Hypothermic Machine Perfusion as an Alternative to Biopsy Assessment in Transplantation of Kidneys Donated After Cardiocirculatory Death: A Pilot Study

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ABSTRACT

Background. Transplantation of kidneys from donation after cardiocirculatory death (DCD) donors is becoming an ever-increasing reality. So far, biopsy histologic assessment is the main parameter for evaluation of graft suitability, but it has several drawbacks and has poor reliability. The aim of this study is to verify if real-time renal resistance (RR) measurement during hypothermic machine perfusion (HMP) can be used as a reliable parameter to evaluate the quality of grafts from DCD and extracorporeal membrane oxygenation (ECMO) donors.

Methods. From January 2015 to September 2018, HMP has been systematically applied to all organs from DCD and ECMO donors. All grafts underwent preimplantation biopsy histologic assessment with Karpinski's score. Single kidney transplants (SKTs) or double kidney transplants (DKTs) were performed according to biopsy score results. Kidneys were considered suitable for transplant if RR reached $\leq$ 1.0 within 3 hours of perfusion. RR trend and postoperative outcome were analyzed considering biopsy score and donor type.

Results. A total of 30 kidneys (15 from DCD and 15 from ECMO donors) were used to perform 26 transplants (22 SKTs and 4 DKTs). Considering RR trend, all grafts were considered suitable for transplant within 1 hour of perfusion. Biopsy confirmed this result in all cases, and median score was 3 (range, 0-7). SKT score kidneys had lower starting RR than DKT ones (1.88 vs 2.88; $P = .04$) but identical final RR (0.58 vs 0.57; $P = .76$). DKT recipients had faster postoperative creatinine reduction than SKT recipients but similar postoperative day 30 value (1.42 vs 1.15 mg/dL; $P = .20$). No differences were found between DCD and ECMO grafts in terms of RR trend and postoperative outcome.

Conclusions. HMP can be an alternative to histologic biopsy assessment for evaluation of transplant suitability of DCD and ECMO kidneys. If acceptability threshold is reached, SKT can be performed in all cases. ECMO donors should be considered like DCD donors.

TO overcome organ shortage, kidney transplantation of donation after cardiocirculatory death (DCD) grafts has become an increasingly popular clinical practice in several European countries over the last decades [1,2]. Although in the past there have been some concerns about the quality of these organs, evidence today shows that DCD kidney transplantation has similar long-term outcome than donation after brain death (DBD) kidney transplantation [1,3,4].

In this scenario, the introduction of extracorporeal hypothermic machine perfusion (HMP) for organ storage and reconditioning has been demonstrated to have several

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benefits, especially in short-term outcome, reducing both primary nonfunction (PNF) and delayed graft function (DGF) rate, thus increasing graft survival [5-7]. For these reasons, the use of HMP for the reconditioning of organs procured from DCD donors is highly recommended in national transplantation programs of several countries, including Italy. Moreover, HMP allows having a real-time measurement of renal resistance (RR) value, whose trend during reconditioning has been shown to have a predictive value of post-transplant outcome stronger than histologic assessment of the graft [8].

After a preliminary experience with DCD transplantation [9], the North Italian Transplant Program, the transplant network to which our center belongs, has recently developed a specific protocol for DCD kidneys transplantation, derived from the experience with extended criteria donors (ECD): after procurement and before HMP, a biopsy of each kidney is performed for the assessment of Karpinski's score [10]; thereafter, according to modified Remuzzi's criteria [11], grafts are allocated to 2 single kidney transplants (SKTs) or to 1 double kidney transplant (DKT). However, several drawbacks related to the execution of the kidney biopsy [12] and its poor prognostic value [13-15] have given rise to some doubts about its reliability, especially in this specific transplantation program [12,16].

The aim of this study is to verify if real-time RR measurement can be used as a reliable parameter to evaluate graft quality and to decide if SKT or DKT should be performed, analyzing the results of a single academic institution.

MATERIALS AND METHODS

We retrospectively analyzed our prospectively collected database from 2015 to present. According to modified DCD Maastricht classification [17], 3 different types of donors were included in this study:

1. Uncontrolled (Maastricht class II) donors who had undergone ineffective attempt of resuscitation and were declared dead after 20 minutes of registered asystole "no-touch period"), as required by the current Italian statutory law. After this period, donors were subjected to locoregional extracorporeal abdomen perfusion for the preservation of abdominal organs until procurement.

2. Controlled (class III) donors in whom withdrawal of life-sustaining therapies is applied because of massive and irreversible brain damage, as agreed within the health care team and with the relatives or representatives of the patients. Also in this case, patients were declared dead and subjected to locoregional extracorporeal abdomen perfusion after 20 minutes of registered asystole.

3. DBD donors who had undergone extracorporeal membrane oxygenation (ECMO) life support protocol after a cardiac arrest and who had developed a subsequent brain death due to extensive postanoxic brain damage (Maastricht IVB class donors or "ECMO" donors).

Immediately after the procurement, a wedge biopsy was performed on the outer profile of the upper pole of all kidneys, and the specimens were held in formalin for a fixating period of at least 4 hours. According to standardized protocols [10], a score between 0 and 12 was assessed for each kidney by a dedicated pathologist, based on histologic appearance of the graft (Table 1). Similarly to what happens with Remuzzi's modified criteria [11] in ECD transplants, when both organs gained a score between 0 and 4, they were allocated to 2 different recipients for SKT; when 1 or both kidneys had a score between 5 and 7, a DKT was recommended; in cases when 1 or both kidneys had a score higher than 7, the organs were considered not suitable for transplant and discarded.

