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Machine Perfusion or Cold Storage in Deceased-Donor Kidney Transplantation

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ABSTRACT

BACKGROUND

Static cold storage is generally used to preserve kidney allografts from deceased donors. Hypothermic machine perfusion may improve outcomes after transplantation, but few sufficiently powered prospective studies have addressed this possibility.

METHODS

In this international randomized, controlled trial, we randomly assigned one kidney from 336 consecutive deceased donors to machine perfusion and the other to cold storage. All 672 recipients were followed for 1 year. The primary end point was delayed graft function (requiring dialysis in the first week after transplantation). Secondary end points were the duration of delayed graft function, delayed graft function defined by the rate of the decrease in the serum creatinine level, primary nonfunction, the serum creatinine level and clearance, acute rejection, toxicity of the calcineurin inhibitor, the length of hospital stay, and allograft and patient survival.

RESULTS

Machine perfusion significantly reduced the risk of delayed graft function. Delayed graft function developed in 70 patients in the machine-perfusion group versus 89 in the cold-storage group (adjusted odds ratio, 0.57; P=0.01). Machine perfusion also significantly improved the rate of the decrease in the serum creatinine level and reduced the duration of delayed graft function. Machine perfusion was associated with lower serum creatinine levels during the first 2 weeks after transplantation and a reduced risk of graft failure (hazard ratio, 0.52; P=0.03). One-year allograft survival was superior in the machine-perfusion group (94% vs. 90%, P=0.04). No significant differences were observed for the other secondary end points. No serious adverse events were directly attributable to machine perfusion.

CONCLUSIONS

Hypothermic machine perfusion was associated with a reduced risk of delayed graft function and improved graft survival in the first year after transplantation. (Current Controlled Trials number, ISRCTN83876362.)

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Two different forms of organ preservation — static cold storage and hypothermic machine perfusion — are used clinically for renal allografts obtained from deceased donors. In static cold storage, the kidney is flushed, cooled with one of several cold preservation solutions, and transported on ice. In hypothermic machine perfusion, after an initial washout of blood, the kidney is connected to a perfusion device, and a solution is pumped continuously through the renal vasculature at temperatures between 1 and 10°C.[1] The typical deceased kidney donor today is older and has been exposed to more concomitant disease than donors were several decades ago; these factors may have a detrimental effect on allograft quality.[2,3] In addition, the use of organs received from donors after cardiocirculatory death is increasing in most countries.[4] Such allografts are known to have significantly higher rates of delayed graft function.[4,5] Evidence suggests that organs that do not function immediately after transplantation have an increased risk of acute rejection, and allograft survival may be inferior.[6,7] In addition, delayed graft function increases the costs of kidney transplantation.[8,9] Retrospective studies have suggested that machine perfusion could result in a better short-term outcome, with lower rates of delayed graft function after transplantation of kidneys from all types of deceased donors.[9-11] Therefore, interest in machine perfusion is increasing. Our international randomized, controlled trial compared machine perfusion with cold-storage preservation in deceased-donor kidney transplantation with a primary end point of delayed graft function.

METHODS

STUDY DESIGN

This investigator-driven, international randomized, controlled study included the Netherlands, Belgium, and the federal state of North Rhine–Westphalia in Germany. All consecutive deceased-donor kidney pairs identified in these regions that met the initial inclusion criteria were eligible for randomization by Eurotransplant, the international organ-exchange organization of Austria, Belgium, Croatia, Germany, Luxemburg, the Netherlands, and Slovenia (Croatia became a member after the present study was completed). Since we aimed to include the whole spectrum of deceased donors, no previous selection of donor types to be included was made. Thus, the study reflects the effect of machine perfusion as compared with cold storage in everyday practice within an international organ-exchange organization. From each donor, one kidney was randomly assigned to machine perfusion and the contralateral organ to cold storage. The organ could be transplanted into any recipient within the Eurotransplant region.[12] Approval for the study was obtained from the ethics review boards in each trial region and from the Eurotransplant Ethical Advisory Committee and Kidney Advisory Committee. Since the random assignment of kidneys to a preservation method was limited to organs isolated before transplantation, no informed consent from recipients was required for this intervention.

An independent scientific steering committee composed of clinicians and scientists from each trial region was solely responsible for the design, conduct, data analysis, and manuscript preparation for this study.

INCLUSION AND EXCLUSION CRITERIA

Organ donors had to be 16 years of age or older. Only kidney pairs from deceased donors were included in the study, either from donation after brain death or donation after cardiocirculatory death. The category for donors without a heartbeat had to be Maastricht category III (awaiting cardiocirculatory death after withdrawal of treatment) or IV (cardiocirculatory death in a brain-dead donor).[13] Kidney pairs were included only if both organs were actually transplanted into two different recipients. If one kidney was transplanted into the same recipient together with another organ, this kidney pair was excluded. The only exclusion criterion for recipients was the death of the patient in the first week after transplantation, since a follow-up of at least 1 week was required to determine the primary end point.

