kidneys were transplanted in another institution, according to the French allocation management rules.

Kidneys from six donors were transplanted en bloc (dual kidney transplantation); however, they were not included in the present study.

The other 44 kidneys were transplanted and included in this study.

Donors' characteristics and ischemia times are reported in Table 1.

The recipients' characteristics and their functional outcomes are reported in Table 2.

Clinical outcomes

There was 1 PNF (2.2%) in this study, while 37 recipients (84.1%) experienced DGF. Serum creatinine levels and MDRD values during the first 2 years of follow-up are shown in Fig. 2. Inulin clearance value was 44.9 ± 12.4 ml/min at 1 year after the transplantation.

Graft survival was 97.6% at 1 and 3 post-transplantation years. Four graft losses were reported: one kidney was removed for venous thrombosis 6 days after the transplantation; other two grafts were lost 48 and 51 months post-transplantation for chronic rejection and another one at 72 months for Berger's disease relapse.

No deaths occurred during the follow-up.

In a linear regression model with stepwise procedure, RI at the end of perfusion was shown to be associated with MDRD variations at 3 and 6 months after transplantation ($P = 0.049$ and $P = 0.002$, respectively) and with inulin clearance at 12 months after transplantation ($P = 0.030$) as shown in Fig. 3.

A logistic regression analysis failed to show an association between RI at the end of perfusion and DGF occurrence.

Regarding RI prediction, a linear regression analysis showed that RI at the beginning of machine perfusion was influenced by the perfusion procedure. The use of *in situ* cold preservation determined higher RI values compared to those of kidneys recovered from donors where NRP had been performed ($P = 0.003$); RI at the beginning of machine perfusion was also influenced by serum creatinine ($P = 0.006$) and asystolic time ($P = 0.054$) (model $R^2 = 0.277$).

At the end of the machine perfusion, RI was only influenced by donor serum creatinine values

($P = 0.008$) and asystolic time ($P = 0.031$) (model $R^2 = 0.123$).

Rejection and histological assessment

The incidence of the AR episodes, including either those histologically confirmed or those highlighted by protocol biopsies, was 18.1% (6 with score 1A, 1 with score 1B and 1 with score 2B). In 11 cases, borderline changes were detected, five of them during a systematic biopsy (three at the three-month biopsies and two at 12-month biopsies). Three patients showed signs of active chronic rejection.

The study included systematic renal biopsy at 3 and 12 months after transplantation but the recipients who underwent them were 59% and 56.8% respectively (the patients did not undergo the biopsy for different

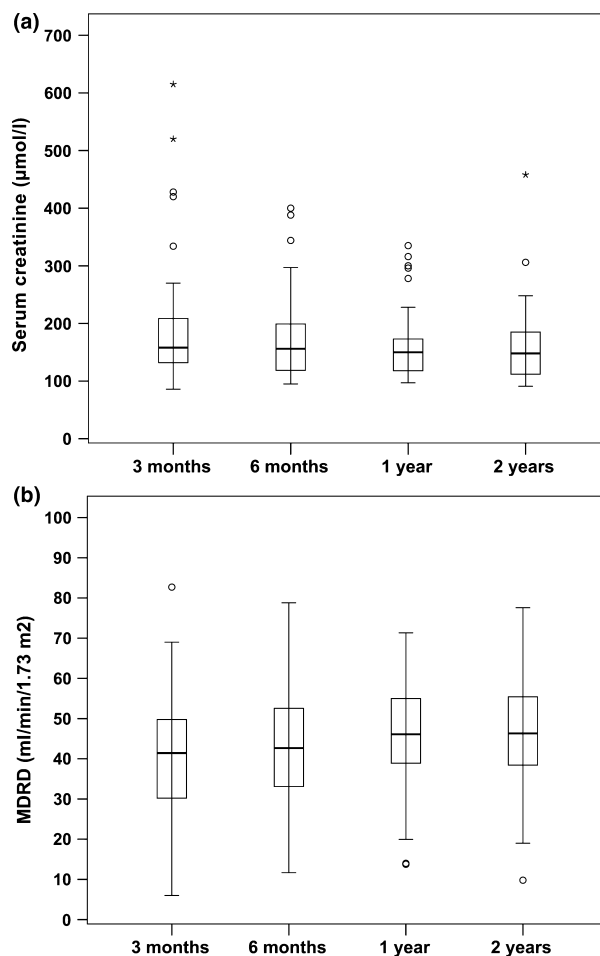


Figure 2 Renal function outcomes in the first 2 years of follow-up. (a) Serum creatinine levels at 3, 6, 12, 24 months after transplantation. (b) Modification of Diet in Renal Disease values at the same time points of the follow-up. *Extreme values