After back-table surgery, each kidney underwent HMP performed by a dedicated team of perfusionists using a Waves pulsatile oxygenated hypothermic perfusion devices (Waters Medical System, LLC; Rochester, Minn, United States). For all perfusions, systolic pressure was set between 35 and 40 mm Hg; 1 L of IGL PERF-GEN (Table 2), a University of Wisconsin–based solution specifically made for HMP, was used as perfusion solution. RR value was real-time calculated by the machine software as the ratio between mean perfusion pressure (mm Hg) and solution flow through the kidney (mL/min) and recorded every minute throughout the perfusion. The solution was oxygen-enriched using atmospheric oxygen through a membrane oxygenator that kept partial oxygen pressure inside the solution (PO_2) around 150 mm Hg. Perfusion temperature was automatically set by the machine at 5°C before kidney placement and strictly controlled throughout the duration of graft reconditioning within a range of ± 2°C.

Kidneys were considered properly reconditioned and thus suitable for transplant as soon as RR value was ≤ 1.0, after at least 3 hours of perfusion. Kidneys were detached from HMP immediately before starting vascular anastomoses. Cold ischemia time was calculated from the start of abdomen perfusion during procurement until the end of HMP. Warm ischemia time was calculated from the end of HMP until kidney reperfusion on recipient.

All transplants were performed by a team of 3 expert and dedicated surgeons: the graft was placed in the left (or right) iliac region, renal vessels were latero-terminally anastomosed to external iliac vessels, and the ureter was anastomosed to the bladder using single U-stitch technique or Lich-Gregoire technique. In case of DKT, 2 separate accesses in both iliac regions were performed in all recipients.

Postoperative immunosuppressive therapy consisted of anti-human thymocyte immunoglobulin (1.5 mg/kg daily) for the first 7 postoperative days (PODs), intraoperative methylprednisolone

Table 1. Detailed Criteria for Histological Score Assessment (Karpinski's Score)

Parameter	Score
A. Glomerular sclerosis	
None	0
< 20% of glomeruli affected	1
20%-50% of glomeruli affected	2
> 50% of glomeruli affected	3
B. Tubular atrophy	
Absent	0
< 20% of tubuli affected	1
20%-50% of tubuli affected	2
> 50% of tubuli affected	3
C. Interstitial fibrosis	
Absent	0
< 20% of parenchyma replaced	1
20%-50% of parenchyima replaced	2
> 50% of parenchyma replaced	3
D. Arterial narrowing	
Absent	0
Wall thickness < lumen diameter	1
Wall thickness = lumen diameter	2
Wall thickness > lumen diameter	3
Renal Biopsy Score	A + B + C +D

Table 2. Composition and Characteristics of Institut Georges Lopez Pulsatile Perfusion Solution PERF-GEN

Parameter	Value
Calcium chloride dihydrate, g/L	0.068
Sodium gluconate, g/L	17.45
Adenine, g/L	0.68
Potassium phosphate monobasic, g/L	3.4
Pentafraction [HES], g/L	50
Magnesium gluconate, g/L	1.13
HEPES, g/L	2.38
Glucose beta D [+], g/L	1.80
Glutathione, g/L	0.92
Ribose D [−], g/L	0.75
Mannitol, g/L	5.4
Sodium hydroxide, pH	qs 7.4
Water for injection, L	qs 1
Osmolarity, mOsm/kg	300
pH	7.4
$[K^+]$, mmol/L	25
$[NA^+]$, mmol/L	100

HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HES, hydroxyethyl starch.

(500 mg) and postoperative prednisone (10 mg daily), mycophenolate mofetil (2 g daily), and tacrolimus (0.035 mg/kg daily) starting when creatinine was $<$ 3 mg/dL or on POD 5 at the latest.

Serum creatinine was measured before transplant (basal value), every day from POD 1 to POD 10, and every other day from POD 12 to POD 30. PNF was defined as the permanent lack of graft function from the time of transplant. DGF was defined as the need for dialysis within the first week after transplant, with subsequent full recovery of graft function.

All variables concerning donors' characteristics, recipients' characteristics, kidney scores, HMP parameters, and postoperative outcome were inserted in a prospectively collected computerized database. Categorical data are presented as absolute value (percent proportion); continuous data are presented as mean (SD). For comparison between categorical variables, Pearson χ^2 test or Fisher exact test was used, as appropriate. For comparison between 2 continuous variables, Mann-Whitney U test was used. A P value $\leq$ .05 was considered statistically significant for all performed tests. Statistical analyses were performed using SPSS 20.0 software (SPSS Inc, Chicago, Ill, United States).

RESULTS

From January 2015 to present we performed 30 HMPs for as many organs: 15 came from DCD donors (11 from Maastricht class II and 4 from Maastricht class III) and 15 came from ECMO donors. Mean donor age was 51.9 (SD, 10) years (range, 31-74 years). Trend of perfusion parameters (flow, temperature, and RR) of the whole group of kidneys is reported in Fig 1. After a mean HMP duration of 391 (SD, 172) minutes, all kidneys reached a RR value lower than 1.0 cutoff point (0.57 [SD, 0.21]) and were thus considered adequately reconditioned and suitable for transplant. No significant differences in reconditioning were observed between the 2 types of grafts (Fig 2), although final RR value in DCD kidneys was slightly lower than in ECMO ones (0.47 [SD, 0.14] vs 0.63 [SD, 0.23]; $P = .08$).