RANDOMIZATION

A randomization scheme based on permuted blocks within regions was used with separate randomization lists for each trial region. A detailed description of the randomization process is available in the Supplementary Appendix, available with the full text of this article at NEJM.org. Surgical teams were allowed to switch preservation methods only if the kidney assigned to machine perfusion had an aortic patch that was too small or if it had too many renal arteries for a reliable con-

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nection to the machine-perfusion device; this switch in preservation methods changed the initial randomization.

LOGISTICS

In each trial region, a team of trained perfusionists was on hand 24 hours per day, 7 days per week to respond when a donor became available. The perfusionists transported the machine-perfusion device to the donor hospital and assisted donor surgeons with connecting one kidney to the machine. No changes were made to the existing Eurotransplant rules for organ allocation or to transportation protocols. Kidneys that underwent machine perfusion as well as those that were preserved with cold storage were transported to their respective recipient center without any monitoring.

HYPOTHERMIC MACHINE PERFUSION

LifePort Kidney Transporter machines (Organ Recovery Systems) were used for perfusion, delivering a pulsatile flow of University of Wisconsin machine preservation solution (Kidney Preservation Solution-1)[14] at 1 to 8°C, with no changes in perfusion settings throughout the preservation period. The systolic perfusion pressure was fixed at 30 mm Hg, and the kidneys underwent machine perfusion from organ procurement until transplantation. To prevent bias in clinical decisions about transplanting or discarding an organ, intravascular resistance and flow readings were never revealed to the transplantation team.

COLD STORAGE

No changes were made to the standard cold-storage protocols. After an initial vascular washout, kidneys were submerged in the preservation solution and stored on melting ice, according to the established Eurotransplant routine.

DATA COLLECTION

Follow-up data were provided by each participating transplantation center through a secure online database hosted by Eurotransplant. A random sample of 10% of all patients was audited externally; no relevant irregularities were found.

STUDY END POINTS

The primary end point was delayed graft function, defined as the requirement for dialysis during the first week after transplantation. The secondary end points were the duration of delayed graft function, primary nonfunction (permanent lack of function of the allograft from the time of transplantation), the area under the curve of the daily serum creatinine level at days 1 to 14, the creatinine clearance at day 14, biopsy-proven acute rejection, toxicity of the calcineurin inhibitor, the length of the recipient's hospital stay, and survival of the graft and patient up to 1 year after transplantation. Data on graft survival were censored at the time of death in patients who died with a functioning allograft. In addition to the primary end point, which was defined in terms of the requirement for dialysis after transplantation, we also examined delayed graft function as a secondary end point. This secondary end point, functional delayed graft function, was defined in terms of the absence of a decrease in the serum creatinine level of at least 10% per day for at least 3 consecutive days in the first week after transplantation, not including patients in whom acute rejection, toxicity of the calcineurin inhibitor, or both developed within the first week.[15] All end points described above were prespecified in the study protocol, except primary nonfunction, which was added post hoc.

STATISTICAL ANALYSIS

This study was powered to detect a reduction in delayed graft function of at least 10%, based on a presumed incidence of 35% among recipients of kidneys that had been preserved by means of cold storage. With a statistical power of 0.8 and a one-sided type I error of 0.05, the minimum required sample size was 300 kidney pairs; this is equivalent to the required sample size for a logistic-regression analysis with a two-sided type I error of 0.05 and similar power.[16] The primary analysis of the primary end point — delayed graft function — consisted of a logistic-regression model, which examined whether machine perfusion as compared with cold-storage preservation, in the context of other relevant factors, influenced the risk of delayed graft function.[7,17] Covariates for this model (see the Supplementary Appendix) were prespecified in the study protocol and were based on relevant literature.[18,19] The final model was determined by entering all covariates together in the analysis, with a built-in normal gamma frailty term for the donor to account for the paired study design.[20] For end-point variables, univariate differences between the groups were assessed with the use of McNemar's test or the Wilcoxon signed-rank test. For demographic variables, differences were

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assessed with the use of Fisher's exact test or the Mann–Whitney test. The Kaplan–Meier method was used to analyze graft and patient survival. Differences between survival curves were determined with the use of log-rank tests. A Cox proportional-hazards model was applied to examine which variables significantly influenced the risk of graft failure.[21] To construct this model, an approach similar to the logistic-regression model for delayed graft function was followed.

We performed prespecified subgroup analyses to determine the treatment effect on the primary end point according to donation after cardiocirculatory death versus donation after brain death and according to expanded-criteria donation versus standard-criteria donation.[22] Expanded-criteria donation was defined as a donor age of 60 years or more or a donor age between 50 and 60 years, with at least two of the following additional donor characteristics: history of hypertension, death due to a cerebrovascular cause, and a serum creatinine level of more than 132 μmol per liter (1.5 mg per deciliter) before removal of the kidney.[23]

All reported P values are two-sided and not adjusted for multiple testing. A P value of 0.05 or less was considered to indicate statistical significance. Analyses were conducted with the use of the SPSS, SAS, and R software packages and were based on all organ pairs that met the inclusion criteria.

