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THE ROLE OF HYPOTHERMIC MACHINE PERFUSION IN SELECTING RENAL GRAFTS WITH ADVANCED HISTOLOGICAL SCORE

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SUMMARY

Background: No definitive data exist on the protective effect of hypothermic perfusion machine (HPM) in the setting of deceased brain death (DBD) kidney transplantation (KT). We aimed at comparing the post-KT clinical course of two preliminarily "balanced" groups of patients undergoing KT with DBD grafts perfused with HPM or preserved with static cold storage (SCS).

Methods: During the period Jan 2014-Sep 2021, 313 patients were transplanted at Sapienza University of Rome. The population was stratified in two groups according to the type of graft preservation: SCS Group (n=218, 69.6%), and HPM Group (n=95, 30.4%). With the intent to compensate for the non-randomized design of this retrospective study, the two groups were "balanced" using a stabilized inverse probability therapy weighting (IPTW).

Results: After IPTW balancing, no differences were observed between the two groups in terms of post-KT delayed graft function (DGF) rates. In detail, need for dialysis within the first week after KT was observed in 17.9 vs. 15.6% of cases in SCS and HPM cases, respectively (P=0.75). HPM cases has a shorter period of ICU stay (P<0.0001) and a shorter, although not statistically relevant, overall hospital stay (P=0.07). When the sub-class of cases requiring a biopsy before KT was explored, more relevant differences were observed. Need for dialysis was more common in SCS cases (23.9 vs. 7.7%; P=0.03). ICU and overall hospital stay were always shorter in the HPM Group (P=0.002 and 0.007, respectively).

Conclusions: The use of perfusion machine positively impacts on the early recovery of DBD renal grafts. This positive phenomenon is more marked in cases requiring biopsy at the time of organ retrieval (i.e., aged donors or presence of relevant donor comorbidities). The routine use of a tailored HPM approach should hesitate in a reduction of post-transplant dialysis rates, and shorter hospital stay.

1. REPLACEMENT THERAPY FOR CHRONIC KIDNEY FAILURE: INCIDENCE AND RISK FACTORS

According to a study published in the Journal of the American Medical Association in 2017, 10% of the global population suffers from chronic kidney disease (CKD). In the surveyed countries, facilities for substitute treatments such as hemodialysis, peritoneal dialysis, and kidney transplantation are available in 95%, 76%, and 75% of cases, respectively [1].
The Italian Society of Nephrology (SIN) reports that CKD affects 2.2 million Italians, with a prevalence of 7%, showing a progressive and steady increase. The main factors contributing to the rising global burden of CKD are population aging, continuous demographic growth, the decline of communicable diseases, and an increase in non-communicable disease risk factors such as obesity, diabetes, hypertension, smoking, and cardiovascular diseases [2].

The Italian Registry of Dialysis and Transplantation Report for the year 2016 describes an incidence rate of dialysis treatment cases at approximately 160 per million population (pmp), with a prevalence of around 750 pmp and a stable trend between 2011 and 2016. The estimate for absolute cases indicates that there are over 45,000 people in substitute treatment in Italy, with around 8,000 patients starting replacement therapy annually. However, only one-sixth of them have access to kidney transplantation [3].

For eligible candidates, kidney transplantation represents the best therapeutic option in terms of quality of life and survival [4-6]. It also offers economic advantages, as dialysis costs exceed those of post-transplant follow-up care [7-8]. Despite the positive outcomes, the main limitation to this therapeutic choice lies in the gap between the availability of deceased donor organs and the increasing number of CKD patients. The waiting time on dialysis significantly impacts both transplantation outcomes and the development of comorbidities [9-13]. Therefore, non-standard donor and living donor transplants represent valid options to increase the donor pool and reduce waiting times for patients with chronic kidney failure [14].

Focusing on the Italian reality, data from the "Activity of Organs, Tissues, and Hemopoietic Stem Cells, Donation & Transplantation" Report, prepared by the Organ Transplantation Information System (SIT) for 2018, show a clear upward trend between 2014 and 2018, with a 24.4% increase in donations and a total of 3,718 transplants, including 2,117 kidney transplants (+20.4%). As of the end of the last year, there were 8,713 patients awaiting a transplant, compared to 8,743 in the previous 12 months. The transplantation quality indices were also extremely positive. For instance, the one-year survival rate for kidney transplant recipients was 97.3%, and 93.0% of patients were able to return to work or were in a condition to do so after the transplant. Nonetheless, the number of patients on the waiting list for kidney transplantation remains stable over the years (6,545 in 2018, compared to 6,683 the previous year) [15].

2. “NON-STANDARD" DONORS

The introduction of "Expanded Criteria Donors" (ECD) or non-standard donors stems from the ongoing need to bridge the gap between the progressively growing number of patients on the transplant waiting list and the limited available donors.
The term "Expanded Criteria Donors" (ECD) first appeared at the American Society of Transplantation meeting in Crystal City in the early 21st century and described a donor aged >60 years or between 50 and 59 years with 2 of the following comorbidities: brain death due to cerebrovascular accident, positive history of hypertension, and serum creatinine >1.5 mg/dL [14, 16].

Considering that a significant percentage (21%) of CKD patients on the waiting list for kidney transplantation are aged over 65, in the United States and much of Europe, allocation models include the use of kidneys from non-standard donors for transplantation in elderly patients, under the "old-for-old" program [17-18].

Several reports describe the reduction in morbidity rates, improvement in quality of life, and life expectancy in elderly CKD patients transplanted with kidneys from non-standard donors compared to patients on dialysis treatment: according to the analysis by Schold and Meier-Kriesche [19], elderly patients (>65 years) would have a greater advantage in receiving a kidney from a non-standard donor compared to younger patients (aged 18 to 39), for whom a standard donor is recommended, regardless of dialysis waiting time. Multivariate analysis of elderly donors compared to younger ones did not show any correlation between donor age and transplant failure in a study that examined 441 patients who underwent kidney transplantation during an eight-year follow-up. Specifically, the comparison between kidneys transplanted from non-standard and standard donors demonstrated no functional differences in these types of donors at one year of follow-up, while significant differences were observed in histology, serum creatinine levels, and estimated glomerular filtration rate after about five years [20].

Survival rates of elderly patients transplanted with kidneys from non-standard donors show contradictory results. Mezrich et al. [21] recently reported that patients >60 years old transplanted with non-standard donor kidneys had a shorter organ and patient survival of less than a year compared to elderly patients transplanted with standard donor kidneys. A similar analysis with 117 recipients indicated a direct correlation between donor age and graft function at 10 years of follow-up [22].

In the USA, the Organ Procurement and Transplantation Network kidney transplantation committee approved the "Kidney Donor Profile Index" (KDPI) score in 2013, a cumulative percentage scale to estimate the risk of graft failure of a particular kidney donor compared to other kidneys from all donors in the previous year. Thus, a donor with an 80% KDPI has a higher expected risk of graft failure than 80% of all kidney donors used in the previous year [23-24]. The KDPI score includes age, height, weight, ethnicity, serum creatinine level, positive history of diabetes and/or hypertension, HCV positivity, cause of death, and whether the donor is donation after cardiac death (DCD). Since then, with this approach, kidneys were not assessed as coming from standard or non-standard donors, but each kidney was evaluated within a range of KDPI between 0% and 100%. Low KDPI scores (closer to 1%) are associated with potentially longer function compared to kidneys with higher KDPI scores [25-26].

An analysis conducted by Stewart DE et al. [27] showed a progressive increase in the percentage of discarded organs (5.1%) starting from 1988, with a peak of 19% in 2009, which stabilized at 18-19% between 2010 and 2015. The renewed interest in donors with higher KDPI scores arises from the need to reduce the percentage of rejected donors. Although they are associated with delayed graft function, longer hospital stays, and more frequent readmissions compared to low KDPI donors, they contribute to improving survival rates in transplanted patients compared to those waiting on the list [28].

Gandolfini et al. [29] subsequently demonstrated that the rejection of kidneys with high KDPI scores could be postponed until the execution of pre-transplant donor biopsies (PTDB) to obtain a more accurate assessment of non-standard donors and the potential organ to be transplanted.

It is known that over 20% of available kidneys come from donors over the age of 65 or those with diabetes and/or arterial hypertension, often considered unsuitable for donation due to the increased risk of early graft failure. The long-term results of non-standard grafts are a consequence of an imbalance between the number of vital nephrons and the metabolic demand of the recipient [30]. The proposal was to estimate the remaining nephron mass through the standardized evaluation of pre-transplant donor biopsies (PTDB).

The indication for PTDB includes the donor's age ≥65 years, estimated creatinine clearance <60 ml/min, or proteinuria >1 g/day. All donors evaluated through PTDB are clearly non-standard.

Each part of the renal parenchyma (vessels, glomeruli, tubules, and connective tissue) is assigned a score ranging from 0 (no alteration) to 3 (alterations present in >50% of the sample) according to the Karpinski-Remuzzi score [31-32]. The scores of each structure are summed to obtain a final score ranging from 0 to 12 for each kidney. Kidneys with a final score ≤4 are assigned as single transplants, and those with a score from 5 to 6 are assigned as dual transplants. When the sum of the scores of the left and right kidney exceeds 12, the kidneys are discarded.

Based on this analysis, comparing our data (in Italy, the evaluation of suboptimal donors through the Karpiski-Remuzzi score obtained from pre-transplant renal biopsies is now standardized) with those from the OPTN / UNOS registry [29, 33], the discard rates for kidneys with KDPI between 80-90% and 90-100% were 36.3% and 62.5%, respectively, compared to only 14.9% and 36.8% in our sample. It is estimated that a strategy based on PTDB could increase the number of kidney transplants from donors with a KDPI of 80-100 by more than 20%, corresponding to an overall increase in transplantation of about 4% considering the entire donor pool. Although non-standard kidney transplantation initially exhibits a lower estimated glomerular filtration rate (eGFR), graft survival at an average follow-up of 3.3 years was similar among different donor types.

Similar results come from the Spanish group led by Martínez-Vaquera [34]. They also consider pre-transplant histological study valuable for selecting functional organs. Performing renal biopsies on ECD is a routine practice in their center without increasing the cold ischemia time of the kidneys. According to a careful analysis of risk factors, it was possible to identify a subgroup of suitable non-standard donors. The comparison between transplanted organs from non-standard donors and discarded ones showed that the unused ones came from donors with a higher average age and more cardiovascular pathologies and a significantly higher biopsy score. The one-year organ survival rates were similar in both donor groups (94% vs. 95%; log-rank test: P=0.38). However, a score >5 was associated with a significant reduction in graft survival compared to a score <5. Moreover, there were no differences in the incidence of complications (vascular thrombosis, urinary fistula, or lymphocele) between recipients from ECD and non-ECD.

Additionally, the work of Sanchez-Escuredo et al. [35] leads to the same conclusions: the introduction of PTDB results in a percentage of discarded donors with higher KDPI (>91%) lower than 19.8%.

Furthermore, employing non-standard donors to expand the donor pool can favor preemptive transplants, avoiding exposure to dialysis and improving the quality of life of the patient, as well as the costs associated with replacement therapy and its consequences. Recently, Chopra et al. [36] evaluated the outcomes in patients over 60 years old who underwent preemptive transplantation with non-standard kidneys compared to those who received a transplant with a KDPI of 35-84% after 1-4 or 4-8 years of dialysis. The risk of graft failure and death-censored graft failure in patients who received preemptive transplantation with suboptimal kidneys was similar to patients with low KDPI kidneys on dialysis for 1-4 or 4-8 years, concluding that the preemptive acceptance of a suboptimal kidney in subjects over 60 years old is not associated with worse outcomes than those with lower KDPI and already on dialysis.

Jay et al. [37] compared the survival of transplanted patients with kidneys with KDPI >85% (both preemptive and non-preemptive) and patients on the waiting list for kidneys with KDPI 0-85%. Based on retrospective data, the study concluded that patients over 60 years old who received a kidney transplant with KDPI >85% had a lower post-transplant mortality within one year compared to patients who remained on the waiting list for a lower KDPI kidney, stating that transplantation with KDPI >85%, especially preemptive, was associated with increased long-term survival compared to remaining on the waiting list for a lower KDPI kidney in patients over 60 years old.