Histologic score assessment of kidney biopsy specimens showed the following results: 1 kidney (3%) had score 0; 4 kidneys (13%) had score 1; 7 kidneys (23%) had score 2; 7 kidneys (24%) had score 3; 2 kidneys (7%) had score 4; 6

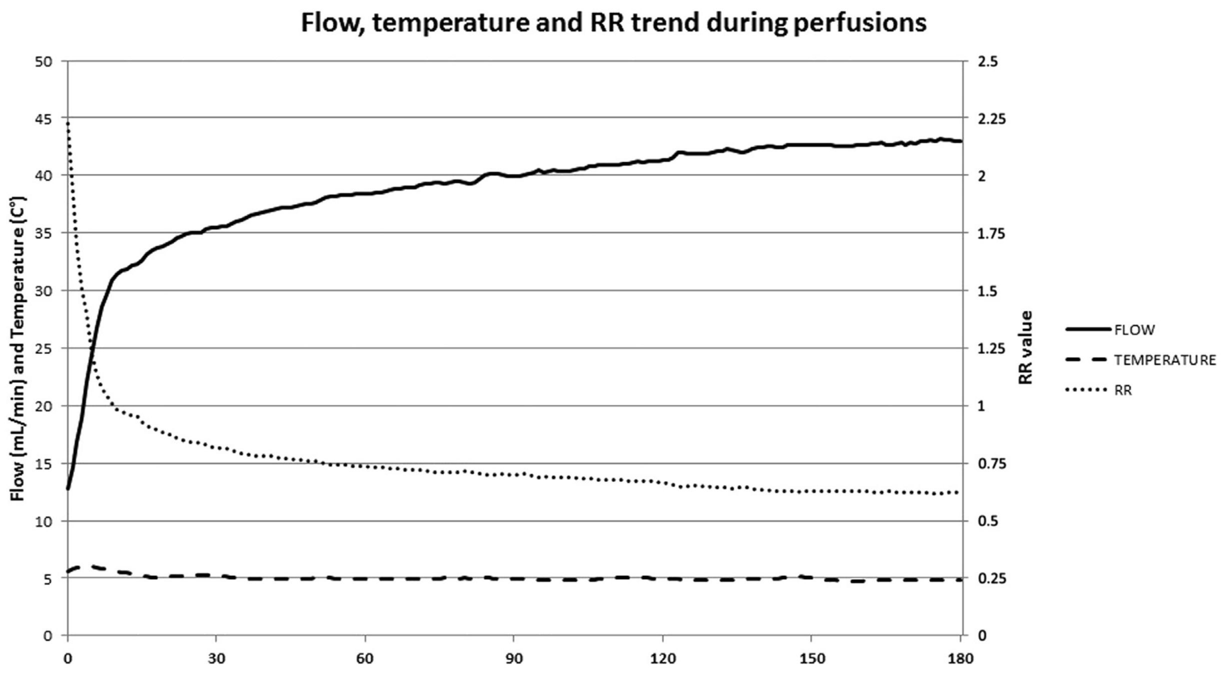

Fig 1. Flow, temperature, and RR trend in the overall series of 30 HMPs during the first 180 minutes (x-axis: min of perfusion). HMP, hypothermic machine perfusion; RR, renal resistance.

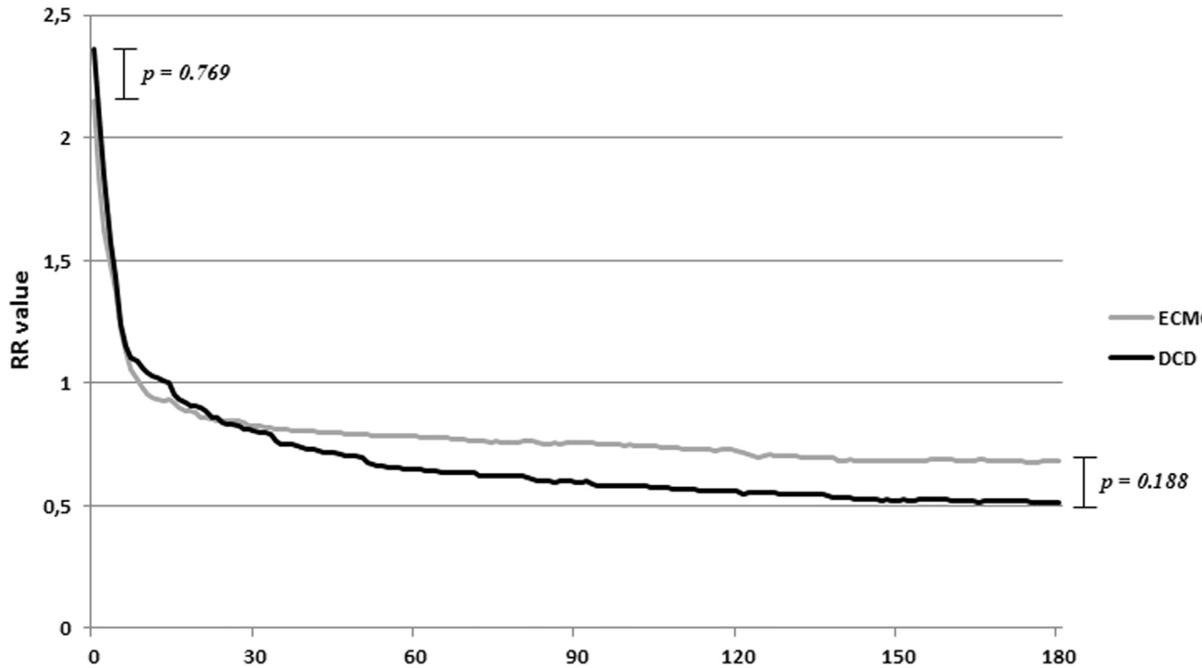

Fig 2. RR trend during HMP of 15 DCD kidneys and 15 ECMO kidneys (x-axis: min of perfusion). Mann-Whitney *U* test showed no statistically significant differences for both initial and final RR value between the 2 groups. DCD, donation after cardiocirculatory death; ECMO, extracorporeal membrane oxygenation; HMP, hypothermic machine perfusion; NS, not significant; RR, renal resistance.