No interim analyses of study end points were carried out. At regular intervals, confidential safety analyses were performed by the trial safety board, which compared the reported rates of adverse events between the two trial groups. The sponsor was not involved in the conduct of the study, the analysis or storage of the data, or the preparation of the manuscript. The scientific steering committee vouches for the accuracy and completeness of the data and analyses.

RESULTS

From November 1, 2005, through October 31, 2006, there were 654 potential deceased kidney donors 16 years of age or older in the three trial regions. Figure 1 shows a flow diagram of the 336 kidney pairs (672 recipients) included in our analysis. In 25 donors (4.6%), preservation methods were switched because of the aberrant vascular anatomy of the kidney assigned to machine perfusion. Vascular anomalies were not observed to have a significant effect on delayed graft function. Aberrant vascular anatomy did not significantly increase the risk of graft failure, and the addition of this factor to the Cox model had no effect on the hazard ratio for graft failure associated with machine perfusion versus cold storage (see the Supplementary Appendix).

The 20 "other reasons for exclusion" of the kidney pairs (Fig. 1) were as follows: 12 adverse events that occurred during the donor procedure, 5 cases in which the donor had one kidney, 2 cases in which the consent for kidney donation was withdrawn just before procurement, and 1 procedure involving a donor after cardiocirculatory death that was planned as a Maastricht category III donation but was changed to a Maastricht category II donation (cardiocirculatory death after unsuccessful resuscitation).

STUDY PATIENTS

Table 1 summarizes the characteristics of the study groups. All kidneys donated after cardiocirculatory death were in Maastricht category III, as defined earlier. There were no significant differences between the two groups with regard to relevant baseline characteristics.

DELAYED GRAFT FUNCTION

Delayed graft function occurred in 70 recipients in the machine-perfusion group (20.8%) as compared with 89 patients in the cold-storage group (26.5%). Table 2 shows the results of analysis using the logistic-regression model. As compared with cold storage, machine perfusion significantly reduced the risk of delayed graft function (adjusted odds ratio, 0.57; P=0.01).

SUBGROUP ANALYSIS

In September 2006, when enrollment of donors in the study was nearly complete, the scientific steering committee expected that an insufficient number of donors would be enrolled at trial completion to conduct a meaningful subgroup analysis for donation after cardiocirculatory death. At the suggestion of the steering committee and with the permission of all centers, the inclusion of additional donors after cardiocirculatory death was extended by an amendment to the protocol, until a total of 82 donors were enrolled on August 17, 2007 (see the Supplementary Appendix for details). Solely for the subgroup analysis involving donation

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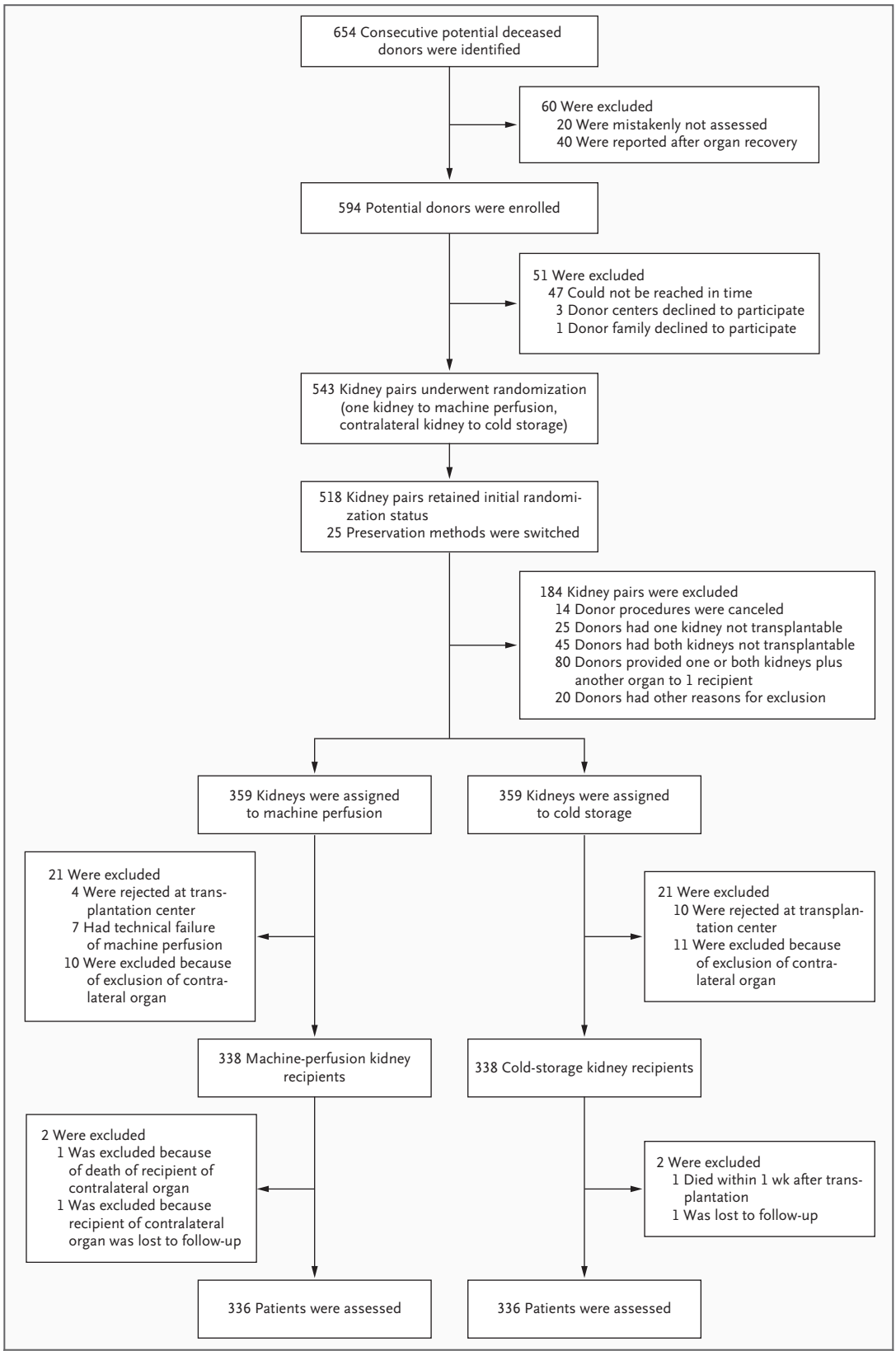

