Time of Cold Storage Prior to Start of Hypothermic Machine Perfusion and Its Influence on Graft Survival

Michal Wszola[a,b], Piotr Domagala[a,*], Agata Ostaszewska[a,b], Lukasz Gorski[a,b], Edyta Karpeta[a], Andrzej Berman[a,b], Maria Sobol[c], Magdalena Durlik[d], Andrzej Chmura[a], and Artur Kwiatkowski[a,b]

[a]Department of General and Transplantation Surgery, The Medical University of Warsaw, Poland; [b]Foundation of Research and Science Development, Otwock, Poland; [c]Department of Biophysics and Human Physiology, The Medical University of Warsaw, Poland; and [d]Department of Transplantation Medicine, Nephrology and Internal Medicine, The Medical University of Warsaw, Poland

ABSTRACT

Background. Hypothermic machine perfusion (HMP) has become a standard method of preservation for kidneys procured from expanded-criteria donors and donors after cardiac death. There are different systems and approaches to the HMP preservation period, with cold storage prior to HMP sometimes taking several hours. This study evaluated whether the time at which kidneys receive HMP had any influence on the outcomes of kidney transplantation.

Methods. In this analysis, patient and graft survival were evaluated over a 1-year post-transplantation period. Patients who received HMP kidneys (n = 379) were divided into 2 groups: those who received kidneys with a cold ischemia time (CIT) prior to HMP <295 minutes (group G1; n = 254) and those who received kidneys with CIT prior to HMP >295 minutes (group G2; n = 125).

Results. Delayed graft function was observed in 31.8% (81/254) of patients in group G1 vs 46.4% (58/125) of patients in group G2 ($P = .007$). One-year graft survival was statistically higher in the group G1 (93.2%; 233/254) vs group G2 (86.5%; 105/125, $P = .029$). Mean 1-year estimated glomerular filtration rate was significantly better in the group G1.

Conclusions. In conclusion, introduction of HMP up to 295 minutes from procurement led to better early and 1-year graft results. Kidneys should receive HMP as soon as possible after retrieval, preferably during procurement.

IN RECENT years, hypothermic machine perfusion (HMP) has become a standard method of preservation for kidneys procured from expanded-criteria donors (ECD) and donors after circulation death (DCD) [1–3]. It has been demonstrated that early and long-term graft survival is superior if HMP is used [4–6], with HMP reducing episodes of delayed graft function (DGF) [7,8], episodes of chronic rejection, and interstitial fibrosis/tubular atrophy [9]. HMP might also improve graft survival due to influences on gene expression involved in ischemia/reperfusion injury [10].

Although there are proven benefits associated with the use of HMP, there are no standards for its implementation, and there are different HMP systems available. One system allows kidneys to receive HMP immediately and throughout transportation to the transplantation center, while other systems allow HMP in the transplantation center but do not have mobile capabilities.

It has been demonstrated that HMP is useful even if kidneys are firstly kept in cold storage (CS) and then, after several hours, receive HMP. HMP should then take at

The analysis was supported by the grant from Foundation for Research and Science Development, Poland (www.fundacjabirn.pl).

*Address correspondence to Piotr Domagala, Department of General and Transplantation Surgery, The Medical University of Warsaw, Poland, Nowogrodzka 59 Street, 02-006 Warsaw, Poland. Tel: +48225021126; Fax: +48225022155. E-mail: piotr.domagal@gmail.com

0041-1345/19
https://doi.org/10.1016/j.transproceed.2019.02.052

least half of the time of CS. To the best of our knowledge, there are no data showing whether time of CS prior to beginning HMP affects kidney transplantation. Therefore, this study evaluated whether the time at which kidneys receive HMP had any influence on the outcomes of kidney transplantation.

MATERIALS AND METHODS

There were 468 kidney transplantations were performed in our center. Kidneys were procured from 257 donors after brain death (DBD). Kidneys from DCD were not procured in our department and were not included into the study. The observation period lasted until March 31, 2015. A total of 84 DBDs met United Network for Organ Sharing criteria of ECDs (32.6%), and 417 kidneys (89%; 417/468) were kept on HMP prior to transplantation. Data on cold ischemia time (CIT)−1 were available from 91% of those kidneys (n = 379), and these were enrolled into the study. Patient and graft survival were analyzed over a 1-year post-transplantation period. All patients completed the 1-year follow-up.