kidneys (20%) had score 5; 2 kidneys (7%) had score 6; and 1 kidney (3%) had score 7. Median score value was 3. No kidneys had a score higher than 7.

During HMP, kidneys with score 0 to 4 showed significantly lower starting RR value than kidneys with score 5 to 7 (1.88 [SD, 1.39] vs 2.88 [SD, 1.55]; $P = .04$),

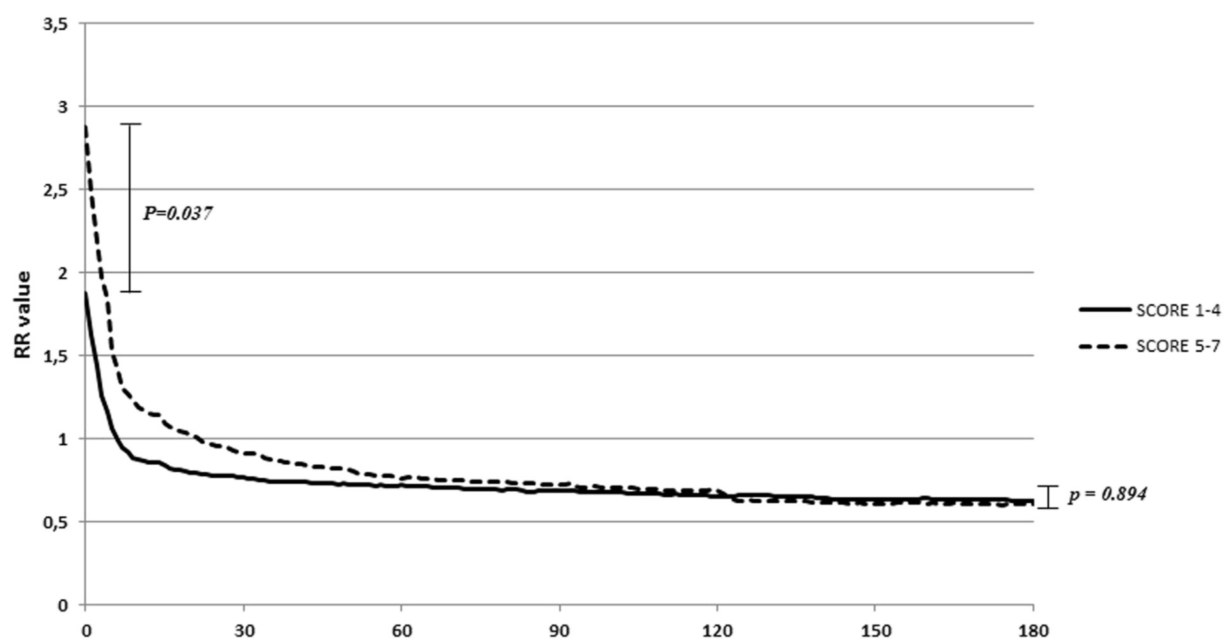

Fig 3. RR trend during HMP of 21 kidneys with score 0-4 and 9 kidneys with score 5-7 (x-axis: min of perfusion). Mann-Whitney *U* test showed a statistically significant difference for starting but not for final RR value between the 2 groups. HMP, hypothermic machine perfusion; RR, renal resistance.

but no significant differences were found for final RR value (0.58 [SD, 0.25] vs 0.57 [SD, 0.14]; P = .76), as shown in Fig 3.

According to biopsy score results, 22 SKTs and 4 DKTs were performed, almost always following Remuzzi's criteria except in 1 case, in which 2 kidneys from the same donor (score 5 and 6, respectively) were transplanted to 2 separate recipients for SKT. This was decided because both kidneys had an excellent RR trend during HMP, reaching in both cases RR < 1 within the very first minutes of perfusion.

Donor-related characteristics, recipient-related characteristics, graft-related characteristics, and postoperative outcome in the whole group and specific for DCD/ECMO donors are reported in Table 3. A higher but not significant DGF rate in recipients of DCD grafts compared with ECMO graft recipients was observed. No other statistically significant differences were found between the 2 groups of recipients. Only 1 case of PNF (4%) was observed in the whole series, which was caused by acute graft thrombosis for thrombotic microangiopathy in a DCD recipient with a history of positive lupus-like anticoagulant and antiphospholipid antibodies, requiring the explantation of the graft.

Postoperative serum creatinine trend in the whole series of transplant patients is reported in Fig 4. Regression line analysis showed an inverse linear correlation between creatinine values and PODs at a constant decrease rate of about 0.21 mg/dL per day (R^2 = 0.930) until lower value (1.36 [SD, 0.52] mg/dL) is reached within POD 30.