Figure 1. Enrollment, Assignment of Kidney Pairs to Machine Perfusion or Cold Storage, Follow-up, and Assessment.

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Table 1. Characteristics of Donors, Recipients, and Transplants and Univariate Differences between the Groups.

Variable	Machine-Perfusion Group (N=336)	Cold-Storage Group (N=336)	P Value*
Donor characteristics			
Age (yr)			
Median	51		
Range	16–81		
Type of donation (no.)			
After brain death	294		
After cardiocirculatory death	42		
Standard criteria	242		
Expanded criteria	94		
Vascular flush solution (no.)			
University of Wisconsin solution	216		
Histidine–tryptophan–ketoglutarate solution	108		
Euro–Collins solution	1		
Not reported	11		
Recipient characteristics			
Age (yr)			0.21
Median	53	52	
Range	11–79	2–79	
Duration of pretransplantation dialysis (yr)			0.59
Median	4.5	4.4	
Range	0.15–18	0.19–24	
Previous transplants (%)†	23	21	0.38
Panel-reactive antibody level (no.)			0.68
0–5%	297	304	
6–84%	35	29	
>84%	4	3	
Immunosuppressive drugs (%)			
Prednisolone	98	99	0.77
Cyclosporine	50	54	0.25
Tacrolimus	49	46	0.39
Azathioprine	1	2	0.18
Mycophenolate mofetil	86	87	0.73
Antithymocyte globulin	14	13	0.82
Interleukin-2 receptor antagonists	42	47	0.18
Transplant characteristics			
No HLA mismatches (% with no mismatches at the HLA-A, B, or DR loci)	16	15	0.90
Cold ischemic time (hr)			0.30
Median	15.0	15.0	
Range	3.5–29.7	2.5–29.7	
Allograft with >1 renal artery (%)	20	22	0.51

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Table 1. (Continued.)

Variable	Machine-Perfusion Group (N=336)	Cold-Storage Group (N=336)	P Value*
Primary end point			
Delayed graft function (%)	20.8	26.5	0.05
Secondary end points			
Functional delayed graft function (%)‡	22.9	30.1	0.03
Primary nonfunction (%)§	2.1	4.8	0.08
Duration of delayed graft function (days)			0.04
Median	10	13	
Range	1–48	1–41	
Creatinine clearance at day 14 (ml/min)			0.25
Median	42	40	
Range	0–171	0–175	
Calcineurin-inhibitor toxicity within 14 days after transplantation (%)	6.3	5.7	0.86
Acute rejection within 14 days after transplantation (%)	13.1	13.7	0.91
Post-transplantation hospital stay (days)			0.78
Median	19	18	
Range	4–392	6–382	

* For baseline characteristics, P values were calculated with the use of Fisher's exact test for discrete variables and the Mann–Whitney test for continuous variables. For end-point variables, P values were calculated with the use of McNemar's test for discrete variables and the Wilcoxon signed-rank test for continuous variables.
† This category was the percentage of recipients who had undergone one or more renal transplantations before the transplantation included in this analysis.
‡ Functional delayed graft function was defined as the absence of a decrease in the serum creatinine level of at least 10% per day for at least 3 consecutive days in the first week after transplantation. This category did not include patients in whom acute rejection, calcineurin-inhibitor toxicity, or both developed in the first week.
§ Primary nonfunction was defined as the permanent lack of function of the allograft from the time of transplantation.