DGF was recognized as a need for dialysis within 7 days after kidney transplantation, regardless of reason (hyperkalemia, high serum urea concentration, hyperhydration). Primary nonfunction (PNF) was defined as permanent loss of graft function immediately after transplantation. Acute rejection (AR) was biopsy proven and diagnosed according to Banff 2009 criteria. CIT was measured from the start of in situ perfusion until the start of vascular anastomosis (CIT−1 and CIT−2): CIT−1 was measured from the start of in situ perfusion until the start of HMP; CIT−2 was measured from the start of HMP until the start of vascular anastomosis.

Data on DBDs (type of donor, time of intensive care unit stay), surgical procedure (time of procedure, CIT, CIT−1, CIT−2), and recipient (age, HLA mismatch, body mass index [BMI], comorbidities, occurrence of DGF, number of hemodialysis sessions post-transplantation, AR occurrence and renal function up to 12 months post-transplantation, including creatinine level and return to hemodialysis) were collected.

Machine Perfusion

Kidneys were procured in hospitals outside our transplantation center and transported on ice to our center (only 61 were placed on machine perfusion in the retrieval center). Immediately following organ recovery and cooling to 4°C, each kidney was placed in a thermally stable container in a preservation solution (University of Wisconsin solution). Upon arrival at our transplant center, both kidneys were simultaneously prepared for machine perfusion, and then each kidney was placed on a perfusion device in sterile disposable cassettes filled with perfusion fluid (1000 mL; KPS−1, University of Wisconsin solution). Systolic pressure was set at 30 mm Hg according to manufacturer's recommendations (LifePort Kidney Transporter, Organ Recovery Systems, Itasca, Ill, United States). The HMP flow was adjusted automatically with the use of the device pressure-driven processor with systolic pressure maintaining at 30 mm Hg. A sterile disposable cassette was placed into a water-ice container, keeping a perfusion temperature of 4–6°C.

Perfusion Measurements

The following parameters were monitored: systolic−diastolic pressure; mean perfusion pressure; flow per minute; and vascular renal resistance (RR) at 1, 2, 3, and 4 hours and thereafter every 4 hours during the perfusion period. Biochemical markers of ischemic organ injury were also investigated−lactic acid dehydrogenase (LDH; spectrophotometric assay) and lactate (GM7 APR)−at the fourth hour of perfusion.

Kidneys were kept perfused during preparation of the iliac fossa of the recipient before implantation. Kidneys were removed from the device just prior to vascular reanastomosis in the recipient.

Kidney Transplantation

Kidney transplantation at our institution is routinely performed retroperitoneally. Vascular anastomosis is performed end-to-side to external iliac vein and artery. Ureterovesical anastomosis is performed by the McKinnon technique. Double-J catheter is not routinely placed during transplantation. Double-J urethral catheter is placed at the discretion of the operating surgeon.

Immunosuppression Therapy

Triple-drug immunosuppression therapy consisting of steroids, calcineurin inhibitor, and anti-proliferative medication is used as standard therapy in our center. During the analysis period, induction immunosuppression was administered in cases of second transplantation, panel reactive antibody > 20%, or for 4 or more mismatches. In most cases, basiliximab was used as an induction therapy.

Statistical Analysis

Statistical analysis was performed using the Statistica 12 package (StatSoft, Medical University of Warsaw, Warsaw, Poland). Categorical variables were summarized through the calculation of frequency and continuous variables were summarized using descriptive statistics (mean, standard deviation, median, and range). Receiver operating characteristic (ROC) curve analysis was performed to find an optimal cutoff value for the ratio for predicting a higher-risk class of considered group of patients [11]. The area under the curve was calculated as a measure of the accuracy of the test. Multiple regression and multiple logistic regression analyses were performed to find the independent predictors of a CIT−1 time. The regression analyses were considered for the following variables: immediate function, DGF, urinary tract infection, AR up to 1 month and 12 months after transplantation, time of hospitalization after transplantation, and 1-year graft survival. Quality variables in 2 groups were compared using χ^2 or Fisher's exact tests. The Student's t test or the Wilcoxon test was applied for testing differences between means and medians, respectively. A critical alpha level for hypothesis testing was set at 0.05.

RESULTS

ECD kidneys made up 31.4% (119/379) of the total number of kidneys transplanted. Mean donor age was 45.9 ± 15 years. Mean recipient age was 48 ± 2 years, and 60.2% of recipients were male. A total of 61.4% of kidneys were retrieved from donors after cerebral incidents. Mean HLA mismatch was 3.48 ± 1.09.