In the comparison of postoperative serum creatinine trend between DCD and ECMO transplants (Fig 5), no substantial differences were found between the 2 patient groups: in both cases, regression line analysis showed inverse linear correlation between creatinine values and PODs, with DCD graft recipients and ECMO graft recipients showing similar creatinine level decrease throughout the 30 post-transplant days (decrease rate: 0.26 mg/dL per day; R^2 = 0.934 and 0.18 mg/dL per day; R^2 = 0.884, respectively). POD 30 creatinine was similar between the 2 groups of patients (1.37 [SD, 0.63] and 1.35 [SD, 0.42] mg/dL, respectively; P = .65).

In the comparison of postoperative serum creatinine trend between recipients of graft with histologic score 0 to 4 (SKT) and recipients of graft with histologic score 5 to 7 (DKT), reported in Fig 6, regression line analysis showed an inverse linear correlation for the first group, with a decrease rate of about 0.21 mg/dL of creatinine per day (R^2 = 0.93), while for the latter it showed a much faster inverse logarithmic correlation (R^2 = 0.92). No statistical differences were found in POD 30 creatinine between the 2 groups of recipients (1.42 [SD, 0.52] and 1.15 [SD, 0.50] mg/dL, respectively; P = .20). In the specific analysis of postoperative outcome of the 2 recipients of single kidneys with DKT score (Fig 7), creatinine trend was much more similar to DKT recipients (inverse logarithmic) than SKT recipients (inverse linear).

DISCUSSION

To overcome organ shortage, over the last decades DCD donors have become an ever-increasing source of good-quality grafts in many European countries, such as Spain, France, England, and the Netherlands. Evidence now shows [1-4] that both short- and long-term outcomes of DCD kidney transplants are almost identical to those of DBD kidney transplants, so that in the abovementioned countries donor pool has significantly increased. At the same time and consequently to the development of this new type of transplantation activity, there has been a remarkable increase in the use of perfusion machines: as a matter of fact, the reconditioning effect of hypothermic pulsatile perfusion over those suboptimal-quality organs has been widely demonstrated to improve transplantation outcomes, especially in the short-term period [5-7], with a consequent

Table 3. Summary of Donor-Related Variables, Graft-Related Variables, Recipient-Related Variables, and Postoperative Outcome for Overall Series, ECD Grafts Transplants, and ECMO Grafts Transplants

Variables	Overall (26 transplants)	DCDs (13 transplants)	ECMOs (13 transplants)	*P* Value (ECD vs ECMO)
Donor's age, mean (SD), y	51.9 (11)	49.1 (10.4)	54.7 (11.2)	.17
Type of transplant, No. (%)				> .99
SKT	22 (85)	11 (85)	11 (85)	
DKT	4 (15)	2 (15)	2 (15)	
Biopsy score, No. (%)				> .99
0-4	21 (70)	11 (73)	10 (67)	
5-7	9 (30)	4 (27)	5 (33)	
Recipient's age, mean (SD), y	50.6 (9.8)	50.3 (7.4)	50.8 (12)	.72
Warm ischemia time, mean (SD), min	37.2 (6.1)	37 (7.5)	37.3 (4.6)	.78
Cold ischemia time, mean (SD), h	15.4 (2.6)	15.9 (2.4)	14.9 (2.8)	.19
PNF, No. (%)	1 (4)	1 (8)	0 (0)	> .99
DGF, No. (%)	8 (32)	6 (50)	2 (15)	.10
Basal creatinine, mean (SD), mg/dL	7.33 (2.86)	8.15 (3.37)	6.59 (2.16)	.22
POD 30 creatinine, mean (SD), mg/dL	1.36 (0.52)	1.37 (0.63)	1.35 (0.42)	.65

DGF, delayed graft function; ECD, extended criteria donor; ECMO, extracorporeal membrane oxygenation; PNF, primary nonfunction; POD, postoperative day.

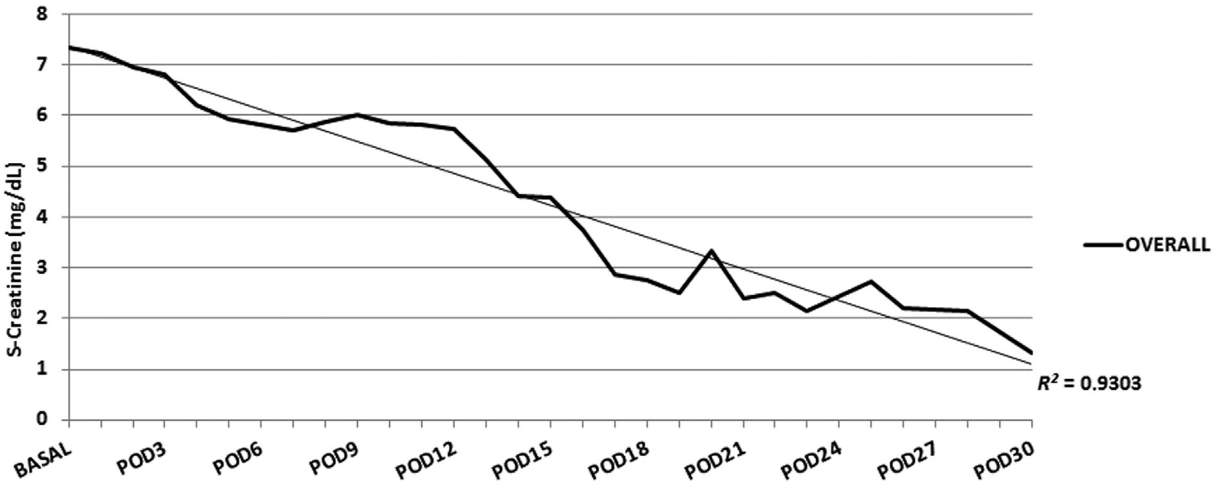

Fig 4. Serum creatinine trend during the first 30 PODs in the whole series of transplant patients. Regression line analysis showed linear correlation between S-creatinine (y) and PODs (x), with $y = -0.21x + 7.57$ ($R^2 = 0.9303$). POD, postoperative day.