after brain death versus donation after cardiocirculatory death, these inclusions were added to the main group of patients to provide more statistical power. Figure 2 shows a forest plot of the treatment effect in the prespecified subgroup analyses. In the main data set, we found no significant difference in the magnitude of the treatment effect on delayed graft function after standard-criteria donation versus expanded-criteria donation (P=0.75) and after donation after brain death versus donation after cardiocirculatory death (P=0.42). In the extended data set, the effect of the preservation method on delayed graft function did not differ significantly between patients who received kidneys from donors after brain death versus patients who received kidneys from donors after cardiocirculatory death (P=0.26).

SECONDARY END POINTS

Functional delayed graft function occurred in 77 recipients in the machine-perfusion group and in 101 recipients in the cold-storage group (22.9% vs. 30.1%, P=0.03). The incidence of primary nonfunction in the cold-storage group (4.8% vs. 2.1%, P=0.08) was more than two times higher than in the machine-perfusion group, but this difference did not reach statistical significance. If delayed graft function developed, its duration was 3 days shorter after machine perfusion as compared with cold storage (10 days vs. 13 days, P=0.04). There were no significant differences between the study groups in creatinine clearance at 14 days after transplantation, length of hospital stay of recipients, the incidence of toxicity of the calcineurin inhibitor, and acute rejection rate in the first 14

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Table 2. Multivariate Analysis of the Risk of Delayed Graft Function and Graft Failure.*

Variable	Odds Ratio (95% CI)	Hazard Ratio (95% CI)	P Value
Delayed graft function			
Machine perfusion vs. cold storage	0.57 (0.36–0.88)		0.01
Panel-reactive antibody level — %	1.01 (0.99–1.02)		0.29
Recipient age — yr	1.01 (0.99–1.03)		0.28
Donor age — yr	1.03 (1.00–1.06)		0.04
ECD donor vs. SCD donor†	1.04 (0.46–2.34)		0.92
Cold ischemic time — hr	1.08 (1.03–1.14)		0.003
HLA mismatches — no.	1.13 (0.94–1.37)		0.18
Duration of pretransplantation dialysis — yr	1.16 (1.03–1.31)		0.01
Second or later transplantation vs. first transplantation	3.01 (1.75–5.18)		<0.001
DCD donor vs. DBD donor	17.2 (8.16–36.2)		<0.001
Graft failure within 1 yr after transplantation‡			
Machine perfusion vs. cold storage		0.52 (0.29–0.93)	0.03
DCD donor vs. DBD donor		0.90 (0.28–2.92)	0.87
Recipient age — yr		0.97 (0.95–1.00)	0.02
Duration of pretransplantation dialysis — yr		1.00 (0.87–1.15)	0.97
Panel-reactive antibody level — %		1.01 (0.99–1.03)	0.31
Cold ischemic time — hr		1.04 (0.97–1.11)	0.25
Donor age — yr		1.05 (1.01–1.10)	0.02
ECD donor vs. SCD donor†		1.18 (0.42–3.27)	0.76
HLA mismatches — no.		1.23 (0.98–1.55)	0.08
Second or later transplantation vs. first transplantation		1.72 (0.88–3.35)	0.11

* A logistic-regression model was used to determine the odds ratio for delayed graft function, and a Cox proportional-hazards model was used to determine the hazard ratio for graft failure. Odds ratios and hazard ratios are associated with a 1-unit increase in each covariate. CI denotes confidence interval, DBD donation after brain death, DCD donation after cardiocirculatory death, ECD expanded-criteria donation, and SCD standard-criteria donation.

† Since donor age was a separate covariate in these models and donor age was also part of the ECD definition, the effect of ECD versus SCD on delayed graft function and the risk of graft failure may appear to be less pronounced than commonly reported.

‡ Data on graft survival were censored at the time of death in patients who died with a functioning allograft.

days after transplantation. Daily serum creatinine values in the first 2 weeks after transplantation were significantly lower in recipients in the machine-perfusion group than in recipients in the cold-storage group (median area under the curve, 1456 [range, 385 to 5782] vs. 1787 [range, 288 to 6500]; P=0.01) (see Fig. S2 in the Supplementary Appendix).