Finally, identifying whether a potential recipient could obtain a survival benefit from a suboptimal kidney transplant is important in order to safely increase the use of this resource not only for elderly recipients. In this regard, the study by Wey et al. [38] analyzed candidates aged between 30 and 40 years with a waiting time of 5 years on the list and calculated the probability of a standard transplant in the 5 years following the offer of a kidney with a KDPI of 95%. The results showed that candidates were unlikely to undergo a standard donor transplant within that time frame. Therefore, due to the low likelihood of transplantation, accepting an offer of a high KDPI kidney (>85%) could maximize the likelihood of a functioning transplant for both young and older patients.

3. KIDNEY PROCUREMENT FOR TRANSPLANTATION

The term "procurement" refers to the removal of organs from the donor for transplantation purposes. The beating-heart multiorgan donor represents the primary source of organs (kidneys, lungs, pancreas, liver, and intestines) and is the only possibility to obtain a heart for most Italian transplant centers. In "suboptimal" donors due to age and/or comorbidities, only the liver or kidneys can be retrieved.

Multiorgan retrieval techniques have evolved from the classical and meticulous in vivo dissection of individual organs to the flexible multiorgan acquisition procedure proposed by Starzl in 1987. In this technique, the portal vein is isolated, the aorta is clamped, and the liver, pancreas, and kidneys are retrieved as a block using a "no-touch" technique. Modern techniques involve rapid retrieval of organs previously cooled through hypothermic perfusion. The advantages of rapid multiorgan retrieval using "no-touch" techniques include better organ preservation, reduced vasospasm, shorter operative times, reduced blood loss, and the possibility of the procedure being performed by less experienced operators. However, the disadvantages include the difficulty in identifying anomalous vessels, particularly multiple renal arteries, which are pulsating in the beating-heart donor but become less visible after cold perfusion.

The various stages of the multiorgan retrieval procedure performed by different teams can be summarized as follows:

1. A median sternolaparotomy is performed, and a self-retaining retractor is placed on the sternum and abdomen. The sternal incision should always be made, even if heart-lung retrieval is not planned, to explore the thoracic cavities and exclude the presence of lymphadenopathy or neoplastic lesions. Additionally, this procedure facilitates cardiac massage in unstable donors. The cardiac surgeon evaluates the heart visually and palpates it, suspends the pericardium at the edges of the sternotomy with sutures, and isolates the superior vena cava, ascending aorta, and pulmonary artery after mobilizing them. The inferior vena cava is also isolated by encircling it with a tape. A tobacco pouch is prepared on the ascending aorta for the infusion of cardioplegic solution. This preparation is necessary in cases of severe donor hypotension and the need for rapid heart retrieval.
2. After ligating the falciform, round, and left triangular ligaments, the liver is retracted upwards and outwards to expose the diaphragmatic pillars and move the esophagus to the left. A longitudinal incision is made on the diaphragmatic pillars between the esophagus and the inferior vena cava to facilitate the exposure of the supra-celiac aorta, which rarely gives rise to collateral branches at this level.
3. The Kocher maneuver is performed to free the lateral edge of the second and third portions of the duodenum from the retroperitoneum. Mobilizing it medially provides easy access to the common bile duct, hepatic artery, gastroduodenal vessels, right gastric artery, inferior renal pole on the right, and left renal vein is also visualized by extending the Kocher maneuver.
4. The inferior mesenteric vein is isolated under the transverse mesocolon, which, in the case of liver retrieval, is cannulated for portal perfusion. A 24-28 French cannula is inserted into the aorta.
5. In concurrent liver and kidney retrieval, the fundus of the gallbladder is incised using an electrobisturi and washed with saline to drain bile and avoid autolysis during the period of hepatic perfusion and hypothermic preservation.
6. If a nasogastric tube is present in the duodenum (useful for sterilizing the duodenal segment in pancreas retrieval), it is removed by the anesthetist.
7. The donor is heparinized with 3 mg/kg intravenously. An angiocatheter is placed on the supra-diaphragmatic aorta and superior vena cava. Five minutes after systemic heparinization and simultaneous to cardioplegia, cold perfusion is initiated with a preservation solution through the infra-

diaphragmatic aorta and, if the portal vein is perfused, also through the inferior mesenteric vein. The perfusion fluid drainage is performed to decompress the splanchnic area by continuously flowing the solution into the inferior vena cava at its junction with the atrium, allowing the liquid to flow out of the field. Typically, an infusion volume of 40 ml/kg is required in adult donors. Cooling can be accelerated by introducing sterile ice into the abdominal cavity.

8. Once the thoracic organs are retrieved, the liver and pancreas are removed after mobilizing them from the retroperitoneum, following ligation of the right gastric artery and gastroduodenal vessels and isolation of the common hepatic artery up to the celiac trunk. The infra-hepatic inferior vena cava is sectioned above the confluence of the renal veins, and the supra-hepatic vena cava is preferably left with a diaphragmatic patch. The common bile duct is sectioned at the upper border of the pancreas. The celiac axis can be retrieved with either the liver or pancreas. After ligating the splenic vessels, the superior mesenteric artery and vein, the gastroduodenal artery, the left gastric artery, and the portal vein are dissected, usually exposing an anomalous right hepatic artery, which originates from the superior mesenteric artery in about 12% of cases. In this case, an aortic patch can be harvested, including the mesenteric artery or celiac trunk, to avoid injuring the right renal artery. Another anomaly to look for during the dissection of the gastroepiploic omentum is the left hepatic artery, which comes from the left gastric artery in about 15% of cases. The isolation of the pancreas is performed by cutting the duodenum distally to the pylorus using a GIA-type stapler. The removal of the stomach by cutting it at the level of the esophagogastric junction facilitates the isolation of the pancreas and celiac trunk. After sectioning the phrenocolic ligament, the spleen and the tail of the pancreas are mobilized medially to the junction of the splenic vein with the superior mesenteric vein and the splenic artery with the celiac axis, taking care not to damage the pancreaticoduodenal arteries. If not done previously, the inferior mesenteric vein, the left gastroepiploic artery, and the right gastric artery are sectioned between ligatures. The sectioning of the common bile duct at the upper border of the pancreas, after an extensive Kocher maneuver, exposes the head of the pancreas. The perfusion of individual organs is then completed on the bench, separating the spleen from the pancreas by sectioning the splenic vessels at the hilum.
9. During multi-organ retrieval, excessive manipulation of the kidneys before supra-renal aortic clamping can lead to portal hypoperfusion and compromise the outcome of liver transplantation. If only the kidneys are being retrieved, we start with points 1-3 as described earlier. If liver and pancreas retrieval has already been performed, interrupting the celiac axis and the superior mesenteric artery facilitates exposure of the retroperitoneum.
10. The incision of the right paracolic gutter completes the medial mobilization of the ascending colon and terminal ileum, and the superior mesenteric artery is ligated distally. We proceed with aortic clamping and the aforementioned cold perfusion. The mobilization of the left colon and sigmoid is performed after sectioning the Toldt's fascia, and the inferior mesenteric vessels are ligated.
11. The kidneys are freed from the psoas and retroperitoneum, preserving the Gerota's capsule, which will be removed at the bench. The ureters are cut just above the bladder dome, avoiding excessive traction. Periureteral adipose tissue must be preserved in the "Golden triangle" area (from the distal portion of the ureter to the lower pole). The ureteric artery runs through this periureteral adipose tissue, being the sole remaining blood supply to the ureter after retrieval and emerging from the

lower edge of the renal artery at the hilum. Injury to this artery can lead to urological complications, including ischemic necrosis of the ureter.

12. The aorta and cava are sectioned below the cannula insertion point after careful inspection for any inferior polar arteries emerging from the common iliacs. The aorta, cava, and ureters are then pulled upward, bluntly interrupting the posterior lumbar vessels in a cranial direction up to the renal hilum. At this point, the major vessels are cut below the diaphragm.
13. The block containing both kidneys is removed and immersed in cold saline and ice, wrapped in a laparotomy sheet. The vascular elements are then isolated on the bench. After flipping the kidney block, the left renal vein is cut at the caval junction, leaving the entire right renal vein attached to the cava.
14. The posterior surface of the aorta is incised longitudinally, exactly in the middle of the emergence of the posterior lumbar arteries, starting from the proximal aorta and respecting any anomalous arteries. This reveals the ostia of the visceral arteries from inside the abdominal aorta.
15. An aortic patch is cut, encompassing the celiac tripod and the superior mesenteric artery. A transverse incision is then made between the ostium of the superior mesenteric artery and the ostia of the renal arteries. A second transverse incision is performed downstream of the renal arteries to leave them with a large patch, and the two kidneys are subsequently separated with a longitudinal incision on the anterior wall between the two arteries.
16. The iliac arteries and veins are harvested while preserving the bifurcation. It is advisable to also harvest the great saphenous veins by cutting them 2 cm above the knee to ensure the execution of any reconstructions at the bench.

The surgeon who will transplant the kidneys may encounter the following problems after a multi-visceral retrieval: a) excessive subhepatic cava sectioning, damaging the right renal vein; b) injury to any aberrant renal arteries originating from the iliacs; c) retrieval of an overly "generous" aortic patch by the liver retriever, compromising the right renal artery [39-40].

4. KIDNEY PERFUSION

The renal parenchyma, when subjected to interrupted blood flow, rapidly undergoes severe suffering due to acute ischemia, leading to irreversible damage unless its cellular metabolism is intervened upon. The term "warm ischemia" refers to the period between the interruption of blood flow and the start of cold perfusion. The "cold ischemia" time corresponds to the hypothermic preservation, and finally, the "second warm ischemia" time describes the interval between the organ's removal from the cold container and revascularization (essentially the duration of vascular anastomosis packaging). It should be noted that the actual warm ischemia period in the deceased donor may be longer than calculated: any cardiac arrests or episodes of prolonged hypotension (mean arterial pressure <80 mmHg) during the observation period may cause hypoperfusion of vital organs, adding to the damaging events that occur during the surgical handling of the harvest.

Organ preservation techniques play a crucial role in the context of the decreasing availability of standard donors (SCD) due to advances in road safety and neurosurgery, and the increasing use of expanded criteria donors (ECD) and donation after cardiac death (DCD) due to the persistent gap between the number of patients on the waiting list and available donors. The latter donors are characterized by brain damage caused by ischemia, which triggers an inflammatory process capable of damaging organs even before their removal or at the moment of reperfusion. Cerebral ischemia leads to hemodynamic instability, hypothermia, coagulopathy, and electrolyte imbalances, which cause renal tubules (in this specific case) to be exposed to a cascade of inflammatory molecules responsible for increased sodium excretion and fibrotic proliferation of the arterial intima [41].

Hypothermia currently forms the basis of solid organ preservation. Oxygen consumption decreases exponentially with decreasing temperature, reaching 5% at 5°C and 3% at 0°C. In kidney-only retrieval, the organ can be perfused in situ or at the bench with a cold solution ranging from 0°C to 4°C. Simple surface cooling, by immersing the organ in perfusion fluid, allows preservation for up to 12 hours, while intrarterial cold drop perfusion experimentally extends preservation to up to 120 hours. Immediately after retrieval, the organ is immersed in cold fluid, and intrarterial perfusion is simultaneously initiated, continued with at least 700 ml until the efflux from the vein is completely clear. The distance between the bag and the organ should be at least 90 cm. After perfusion, the kidney, wrapped in a laparotomy pad to reduce transport microtraumas, is placed in a sterile bag containing the perfusion fluid. Two additional individually sealed sterile bags are included, and all are placed in melting ice in a thermal container, with occasional ice added to maintain optimal preservation for more than 45 hours, although it is usually preferred not to exceed 24 hours.

Hypothermic perfusion and preservation aim to: a) prevent intravascular coagulation; b) prevent cellular edema with mannitol and glucose, sucrose and raffinose, lactobionate and gluconate, depending on the fluid used; c) maintain pH with phosphate, citrate, histidine, and bicarbonate; d) prevent damage from oxygen-derived free radicals that result from collateral metabolism and redox reactions through allopurinol, glutathione, ascorbate, mannitol, histidine, and tryptophan; e) moderate cellular calcium with diltiazem, verapamil, trifluoperazine; and f) provide nutrients with glucose, ATP, and adenosine.

