Controlled Donation After Circulatory Death Using Normothermic Regional Perfusion Does Not Increase Graft Fibrosis in the First Year Posttransplant Surveillance Biopsy

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Abstract

Objectives: **The number of kidney transplants obtained from controlled donations after circulatory death is increasing, with long-term outcomes similar to those obtained with donations after brain death. Extraction using normothermic regional perfusion can improve results with controlled donors after circulatory death; however, information on the histological impact and extraction procedure is scarce.**

Materials and Methods: **We retrospectively investigated all kidney transplants performed from October 2014 to December 2019, in which a follow-up kidney biopsy had been performed at 1-year follow-up, comparing controlled procedures with donors after circulatory death and normothermic regional perfusion versus donors after brain death. Interstitial fibrosis/tubular atrophy was assessed by adding the values of interstitial fibrosis and tubular atrophy, according to the Banff classification of renal allograft pathology.**

Results: **When we compared histological data from 66 transplants with donations after brain death versus 24 transplants with donations after circulatory death and normothermic regional perfusion, no differences were found in the degree of fibrosis in the 1-year follow-up biopsy (1.7 ± 1.3 vs 1.7 ± 1.1; *P* = .971) or in the ratio of patients with increased fibrosis calculated as interstitial fibrosis/tubular atrophy >2 (18% vs 13%; *P* = .522). In our multivariate analysis, which included acute rejection, expanded criteria donation, and the type of donation, no variable was independently related to an increased risk of interstitial fibrosis/tubular atrophy >2.**

Conclusions: **The outcomes of kidney grafts procured in our center using controlled procedures with donors after circulatory death and normothermic regional perfusion were indistinguishable from those obtained from donors after brain death, showing the same degree of fibrosis in the 1-year posttransplant surveillance biopsy. Our data support the conclusion that normothermic regional perfusion should be the method of choice for extraction in donors after circulatory death.**

Key words: *Delayed graft function, Extended criterial donor, Interstitial fibrosis/tubular atrophy*

Introduction

With the persistently high number of patients on wait lists for solid-organ transplants, organs from donors who were previously considered unsuitable for transplant are now used. These organs include those from donors after circulatory death (DCDs), including controlled donors after circulatory death (cDCDs) and uncontrolled donors after circulatory death (uDCDs).[1] The use of DCDs has made it possible to increase the number of available organs and thus limit the number of patients on kidney transplant wait lists. Recently, in several countries such as the United Kingdom, the Netherlands, and Spain, the number of kidney transplants performed from DCDs has increased considerably.[2,3]

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Acknowledgements: *This work was sponsored by the Ministry of Science and Innovation, the Carlos III Health Institute and the European Regional Development Fund Research Network for Kidney Diseases ("RedInRen" RD16/0009/0027), and Networks for Cooperative Research Health Results ("RICORS2040" RD21/0005/0010). The authors have no declarations of potential conflicts of interest.*

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Experimental and Clinical Transplantation (2022) 12: 1069-1075

DOI: 10.6002/ect.2022.0171

The ischemia time with use of organs from DCDs has raised concerns that the results obtained with kidneys from DCDs are significantly worse than results with kidneys from donors after brain death (DBDs). However, although the frequency of primary nonfunction and delayed graft function (DGF) is higher in DCDs (especially in uDCDs), long-term renal graft survival rates are comparable to rates for DBDs.[4,5] To limit the ischemic damage, improve graft results, and increase the number of usable abdominal organs, the use of normothermic regional perfusion (NRP) has been advocated instead of super-rapid extraction, not only for uDCDs but also for cDCDs. Normothermic regional perfusion allows "in situ" perfusion of abdominal organs with oxygenated blood, restoring the energy substrates of the cells and hence limiting ischemic damage. The use of NRP also allows the organ extraction procedure to be performed less urgently, reducing surgical injuries of the kidney that commonly occur in rapid laparotomy.[6] In addition, the use of NRP does not hinder combined retrieval of abdominal and thoracic organs, allowing for maximum utilization of donor organs.[7,8]

In a preliminary noncomparative study, the use of NRP increased the number of organs transplanted effectively in our center, with excellent survival rates.[9] A recent Spanish multicenter study involving 770 kidney transplants from DCDs confirmed that the use of NRP reduced the risk of DGF and improved graft survival at 1 year posttransplant compared with super-rapid extraction.[10]

Apart from short- and long-term data on function and survival of renal grafts from DCDs, information is scarce on the histological changes that develop in these grafts. The degree of fibrosis and inflammation in the first year posttransplant can provide relevant prognostic information on the subsequent evolution of kidney grafts.[11,12] Therefore, an analysis of how DCDs affect the development of fibrosis in the graft is of the utmost interest to better understand its subsequent evolution. In addition, this histological information may allow identification of subgroups of patients with different evolution of fibrosis.