PATIENT AND GRAFT SURVIVAL

In the cold-storage group, one patient died within 1 week after transplantation because of cardiac arrhythmia and was therefore excluded from the study along with the recipient of the contralateral kidney. At 1 year after transplantation, patient survival was 97% in both groups. Between 7 days and 1 year after transplantation, 11 patients in the machine-perfusion group died and 9 patients in the cold-storage group died (Table 3). One-year graft survival (Fig. 3) in the machine-perfusion group was significantly higher than in the cold-storage group (94% vs. 90%, P=0.04). Cox regression analysis (Table 2) showed that machine perfusion significantly reduced the risk of graft failure in the first year after transplantation, with a hazard ratio of 0.52 (P=0.03). A post hoc analysis in which delayed graft function was added as a time-dependent covariate to the Cox model indicated that re-

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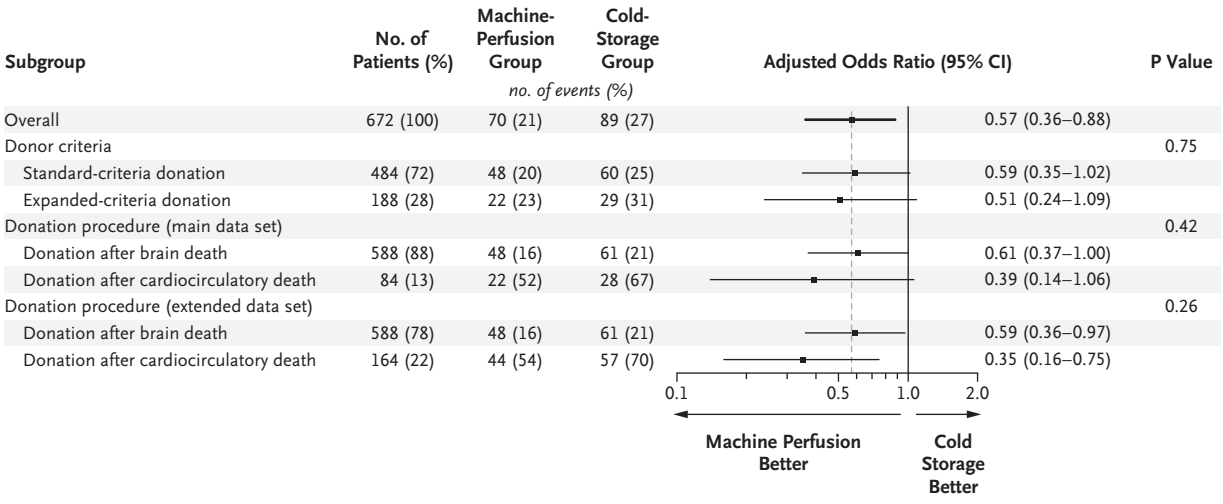

Figure 2. Forest Plot of the Treatment Effect in Prespecified Subgroup Analyses.

In the main data set, there was no significant difference in the magnitude of the treatment effect on delayed graft function after standard-criteria donation versus expanded-criteria donation (P=0.75) and after donation after brain death versus donation after cardiocirculatory death (P=0.42). The extended data set consisted of the main data set plus the additional 80 recipients of kidneys from donors after cardiocirculatory death who were enrolled after the inclusions had ended. This extended data set of 752 recipients was used solely to provide more statistical power for a meaningful subgroup analysis of donation after cardiocirculatory death versus donation after brain death. In the extended data set, the effect of the preservation method on delayed graft function did not differ significantly between patients who received kidneys from donors after brain death versus patients who received kidneys from donors after cardiocirculatory death (P=0.26). P values are for the interaction between the treatment effect (machine perfusion vs. cold storage) and any subgroup variable.

cipients with delayed graft function had a significantly increased risk of graft failure (hazard ratio, 1.69; P<0.001); when this was applied, the hazard ratio for graft failure with machine perfusion versus cold storage increased to 0.60, and this covariate became nonsignificant in the model (P=0.08) (see the Supplementary Appendix).

ADVERSE EVENTS

Table 3 summarizes reported adverse events and deaths. No serious adverse events directly attributable to machine perfusion were observed.

DISCUSSION

Static cold storage is the easiest and most widely used preservation method in kidney transplantation. In the United States, it is used in 80% of these procedures, and in Eurotransplant countries it is used in approximately 100%.[24,25] Although retrospective studies have suggested that machine perfusion is superior,[9-11] these registry analyses are biased because of the selection of donor kidneys to be perfused or allografts that are discarded on the basis of perfusion variables. Several prospective studies have either lacked adequate randomization or have had equivocal results because of small sample sizes.[26-30] The present study indicates that machine perfusion significantly reduces the risk of delayed graft function; these findings are probably related to the study's size and strictly paired design.