Although universally widespread, hypothermic preservation has limitations regarding the integrity of renal cells and the restoration of cellular homeostasis, which can be summarized as follows:

1. Lowering of intracellular pH and blocking of glycolysis: tissue hypoxia rapidly reduces ATP levels, leading to anaerobic metabolism to support cellular metabolic processes, leading to the production of lactic acid and acidosis. Severe acidosis activates phospholipases and proteases, causing lysosomal damage and cell death. Maintaining pH values between 6.9-7.0 would be protective by inhibiting phosphofructokinase and subsequent glycolysis block [42].
2. Blocking fatty acid oxidation;
3. Blocking active ion transport mechanisms: altered function of the Na+/K+ ATP-dependent pump causes passive entry of sodium into cells (attracted by the negative charges of cytoplasmic proteins), creating a hyperosmolar environment with resulting water passage and cellular swelling. Moreover,

there is an increased intracellular calcium ion (Ca++) concentration due to malfunctions in the Na+/Ca++ and Ca++ ATPase pump: calcium accumulation leads to calpain activation and transduction of the signal mediated by protein kinase C, which damages the cytoskeleton by direct damage to spectrin [43].

4. Preservation of passive ionic diffusion;
5. Generation of free radicals: predominantly reactive oxygen species (ROS) through the enzyme xanthine oxidase. ROS are responsible for irreversible damage to lipids, proteins, and nucleic acids, especially during the reperfusion phase of organs.
6. Enzymatic activation: Hypoxia observed during organ preservation activates matrix metalloproteinases, leading to matrix protein degradation and rupture of bonds with endothelial cells, activation of the caspase family mediating cellular apoptosis, and release of iron from cytochrome P 450 responsible for ROS synthesis [44].

4.1 Hypothermic Machine Perfusion (HMP)

Pulsatile perfusion has been the first method of organ preservation since the inception of the first transplants. The implementation of perfusion solutions and their diffusion, replacing HMP, occurred subsequently based on the same results obtained by both methods but at lower costs. Due to modifications in the donor pool, HMP has recently been reintroduced in an attempt to improve the quality of "non-standard" organs.

One example of a device used to illustrate its operation is the LifePort® Kidney Transporter (Organ Recovery Systems, Brussels, Belgium. Figure 1), but other devices are available on the market (the KidneyAssist®, OrganAssist; Groningen, Netherlands, and the Waters RM3® system, Rochester, Minnesota), which generally have the same characteristics with minimal variations in perfusion temperature (4°C-10°C) and flow type (pulsatile vs. non-pulsatile).

The device includes an insulated housing inside which the kidney is immersed in perfusion fluid and surrounded by a casing containing sterile ice. The renal artery is connected to a circuit that allows for pulsatile but continuous flow of perfusion fluid through a cannula connecting the kidney to it. The fluid exits through the renal vein and is collected via aspiration from the reservoir to continue the process. The perfusion circuit is a sealed fluid path that draws from the perfusate bath and delivers it to the kidneys. It also has a pressure sensor to control the systolic and diastolic pressure of the set perfusion process (diastolic pressure is determined by the kidney's resistance to flow). Parameters such as flow, resistance, and temperature are monitored through an external display connected to the device's computer [45].

Currently, the Belzer® solution (KPS-1, Organ Recovery Systems, Brussels, Belgium) is commonly used for kidney perfusion during HMP. It is a gluconate-based perfusate containing hydroxyethyl starch and, unlike the cold storage (CS) solution used in static perfusion, it has a low potassium concentration to reduce vasoconstriction.

Since the early studies on the preservation of organs from deceased donors, it became evident to Calne et al. [46] that cold ischemia played a fundamental role in both short-term and long-term graft survival, even more than immunological reactions.

The damage caused by cold ischemia manifests as "delayed graft function" (DGF), which refers to the need for dialysis within the first week after transplantation. The mechanism of DGF is not solely limited to ischemic damage to renal tubules but is associated with a constellation of thrombotic and inflammatory effects mediated by cytokines and both innate and adaptive immune responses. Therefore, kidneys that develop DGF after transplantation have an increased risk of acute rejection, longer hospital stays, and higher overall costs. However, the impact of the duration of DGF on transplant outcomes is limited and controversial. In a cohort analysis of 1412 recipients, DGF had a duration of more than 15 days in 25% of patients, and it was an independent factor for reduced transplant survival [47].

The use of kidneys from expanded criteria donors (ECD) has become common practice in the last decade, with 70% of these organs being transplanted into patients aged 50 or older [48]. HMP can reduce ischemia-reperfusion injuries and offer an additional opportunity to evaluate the quality of the organ to be transplanted through the study of device parameters, especially for this type of donors.

According to a study by Lazaro et al. in 2016 [49], which involved 12 mini pigs subjected to 45 minutes of vascular clamping, followed by right nephrectomy and preservation of half of them using HMP or CS before autotransplantation, the overall renal ischemia resulted in significant histological damage compared to the contralateral control kidneys. The structural renal damage was characterized by tubular necrosis, mesangial expansion, tubular dilation, interstitial inflammation, and hyaline deposits in renal tubules. Hypothermic preservation before autotransplantation was associated with increased caspase-3 activation, which mediates apoptotic pathways, and the production of TGF-β1, responsible for extracellular matrix proteins leading to interstitial fibrosis and glomerulosclerosis [50]. The use of HMP significantly decreased the levels of activated caspase-3 and TGF-β1 in the kidneys compared to controls.

In a review published this year by Tingle et al. [51], comparing CS and HMP, sixteen studies (2,266 participants) were included. The analysis confirms that:

- The use of HMP instead of static hypothermic preservation reduces the risk of DGF by approximately 23%, with strong evidence of a reduction in its duration. In detail, the study by Moers in 2009 conducted subgroup analysis to compare standard criteria donors (SCD) with ECD. The incidence of DGF was lower with HMP versus SC in both subgroups, SCD (OR=0.59, 95% CI=0.35-1.02), and ECD (OR=0.51, 95% CI=0.24-1.09).
- Three studies (Moers 2009; Tedesco-Silva 2017; Zhong 2017) observed significantly faster short-term graft functional recovery with HMP, perfectly in line with the lower incidence of DGF reported in the HMP group by these studies.
- Based on evidence from seven studies, it was not demonstrated that HMP has an impact on the incidence of PNF (primary non-function) (RR=0.88, 95% CI=0.58-1.33; P=0.55): PNF is an unavoidable event unaffected by HMP, given its multifactorial nature.
- There is clear evidence that HMP positively impacts both short- and long-term transplant survival, for both donation after brain death (DBD) and donation after cardiac death (DCD) donors. This is predictable since previous research has shown that DGF is associated with higher graft loss rates (Yarlagadda 2009). The significant improvements observed in short-term organ function are similar to the significant improvements seen in the incidence of DGF.
- The effect of HMP on other studied variables (incidence of acute rejection, patient survival, hospital stay, long-term organ function) remains under investigation.

Lastly, HMP can be rational for use in elderly recipients with a long history of dialysis and associated complications who do not have the option of a living donor. Patients on the waiting list may need further clinical and instrumental assessments, prolonging the graft's ischemic time due to their fragile clinical condition. A study published by the Birmingham group in 2015 [52] supports this concept: the use of HMP can allow a longer ischemic time without increasing the percentage of DGF since kidneys treated with HMP seem to have similar potential to kidneys in CS, despite longer cold ischemia times during transplantation. Additionally, for ECD donors matched with this type of recipients, comparing HMP parameters can be helpful in the qualitative evaluation of the graft.

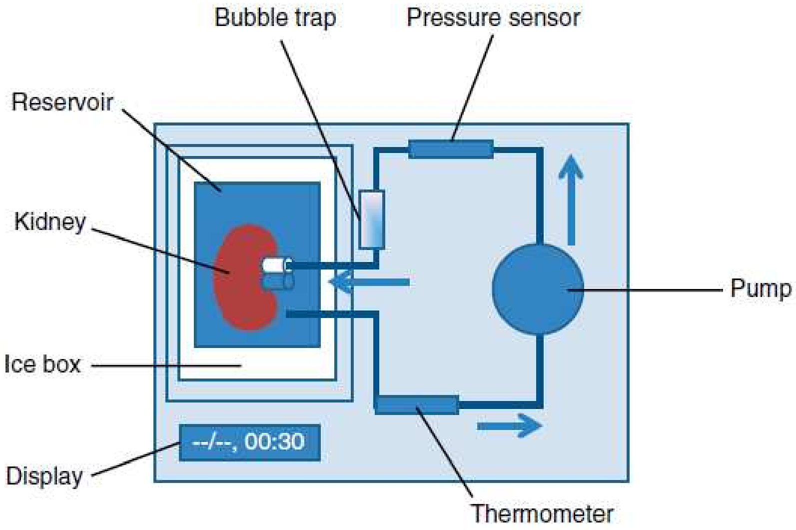

Figure 1. Diagram of the hypothermic pulsatile perfusion device

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5. KIDNEY TRANSPLANTATION

The technique of kidney transplantation has now become a standardized procedure, similar across all transplant centers, differing only in some technical details of vascular anastomoses and reconstruction of the urinary tract. The kidney is placed in an ectopic position, either in the right or left iliac fossa, extraperitoneally.

This choice is motivated by the following reasons: a) The simplicity and speed of access to the virtual space that will host the graft. b) The rapidity of anastomosis of the renal vessels to the recipient's iliac vessels, which are easily isolated, have a high flow rate, and a similar caliber. c) The possibility of keeping the organ in an extraperitoneal location, which reduces the potential for infection. d) The short distance between the transplanted organ and the bladder allows the use of the proximal part of the ureter, which is better vascularized (the ureteral vessels from the distal third of the ureter coming from the vesical artery are interrupted during retrieval). e) The superficial position of the organ allows for easy evaluation of its dimensions, consistency, and tension on the abdominal wall. Furthermore, invasive examinations with reduced risks, such as biopsy or the placement of a nephrostomy catheter, can be performed. The location of the transplanted kidney also makes it easier to perform reoperations on the renal vessels, revise the urinary tract, or remove the organ.

The patient is positioned supine on the operating table, and a catheter is placed in the internal jugular or subclavian vein to assess the recipient's hydration status by measuring central venous pressure and to administer fluids rapidly. The side of the incision depends on the presence of scars from previous surgeries or the location of a possible peritoneal catheter. If there is no specific preference, the right iliac fossa is often chosen because the vessels are more superficial in that position.

The skin incision is oblique, starting from the iliac region with an upward concavity. The upper portion of the wound is located 3 cm medially to the anterior superior iliac spine and directed towards the pubic symphysis. Some surgeons prefer an alternative J-shaped skin incision that starts just above the inguinal ligament and runs along the lateral border of the rectus abdominis muscle because it provides more medial access and allows for better visualization of the bladder. The skin incision corresponds to an incision on the fascia of the external oblique muscle. A pararectal incision is then made to reach the transversalis fascia, which is incised, and the inferior epigastric vessels are ligated.

In women, the round ligament is interrupted, while in men, the spermatic cord is isolated, and its section is only necessary in special cases to prevent angulations of the transplanted ureter. The iliac vessels are reached by medially displacing the peritoneum, which should be carefully avoided from being injured. To prevent postoperative lymphorrhea, it is recommended to perform limited isolation of the iliac vessels and ligate all perivascular lymph collectors with transfixing stitches. The most critical surgical risk occurs during the isolation of the external iliac vein and the ligation of its small tributary vessels. During the anastomosis, the kidney is wrapped in a moist laparotomy pad soaked with cold preservation fluid in which it was previously immersed.

Before starting the arterial anastomosis, it is necessary to ensure the presence of atherosclerotic plaques, any "flaps" inside the renal artery, or dissection of the muscular tunics. In these cases, it is advisable to resect the involved segment of the artery until reaching a healthy zone, and if necessary, perform a bench reconstruction to extend the artery. If the dissection of the arterial tunics continues proximally, it is prudent to place at least 3 sutures of 6/0 or 7/0 Prolene at a distance of about 120 degrees from each other to fix the intima. In our standard technique, the arterial anastomosis is performed by suturing the renal artery termino-laterally to the recipient's external iliac artery with two semi-continuous stitches using 6/0 or 7/0 Prolene with a double needle.