One-year graft survival was 91%, and mean patient survival was 96%. DGF was observed in 36.6% of patients, and PNF was observed in 2.9% of patients. AR was observed in 9.1% of patients up to the first month after transplantation and in 18.5% up to 1 year after transplantation.

We noticed a positive, statistically significant correlation between 1-year graft survival and CIT−1 time. The Spearman's

rank correlation coefficient was 0.1964. ROC curves [12] were used to determine an optimal cutoff point of CIT–1 time for the group of patients with better 1-year graft survival. The threshold cutoff was 295 minutes. The area under curve for our data was 0.61, the positive predictive value was 0.94, the negative predictive value was 0.16, and the F_1-score was 0.689 (Fig 1).

According to the ROC cutoff, patients who received HMP kidneys (n = 379) were divided into 2 groups: those who received kidneys with a CIT < 295 minutes (group G1; n = 254) and those who received kidneys with CIT > 295 minutes (group G2; n = 125).

Donor Analysis

There were 33.1% (84/254) of kidneys from ECDs in the group G1 and 28% (35/125) in the group G2 (P = .31). There were no differences between the groups regarding donor age, creatinine level, intensive care unit stay, and BMI (Table 1).

Perfusion Parameters

Initial mean RR during perfusion was significantly higher in the group of kidneys that received HMP sooner (group G1). Mean RR in this group then decreased over approximately 1 hour and was significantly lower until the end of perfusion (Fig 2). Levels of unspecific marker of ischemic injury LDH were significantly higher in the group G2 at the fourth hour of perfusion (Fig 3).

Immunosuppression Therapy

Induction therapy was administered to 52.7% (200/379) of patients. All patients received triple-drug immunosuppression therapy consisting of: calcineurin inhibitors (tacrolimus [89.4%; 339/379] and cyclosporine [10.5%; 40/379]); antiproliferative drugs (mycophenolate mofetil acid [82.5%; 313/379], mycophenolate mofetil sodium [16.3%; 62/379], and azathioprine [1%; 4/379]). All patients received steroids. There were no differences in immunosuppressive therapy between groups G1 and G2.

Recipients

Regarding age, sex, HLA mismatch, BMI, type and time of dialysis prior to transplantation, arterial hypertension, and chronic heart insufficiency, patients who received kidneys kept in CS for < 295 minutes prior to HMP (group G1) did not differ from patients who received kidneys kept in CS prior to HMP > 295 minutes (group G2) (Table 2).

CIT Parameters

Mean CIT was 28.8 ± 8 hours, and mean CIT–1 for all patients was 249 ± 250 minutes. Mean CIT–1 for groups G1 and G2 was 116 ± 74 vs 521 ± 284 minutes, respectively (P < .0001). Mean CIT did not differ between the groups and was 28.3 ± 8 vs 30 ± 9 hours (P = .08) in groups G1 and G2, respectively. Time of HMP was significantly longer in the group G1 (26.7 ± 9.2 hours) vs group G2 (22.7 ± 9.6 hours; P < .001), and correlation of CIT–2/CIT–1 was also significantly higher for group G1 vs group G2 (22.5 vs 3.64; P < .001), but the percentage of kidneys receiving HMP for more than 50% of CIT–1 was not statistically different in both groups (100% for group G1 vs 98% for group G2; P = .85). According to Goldstein et al [11] HMP should take at least half of the time of CS.

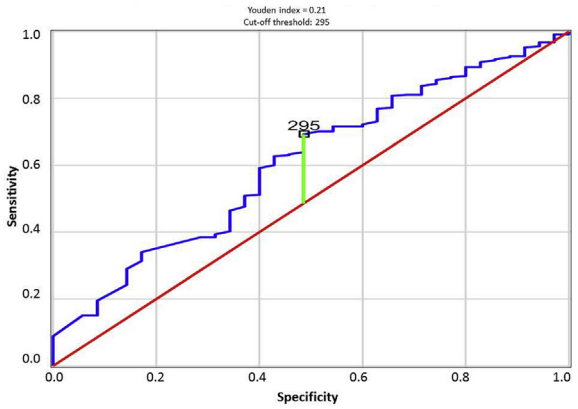

Fig 1. Receiver operating characteristic curve analysis. Youden index = 0.21. Cutoff threshold: 295.