To our knowledge, there are no studies on the effects of ischemia on the degree of long-term renal fibrosis in DCDs treated with NRP. A recent US study of cDCDs using super-rapid extraction showed that this practice increases the risk of interstitial fibrosis and tubular atrophy (IFTA) in the first year posttransplant compared with results shown with DBDs.[13] A previous French study found increased progression of IFTA at 1 year posttransplant in renal grafts obtained from uDCDs.[14] Our hypothesis is that the degree of chronic damage at the first year posttransplant in renal grafts from cDCDs that used NRP, an emerging technique that offers good results in terms of renal graft survival in cDCDs, would be comparable to that shown in grafts from DBDs.

Materials and Methods

All transplants performed in our center from October 2014 to December 2019, in which a follow-up biopsy had been performed in the first year posttransplant and an adequate sample had been obtained for histological study, were considered candidates for inclusion in this study. Living-donor transplants, uDCD transplants, cDCD transplants that used the super-rapid technique, and hypersensitized recipients were excluded. Since 2012, our center has routinely performed surveillance biopsies at 1 year after transplant for all kidney transplant recipients who agree to the procedure. This study was conducted following the guidelines of the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of Cantabria (2020.388).

Renal graft biopsies were reviewed and classified according to the Banff classification system by 2 expert pathologists.[15] We assessed IFTA by adding the ci + ct values, which ranged between 0 and 6. Patients with irreversible brain injury or heart disease, pulmonary involvement, or terminal neurodegenerative disease in which the treatment team had made the decision to withdraw life-sustaining measures were considered as potential cDCDs. Functional warm ischemia time was defined as the time from systolic blood pressure <60 mm Hg to the onset of NRP (including a 5-min standoff period). To accept the kidney grafts, the maximum period of functional warm ischemia was 60 minutes.

To perform the NRP, a Maquet Rotaflow extracorporeal membranous oxygenation system was used. After specific informed consent was obtained from the legal representatives of the potential donors to perform premortem interventions, 400 to 500 U/kg of heparin were administered and the femoral vessels were cannulated before treatment withdrawal using the Seldinger technique in the intensive care unit. To avoid cerebral and coronary perfusion during NRP, an endoaortic balloon occlusion was placed into the

groin. The target flows to be maintained during abdominal NRP were between 2 and 2.4 L/min. In addition, a pressure of 60 to 65 mm Hg in the femoral arterial cannula, a temperature of 37 °C, and a supply of bicarbonate to keep the pH between 7.35 and 7.45 were maintained.

At the judgment of the attending transplant team, a perfusion machine was used as a method of preservation to limit the damage associated with cold ischemia in those kidneys in which time was expected to be greater than 12 hours.

Immunosuppressive therapy for recipients in our center consisted of tacrolimus, mycophenolate mofetil, and prednisone. Until July 2016, all cDCD recipients received thymoglobulin induction treatment with delayed introduction of tacrolimus, and from August 2016 induction therapy was changed to basiliximab with immediate tacrolimus initiation. Demographic and clinical variables related to the recipient, donor, and transplant process were collected retrospectively from the prospectively maintained renal transplant database of the Nephrology Department. Delayed graft function was defined as the need for at least 1 dialysis treatment during the first week after kidney transplant. Renal function was recorded at day 10 and at month 1, 3, and 12 posttransplant; glomerular filtration rate at the first year was estimated by using the CKD-EPI equation.

Continuous variables are presented as means ± SD and compared by *t* test, and qualitative variables are expressed as percentages and analyzed by chi-square test. We compared patient and graft survival rates using Kaplan-Meier analysis. We analyzed the relationship between IFTA and continuous variables using Pearson correlation and logistic regression. Statistical analysis was performed using SPSS.

Results

Between October 2014 and December 2019, 246 renal transplants were performed in our center. Of the total number of nonhypersensitized DBD transplants (n = 125), 68 kidney biopsies were performed 1 year later (54.4%), with inadequate biopsy samples retrieved for 2 of them. Of the total number of nonhypersensitized asystole cDCD kidney transplants obtained with NRP (cDCD/NRP; n = 49), 24 recipients (49.0%) underwent renal biopsy 1 year later. We found no differences in 1-year patient survival (95.8% vs 95.6%; $P = .980$) and 1-year graft survival (94.3% vs 91.8%; $P = .903$) between the grafts obtained from DBDs and cDCDs/NRP.