The renal vein is anastomosed termino-laterally to the external iliac vein using two semi-continuous stitches with 6/0 or 5/0 Prolene with a double needle. The incision on the external iliac vein, performed between two soft angiostats (as for the artery), must take into account the size of the renal vein. A relatively frequent finding is the presence of a valve in the external iliac vein; it is prudent to resect its leaflets and suture them to avoid "flaps" that could cause potential thrombosis. On the renal vein, a large caval patch should be left. To avoid trapping the contralateral venous wall or the adventitia intrusion into the suture, in addition to the two sutures between the renal vein and the external iliac vein, positioned at 180 degrees, a third temporary suture at 90 degrees, on the contralateral side, can be helpful and should be maintained during the execution of the anastomosis on the anterior wall. Subsequently, the kidney is rotated to facilitate the construction of the anastomosis using an extroflection technique on the medial side. At declamping, the kidney is irrigated with warm saline, and the pressure values are monitored to ensure adequate organ filling. In the case of bleeding from the anastomosis, it is preferable to apply immediate hemostatic stitches rather than re-clamping the vessels. Further warm ischemia can be detrimental to the functional recovery of the organ.

The urinary tract can be reconstructed with various techniques, but those that involve creating a ureterocystoneostomy with an antireflux tunnel are currently the most common. The technique of ureterocystoneostomy according to Leaddbetter-Politano involves a wide opening of the bladder: two small incisions are made on the mucosal wall of the base, between which a subcutaneous tunnel is created, and the ureter is drawn through it after passing through the bladder wall. The proximal mucosal incision is sutured with an absorbable 5/0 or 6/0 stitch, and the distal end of the ureter is spatulated and anastomosed with absorbable 5/0 or 6/0 stitches, sinking the two ureteral and bladder mucosas. The bladder is subsequently sutured with absorbable 3/0 or 4/0 stitches, and endoureteral stents are not necessary. The technique of extravescical ureterocystoneostomy according to Gregoire-Lich is more common because it involves only a small opening of the bladder, corresponding to the ureteral orifice. The bladder is distended by filling it with physiological solution and antibiotics. The serous and muscular tunics of the dome are incised for 3-4 cm, and a continuous solution is made in the mucosa that protrudes to the anterior outside of the incision. The spatulated ureter is anastomosed to the bladder dome with 6-8 detached absorbable 5/0 or 6/0 stitches, and the muscular tunic is reconstructed, sinking the ureter with a three-layered detached suture of 4/0 of the same material to create an antireflux valve. The advantage of using the Gregoire-Lich technique over the Leadbetter-Politano one is the small bladder incision, which minimizes the risks of contamination and dehiscence, which can be high in defunctionalized bladders.

A soft drain, such as a Redon drain or one equipped with a closed system, is left in place in the perirenal and paravesical space, with the latter being removed between the sixth or seventh postoperative day. The bladder catheter is generally removed before removing the lower drain (fifth or sixth postoperative day). [39, 53]

6. SCOPE OF THE STUDIES

We hypothesized that the post-KT graft function recovery was faster in patients receiving deceased brain death (DBD) single grafts perfused with hypothermic perfusion machine (HPM) with respect to patients with DBD single grafts preserved in static cold storage (SCS).
We aimed at comparing the post-KT clinical course of two preliminarily "balanced" groups of patients undergoing KT with DBD grafts perfused with HPM or preserved with SCS.

Regarding the subpopulation of deceased donors with Karpinski score > 3 the main objective of the study was to investigate the role of HMP in terms of delayed graft function (DGF), recovery of organ function through reduction of serum creatinine (sCr), and organ survival at six months post-transplant.
The secondary aim was to use HMP to determine a threshold value of the initial renal resistance index (RR) capable of selecting patients at risk for poor functional recovery of the graft.

7. MATERIALS AND METHODS

7.1 Entire population of transplanted kidneys

Study Design
This is a retrospective monocenter observational study investigating the data of patients undergoing a KT using a DBD graft.
The present study was approved by the Local Ethics Board of Sapienza University of Rome. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed to create the study.

Population
During the period January 2014 – September 2021, 313 patients were transplanted at Sapienza University of Rome. The population was stratified in two groups according to the type of graft preservation: SCS Group (n=218, 69.6%), and HPM Group (n=95, 30.4%).

Outcomes measures
The main outcome of the study was the recovery of the transplanted graft after single KT defined as the absence of a post-transplant delayed graft function (DGF) defined at day 7 after KT.
Secondary outcomes were: a) urine outputs at day 2 and 7, b) intensive care unit (ICU) stay duration; c) hospital stay duration. The last follow-up date was October 2021.
DGF was defined in two different ways: a) need for dialysis within seven days from KT (x); and b) creatinine reduction ratio at day 7 (CRR7) ≤70%. CRR7 was calculated using the following equation: $CRR7(\%) = ([sCr0–sCr7]\times 100)/sCr0$.
sCr0 corresponded to the sCr levels immediately before KT and no later than six hours after the last dialysis; Cr7 corresponded to the sCr levels at post-KT day 7.
Cold ischemia time (CIT) was calculated from the cross-clamp time until the end of the HMP.

The quality of the grafts was calculated using the Kidney Donor Profile Index (KDPI). The last available scaling factor was used for calculating the KDPI (year=2020; number=1.28654071484779).

A pre-KT biopsy of the graft with the intent to establish the transplantability of the organ was done in al the following cases: a) donor age ≥60 years; or b) donor age of 50-59 years with two or more of the following comorbidities: a history of hypertension, death resulting from cerebrovascular accident (CVA), and terminal sCr ≥1.5 mg/dL.

The histological graft quality was assessed by a group of expert pathologists using the Karpinski Score. In all the cases, the histological quality information was available before the beginning of the KT. Grafts presenting a Karpinski Score ≤3 were judged to be usable for a single KT. In case of a Score ranging 4-6, we decided case by case to use the graft for a single KT according to the following parameters: a) absence of diffuse gross vascular atherosclerosis in the graft; b) absence of severe microscopically assessed pyelonephritis. In no case, we performed a double KT. When the Score was ≥7, the kidney was directly discarded.

The LifePort® Kidney Transporter machine (Organ Recovery Systems, Brussels, Belgium) and KPS-1 solution (Organ Recovery Systems, Brussels, Belgium) were used for HMP. Renal resistances (mmHg/mL/min), flow (ml/min), and temperature (°C) were recorded at different time points: at the beginning of perfusion, at 20 minutes, 40 minutes, 60 minutes, and 120 minutes. All kidneys were perfused for at least 120 minutes. The cold ischemia time (CIT) was calculated from cross-clamp until the end of HMP.

There were no technical problems related to arterial cannulation, nor accidental decannulation during perfusion.

During the postoperative period, the following recipient data were collected: daily sCr, the need for dialysis, and length of hospital stay. DGF was defined in two different ways: a) the need for dialysis within 7 days of transplantation; b) the percentage reduction of creatinine on the seventh postoperative day (CCR7) ≤ 70%. CCR7 was calculated using the following equation: CCR7 (%) = ([sCr0-sCr7] × 100) / sCr0). sCr0 corresponds to sCr levels immediately before transplantation and no later than six hours after the last dialysis, while sCr7 corresponds to sCr levels on the seventh postoperative day.

Graft loss was defined as the return to dialysis or sCr clearance < 15 ml/min/1.73m2 at the last assessment. None of the kidneys in the machine perfusion group were removed due to technical problems nor were there any rate of arterial thrombosis.

Statistical analysis

Continuous variables were reported as medians and interquartile ranges (IQR). Categorical variables were described as numbers and percentages. Comparisons between groups were made using Fisher's exact test or chi-square test for categorical variables, as appropriate. Mann-Whitney was used for continuous variables. Missing data relative to study covariates always involved less than 10% of patients. Missing data were reported in detail in **Supplementary Table 1**. In all the cases, missing data were handled with a single imputation method. In detail, a median of nearby points imputation was adopted. The median instead of the mean was adopted due to the skewed distribution of the managed variables.

The entire population was divided in two groups according to the use of HPM.

With the intent to compensate for the non-randomized design of this retrospective study, the two groups were “balanced” using a stabilized inverse probability therapy weighting (IPTW).

A propensity score for each patient on the original population was generated. The score was created using a multivariate logistic regression model considering the need for dialysis within the first week after KT (no versus yes) as the dependent variable. We selected 26 possible clinically relevant confounders as covariates: patient age, patient male sex, patient body mass index (BMI), re-transplantation, pre-emptive KT, dialysis duration, patient arterial hypertension, patient type-2 diabetes mellitus (T2DM), patient cytomegalovirus-antibody (CMV-Ab) positivity, patient hepatitis B surface-antibody (HbS-Ab) positivity, patient hepatitis C virus-antibody (HCV-Ab) positivity, donor age, donor male sex, donor BMI, donor CMV-Ab positive, CVA as cause of donor death, donor hypotension(s), donor cardiac arrest(s), donor history of arterial hypertension, donor history of T2DM, biopsy, surgical complexity caused by multiple anastomoses, donor serum creatinine (sCr), donor ICU stay, KDPI, cold ischemia time (CIT).

All the covariates were available before the end of the KT procedure to avoid the risk of a possible immortal time bias in covariate selection.

With the intent to reduce the artificial modification of the sample size in the pseudo data, we used stabilized weights (SW) according to the formula:

SW = p /PS for the study group, and SW = (1-p)/(1-PS) for the control group

where p is the probability of etiology without considering covariates and PS is the propensity score.

Because p-values can be biased from population size, results from the comparisons between covariates subgroups were reported as effect size (Cohen's D value): values lower than |0.1| indicated very small differences between means, values between |0.1| and |0.3| indicated small differences, values between |0.3| and |0.5| indicated moderate differences, and values greater than |0.5| indicated considerable differences.

Multivariable logistic regression analysis was run after the stabilized IPTW to identify the risk factors for DGF after KT. According to previous research papers focused on the same argument, the investigated variables were selected using a 'full model' approach. A backward Wald method was finally adopted for constructing the final model. Odds ratios (OR) and 95.0% confidence intervals (CI) were reported for significant variables. Variables with a $p<0.05$ were considered statistically significant. Statistical analyses and plots were run using the SPSS statistical package version 27.0 (SPSS Inc., Chicago, IL, USA).

7.2 Subpopulation of kidneys with Karpinski score > 3

We conducted a retrospective analysis of data from 92 kidneys from brain-dead donors evaluated for single kidney transplantation between November 1, 2017, and December 31, 2018. Exclusion criteria were: a) kidneys transplanted without HMP treatment (n=44); b) kidneys perfused with Karpinski score ≤ 3 (n=19); and c) grafts discarded due to poor quality (n=6). Finally, 23 transplanted kidneys were enrolled for the present study.

Statistical Analysis

Continuous variables were reported as medians and interquartile ranges (IQR). Categorical variables were reported as numbers and percentages. The Mann-Whitney U test and Fisher's exact test were used to compare continuous and categorical variables, respectively.

The performance of different variables (initial RR, initial flow, donor serum creatinine, and Karpinski score) in predicting DGF defined as CCR7 ≤ 70% was tested using Receiver Operating Characteristic (ROC) curve analysis. Different threshold values of initial RR were studied in terms of sensitivity, specificity, and diagnostic odds ratio (DOR). We arbitrarily studied RR values of 0.5, 0.75, and 1.0 mmHg/mL/min, approximately corresponding to the fourth, seventh, and ninth decile, respectively. A higher DOR value indicated better test performance.

The probability of transplant survival was estimated using the Kaplan-Meier method. The log-rank test was used to compare survival outcomes. The last follow-up was conducted on June 30, 2019.

Variables with $p < 0.05$ were considered statistically significant. The statistical analyses were performed using SPSS statistical package version 24.0 (SPSS Inc., Chicago, IL, USA).

8. RESULTS

8.1 Entire population of transplanted kidneys

Between January 2014 and September 2021, 313 KT patients merging the inclusion criteria of the study were transplanted.

The pre-KT characteristics of the investigated population were reported in the **Table 1**.