reduction of hospitalization time and costs [18], so that the use of HMP has become nearly mandatory. The modulation in the expression of several genes related to the inflammatory cascades [19-21] and the consequent protection from ischemia/reperfusion injury and reduction of cellular apoptosis [22] seems to be the biological explanation to the beneficial effects of HMP. Furthermore, the use of HMP grants another remarkable advantage, which is to have a real-time measurement of RR values throughout perfusion of the organ. In a work recently published by our group [8], we have demonstrated that RR absolute value and, mostly, its trend during organ reperfusion can predict postoperative outcome in both transplantation of DCD and ECD organs. As a matter of fact, the use of organs that reach RR value 1.0 or lower at the end of HMP allowed avoidance of PNF cases; furthermore, those grafts that reached RR 1.0 within the first hour of HMP had a significantly lower DGF rate. The main purpose of this study was to evaluate if perfusion parameters assessed during HMP can be sufficient to evaluate the suitability for transplantation of DCD and ECMO organs. As a matter of fact, one of the pivotal issues in transplantation of DCD kidneys is finding a reliable

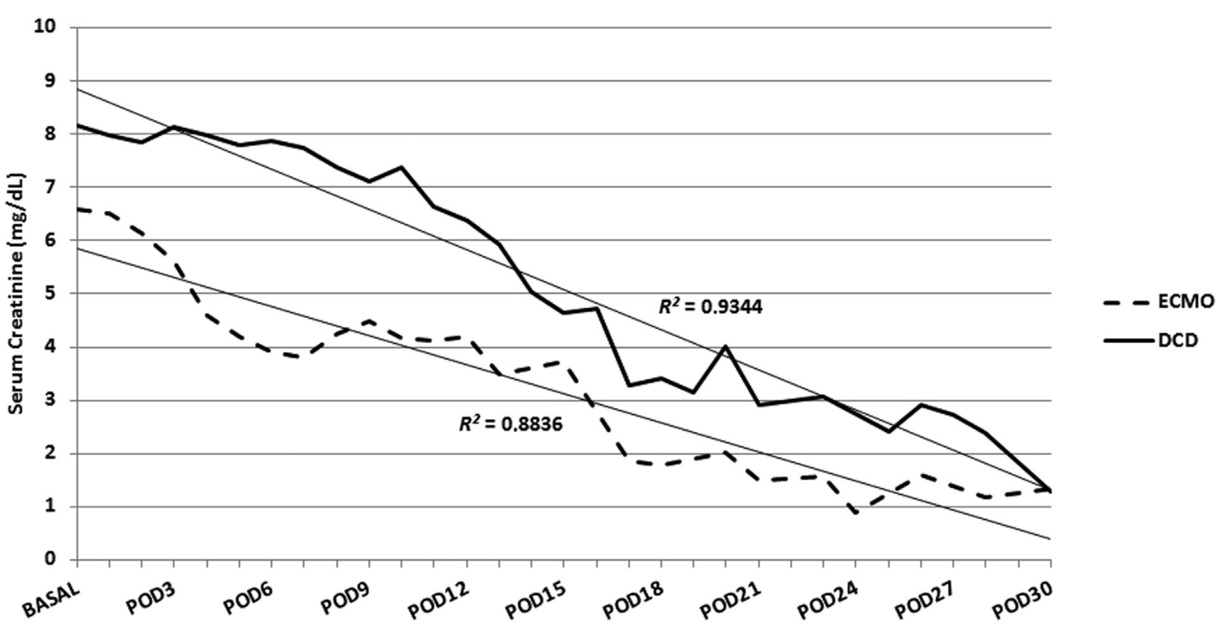

Fig 5. Serum creatinine trend during the first 30 PODs in DCD and ECMO grafts transplant patients. Regression line analysis showed linear correlation between S-creatinine (y) and PODs (x) in both DCD and ECMO group, with $y = -0.26x + 9.14$ ($R^2 = 0.9305$) and $y = -0.18x + 6.04$ ($R^2 = 0.8836$). DCD, donation after cardiocirculatory death; ECMO, extracorporeal membrane oxygenation; POD, postoperative day.