Early Post-Transplant Period

DGF was observed in 31.8% (81/254) of patients in the group G1 vs 46.4% (58/125) in the group G2 (P = .00003). Odds ratio for DGF after kidney transplantation was 1.5-fold more probable if CIT–1 for kidney was longer than 295 minutes. Patients in the group G1 who had DGF underwent a mean of 2.69 ± 1.84 sessions of hemodialysis after kidney transplantation vs 3.6 ± 3.01 sessions in the group G2, but this difference did not reach statistical significance (P = .07). PNF did not differ between the groups and assumed 1.5% (4/254) vs 4.8% (6/125; P = .18) for groups G1 and G2, respectively. Biopsy proven AR up to 1 month was seen in 8.3% (21/254) of group G1 vs 10.4% (13/125) of group G2 patients (P = .48). The mean time of hospitalization was shorter in the group G1 (13.8 ± 8 days) vs group G2 (17.4 ± 18 days; P = .049), resulting in savings of €825 per patient in the group G1. Mean creatinine levels and mean glomerular filtration rate (GFR) were also

Table 1. Donor Characteristics (mean ± SD)

	Group G1 (n = 254)	Group G2 (n = 125)	P Value
Age (years)	46.5 ± 15.4	44.8 ± 15.3	.32
Male sex (%)	60%	63%	.95
BMI (kg/m^2)	25.8 ± 5.6	25 ± 5.1	.1
ECDs kidneys (%)	33.1%	28%	.31
ICU stay (days)	4.42 ± 2.74	4.59 ± 3.28	.64
Creatinine level (mg/dL)	1.67 ± 1.24	1.72 ± 1.29	.68
Kidney Donor Risk Index	1.42 ± 0.5	1.38 ± 0.46	.52

Abbreviations: BMI, body mass index; ECD, expanded-criteria donor; ICU, intensive care unit; SD, standard deviation.

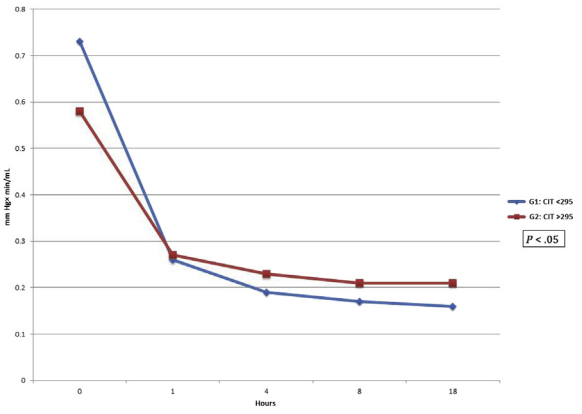

Fig 2. Renal resistance (RR) during perfusion. CIT, cold ischemia time.

significantly lower in the group G1 compared with the group G1 (Fig 4).

One-Year Graft Results

All patients completed the 1-year observation period. The mean time of observation did not differ between the groups (1005 ± 448 days in the group G1 vs 1067 ± 563 days in the group G2; $P = .28$). Patient survival did not differ between the groups and was 97.6% (248/254) in the group G1 vs 95.2% (119/125) in the group G2 ($P = .26$). One-year graft survival was statistically higher in the group G1 compared with the group G2 (94% (239/254) vs 84% (105/135), respectively; $P = .02$). Odds ratio for graft loss during the first year post-transplantation was 3.01 higher if CIT−1 was longer than 295 minutes. Mean serum creatinine levels 1 year post-transplantation were significantly lower in the group G1, and estimated GFR at 1 year post-transplantation was 54 ± 38 mL/min in the group G1 vs 46 ± 23 mL/min in group G2 ($P = .01$; Fig 4). Biopsy proven AR up to 1 year was seen in 16.6% of the group G1 patients vs 21.2% of the group G2 patients ($P = .26$).

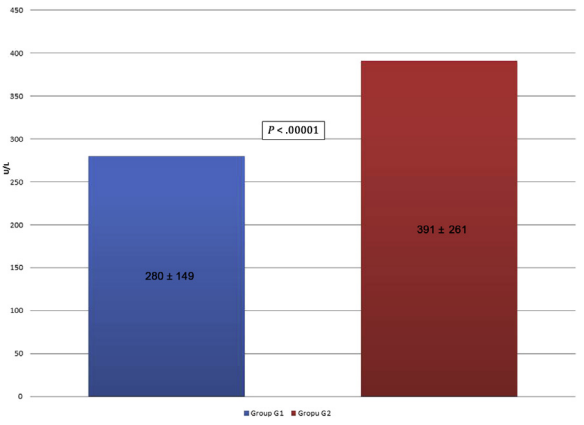

Fig 3. LDH concentration at the fourth hour of perfusion.