Comparing the SCS and HPM Group, only few differences were observed. As an example, the KT recipients were similar in the two groups in terms of age, sex, underlying renal disease, re-transplantation rates, and history of arterial hypertension or T2DM. Only BMI and duration of dialysis were different, with the HPM cases being with higher BMI values and shorter dialysis duration periods.

As for the donor characteristics, only the history of T2DM was statistically relevant, with higher rates in the HPM Group. On the opposite, the two groups were similar in terms of donor age, sex, BMI, cause of death, hemodynamic instability, history of arteria hypertension, serum creatinine at the time of procurement, ICU length of stay, KDPI, and need for biopsy.

Lastly, CIT evaluated at the time of KT was similar between the two groups.

Stabilized IPTW effect

Despite the two groups showed only limited initial differences, with the intent to minimize further the effect of selection biases caused by the non-randomized design of this retrospective study, the two groups were “artificially” balanced using a stabilized IPTW method. As reported in **Table 2**, the “balancing “was efficaciously done for the 26 potential confounders adopted.

In detail, before the IPTW “balancing” eight variable showed very small differences, 17 small, and one moderate differences. After the IPTW, 24 variables showed very small differences, and only two a small difference. Thanks to the stabilized IPTW, no modification of the initial sample size was observed in the pseudo population.

Post-KT clinical course in the two "balanced" Groups

The results of the post-transplant clinical course observed in the groups after their IPTW balancing are reported in **Table 3**

DGF rates

No differences were observed between the two groups in terms of post-KT DGF rates. In detail, need for dialysis within the first week after KT was observed in 17.9 vs. 15.6% of cases in SCS and HPM cases, respectively (P=0.75). Similarly, a CCR7 <70% was reported in 22.9 vs. 19.8% of SCS vs. HPM cases (P=0.66) (**Figure 1**).

Urine output

Contrarily, urine output was different in the two groups, with higher values at day 2 (P=0.046) and lower values at day 7 (P=0.003) in the HPM Group.

Length of stay

As for the length of stay, HPM cases has a shorter period of ICU stay (P<0.0001) and a shorter, although not statistically relevant, overall hospital stay (P=0.07).

Kidneys requiring biopsy

When the sub-class of cases requiring a biopsy before KT was explored, more relevant differences were observed. As an example, need for dialysis was more common in SCS cases (23.9 vs. 7.7%; P=0.03). The median decrease of creatinine at day 7 was higher, although not statistically relevant, in the HPM Group (62 vs. 53% of decrease; P=0.07). ICU and overall hospital stay were always shorter in the HPM Group (P=0.002 and 0.007, respectively).

Risk factors for DGF (need for dialysis)

Using a multivariable logistic regression analysis, the risk factors for DGF was explored in the entire post-IPTW population (**Table 4**). CVA as cause of death (OR=2.317; P=0.04) was the only significant factor. Duration of dialysis only merged relevance (OR=1.067; P=0.07).

When a sub-analysis focused only on patients with grafts biopsied before KT was done, HPM use merged the statistical relevance as a protective factor (OR=0.308; P=0.06). On the opposite, duration of dialysis merged the statistical relevance as a risk factor (OR=1.109; P=0.07).

8.2 Subpopulation of kidneys with Karpinski score > 3

Population

From November 2017 to December 2018, 92 kidneys were considered for single transplantation; of these, 29 had a Karpinski score > 3. After evaluating the grafts during bench surgery, six kidneys were discarded due to the presence of widespread atherosclerosis (n=2) and severe pyelonephritis (n=4).

Tables 5 and **6** show the characteristics related to donors, recipients, perfusion, and transplantation.

Donor Characteristics

Regarding donor characteristics, the median Kidney Donor Risk Index and age were 1.68 (IQR=1.36-2.17) and 67 years (IQR=60-74), respectively. The median sCr at donation was 1.3 mg/dL (IQR=0.8-1.6). Of the 23 perfused kidneys, 16 (69.6%), 5 (21.7%), and 2 (8.7%) had a Karpinski score of 4, 5, and 6, respectively. The mean age of recipients and cold ischemia time was 61 years (IQR=53-68) and 380 minutes (IQR=298-419), respectively.

HMP data

Regarding HMP data, the median perfusion duration was 247 minutes (IQR=125-330). In no case was it less than 120 minutes. At the start of perfusion, the median RR and flow were 0.57 mmHg/mL/min (IQR=0.46-0.87) and 46 ml/min (IQR=0-56), respectively. After two hours, the same parameters were 0.28 mmHg/mL/min (IQR=0.23-0.36) and 87 ml/min (IQR=65-110), respectively. All kidneys were perfused for at least two hours, and the longest perfusion time was 7.4 hours.

Early outcomes

During the first week after transplantation, only two patients (8.7%) required dialysis, while 16 (69.6%) patients showed a reduction in sCr, defined as CCR7 ≤ 70%. The mean length of hospital stay was 11 days (IQR=9-13).

Late outcomes

At a mean follow-up of 10 months (IQR=7-13), two kidneys were lost (8.7%). Specifically, these had Karpinski scores of 4 and 5 and showed initial RR of 0.68 and 0.90 mmHg/mL/min. The cause of graft loss was primary non-function (PNF) in both cases. The six-month transplant survival rate was 91.3%.

Threshold values of RR for predicting DGF

We studied five different characteristics related to donors, grafts, and HMP to identify predictors of DGF defined as CCR7 ≤ 70% (**Table 7**).

Initial RR showed the best diagnostic capability, with an AUC of 0.83 (P=0.02). The diagnostic capacity of renal resistances remained substantially constant after two hours of perfusion, with an AUC of 0.81 (P=0.03). Flow during perfusion, donor age, and Karpinski Score did not predict post-transplant DGF.

Three different threshold values of initial RR were studied, corresponding to values of 0.5, 0.75, and 1.0 mmHg/mL/min, approximately corresponding to the fourth, seventh, and ninth decile. The cutoff of 0.5 mmHg/mL/min showed very high sensitivity and specificity (82.4 and 83.3, respectively), with an excellent DOR value of 23.4.

Based on this value, we divided the 23 cases into two groups based on the resistances shown at the beginning of perfusion: the group with low RR A (initial resistances <0.5 mmHg/mL/min; n=8) and high RR B (initial resistances ≥0.5 mmHg/mL/min; n=15).

In Table 5, we reported the specific characteristics of donors for the 23 cases stratified into the two groups based on initial RR.

It is interesting to note that no differences were observed between the two groups regarding donor characteristics, except for a slightly lower median peak sodium value observed in the group with high RR (150 vs. 152 mEq/L; P=0.02). Overall, donors with high RR had a higher mean KDRI value (1.68 vs. 1.47; P=0.43) and a greater number of grafts with Karpinski scores of 5-6 vs. 4 (40 vs. 12.5%; P=0.35). Additionally, the mean age was higher in the high RR group (67 vs. 60 years; P=0.21).

In Table 6, we reported the characteristics of recipients and perfusions of the 23 transplanted kidneys stratified into the two groups based on initial RR. In this case, no differences were observed between the two groups regarding recipient characteristics. Overall, the mean age of recipients was higher in the high RR group (63 vs. 58 years; p=0.21).

The median CIT was slightly lower in the low RR group (375 vs. 380 min, P=1.00), while the perfusion duration was markedly lower, although not significant (183 vs. 267; P=0.20). In all cases, the organs were perfused for at least two hours, with the longest perfusion time being 7.4 hours.

All variables related to HMP were reported in **Table 6** and **Figure 2.** In **Figure 3**, we showed the trend of RR in all grafts subjected to HMP.

As expected, significant differences were observed in terms of renal flow and RR. At the beginning of HMP, kidneys in the high RR group had a higher mean RR (0.76 vs. 0.43 mmHg/mL/min; $P < 0.001$) and lower flow (32 vs. 53 mL/min; $P = 0.008$). Although a trend was observed in all cases with decreased RR and a concomitant increase in flow, significant differences were already observable after two hours of perfusion, with high RR cases remaining high (0.33 vs. 0.23 mmHg/mL/min; $P = 0.04$) and lower flow (80 vs. 98 mL/min; $P = 0.03$).

After perfusion, all kidneys were successfully transplanted. Regarding graft recovery post-transplant, the median sCr value on the seventh postoperative day was lower, although not statistically significant, in the low RR group (1.7 vs. 3.4 mg/dL; P=0.29). According to the classic definition of DGF, i.e., the need for dialysis during the first week after transplantation, no differences were observed between the groups, with 2 cases out of 15 (13.3%) compared to zero cases in the high RR and low RR groups, respectively (P=0.53). However, when DGF was defined as CCR7 ≤ 70%, the number of cases in the high RR group was statistically significant (86.7 vs. 13.3%; P=0.03).

The mean length of hospital stay was shorter in the low RR group, although no significant difference was observed (11 vs. 12 days, p=0.27).

Both lost kidneys after transplantation had initial RR > 0.5 mmHg/mL/min. Consequently, the six-month transplant survival rate was 100.0 vs. 86.7% in the low RR and high RR groups, respectively (P=0.29) (**Figure 4**).

DISCUSSION

In the present studies we observed that the use of HMP does not appear to significantly impact the reduction of Delayed Graft Function (DGF) and the decrease in serum creatinine levels on the seventh postoperative

day compared to static cold storage (SCS). However, there is a different scenario when it comes to urine output. In cases subjected to HMP, there was indeed a higher urine output on the second postoperative day compared to SCS, followed by subsequent normalization and, consequently, a reduction in urine output values on the seventh day.
Similarly, the use of HMP seems to have a positive impact in terms of reducing ICU stay and overall hospital stay.

When considering the subpopulation of organs for which a biopsy was necessary to assess organ suitability for transplantation, the impact of HMP becomes statistically more significant. In this subgroup, which currently represents the largest donor category (known as ECD), we observed a higher incidence of DGF, defined as the need for dialysis in the first week post-transplant, in the SCS subgroup compared to kidneys subjected to HMP.
Similarly, but with greater statistical significance, we noted a reduction in creatinine levels on the seventh postoperative day, as well as a decrease in ICU stay and total hospital stay in the subgroup of kidneys subjected to HMP.
In these studies, we observed that the use of "in-house" HMP (performed after organ transport to the transplant center following static cold storage) allows for very low rates of DGF and graft loss at 6 months (2/23, 8.7%), even in kidneys with high histological scores (Karpinski > 3). Moreover, we confirmed the importance of HMP in ECDs, showing the significant impact of information (i.e., RR at the end of perfusion) from this process as a useful selection tool.
Previous preclinical and clinical studies have analyzed the mechanisms justifying the advantage of using HMP. A correlation has been observed between HMP and endothelial integrity protection [58]: Chatauret et al. demonstrated that non-oxygenated perfusion increases eNOS phosphorylation through an AMPK-dependent pathway in the renal cortex at the end of the procedure and in the renal artery after reoxygenation [59]. The efficacy of HMP in reducing ischemia/reperfusion damage by decreasing the expression of proinflammatory cytokines and adhesion molecules like ICAM-1 has also been emphasized [60], as well as the findings from a systematic Cochrane review based on sixteen studies (N=2,266) comparing HMP with static cold storage, which showed an overall 22.0% reduction in the risk of DGF in DBD donors (P=0.006) [51].
Regarding the role of RR in organ selection during perfusion, the evidence from the literature is weak, mainly due to the selection biases present in studies investigating this aspect. Several kidneys have been systematically discarded based on arbitrarily defined RR threshold values [61]. However, we should emphasize that no organs in our series were rejected due to poor RR reduction; they were evaluated and deemed unsuitable before perfusion due to the presence of unfavorable microscopic and macroscopic conditions (e.g., severe pyelonephritis, widespread atherosclerosis).
A study by Ding C.G. et al., including 76 grafts from DCD donors, showed that RR at the end of HMP was an independent predictor of DGF (OR=3.12; P=0.01) and organ survival (hazard ratio [HR]=2.06; P=0.03) [62]. The same group even proposed a scoring model to identify the risk of DGF based on a combination of HMP duration (OR=1.17; P=0.043), RR (OR=2.19; P<0.001), and flow rates (OR=0.93; P=0.01) [63].
Sandal et al. identified two different RR threshold values at the end of HMP capable of predicting graft failure risk, namely 0.2 (HR=2.42; P=0.04) and 0.4 (HR=2.67; P=0.07) mmHg/mL/min [56].