The median follow-up time was 3.7 ± 1.4 years. Clinical variables and patient-related variables included in the analysis are listed in Table 1. In the cDCD/NRP group, there were fewer donors who died from stroke, donor creatinine levels were lower, and induction treatment and machine perfusion were used more frequently; no significant differences were shown with remaining variables.

Table 1. Variables of Renal Transplants From Donors After Brain Death and Controlled Donors After Circulatory Death

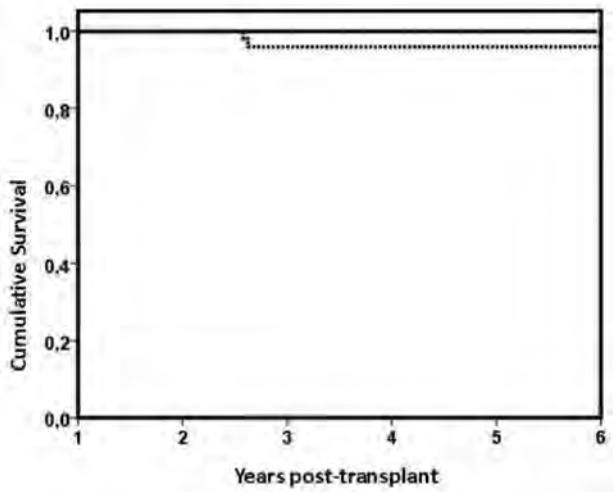
	DBD (n = 66)	cDCD (n = 24)	*P*
Recipient age, y	53 ± 11	53 ± 13	.979
Male recipient	60.6%	62.5%	.871
Diabetes as cause of ESRD	30.3%	16.7%	.196
Preemptive transplant	18.2%	8.3%	.254
Time on RRT	1.1 ± 1.4	1.5 ± 2.2	.301
Previous transplant	16.7%	25.0%	.372
Virtual PRA	6 ± 17	2 ± 8	.206
Donor age, y	50 ± 14	48 ± 14	.572
Male donor	64.6%	58.3%	.586
Donor terminal creatinine, mg/dL	0.89 ± 0.41	0.70 ± 0.33	.044
Cause of death			
Anoxia	15.2%	41.7%	
CVA	60.6%	33.3%	
Trauma	19.7%	8.3%	.003
Other	4.5%	16.7%	
ECD	33.3%	16.7%	.125
f-WIT, min		14.8 ± 8.3	
CIT, h	16.8 ± 5.8	13.7 ± 8.9	.126
HLA mismatching	4.3 ± 1.1	4.3 ± 1.4	.923
Machine perfusion	3.0%	50.0%	<.001
Induction therapy	54.5%	100.0%	<.001
Thymoglobulin	28.8%	45.8%	.129
DGF	12.1%	12.5%	.961
Creatinine day 10, mg/dL	2.09 ± 1.51	1.87 ± 1.36	.523
Creatinine month 1, mg/dL	1.31 ± 0.51	1.29 ± 0.37	.859
Creatinine month 3, mg/dL	1.32 ± 0.47	1.33 ± 0.33	.880
Creatinine year 1, mg/dL	1.29 ± 0.47	1.32 ± 0.39	.789
GFR year 1, mL/min	62 ± 18	59 ± 18	.521
Albuminuria, mg/g	78 ± 141	98 ± 161	.578
Acute rejection during year 1	21.2%	8.3%	.158
IFTA score	1.7 ± 1.3	1.7 ± 1.1	.971
IFTA >2	18.2%	12.5%	.522

Abbreviations: cDCD, controlled donor after circulatory death; CIT, cold ischemia time; CVA, cerebrovascular accident; DBD, donor after brain death; DGF, delayed graft function; ECD, expanded criteria donors; ESRD, end-stage renal disease; f-WIT, functional warm ischemia time; GFR, glomerular filtration rate; IFTA, interstitial fibrosis/tubular atrophy; PRA, panel reactive antibody; RRT, renal replacement therapy; WIT, warm ischemia time

In relation to the progression of renal graft, we did not observe any differences in DGF rates or creatinine levels throughout the first year between DBD and cDCD/NRP recipients. There were also no significant differences in patient survival (3-year survival 96.0% vs 94.4%; $P = .592$) and graft survival (3-year survival 96.5% vs 100%; $P = .382$) comparing DBD and cDCD/NRP recipients who had undergone