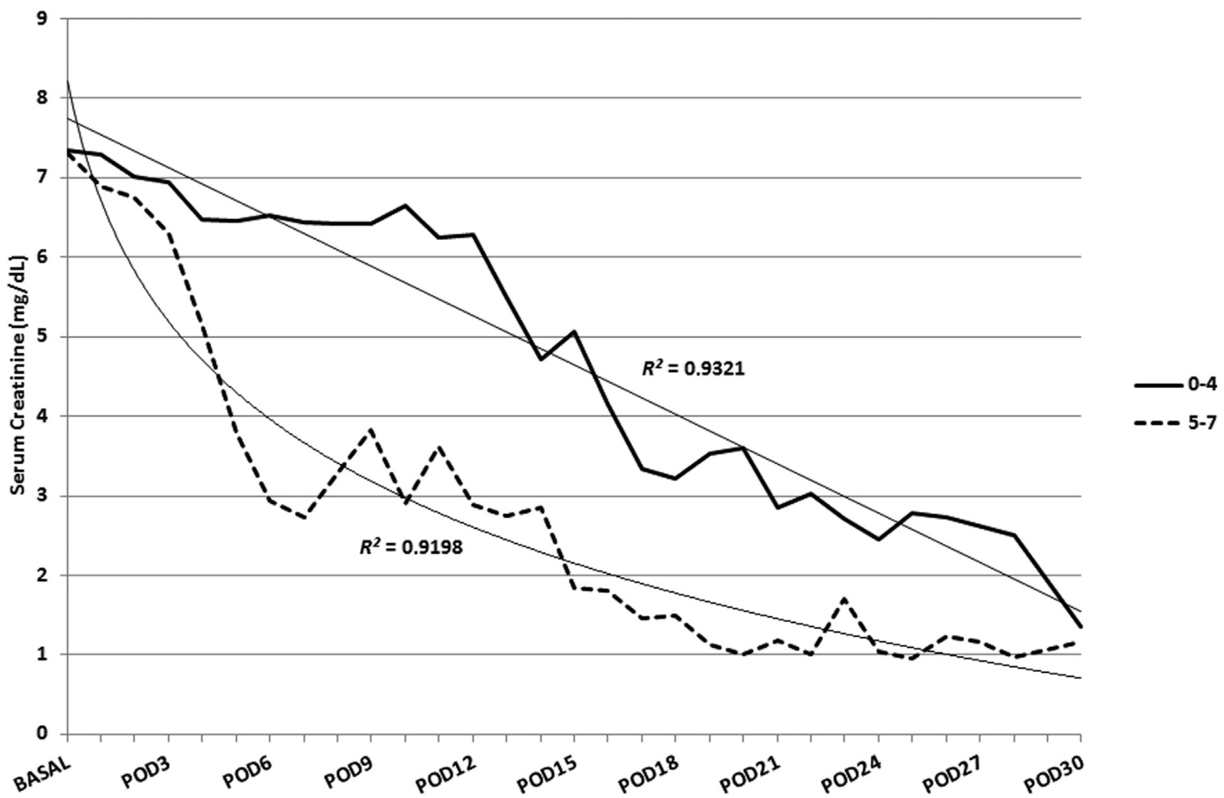

Fig 6. Serum creatinine trend during the first 30 PODs in patients receiving grafts with histological score 0-4 and 5-7. Regression line analysis showed linear correlation between S-creatinine (y) and PODs (x) in both 0-4 group, with $y = -0.21x + 7.95$ ($R^2 = 0.9321$), and logarithmic correlation for 5-7 group, with $y = -2.2\ln(x) + 8.23$ ($R^2 = 0.9198$). POD, postoperative day.

parameter to evaluate the quality of this type of grafts. Estimated glomerular filtration rate, commonly used as a reference parameter in DBD kidney donation, may often be misleading in these situations, since in most cases pre-procurement serum creatinine levels are higher than normal because of acute kidney injury due to cardiac arrest and may not reflect the real filtration rate once renal activity is restored. In the northern Italy transplantation network, as consequence of the experience gained from ECD kidney transplantation programs, both DCD and ECMO kidneys are routinely subjected to biopsy for the histologic assessment with Karpinski's score [10], and the grafts are intended for SKT or DKT on the basis of Remuzzi's modified criteria [11]. Nevertheless, this strategy has several drawbacks and generates some criticism on its effectiveness. Indeed, biopsy specimen evaluation is a time-consuming procedure, leading sometimes to high cold ischemia time that is pivotal in DCD transplantation [23]. Moreover, histologic evaluation is limited only to a small portion of the kidney that may not be representative of the whole organ, with the limitation of an operator-dependent procedure. Finally, the execution of renal biopsy can lead to serious post-transplant complications (hematomas, bleeding, arteriovenous fistulas), which in the worst cases can cause the loss of the organ. In addition to the above reported drawbacks, biopsy specimen score does not seem to have a predictive value of post-transplant outcomes [8,12,13], and some authors [12,16] raise the doubt that it may even lead to the discarding of kidneys that would instead be suitable for transplant. In the present study, 21 of 30 analyzed kidneys had an SKT score, and more than 90% of them had a score between 0 and 3, meaning that in those cases histologic assessment was basically unnecessary for decision making about graft suitability for SKT. No kidney had a score greater than 7, and thus no kidney was ever discarded on the basis of histologic assessment. This can be easily understood considering the young age of the donors. As a matter of fact, Karpisky's score is basically aimed to evaluate kidney chronic damage that these kind of grafts do not have. Even more interesting, RR reconditioning trend during HMP showed no differences (apart from starting value) between graft with an SKT score and grafts with DKT score, and in all cases suitability for transplant (RR $<$ 1.0) was assessed within 30 minutes from the beginning of perfusion, several hours before the biopsy histologic analysis confirmed the same indication. This behavior is typical of young and good-quality organs that, without chronic damage, are easily reconditioned. In postoperative evaluation, recipients of DKT had a much faster creatinine decrease than recipients of SKT, although in both cases serum creatinine reached acceptable value