An Italian study based on 35 kidney transplants using ECD demonstrated that recipients of kidneys with RR ≤1.0 within one hour of HMP showed a lower percentage of PNF/DGF (11 vs. 44%; P=0.03) and a faster reduction in sCr post-transplant (X day post-transplant: 1.79 mg/dL vs. 4.33 mg/dL, P=0.02) [55].

The results we obtained are fully in line with all these previous experiences. In our series, the initial RR showed an excellent AUC=0.83 for diagnosing DGF, with a cut-off of 0.5 mmHg/mL/min, with excellent sensitivity=82.4 and specificity=83.3. In more detail, investigating this threshold value, we observed that kidneys with higher initial RR (>0.5 mmHg/mL/min) maintained elevated RR and lower flows even after two hours of perfusion, although all showed significant improvement in these parameters. This data suggests that the value obtained at the beginning of perfusion plays a discriminating role in evaluating graft quality.

Regarding the postoperative course, initial RR values correlated with a faster recovery of transplant function, as defined by better CCR7 results observed in the low RR group (CCR7 ≤70%: 13.3 vs. 87.6%; P=0.03). The same conclusion was not observed in terms of the need for post-transplant dialysis and hospitalization duration, although a reduction trend was reported; the non-significance of these data could be due to the small number of cases investigated.

The uniqueness of our study compared to previous experiences is the selective analysis of the role of RR during HMP to select kidneys suitable for single transplantation from DBD donors with high histological scores (Karpinski ≥3).

The decision to use these organs for single transplantation partly goes beyond the proposals of the Remuzzi score, in which organs available for single transplantation possess a Karpinski score of ≤3 [31]. However, there is growing evidence that the attempt to minimize the extension of the score for single transplantation could be achieved under specific conditions, as observed in some experiences.

In fact, an Italian study proposed modifying the decision-making protocol, considering scores of 4-5 suitable for single transplantation if the donor's glomerular filtration rate was ≥60 mL/min [64].

Another study comparing the outcomes of kidneys allocated for clinical reasons versus the histological-clinical protocol showed no differences in long-term graft survival and sCr values [65].

In the study by Bissolati et al., where histological characteristics were investigated in the context of reconditioned organs, the Karpinski score was not correlated with the PNF/DGF rate (P=0.87) and postoperative sCr trend (P=0.80) [55].

These findings are in line with our results, which, when observed after perfusion, were satisfactory even though non-standard organs were used for single transplantation. Additionally, initial RR values had a greater impact on clinical outcomes than initial histology.

All these aspects should allow some evaluation of the results obtained. For example, initial RR could act as a non-invasive method for evaluating organ quality, offering valuable additional information compared to biopsy alone. Evaluating RR could potentially better reflect the characteristics of the entire cortex compared to biopsy, which can only examine a small quantity of glomeruli, tubules, and vessels. This limitation is well described in some studies, where the risk of erroneously discarding organs due to renal biopsy results is emphasized [65,66].

Another consideration is the ability of HMP to reduce the process of deterioration in perfused organs compared to CS, primarily through minimizing ischemia-reperfusion damage. This would lead to the evaluation and possible use of additional non-standard kidneys without risking worse outcomes.

Obviously, all these reflections require further studies and larger series, aiming to build mathematical models aimed at "weighing" the actual role of hypothermic reconditioning and the selective role of RR in combination with other histological characteristics.

The studies presented has some limitations. Firstly, these are a preliminary experience based on a small number of patients. Unfortunately, this condition is common in all published casistics on the subject, so multicenter studies are needed to increase the study population. The other limitation is that they are a single-center studies and, as such, potentially influenced by local prerogatives.

10. CONCLUSIONS

The use of perfusion machine positively impacts on the early recovery of DBD renal grafts. This positive phenomenon is more marked in cases requiring biopsy at the time of organ retrieval (i.e., aged donors or presence of relevant donor comorbidities). The routinary use of a tailored HPM approach should hesitate in a reduction of post-transplant dialysis rates, and shorter hospital stay.

The use of in-house HMP for preconditioning organs with Karpinski >3 allows for very low rates of DGF and graft loss at six months. Initial RR values represent a useful parameter for selecting non-standard organs to be used for single kidney transplantation, even in the presence of high histological scores.

11. TABLES

Table 1. Pre-KT characteristics of the investigated population divided in the two groups.

Variables	**SCS (n=218, 69.6%)**	**HPM (n=95, 30.4%)**	**P-value**
	Median (IQR) or n (%)		
	Patient		
Age, years	53 (44-61)	53 (45-62)	0.52
Male sex	130 (59.6)	51 (53.7)	0.38
Caucasian	201 (92.2)	91 (95.8)	0.33
Height, cm	168 (163-175)	167 (160-172)	0.10
Weight, kg	70 (61-77)	72 (63-79)	0.16
BMI	24.2 (22.1-26.4)	25.4 (22.8-28.1)	0.004
Underlying renal disease			
ADPKD	40 (18.3)	18 (18.9)	0.88
GN	97 (44.5)	43 (45.3)	0.90
PN	5 (2.3)	3 83.2)	0.70
Nephroangiosclerosis	31 (14.2)	15 (15.8)	0.73
VUR	9 (4.1)	4 (4.2)	1.00
Unknown	15 (6.9)	2 (2.1)	0.11
Other	21 (9.6)	10 (10.5)	0.84
Re-transplantation	31 (14.2)	12 812.6)	0.86
Pre-emptive KT	11 (5.0)	3 (3.2)	0.56
Dialysis duration, years	3 (2-6)	3 (1-4)	0.004
Arterial hypertension	181 (83.0)	81 (85.3)	0.74
T2DM	21 (9.6)	11 (11.6)	0.69
CMV-Ab positive	204 (93.6)	84 (88.4)	0.17
HbS-Ab positive	85 (39.0)	32 (33.7)	0.45

HCV-Ab positive	6 (2.8)	6 (6.3)	0.20
Donor			
Age, years	55 (43-66)	55 (47-65)	0.34
Male sex	105 848.2)	50 852.6)	0.54
Height, cm	170 (164-175)	170 (165-175)	0.23
Weight, kg	75 (66-86)	75 (72-83)	0.72
BMI	26.1 (24.2-28.9)	26.0 (24.6-27.8)	0.47
CMV-Ab positive	192 888.1)	87 (91.6)	0.43
HCV-Ab positive	0 (-)	1 (1.1)	0.30
Cause of death			
CVA	160 (73.4)	73 (76.8)	0.58
Anoxia	14 (6.4)	5 (5.3)	0.80
Trauma	38 (17.4)	17 (17.9)	1.00
Other	6 (2.8)	0 (-)	0.18
Hypotension episode(s)	35 (16.1)	17 (17.9)	0.74
Cardiac arrest episode(s)	26 (11.9)	8 (8.4)	0.43
Arterial hypertension	63 (28.9)	27 (28.4)	1.00
T2DM	12 (5.5)	13 (13.7)	0.02
Biopsy	91 (41.7)	42 (44.2)	0.71
Surgical complexity	14 (6.4)	12 (12.6)	0.08
sCr, mg/dL	0.9 (0.6-1.1)	0.9 (0.8-1.1)	0.08
ICU stay, stay	4 (3-6)	4 (2-5)	0.17
KDPI	1.11 (0.85-1.54)	1.13 (0.94-1.54)	0.27
Transplantation			
CIT, minutes	715 (600-870)	717 (610-868)	0.85
Induction with thymoglobulin	13 (6.0)	3 (3.2)	0.41

Abbreviations: SCS, static cold storage; HPM, hypothermic perfusion machine; n, number; IQR, interquartile ranges; BMI, body mass index; ADPKD, autosomal dominant polycystic kidney disease; GN, glomerulonephritis; PN, pyelonephritis; VUR, vesicoureteral reflux; KT, kidney transplantation; T2DM, type-2 diabetes mellitus; CMV, cytomegalovirus; Ab, antibody; HbS, hepatitis B surface; HCV, hepatitis C virus; CVA, cerebrovascular accident; sCr, serum creatinine; ICU, intensive care unit; KDPI, kidney donor profile index; CIT, cold ischemia time.

Table 2. Effect of stabilized IPTW in the population on the variables used for balancing the two groups.

Variables	Pre-IPTW			Post-IPTW		
	SCS (n=218)	**HPM (n=95)**	**Cohen's D-value**	**SCS (n=218)**	**HPM (n=95)**	**Cohen's D-value**
	Mean (±SD)			**Mean (±SD)**		
Patient age	0.78±0.42	0.76±0.43	0.05	51.94±12.43	51.52±11.88	0.05
Patient male sex	51.71±12.21	53.12±12.12	-0.12	0.59±0.49	0.60±0.49	0.03
Patient BMI	0.59±0.49	0.54±0.50	0.10	24.64±3.43	24.59±3.72	-0.02
Re-transplantation	24.31±3.42	25.67±3.95	-0.36	0.14±0.34	0.13±0.33	0.01
Pre-emptive KT	0.14±0.35	0.13±0.34	0.04	0.04±0.21	0.03±0.18	0.03
Dialysis duration	0.05±0.22	0.03±0.17	0.12	4.40±3.74	4.97±4.30	0.06
Patient arterial hypertension	4.73±4.02	3.70±3.33	0.29	0.84±0.37	0.86±0.34	-0.14
Patient T2DM	0.83±0.38	0.86±0.35	-0.10	0.10±0.30	0.09±0.28	-0.06
Patient CMV-Ab positive	0.09±0.29	0.12±0.33	-0.08	0.92±0.27	0.92±0.27	0.04
Patient HbS-Ab positive	0.94±0.24	0.88±0.33	0.19	0.38±0.49	0.42±0.50	-0.01
Patient HCV-Ab positive	0.38±0.49	0.37±0.48	0.02	0.04±0.20	0.04±0.19	-0.09
Donor age	0.03±0.17	0.06±0.24	-0.14	53.57±16.53	53.29±16.25	0.02
Donor male sex	52.89±16.38	55.87±15.73	-0.19	0.49±0.50	0.53±0.50	0.02

Donor BMI	0.49±0.50	0.51±0.50	-0.06	26.51±3.70	26.32±3.41	-0.08
Donor CMV-Ab positive	26.63±3.70	26.36±3.35	0.08	0.89±0.31	0.90±0.30	0.05
CVA as donor cause of death	0.88±0.33	0.92±0.27	-0.15	0.74±0.44	0.71±0.46	-0.04
Donor hypotension(s)	0.73±0.45	0.78±0.41	-0.13	0.16±0.36	0.14±0.35	0.06
Donor cardiac arrest(s)	0.16±0.37	0.18±0.38	-0.05	0.11±0.31	0.11±0.32	0.04
Donor arterial hypertension	0.12±0.33	0.08±0.27	0.15	0.30±0.46	0.31±0.47	-0.01
Donor T2DM	0.30±0.46	0.27±0.44	0.07	0.08±0.28	0.08±0.27	-0.03
Biopsy	0.06±0.23	0.13±0.34	-0.24	0.43±0.50	0.43±0.50	0.01
Surgical complexity	0.41±0.49	0.47±0.50	-0.12	0.08±0.28	0.08±0.27	0.00
Donor sCr	0.06±0.24	0.13±0.34	-0.22	1.02±0.80	1.02±0.57	0.02
Donor ICU stay	1.00±0.72	1.10±0.72	-0.14	5.24±5.23	4.75±3.97	0.00
KDPI	5.44±5.85	4.71±4.26	0.15	1.24±0.44	1.23±0.47	0.11
CIT	1.21±0.43	1.30±0.50	-0.18	728.90±196.49	730.09±242.69	0.02

Abbreviations: IPTW, inverse probability therapy weighting; SCS, static cold storage; HPM, hypothermic perfusion machine; n, number; SD, standard deviation; BMI, body mass index; KT, kidney transplantation; T2DM, type-2 diabetes mellitus; CMV, cytomegalovirus; Ab, antibody; HbS, hepatitis B surface; HCV, hepatitis C virus; CVA, cerebrovascular accident; sCr, serum creatinine; ICU, intensive care unit; KDPI, kidney donor profile index; CIT, cold ischemia time.