The relatively large number of exclusions in our study is typical for a paired study in organ preservation, since logistics necessitated that randomization occur at a very early stage in the donation cascade, when a patient in an intensive care unit (ICU) was a potential kidney donor. Only after both kidneys had actually been transplanted could we determine whether a donor would meet the inclusion criteria. The exclusion of donors from whom one kidney was discarded may have led to a mild bias toward the "better" kidney donors in our study. The same might be true regarding donors who were not included because the donor hospital could not be reached in time by the perfusionist. Theoretically, such donors may have been patients in the ICU who had more unstable conditions. Conversely, excluding donors from whom com-

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Table 3. Adverse Events and Deaths Reported in the First Year after Transplantation.*

Variable	Machine-Perfusion Group (N=336)	Cold-Storage Group (N=336)
	no. of events (%)	
Adverse events		
During donor procedure†		
Vascular anatomy of both kidneys unsuitable for machine perfusion	7 (2)	
Surgical team insisted on using machine perfusion for both kidneys	4 (1)	
Surgical team declined to cooperate with study	3 (1)	
13-yr-old donor mistakenly underwent randomization	1 (<1)	
Renal polar artery overlooked during procurement‡	1 (<1)	
During organ preservation		
Technical failure or malfunction during machine perfusion§	7 (2)	NA
Delayed delivery of cross-match material¶	1 (<1)	0
Serious — in recipients		
Any serious event	77 (23)	88 (26)
Severe urinary tract infection	11 (3)	10 (3)
Sepsis due to any cause	9 (3)	10 (3)
Diabetes mellitus	9 (3)	10 (3)
Severe respiratory tract infection	8 (2)	14 (4)
Postoperative bleeding	8 (2)	8 (2)
Peritonitis	6 (2)	5 (1)
Any arterial thrombosis	6 (2)	4 (1)
Any venous thrombosis	6 (2)	4 (1)
Any cancer	4 (1)	9 (3)
Severe gastrointestinal tract infection	4 (1)	5 (1)
Cardiac decompensation	3 (1)	3 (1)
Myocardial infarction	2 (1)	2 (1)
Ileus	1 (<1)	3 (1)
Gastrointestinal bleeding	0	2 (1)
Minor — in recipients		
Any minor event	170 (51)	148 (44)
Uncomplicated urinary tract infection	43 (13)	47 (14)
Cytomegalovirus infection or reactivation of infection	23 (7)	29 (9)
Uncomplicated gastrointestinal tract infection	22 (7)	21 (6)
Seroma	20 (6)	13 (4)
Ureteral stenosis (graft)	12 (4)	5 (1)
Anemia	11 (3)	8 (2)
Electrolyte disturbances	9 (3)	5 (1)
Leukopenia	7 (2)	2 (1)
Wound abscess	5 (1)	3 (1)
Hydronephrosis of unknown cause (graft)	5 (1)	2 (1)
Mild cardiac arrhythmia	5 (1)	2 (1)
Incisional hernia	4 (1)	5 (1)
Upper respiratory tract infection	2 (1)	6 (2)
Renal capsular hematoma due to biopsy‖	2 (1)	NA

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Table 3. (Continued.)

Variable	Machine-Perfusion Group (N=336)	Cold-Storage Group (N=336)
	no. of events	
Deaths		
Any cause	11	9
Multiorgan failure due to sepsis	4	2
Gastrointestinal bleeding	2	0
Death from unknown cause	2	0
Pneumonia	1	2
Malignant condition	1	1
Pulmonary embolism	1	0
Myocardial infarction	0	2
Cerebral abscess	0	1
Uncontrolled bleeding	0	1

* All serious adverse events except the study end points are listed in this table. No serious adverse events directly attributable to machine perfusion were reported. Of all minor adverse events, only those that occurred in 1% or more of all patients are listed. No statistical tests were performed on the data in this table. NA denotes not applicable.
† All these events led to exclusion of the kidney pair from the study.
‡ One kidney was unsuitable for transplantation because of the insufficient length of the remaining polar artery.
§ None of these events rendered the graft unsuitable for transplantation. When machine perfusion failed, the kidney was automatically preserved by means of cold storage inside the machine.
¶ Transplantation was postponed for 3 hours because of a delayed cross-match.
‖ For an amendment to the study protocol that addressed additional research questions not reported in this article, cortical-biopsy specimens were obtained from several machine-perfused kidneys. Capsular hematomas did not compromise the function of these kidneys.

bined kidney–pancreas transplantations were performed may have slightly biased the data in the opposite direction, since, in general, only the most optimal donors are considered for these procedures. In a small number of patients, the initial randomization was switched because of the vascular anatomy. It is unlikely that this practice has significantly biased the study's outcomes, since aberrant vascular anatomy did not have a significant effect on delayed graft function or on the risk of graft failure, and the observed effect of the machine perfusion versus cold-storage covariate did not change when this factor was added to the Cox model.

The effect of machine perfusion on delayed graft function in our study is slightly stronger than the associations observed in retrospective studies and meta-analyses (odds ratios, 0.62 to 0.73).[9,10] The median cold ischemic time in both treatment groups was relatively short as compared with that in other data sets[25]; this may explain why the incidence of delayed graft function in the cold-storage group in this study was 8.5% lower than the originally anticipated incidence of 35.0%. In addition, the effect of machine perfusion may have been stronger if cold ischemic times had been longer.[25] Machine perfusion was associated with a more pronounced decrease in functional delayed graft function than that observed in the primary end point. Hence, the magnitude of the beneficial short-term effect of machine perfusion may, in part, depend on how delayed graft function is defined.