Table 2. Recipient Characteristics (mean ± SD)

	Group G1 (n = 254)	Group G2 (n = 125)	*P* Value
Age (years)	48.45 ± 13.8	47.8 ± 13.7	.66
Male sex (%)	61.0%	63.4%	.96
BMI (kg/m^2)	24.6 ± 3.94	24.55 ± 3.47	.82
HD treatment prior to transplant (months)	40 ± 32	40 ± 33	.97
Arterial hypertension (%)	89.0%	91.7%	.44
Ischemic heart disease (%)	24.2%	25.0%	.86
Triple drug immunosuppression (%)	100%	100%	1.00
Induction therapy (%)	56.3%	48%	.13
Mean HLA mismatch (A,B,DR)	3.48 ± 1.1	3.48 ± 1.07	.99

Abbreviations: BMI, body mass index; HD, hemodialysis; SD, standard deviation.

Multivariate Analysis

In multivariate logistic analysis, we included following variables donor age, recipient age, DGF, ECD, CIT, and CIT group < 295. The independent variables that were not significant in the univariate analysis, donor age, recipient age, ECD, and CIT time, were not significant in the multivariate analysis too (Tables 3 and 4). In the multivariate logistic analysis, DGF was found to be statistically significant factors for 1-year graft survival. The beta coefficient for DGF was equal to 0.455. In the second model, we included donor age, recipient age, ECD, and CIT time, group CIT < 295. Here statistically significant was group CIT < 295 ($P = .047$) and beta coefficient, respectively, 0.356 (Table 5).

Analysis of Graft Loss

Within the first year post-transplantation, 15 patients from G1 have lost graft−6% (15/254)−and 30 patients from the G2 group−16% (30/135) ($P = .02$). We collected data of 12

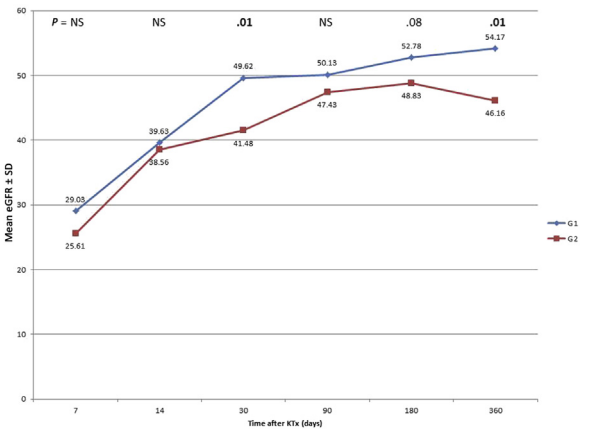

Fig 4. Mean eGFR (Modification of Diet in Renal Disease) after transplantation. eGFR, estimated glomerular filtration rate; KTx, kidney transplantation; NS, not significant; SD, standard deviation.

Table 3. Univariate Analysis

	P
Recipients' age	.1137
ECD	.2528
Donors' age	.7883
CIT	.1556
Group CIT < 295	.0178
DGF	.0051

Abbreviations: CIT, cold ischemia time; DGF, delayed graft function; ECD, expanded-criteria donor.

graft losses from the G1 group (80%) and 23 in the G2 group (77%). There was no statistical difference observed between the groups (Table 6).

DISCUSSION

The results of this study showed that kidneys that were put on HMP up to 295 minutes from beginning of CIT had higher initial renal resistance with a better decrease in renal resistance during perfusion and lower mean level of LDH in the fourth hour of the perfusion. Patients who received kidneys put on HMP up to 295 minutes from procurement had lower incidences of DGF, a shorter hospitalization stay, better 1-year graft survival, serum creatinine levels, and estimated GFR (Modification of Diet in Renal Disease) 1 year post-transplantation. Odds ratio for graft loss during the first year post-transplantation is 3 times higher if the patient received a kidney kept in CS for more than 295 minutes prior to machine perfusion.