Table 3. Post-transplant clinical course observed in the two groups (pseudo populations after IPTW balancing).

Variables	SCS (n=218)	HPM (n=95)	P-value
	Median (IQR) or n (%)		
DGF (need for dialysis)	39 (17.9)	15 (15.6)	0.75
DGF (CCR7 <70%)	50 (22.9)	19 (19.8)	0.66
CCR7	66 (35-76)	66 (35-82)	0.12
Urine output at day 2, mL	4100 (2401-5502)	4193 (3500-6215)	0.046
Urine output at day 7, mL	3500 (3000-4543)	3500 (2628-4000)	0.03
ICU stay, days	2 (1-3)	1 (1-2)	<0.0001
Hospital stay, days	12 (10-18)	11 (9-15)	0.07
Only biopsied cases			
Variables	**SCS (n=92)**	**HPM (n=39)**	**P-value**
	Median (IQR) or n (%)		
DGF (need for dialysis)	22 (23.9)	3 (7.7)	0.03
DGF (CCR7 <70%)	30 (32.6)	8 (20.5)	0.21
CCR7	53 (18-66)	62 (36-79)	0.07
Urine output at day 2, mL	4100 (1620-4776)	4100 (3127-6301)	0.10
Urine output at day 7, mL	3500 (3000-4430)	3370 (2650-3800)	0.15
ICU stay, days	2 (1-3)	1 (1-2)	0.002
Hospital stay, days	12 (11-21)	11 (8-14)	0.007

Abbreviations: n, number; IQR, interquartile ranges; DGF, delayed graft function; CCR7, creatinine reduction ratio at day 7; ICU, intensive care unit.

Table 4. Multivariable logistic regression analysis for the risk factors of DGF (need for dialysis within the first post-KT week) (pseudo populations after IPTW balancing). Backward Wald method.

Variables	Beta	SE	Wald	OR	95.0%CI	P
Entire population*						
CVA as cause of donor death	0.840	0.400	4.423	2.317	1.059-5.071	0.04

Duration of dialysis	0.065	0.036	3.290	1.067	0.99-1.059	0.07
Only biopsied cases**						
HPM	-1.176	0.623	3.564	0.308	0.091-1.046	0.06
Duration of dialysis	0.104	0.057	3.295	1.109	0.992-1.241	0.07

-2LogLikelihoods: 280.67 (*); 121.80 (**)
Variables initially introduced into the mathematical models: patient age, patient BMI, retransplantation, preemptive transplantation, duration of dialysis, donor age, donor BMI, CVA as donor cause of death, anoxia as donor cause of death, donor cardiac arrest(s), donor history of hypertension, donor history of diabetes, biopsy, surgical complexity due to multiple anastomoses, CIT, HPM use.
Abbreviations: SE, standard error; OR, odds ratio; CI, confidence intervals; CVA, cerebrovascular accident; BMI, body mass index; HPM, hypothermic perfusion machine; CIT, cold ischemia time.

Table 5. Donor Characteristics in the Entire Population and the 2 Groups.

Variables	**Entire Population (n=23)**	**Low-RR Group (n=8)**	**High-RR Group (n=15)**	**P**
KDRI	1.68 (1.36-2.17)	1.47 (1.30-2.00)	1.68 (1.38-2.22)	0.43
KDPI	92 (80-99)	85 (76-97)	92 (81-99)	0.51
Karpinski Score 4 (%)	69.6	100	66.7	0.27
Karpinski Score 5 (%)	21.7	0	20.0	0.35
Karpinski Score 6 (%)	8.7	0	13.3	0.62
Glomerulosclerosis	1 (1-1)	1 (1-1)	1 (1-1)	0.98
Fibrosis	1 (1-1)	1 (1-1)	1 (1-1)	0.64
Arteriosclerosis	1 (1-2)	1 (1-1)	1 (1-2)	0.73
Tubular Atrophy	1 (1-1)	1 (1-1)	1 (1-1)	0.55
Age (years)	67 (60-74)	60 (56-78)	67 (61-74)	0.21
Male Gender (%)	52.2	75.0	40.0	0.19
Weight (kg)	80 (73-90)	83 (76-90)	80 (73-90)	0.47
Height (cm)	170 (164-180)	179 (165-180)	168 (164-177)	0.21
BMI	27 (26-31)	28 (26-31)	27 (26-31)	1.00
Serum Creatinine (mg/dL)	1.3 (0.8-1.6)	1.2 (0.9-1.8)	1.4 (0.8-1.4)	0.68
Peak Plasma Sodium (mEq/L)	150 (145-151)	152 (150-161)	150 (144-150)	0.02
UTI Stay (days)	5 (2-5)	4 (2-7)	5 (2-5)	0.83
Vasoactive Score (VAS)	27 (0-60)	30 (11-61)	5 (0-50)	0.33
Hypertension (%)	52.2	50.0	53.3	1.00
DM Type 2 (%)	30.4	50.0	20.0	0.18
Smoking Habit (%)	43.5	50.0	40.0	0.69
Known Heart Disease (%)	17.4	12.5	20.0	1.00
Known Liver Disease (%)	8.7	25.0	0	0.11
Known Dyslipidemia (%)	17.4	12.5	20.0	1.00
Cause of Death				0.52

Variables	Entire Population (n=23)	Low-RR Group (n=8)	High-RR Group (n=15)	P
Anoxia (%)	4.3	0	6.7	1.00
Cranial Trauma (%)	21.7	12.5	26.7	0.13
CVA (%)	73.9	87.5	66.7	0.27
Hypotension Episodes	26.1	50.0	13.3	0.13
Cardiac Arrest Episodes	13.0	25.0	6.7	0.27

(Note: Values are expressed as medians (IQR) or percentages, as appropriate. Abbreviations: RR - Renal Resistances; KDRI - Kidney Donor Risk Index; KDPI - Kidney Donor Profile Index; BMI - Body Mass Index; UTI - Intensive Care Unit Stay; VAS - Vasoactive Score; DM 2 - Type 2 Diabetes Mellitus; CVA - Cerebrovascular Accident)

Table 6. Recipient and Perfusion Characteristics in the Entire Population and the 2 Groups.

Variables	Entire Population (n=23)	Low-RR Group (n=8)	High-RR Group (n=15)	P
Age (years)	61 (53-68)	58 (53-64)	63 (57-69)	0.21
Male Gender (%)	56.5	62.5	53.3	1.00
Weight (kg)	71 (70-76)	71 (56-77)	71 (70-76)	0.89
Height (cm)	165 (160-165)	165 (161-165)	165 (160-165)	0.86
BMI	24 (23-27)	24 (20-28)	24 (24-27)	0.39
Underlying Disease				0.13
ADPKD (%)	17.4	25.0	6.7	0.13
Arteriosclerosis (%)	8.7	0	13.3	0.73
DM Type II (%)	8.7	12.5	6.7	1.00
Glomerulonephritis (%)	13.0	25.0	6.7	0.07
Lupus (%)	8.7	25.0	0	0.03
Re-Tx (%)	8.7	12.5	6.7	1.00
Other (%)	13.0	12.5	20.0	0.27
Unknown (%)	21.7	0	26.7	0.01
Dialysis Duration (years)	5 (4-5)	5 (3-8)	5 (4-5)	0.74
Hypertension (%)	78.3	87.5	73.3	0.62
DM Type II (%)	8.7	12.5	6.7	1.00
CIT (min)	380 (298-419)	375 (269-479)	380 (300-405)	1.00
Perfusion Time (min)	247 (125-330)	183 (126-311)	267 (156-365)	0.20
Flow (mL/min)				0.008
0 min	46 (30-56)	53 (50-78)	32 (17-53)	
20 min	78 (51-98)	96 (81-124)	53 (35-82)	

Variables	Entire Population (n=23)	Low-RR Group (n=8)	High-RR Group (n=15)	P
40 min	81 (60-100)	95 (90-126)	65 (50-97)	
60 min	85 (60-105)	95 (89-127)	71 (54-102)	
120 min	87 (65-110)	98 (89-128)	80 (60-92)	
Flow Delta (%)	+124 (+71 – +200)	+61 (+14 – +142)	+140 (+96 – +231)	0.001
RR (mmHg/mL/min)				<0.001
0 min	0.57 (0.46-0.87)	0.43 (0.31-0.48)	0.76 (0.57-1.35)	
20 min	0.34 (0.26-0.53)	0.27 (0.31-0.48)	0.50 (0.32-0.73)	
40 min	0.28 (0.22-0.47)	0.23 (0.20-0.26)	0.43 (0.26-0.57)	
60 min	0.28 (0.22-0.41)	0.23 (0.20-0.27)	0.35 (0.25-0.43)	
120 min	0.28 (0.23-0.36)	0.23 (0.19-0.28)	0.33 (0.26-0.40)	
RR Delta (%)	-60 (-67 – -48)	-41 (-57 – -6)	-63 (-70 – -54)	0.001
sCr (mg/dL)				0.83
at Tx	6.1 (4.6-7.9)	6.2 (5.5-7.6)	6.1 (4.1-8.9)	
7 days after Tx	2.2 (1.5-5.4)	1.7 (1.5-3.4)	3.4 (1.5-5.4)	
CCR7 (%)	57.9 (31.6-72.7)	72 (52-78)	46 (29-63)	0.07
Dialysis Need 1st week	2 (8.7)	0 (-)	2 (13.3)	0.53
Hospital Stay (days)	11 (9-13)	11 (9-12)	12 (9-15)	0.27

(Note: Values are expressed as medians (IQR) or percentages, as appropriate. Abbreviations: RR - Renal Resistances; BMI - Body Mass Index; ADPKD - Autosomal Dominant Polycystic Kidney Disease; DM II - Type II Diabetes Mellitus; Tx - Transplantation; CIT - Cold Ischemia Time; sCr - Serum Creatinine; CCR7 - Creatinine Clearance Rate at 7 Days Post-Transplant)

Table 7. Prediction of CRR7 ≤70% and RR threshold values investigation.

Variables	**AUC**	**SE**	**95%CI**		**P**
			Lower	**Upper**	
RR initial time	0.83	0.09	0.67	1.00	0.02
RR 120 min	0.81	0.12	0.58	1.00	0.03
Flow initial time	0.26	0.11	0.05	0.47	0.09
Karpinski Score	0.71	0.11	0.49	0.92	0.14
Donor sCr	0.62	0.15	0.34	0.91	0.38
Initial RR	**Sensitivity**		**Specificity**		**DOR**
0.5 mmHg/mL/min	82.4		83.3		23.4
0.75 mmHg/mL/min	52.9		100.0		∞
1.0 mmHg/mL/min	29.4		100.0		∞

Abbreviations: AUC, area under the curve; SE, standard error; CI, confidence intervals; RR, renal resistances; sCr, serum creatinine; DOR, diagnostic odds ratios.

Supplementary Table 1. Missing data in the entire population (N=313).

Variables	Missing data (n)	(%)
Patient		
Age	0	0.0
Sex	0	0.0
Caucasian	0	0.0
Blood group	11	3.5
BMI	18	5.8
Renal disease	0	0.0
Re-KT	12	3.8
Pre-emptive	24	7.7
Years dyalisis	27	8.6
Arterial hypertension	22	7.0
T2DM	14	4.5
Donor		
Age	0	0.0
Sex	0	0.0
Caucasian	0	0.0
Blood group	11	3.5
BMI	28	8.9
Cause of death	28	8.9
Arterial hypertension	30	9.6
T2DM	31	9.9
sCr	21	6.7
HCV status	0	0.0
Transplantation		
CIT	25	8.0
Use of HPM	0	0.0
sCr time of KT	0	0.0
sCr time 7th post-KT day	0	0.0
Dyalisis within the first week	0	0.0

Abbreviations: BMI, body mass index; KT, kidney transplantation; T2DM, type-2 diabetes mellitus; sCr, serum creatinin; HCV, hepatitis C virus; HBV, hepatitis B virus; CIT, cold ischemia time; HPM, hypothermic perfusion machine.