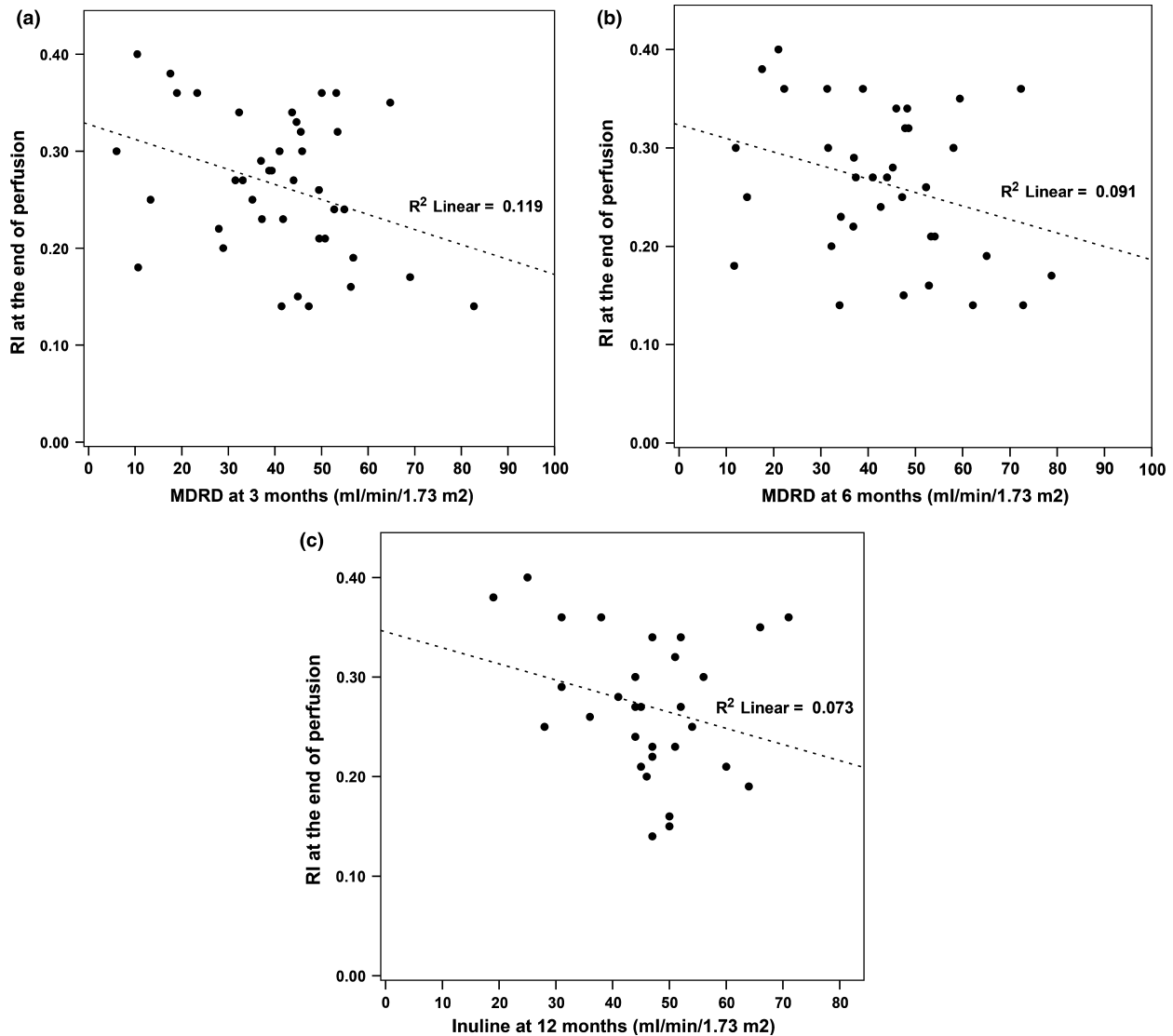


Figure 3 Correlation between resistive index (RI) at the end of the perfusion and the functional outcomes. (a) Correlation between RI and Modification of Diet in Renal Disease (MDRD) at 3 post-transplant months. (b) Correlation between RI and MDRD at 6 post-transplant months. (c) Correlation between RI and inulin clearance at 12 post-transplant months.

reasons, such as refusal or anticoagulant treatment; while in five patients, the material was not sufficient for the histological assessment). Despite this small sample, it was possible to show an association between 3-month interstitial fibrosis and RI at the end of perfusion ($P = 0.022$ and $b = 0.52$; $R^2 = 0.229$ by stepwise forward linear regression considering donor characteristics as previously reported). No associations were shown between RI and 12-month interstitial fibrosis or graft loss.