HMP has become a routine method of kidney preservation prior to kidney transplantation, especially in ECD and DCD kidneys. Nevertheless, there are several systems of perfusion, and there is a lack of standardization for this procedure. Sometimes kidneys are procured and then transported in CS to the transplantation center where they receive HMP. This nonstandardized method of HMP might be the reason why different authors receive different results using HMP. Watson et al could not see a statistical difference in terms of incidence of DGF and 1-year graft survival with use of HMP [12], while Jochman et al reported a clear difference [1]. This confusing data may be explained when one looks at the method used for HMP. Watson et al introduced HMP at the transplant center [12] while Jochmans et al put kidneys on HMP immediately after retrieval from the donor [1]. It seems logical that HMP should start immediately after retrieval. To the best of our knowledge, there are no publications to date that answer if such an approach would bring benefits for kidneys and patients.

Table 4. Multivariate Analysis With DGF

	Degree of freedom	Wald stat.	*P*
Free term	1	13.88082	.000195
ECD (Y–1; N–0)	1	0.09616	.756485
Group CIT < 295	1	3.45377	.063108
DGF (Y–1; N–0)	1	4.36468	.036691
Donors' age	1	0.80990	.368149
Recipients' age	1	0.00721	.932329
CIT	1	0.29314	.588215

Abbreviations: CIT, cold ischemia time; DGF, delayed graft function; ECD, expanded-criteria donor.

Table 5. Multivariate Analysis Without DGF

	Degree of freedom	Wald stat.	*P*
Free term	1	13.60281	.000226
ECD (Y–1; N–0)	1	0.01084	.917070
Group CIT < 295	1	3.91017*	.047995
Donors' age	1	1.19646	.274031
Recipients' age	1	0.01124	.915553
CIT	1	0.13665	.711630

Abbreviations: CIT, cold ischemia time; DGF, delayed graft function; ECD, expanded-criteria donor.

It may be confused that donor age and ECD status, which is mostly determined by donor's age, did not correlate with 1-year graft survival in our analysis, as it was described by others [13]. The explanation could be the fact that all analyzed kidneys were stored prior to transplantation in hypothermic machine perfusion but the hypothermic machine perfusion has been started after the period of simple cold storage.

We have calculated that if CS prior to HMP was shorter than 295 minutes, it may influence early and 1-year graft survival. The major difference between our groups was time of CS prior to start of HMP. What was observed and seemed surprising was that mean initial RR during perfusion was higher when kidneys were put on MP earlier (0.77 in G1 group vs 0.58 in G2). When we analyze our subgroup of kidneys with CIT–1 below 60 minutes, mean initial renal resistance was 0.85 mm Hg, which is higher than in G1 group (where RR was 0.77 mm Hg). The drop down of RR during first and further hours of perfusion compensate totally this initial high number. In our opinion, it shows that retrieval procedure is a great shock for kidneys due to direct contact to ice during procurement. It may explain the results' high initial renal resistance, and we should think on further improvement on retrieval techniques. One may also consider whether longer CIT–2 time could influence that situation. CIT–2 was longer in group G1, but overall CIT was not statistically different in both groups, and the percentage of kidneys with a CIT–2/CIT–1 ratio > 50% was

Table 6. Graft Loss Analysis

	G1 (n = 15)	G2 (n = 30)	*P*
Urinary tract infection (%)	13.3% (2/15)	16.6% (5/30)	1
Surgical site infection (*organ involved*)	13.3% (2/15)	10% (3/30)	1
Acute rejection	6.6% (1/15)	6.6% (2/30)	1
Chronic rejection	6.6% (1/15)	13.3% (4/30)	.65
Bleeding	6.6% (1/15)	13.3% (4/30)	.65
Arterial thrombosis	13.3% (2/15)	6.6% (2/30)	.59
Venous thrombosis	13.3% (2/15)	6.6% (2/30)	.59
Noncompliance	6.6% (1/15)	3.3% (1/30)	1
Unknown	20% (3/15)	23% (7/30)	1

similar in both groups, allowing the assumption that the shorter CIT−1 in group G1 had a major impact on early and 1-year graft survival, which was also seen in the logistic regression analysis. The reason for unfavorable results of early and 1-year graft survival if CS was longer than 295 minutes might be answered on a pathophysiological level on endothelium activation. The beneficial effect of HMP in kidney protection during the preservation and reperfusion period is due to better endothelium protection and maintaining vasorelaxation [14]. Flow cessation within 2 hours diminishes the production of endothelial protective molecules such as Kruppel-like factor−2 [15]. Ischemic injury, which may lead to higher production of transforming growth factor β and enhanced process of interstitial fibrosis [16], is more pronounced in kidneys kept in CS [9]. Therefore, it seems to be inevitable to have perfusion systems that allow a quick start of HMP after procurement. It also seems that there is an urgent need to establish standards for pulsatile preservation of kidneys and other organs prior to transplantation.