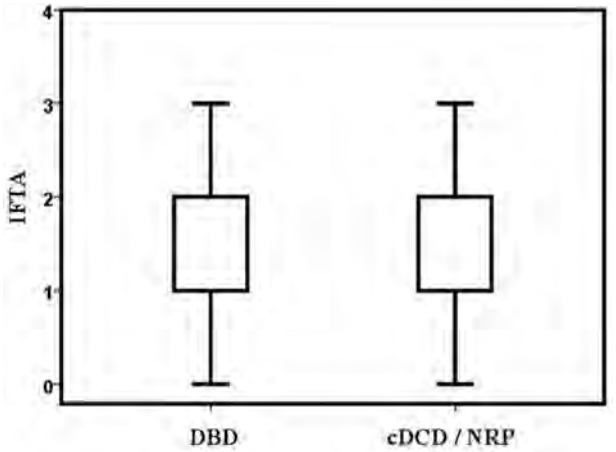
renal biopsy at 1 year included in the analysis (Figure 1). Moreover, no differences were found between groups on the degree of fibrosis in the 1-year follow-up kidney biopsy (Figure 2), nor in the rate of patients with increased fibrosis assessed as IFTA >2 (Table 1).

Figure 1. Renal Graft Survival Censored by Death



Solid line, recipient of controlled donor after circulatory death with normothermic regional perfusion; dashed line, recipient of donor after brain death. Log-rank *P* = .391.

Figure 2. Box Plot With Interstitial Fibrosis/Tubular Atrophy Values Comparing Recipient Groups (*P* = .971)



Abbreviations: cDCD/NRP, controlled donor after circulatory death with normothermic regional perfusion; DBD, donor after brain death; IFTA, interstitial fibrosis/tubular atrophy

Correlations between continuous variables and IFTA are listed in Table 2.

Table 3 shows the variables associated with a high degree of fibrosis in the first year posttransplant biopsy. Receiving a cDCD graft was not associated with increased risk of fibrosis. The donor's final creatinine level approached borderline statistical significance, as well as grafts from donors with expanded criteria, with the development of acute rejection throughout the first year posttransplant. In our multivariate analysis, which included acute rejection (odds ratio [OR] = 3.375; 95% CI, 0.874-13.028; *P* = .078), donor expanded criteria (OR = 3.102; 95% CI, 0.905-10.630; *P* = .072), and type of donation (DBD vs cDCD/NRP) (OR = .966; 95% CI, 0.597-1.563; *P* = .888), there were no independent variables related to higher risk of IFTA >2.

Table 2. Correlation Between Continuous Variables and Interstitial Fibrosis/Tubular Atrophy

	r	*P*
Donor age	0.111	.296
Donor terminal creatinine	0.200	.060
CIT	0.001	.992
HLA mismatching	0.049	.648
No. of dialysis treatments posttransplant	0.175	.098
Creatinine year 1	0.305	.003
GFR year 1	-0.334	.001
Albuminuria year 1	0.163	.127

Abbreviations: CIT, cold ischemia time; GFR, glomerular filtration rate

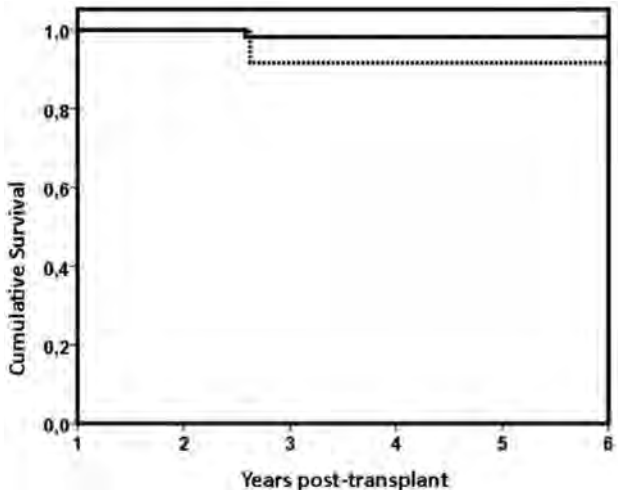
Table 3. Variables Related to Higher Risk of Fibrosis in the First Year Posttransplant (Interstitial Fibrosis/Tubular Atrophy >2).