Vascular lesions (cv) according to the Banff 2007 classification and interstitial fibrosis measured by color image analysis showed cv lesions (score 2) in 50% of the patients and interstitial fibrosis score in 33–34%.

Discussion

Kidneys from uDCD can be a useful source of grafts when selecting criteria, advanced resuscitation and preservation procedures are used [6–8]. In the present study, we used the selecting criteria and resuscitation procedures recommended by the French Agency of Biomedicine, which is the French national regulatory authority for organ and tissue procurement and transplantation. Before using pulsatile machine perfusion, it was almost impossible to select and preserve the kidneys from uDCD before transplantation, while in the last few years, several studies have shown that the outcomes of kidney grafts from uDCD are comparable with those of

kidney grafts from controlled donors [3,9,10]. In the present study, survival rate was 97.6% at 2 years; there was a unique case of PNF, and DGF rate was 84.1% as reported in other series [11–14]. However, DGF did not seem to negatively influence long-term outcomes in kidney grafts from uDCD as reported in series of kidney grafts from brain dead donors [15].

Renal function estimated by MDRD and inulin clearance at 1 year showed encouraging results, comparable to those of kidneys from controlled donors. Although pulsatile machine perfusion is considered useful to preserve kidney grafts, it is still debated whether RI could predict graft outcome [16]. Nyberg *et al.* [17] have shown as RI influences early and late outcomes of kidney grafts from deceased donors and created an algorithm where grade C and D kidneys (marginal kidneys according to the deceased donor score) were pumped and those with $RI \geq 0.5$ were discarded.

It is important to remark that in our study, we decided to discard the kidneys with $RI \geq 0.5$ [17] and to perform dual kidney transplantation for kidneys with RI comprised between 0.35 and 0.5 mmHg/ml/min on the basis of our previous experience [18]. This decision may have determined the impossibility to show an association between RI and PNF or DGF, but it can also explain the reported low rate of PNF.

The results reported in the present study confirm those already published [18] on dual kidney transplantation.

Renal function was correlated to RI values at the end of perfusion: lower values of RI at the end of perfusion were associated with higher values of MDRD at 3 and 6 months after transplantation and with higher values of inulin clearance at 1 year. This correlation has been shown only in the first period after transplantation, and it might be due to the large number of variables influencing renal function in the long-term follow-up as well as to the small group of patients included in the present study.

To our knowledge, for the first time, the factors which may influence RI in uDCD were investigated. Indeed, flow characteristics of uDCD kidneys were influenced by the preservation procedure (showing indirectly the superiority of NRP compared to the *in situ* cold perfusion), asystolic time, and donor creatinine values, which

induces or indicates donor renal damage, respectively. The preservation procedure influenced RI and consequently also renal function after the transplantation.

This study confirmed that good clinical outcomes could be obtained transplanting selected uDCD kidneys. Moreover, the incidence of acute rejection episodes did not differ from that reported in expanded criteria donors (ECD) [1,6,11–14]. Interstitial fibrosis increased in uDCD kidneys with impact on renal function has been reported in the literature [19]. In the present study, an association between 3-month interstitial fibrosis and RI at the end of perfusion has been shown. However, it was not higher than in ECD and did not seem to influence graft survival at 1 post-transplant year as previously shown [20]. The very low rate of PNF and the high kidney survival rate confirmed [3,10,11] that using the machine perfusion, it is possible to select uDCD kidneys pre-operatively and to prevent the deleterious effects of warm ischemia improving renal function after the transplantation.

Authorship

XM: Designed research protocol and collected data, contributing to writing of the article. FD: performed the statistical analysis. PP: designed research protocol and wrote the article. OT: contributed to writing of the article. TR: management of the donors. CD: management of the donors. AF: management of the donors. MR: performed the histological studies. VMY: performed the histological studies. WH: contributed to writing of the article. EM: management of the grafted patients. LB: recovery of kidneys and their transplantation. RC: designed research protocol and contributed to collect data and to writing of the article.

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Conflict of interest

All authors disclose no conflict of interests.

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