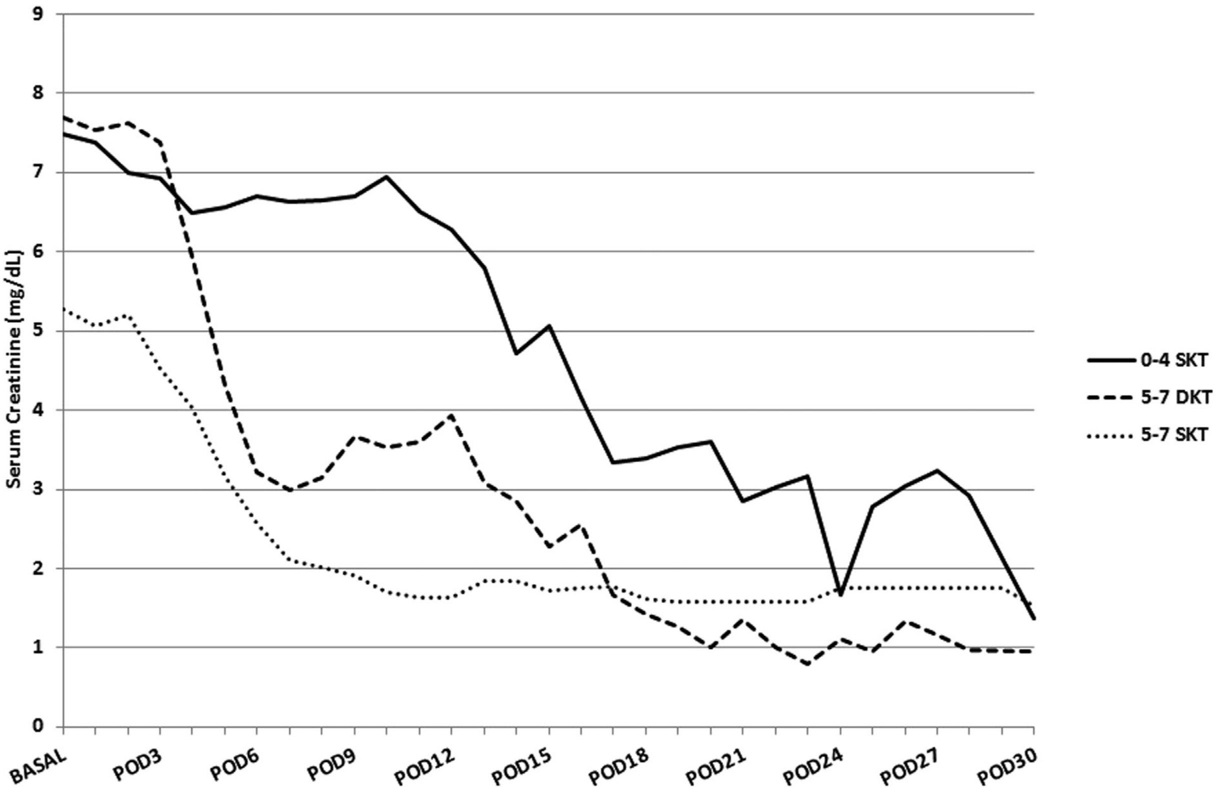

Fig 7. Serum creatinine trend during the first 30 PODs in SKT recipients of grafts with score 0-4, DKT recipients of grafts with score 5-7, and SKT recipients of grafts with score 5-7. DKT, double kidney transplant; POD, postoperative day; SKT, single kidney transplant.

within POD 30. We may thus speculate that in these patients, DKT was probably unnecessary and that an SKT could have been enough to achieve an equally good postoperative result. In this perspective, biopsy has led to an excessive expenditure of good-quality organs. As a further confirmation of this hypothesis, the 2 recipients of a single kidney with DKT score had a regular post-transplant course; no DGF or PNF was registered, and in both cases serum creatinine normalized within 30 days from transplant. As a secondary aspect of this study, 2 different types of donors have been considered and compared: in addition to standard controlled and uncontrolled DCD donors, in which abdominal perfusion through loco-regional ECMO is performed after death declaration with cardiac criteria, we have also considered patients who have undergone ineffective ECMO life support protocol and have sustained massive brain past-anoxic damage after cardiac arrest. In this type of donor, since heart electric activity is restored, cardiac death criteria cannot be applied, and thus the death assessment is carried out through the neurologic criteria (DBD). However, as in DCD, abdominal organs perfusion is primarily maintained through ECMO machine, since the cardiac pump function is almost nil. In the present study we demonstrated that these 2 types of donors are very similar: no significant differences were found in demographic characteristics or in histologic appearance of the grafts. Furthermore, RR reconditioning trends of the 2 graft types during HMP were almost identical: starting values showed no significant differences, and in all cases RR quickly reached the threshold of 1.0 within the first 30 minutes of perfusion. Fully reconditioned RR value at steady state was slightly lower, although not significantly, in grafts from DCD donors than in grafts from ECMO donors, probably as a result of the younger age of the first ones compared with the latter. In postoperative outcome evaluation, the 2 groups of recipients were homogeneous for what concerns age, SKT/DKT, cold and warm ischemia times, and basal creatinine. We found a difference, although not statistically significant, in DGF rate, which was higher in recipients of DCD kidneys (50%) than in recipients of ECMO kidneys (15%), probably because of a most pronounced acute tubular necrosis (not assessed in the biopsy score) in the first type of grafts. Nevertheless, in both groups of patients, the postoperative creatinine trend showed a progressive and linear reduction of serum levels up to the normalization within POD 30, with a similar decrease rate. In conclusion, this study shows that real-time RR evaluation through HMP can replace histologic assessment in the evaluation of graft suitability for transplant. If RR value reaches the threshold of 1.0, SKT can be performed with excellent postoperative results. Kidneys from ECMO donors behave very similarly to kidneys from DCD ones and should be considered as belonging to the same category of donors. The results of this retrospective study should be confirmed by larger prospective studies.

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