	IFTA ≤2 (n = 75)	IFTA >2 (n = 15)	*P*
Recipient age, y	53 ± 11	53 ± 14	.922
Male recipient	62.7%	53.3%	.498
Diabetes as cause of ESRD	28.0%	20.0%	.522
Preemptive transplant	13.3%	26.7%	.193
Time on RRT	1.3 ± 1.7	0.7 ± 1.00	.159
Previous transplant	21.3%	6.7%	.185
Virtual PRA	5 ± 15	7 ± 19	.668
Donor age, y	49 ± 13	52 ± 15	.370
Male donor	62.2%	66.7%	.742
cDCD	28.0%	20.0%	.522
Donor terminal creatinine, mg/dL	0.79 ± 0.36	1.06 ± 0.52	.073
CVA as cause of death	52.0%	60.0%	.571
ECD	25.0%	46.7%	.092
CIT, h	16.2 ± 6.9	14.9 ± 6.6	.504
HLA mismatching	4.3 ± 1.2	4.4 ± 1.1	.568
Machine perfusion	16.0%	13.3%	.795
Induction therapy	66.7%	66.7%	1.000
Thymoglobulin	36.0%	20.0%	.230
DGF	10.7%	20.0%	.314
No. of dialysis treatments posttransplant	0.20 ± 0.64	0.53 ± 1.14	.283
Creatinine day 10, mg/dL	1.89 ± 1.34	2.79 ± 1.89	.123
Creatinine month 1, mg/dL	1.25 ± 0.46	1.56 ± 0.48	.028
Creatinine month 3, mg/dL	1.26 ± 0.37	1.68 ± 0.59	.024
Creatinine year 1, mg/dL	1.24 ± 0.41	1.60 ± 0.55	.004
GFR year 1, mL/min	64 ± 18	47 ± 14	.001
Albuminuria, mg/g	72 ± 130	147 ± 211	.218
Acute rejection during year 1	14.7%	33.3%	.084

Abbreviations: cDCD, controlled donor after circulatory death; CIT, cold ischemia time; CVA, cerebrovascular accident; DGF, delayed graft function; ECD, expanded criteria donor; ESRD, end-stage renal disease; GFR, glomerular filtration rate; IFTA, interstitial fibrosis/tubular atrophy; PRA, panel reactive antibody; RRT, renal replacement therapy

Patients with greater degree of fibrosis on follow-up biopsy at 1 year posttransplant showed poorer renal function from the first month throughout the first year posttransplant (Table 3) and slightly worse death-censored graft survival, although this did not reach statistical significance (3-year survival of 98.2% vs 91.7%; $P = .237$) (Figure 3).

Figure 3. Death-censored renal graft survival Comparing Patients With Interstitial Fibrosis/Tubular Atrophy ≤2 or >2



Solid line, interstitial fibrosis/tubular atrophy (IFTA) ≤2; dashed line, IFTA > 2. Log-rank $P = .237$.

Discussion

The cDCD procedure is a type of multiorgan procurement that allows simultaneous extraction of thoracic and abdominal organs with excellent results.[7,8] With experiences gained since the beginning of the asystole donation program in our center in 2014 and the successful results achieved, which are consistent with those found across Spain and other countries, this type of donation has expanded.[3]

No significant differences were found in terms of patient or graft survival when we compared outcomes between grafts from DBDs or cDCDs/NRP. These data support those published by British and Dutch registries.[16,17] The use of NRP has been promoted in our center from the beginning of the program; however, a few renal grafts were obtained by super-rapid extraction and were excluded from our study. Rates of DGF for cDCD/NRP recipients were not significantly higher than those for DBD recipients (12% vs 13%; $P = .961$). However, rates of DGF for cDCD recipients published by other groups have been higher (49% in the British study and 42% in the Dutch) and always significantly higher than rates for DBD recipients.[16,17] Previously published studies have suggested that those centers that used NRP showed a lower DGF rate (18%-40%) than those that used super-rapid extraction (48.5% in the British registry and >60% in the Dutch).[18] A Spanish study that retrospectively compared 865 cDCD/NRP renal transplants versus 1437 transplant with super-rapid extraction revealed that this technique increased the risk of DGF (OR = 1.97; 95% CI, 1.43-2.72; $P < .001$).[10] Compared with previous findings, the progression of renal function from day 10 and throughout the first year posttransplant was indistinguishable between groups (cDCD/NRP vs DBD) in our study (Table 1).