The treatment effect on the primary end point did not differ between subgroups of deceased donors. On the basis of the evidence from this and other studies,[11] it is probably most legitimate to assume that the effect of machine perfusion as compared with cold storage on delayed graft function is at or near the overall odds ratio of 0.57 in various subgroups. With this assumption, machine perfusion can be considered to have a beneficial effect on the short-term outcome in all common types of deceased-donor kidney transplantation.

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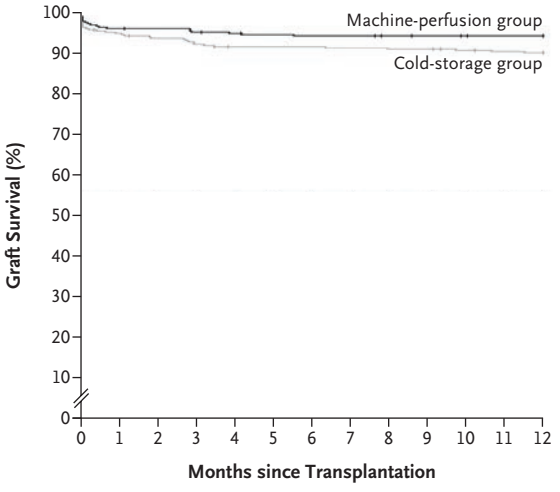

No. at Risk													
Machine-perfusion group	336	323	322	319	317	315	314	314	312	311	310	309	309
Cold-storage group	336	318	313	308	304	304	304	303	302	302	299	299	296

Figure 3. Graft Survival after Transplantation.

The rate of graft survival at 1 year in the machine-perfusion group was significantly higher than the rate in the cold-storage group (94% vs. 90%, P=0.04). Data on graft survival were censored at the time of death in patients who died with a functioning allograft.

Nevertheless, there is a higher incidence of delayed graft function among recipients of kidneys donated after cardiocirculatory death and with expanded-criteria donation.[31] Hence, the absolute number of patients who would actually benefit from machine perfusion might be larger in these subgroups.

Machine perfusion was associated with a significant decrease in graft loss, which became apparent within 1 year after transplantation. The post hoc addition of delayed graft function as a covariate to the Cox model suggests that delayed graft function renders a kidney recipient more at risk for graft failure. In addition, it was linked to an increase in the hazard ratio for graft failure associated with machine perfusion versus cold storage, and this covariate became nonsignificant in the model. Therefore, we think that the reduction in delayed graft function associated with machine perfusion contributes to the improvement in graft survival.

The number of patients with primary nonfunction was reduced by half in the machine-perfusion group as compared with the cold-storage group. However, this difference was not statistically significant, which may be explained by the low overall incidence of primary nonfunction. In this trial, characteristics of machine perfusion were not allowed to be used as a diagnostic tool to identify kidneys that were at risk for a poor outcome. Although evidence is scarce, attention to these variables, as well as to perfusate viability markers, might further increase the effect of machine perfusion on transplantation outcomes.[32]

In conclusion, the present trial showed that hypothermic machine perfusion reduced the incidence of delayed graft function in the kidneys obtained from the most common types of deceased donors. In addition, machine perfusion reduced the duration of delayed graft function, when it occurred. Machine-perfused renal allografts had a lower risk of graft failure in the first year after transplantation and, as a result, these kidneys showed an improved 1-year graft survival as compared with kidneys preserved by static cold storage.

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APPENDIX

The following persons participated in the trial: **Former Director of Eurotransplant** — B. Cohen; **Perfusionists** — I. Abou Habaga, M. Bijvoet, A. de Boer, L. Boneschansker, J. Bronkhorst, J.W. Buikema, E. Dierselhuis, M. Drescher, A. Gallinat, B. Ganske, R. Grond, J. Hamminga, F. Heisterkamp, M. Hellemons, L. van Hessem, L. van den Heuvel, A. Hosman, W. Jager, H. Jansen, M. Kilsdonk, E. Kingma, M. Lambregts, S. Lehnick, B. Lier, N. Luiting, M. Mellema, J. Munster, L. van Nunspeet, H. Oosterhuis, D. Reimer, A. Romeijn, C. Roosendaal, A. de Rotte, S. Schipperijn, M. Schlusen, T. Schwert, J. Siebe, J. Sierink, J. Sprakel, R.J. Sprong, M. Stakelbeek, T. Stege, W. Stomp, I. Thies, T.L. Trang, P.-J. Vlaar, G. de Vries, E. de Vries, M. Wiegman, L. Wierenga, J. Wind, E. Witte; **Eurotransplant Duty Desk Officers** — M. Bekker, C. van den Berg, T. Berger, M. Blikkendaal, L. Boogert, E.J. Bos, D. da Costa, S. Driessen, H. Duijsens, K.

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