The analysis is not free from limitations as this is retrospective, single-center, cohort study. The negative predictive value for ROC analysis is relatively low, which suggests obviously that many factors play a role in 1-year graft survival and that reduced ischemia time before starting HMP may not be sufficient to provide satisfactory results.

In conclusion, kidneys should be put on HMP as soon as possible after retrieval, preferably during procurement, and this study showed that introduction of HMP up to 295 minutes from kidney procurement leads to better early and 1-year graft outcomes. All perfusion devices should be capable of transportation and should be designed to be able to use during retrieval to limit the adverse effect of CS.

ACKNOWLEDGMENTS

The authors wish to thank to Prof. Leszek Paczek and the team of doctors from the Department of Immunology, Transplantology, and Internal Diseases, Medical University of Warsaw, Warsaw, Poland, for their effort in medical care in some of patients in this study.

REFERENCES

[1] Jochmans I, Moers C, Smits JM, et al. Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial. Ann Surg 2010;252:756–64.

[2] Treckmann J, Moers C, Smits JM, et al. Machine perfusion versus cold storage for preservation of kidneys from expanded criteria donors after brain death. Transpl Int 2011;24:548–54.

[3] Kwiatkowski A, Wszola M, Kosieradzki M, et al. Machine perfusion preservation improves renal allograft survival. Am J Transplant 2007;7:1942–7.

[4] Kwiatkowski A, Wszola M, Kosieradzki M, et al. The early and long term function and survival of kidney allografts stored before transplantation by hypothermic pulsatile perfusion. A prospective randomized study. Ann Transplant 2009;14:14–7.

[5] Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med 2009;360:7–19.

[6] Kosieradzki M, Danielewicz R, Kwiatkowski A, et al. Early function of kidneys stored by continuous hypothermic pulsatile perfusion can be predicted using a new "viability index. Transplant Proc 2002;34:541–3.

[7] Danielewicz R, Kwiatkowski A, Polak W, et al. An assessment of ischemic injury of the kidney for transplantation during machine pulsatile preservation. Transplant Proc 1997;29:3580–1.

[8] Schold JD, Kaplan B, Howard RJ, Reed AI, Foley DP, Meier-Kriesche HU. Are we frozen in time? Analysis of the utilization and efficacy of pulsatile perfusion in renal transplantation. Am J Transplant 2005;5:1681–8.

[9] Kwiatkowski A, Wszola M, Perkowska-Ptasinska A, et al. Influence of preservation method on histopathological lesions of kidney allografts. Ann Transplant 2009;14:10–3.

[10] Wszola M, Kwiatkowski A, Domagala P, et al. Preservation of kidneys by machine perfusion influences gene expression and may limit ischemia/reperfusion injury. Prog Transplant 2014;24:19–26.

[11] Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–77.

[12] Watson CJ, Wells AC, Roberts RJ, et al. Cold machine perfusion versus static cold storage of kidneys donated after cardiac death: a UK multicenter randomized controlled trial. Am J Transplant 2010;10:1991–9.

[13] Metzger RA, Delmonico FL, Feng S, Port FK, Wynn JJ, Merion RM. Expanded criteria donors for kidney transplantation. Am J Transplant 2003;3(Suppl. 4):114–25.

[14] Harrison DG, Widder J, Grumbach I, Chen W, Weber M, Searles C. Endothelial mechanotransduction, nitric oxide and vascular inflammation. J Intern Med 2006;259:351–63.

[15] Gracia-Sancho J, Villarreal Jr G, Zhang Y, et al. Flow cessation triggers endothelial dysfunction during organ cold storage conditions: strategies for pharmacologic intervention. Transplantation 2010;90:142–9.

[16] Kedzierska K, Domanski M, Sporniak-Tutak K, Dolegowska B, Ciechanowski K. Oxidative stress and renal interstitial fibrosis in patients after renal transplantation: current state of knowledge. Transplant Proc 2011;43:3577–83.