The limited follow-up time period (~3 years) may be the underlying cause as to why no differences were observed in the evolution of renal grafts between cDCD/NRP and DBD groups. Thus, having histological information at 1 year posttransplant may be useful as a substitute marker of later evolution. As referred to in the introduction, the degree of fibrosis in the first year posttransplant biopsy provides information on later renal graft outcome.[11,13] Accordingly, the most important finding of our study was that no significant differences were found in the degree of fibrosis on the follow-up surveillance biopsy in the first year posttransplant between renal grafts from cDCDs/NRP and DBDs. Our multivariate analysis confirmed that cDCD/NRP was not associated with an increased risk of fibrosis in the first year posttransplant. In light of these data and given that there is no higher subclinical fibrosis, we can speculate that cDCD/NRP grafts will have a long-term progression (beyond the 3 years of follow-up period that we recorded) comparable to DBD grafts.

This finding partially contradicts the recently published finding from van der Windt and colleagues.[13] This group at the University of Pittsburgh compared the histological results of follow-up biopsies at 1 year posttransplant between 87 cDCD recipients (with probable super-rapid extraction) and 246 DBD recipients, finding an IFTA significantly higher in the cDCD group (2.42 ± 1.26 vs 1.98 ± 1.19; $P = .004$).[13] However, we suggest that our results are different because of the protective effects of NRP in the kidney, which minimizes ischemic damage and enables a better preservation of the kidney after potential donor death. In fact, the

rate of DGF published by our group was lower than those reported by super-rapid extraction in the scientific literature.[2,9,10]

Furthermore, the association between cDCD and IFTA reported by van der Windt and colleagues[13] was independent of other donor-related variables such as rejection, cold ischemia time, and DGF. Despite the fact that their study is broader than ours, the data cannot be extrapolated to our population, since their cDCD recipients showed higher incidence of DGF (39% vs 19%; $P = .0001$) and worse renal function 1 year posttransplant (40 ± 16 vs 48 ± 19 mL/min; $P = .0005$), whereas, in our cDCD/NRP and DBD recipients, the incidence of DGF and renal function were indistinguishable. The difference in degree of fibrosis between our cDCD/NRP recipients (1.8 ± 1.4) and the cDCD recipients of the Pittsburgh study (2.42 ± 1.26) could be due to many factors, not only related to donor characteristics (slightly younger, but hypertensive in the Pittsburgh study) but also to posttransplant management. Nevertheless, the use of NRP by our team could explain, at least partially, both the better renal function and the lower degree of fibrosis at 1 year after transplant.[13]

Although donor characteristics, mainly age, determine the degree of fibrosis in preimplant or early posttransplant biopsies, fibrosis in the first year posttransplant is determined not only by the degree of prior fibrosis but also by events, primarily inflammatory, for graft experiences throughout the first year.[19] We did not find an independent relationship between fibrosis and donor-related variables such as age, renal function, and expanded criteria in our study. It is possible that a stricter donor selection procedure in our center could explain the lack of relationship between fibrosis and donor characteristics. The relationship between donor age and fibrosis at 1 year posttransplant has been reported in the literature,[14,20,21] although not by all.[22] Later events can influence the development of fibrosis at 1 year posttransplant.

Events occurring throughout the first year posttransplant can also influence subsequent fibrosis. The appearance of DGF has been reported as a risk factor for fibrosis by some authors, but not by others.[20,22,23] Other prior analysis have consistently shown than development of clinical[21,24] or subclinical[20,25,26] rejection during the first year posttransplant is an important factor for fibrosis progression.

Several limitations of our study should be noted. Because most of the organs obtained from cDCDs were extracted by NRP, it was not possible to compare these results with a super-rapid extraction group. The lack of sequential biopsies did not allow us to determine whether the degree of fibrosis of the early damage was related to donor characteristics and whether the NRP extraction method influenced subsequent fibrosis events. Finally, our study population was small, and a larger multicenter study would be needed to confirm these findings.

In conclusion, the posttransplant evolution of renal grafts procured in our center by cDCD/NRP was indistinguishable from those obtained by DBD. The fact that a surrogate marker, the degree of fibrosis in the first year posttransplant, showed the same results when we compared cDCD/NRP versus DBD confirmed that using NRP in cDCDs helps to minimize the damage induced by warm ischemia related to the asystole period. From our data, we can conclude that NRP should be the method of choice for extraction in cDCDs. It would be advisable to conduct a multicenter study that compares the degree of fibrosis in follow-up biopsies between cDCD grafts obtained by NRP and the super-rapid technique.

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