12. FIGURES

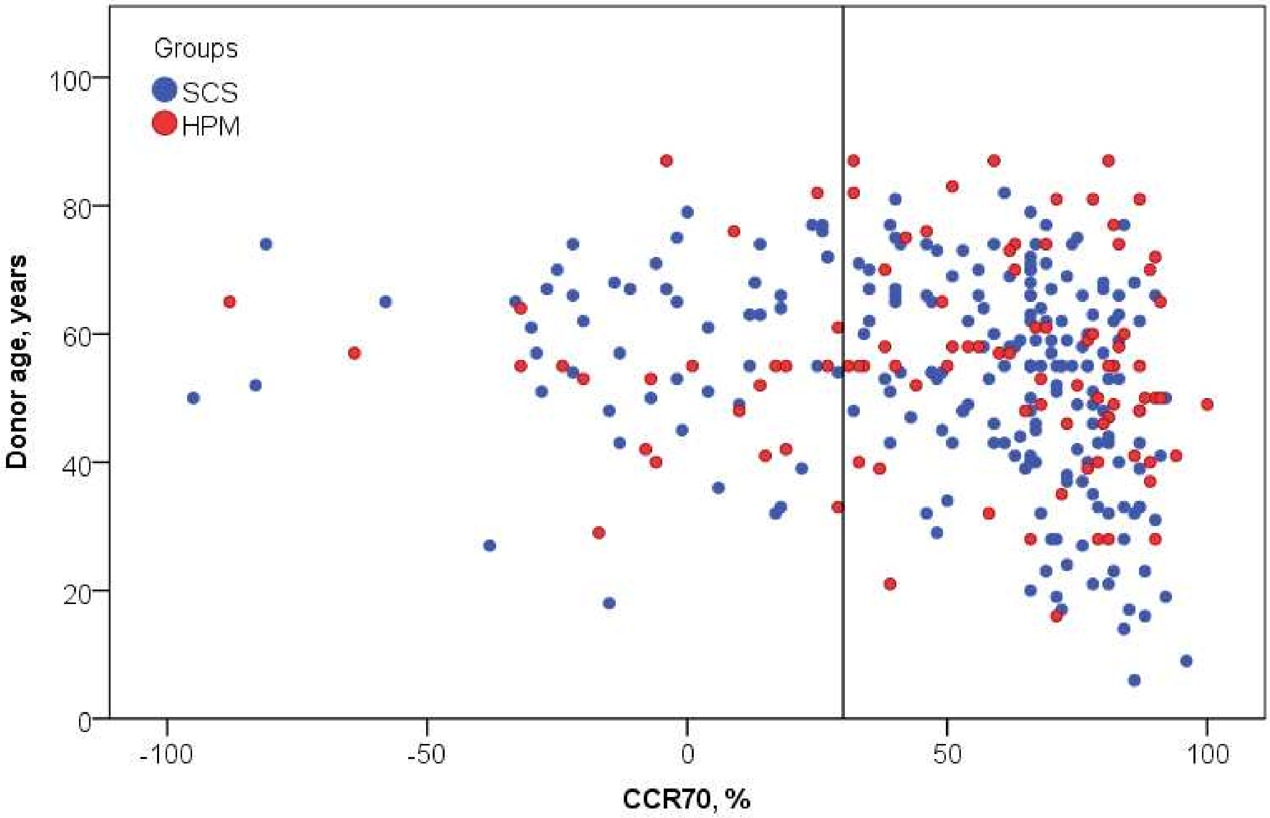

Figure 1. Distribution of SCS and HPM cases according to donor age and modification of serum creatinine at day 7 after transplantation.

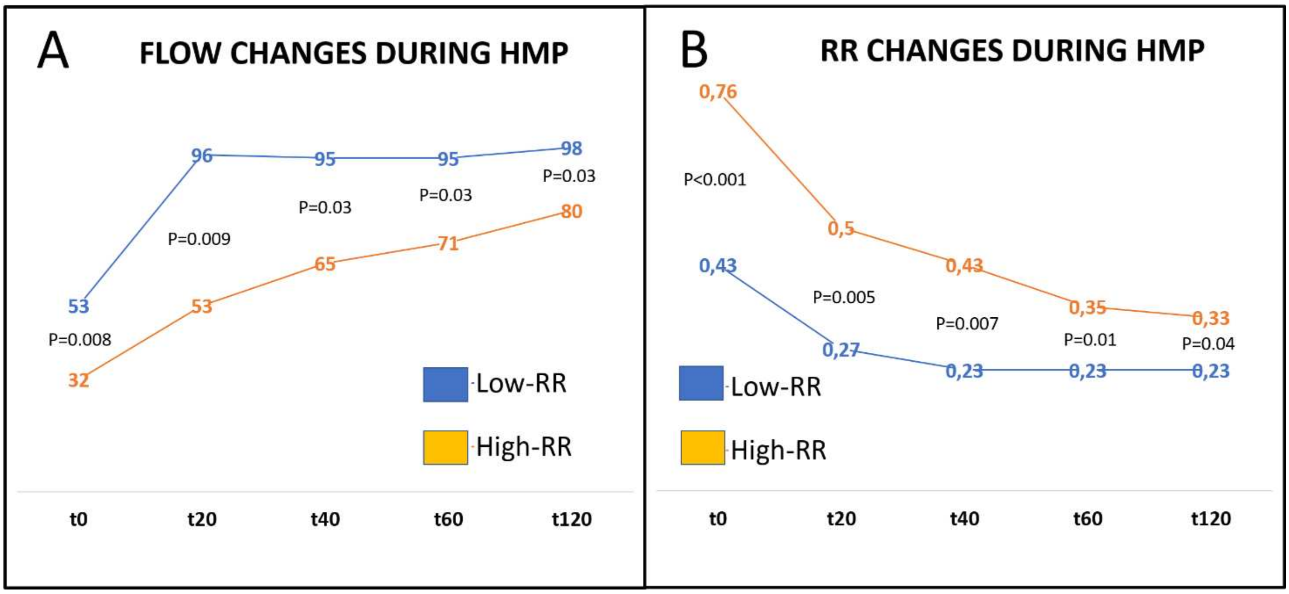

Figure 2. Changes in flow and renal resistances during HMP in low-RR and high-RR groups.

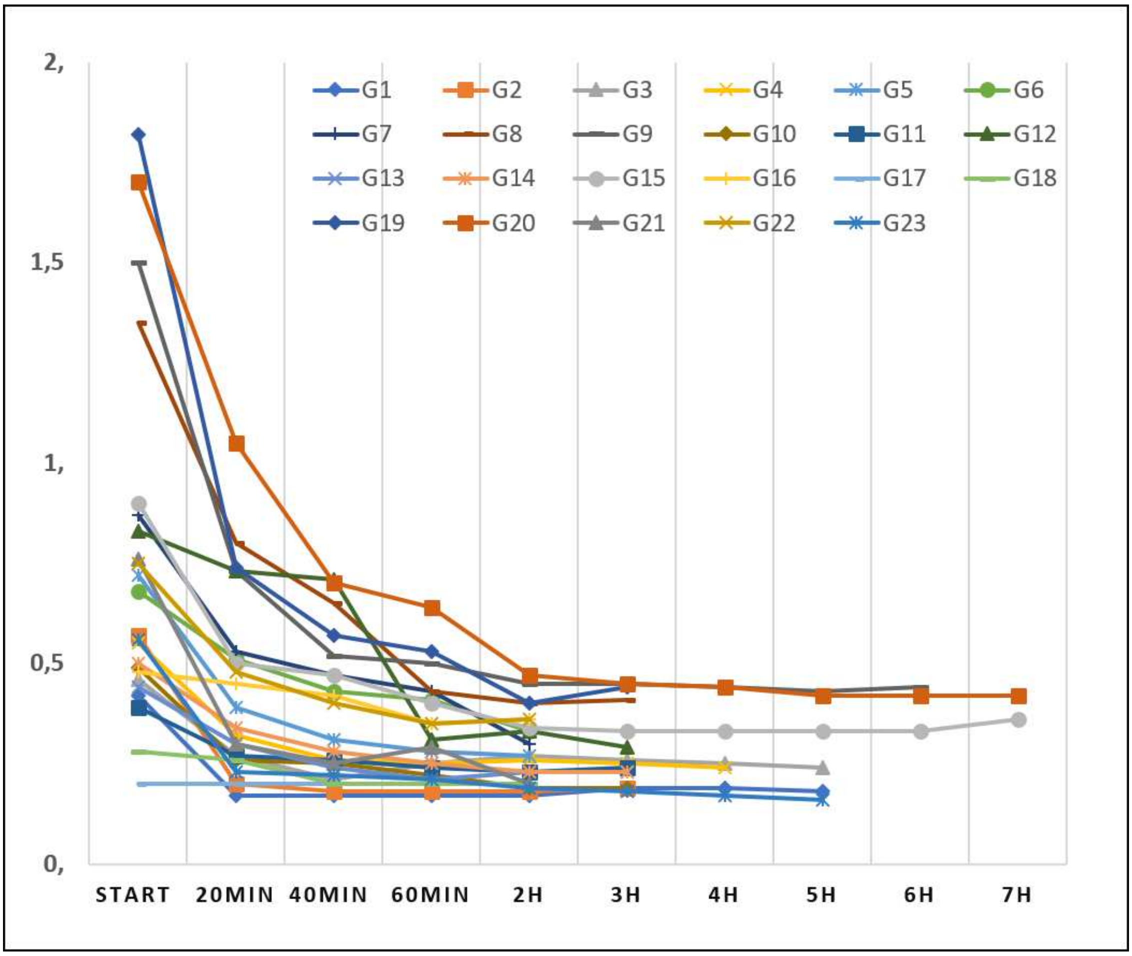

Figure 3. Variations in renal resistances in the 23 transplanted patients.

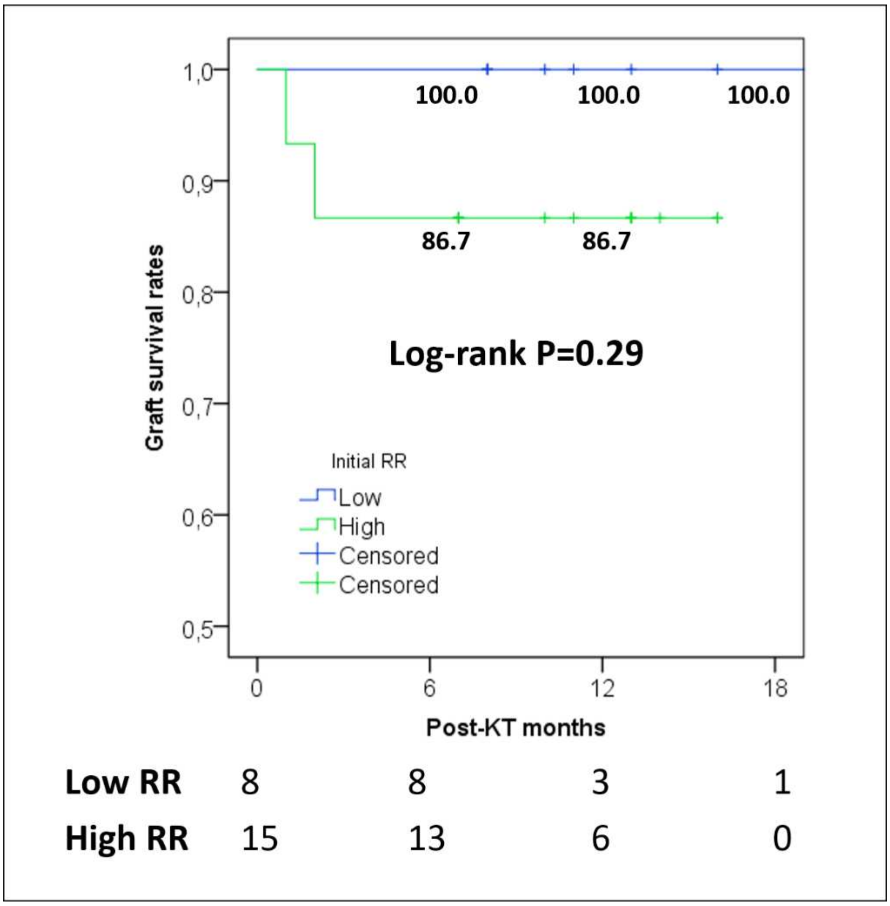

Figure 4. 6-month graft survival rates in the 2 groups.

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