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# Impact of Extraction Time During Donation After Circulatory Death Organ Procurement on Kidney Function After Transplantation in The Netherlands

Erika van Straalen, MSc,[1] Elsaline Rijkse, MD, PhD,[1] AnneLoes van Staa, MD, PhD,[2] Paul M. Rebers,[1] Hanneke J.A.M. Hagenaars,[1] Jacqueline van de Wetering, MD, PhD,[3] Jan N.M. Ijzermans, MD, PhD,[1] and Robert C. Minnee, MD, PhD[1]

**Background.** In The Netherlands, 60% of deceased-donor kidney offers are after donation after circulatory death. Cold and warm ischemia times are known risk factors for delayed graft function (DGF) and inferior allograft survival. Extraction time is a relatively new ischemia time. During procurement, cooling of the kidneys is suboptimal with ongoing ischemia. However, evidence is lacking on whether extraction time has an impact on DGF if all ischemic periods are included. **Methods.** Between 2012 and 2018, 1524 donation after circulatory death kidneys were procured and transplanted in The Netherlands. Donation and transplantation-related data were obtained from the database of the Dutch Transplant Foundation. The primary outcome parameter was the incidence of DGF. **Results.** In our cohort, extraction time ranged from 14 to 237 min, with a mean of 62 min (SD 32). In multivariate logistic regression analysis, extraction time was an independent risk factor for incidence of DGF (odds ratio per minute increase 1.008; 95% confidence interval, 1.003-1.013; $P=0.001$). The agonal phase, hypoperfusion time, and anastomosis time were not independent risk factors for incidence of DGF. **Conclusions.** Considering all known ischemic periods during the donation after the circulatory death process, prolonged kidney extraction time increased the risk of DGF after kidney transplantation.

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The best treatment for end-stage renal disease is kidney transplantation.[1] In 2020, 1365 new patients were waitlisted for kidney transplantation in The Netherlands. Because the number of living kidney donors (375 in 2020) is insufficient, we largely depend on deceased kidney donation.[2]

In The Netherlands, 60% of the donors donate after circulatory death (DCD).[2] The early graft failure is significantly more likely in kidney recipients from DCD donors than brain death donors (DBDs).[3] Therefore, limiting damage from the warm ischemia of the DCD donation is of crucial importance.

Cold ischemia time (CIT) and warm ischemia time (WIT) are known risk factors for reperfusion injury. Limiting ischemia times is important to preserve graft function. Over the years, the preservation process has been further optimized. However, during procurement, cooling of the kidneys may be suboptimal, which may induce additional ischemia damage. This so-called extraction time is a relatively newly described ischemia period of interest in organ donation and is defined as the interval between the start of the cross-clamp and placement of the donor kidney on ice. Little is known about the impact of this extraction time on graft outcomes. The duration of kidney extraction time is highly associated with the number of procured organs. In recent years, multiorgan DCD donation has increased (60% in 2020 versus 29% in 2010), which consequently also affects extraction time.[2,4] Therefore, evaluation of the association between the duration of the extraction time and graft outcomes is important.

Osband et al[5] showed in 576 transplantations from deceased donors (both DBD and DCD donors) that longer kidney extraction time affects early graft failure and primary

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*[1] Division of Hepatopancreatobiliary and Transplant Surgery, Department of Surgery, Erasmus Medical Center Transplant Institute, Rotterdam, The Netherlands.*

*[2] Research Center Innovations in Care, Rotterdam University of Applied Sciences, The Netherlands.*

*[3] Department of Nephrology, Erasmus Medical Center Transplant Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands.*

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*Correspondence: Erika van Straalen, MSc, Division of Hepatopancreatobiliary and Transplant Surgery, Department of Surgery, Erasmus Medical Center Transplant Institute, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands. (e.vanstraalen@erasmusmc.nl).*



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outcome of kidney transplantation. Up to 60 min of extraction time, the delayed graft function (DGF) incidence was 27.8%. However, when the extraction time exceeded 120 min, the rate doubled to nearly 60%. This trend was also seen for primary nonfunction (PNF), although not statistically significant.[5] The impact of prolonged extraction time is also seen after liver transplantation, especially in DCD instead of DBD.[6-8]

In a Eurotransplant cohort of almost 14 000 transplant recipients, prolonged extraction time was only associated with increased graft failure in DCD donors.[9] In a multicenter cohort between 2002 and 2016, Maassen et al[10] showed the association with DGF after transplantation in the United States from DCD donors and with DGF and graft loss in transplants in The Netherlands from a deceased donor.

Even before the start of the first WIT, organs are already less oxygenated, triggering anaerobic metabolism. The impact and importance of this agonal phase after withdrawal of treatment in controlled DCD has been recognized.[11,12] In a letter to the editor, we pointed to the importance of the agonal phase for graft function.[13] Ischemic periods before the WIT have not been included in previous studies analyzing the impact of extraction time on posttransplant outcomes.[9,10] All different ischemic periods are known to affect the outcome of kidney transplants.

The aim of our research was to investigate whether the duration of kidney extraction time independent of all known donation-related ischemic periods was associated with DGF after kidney transplantation.

## MATERIALS AND METHODS

Between 2012 and 2018, DCD donor and recipient characteristics with intraoperative procurement information and transplant outcomes were obtained from the prospectively collected database of the Dutch Transplant Foundation. This nonprofit organization manages patient-oriented allocation of deceased-donor organs to achieve the best possible match between available donor organs and patients on the transplant waiting list in The Netherlands. The study was approved by the local Ethics Committee and was conducted in accordance with the provisions of the Declaration of Helsinki (MEC 2018-1401) and waived the need for informed consent. The surgeons from the different multiorgan procurement teams in The Netherlands were certified for this procedure. Cases were excluded if the age is younger than 18 y, in cases with missing values regarding extraction time and DGF and extraction times <10 min (extraction times <10 min seem technically impossible and are probably input errors). In 2018, a pilot trial was conducted in The Netherlands in which transplant kidneys were treated with normothermic machine perfusion. These pilot cases were also excluded.[14]

The entire DCD process was divided into different ischemic periods (Figure 1). The DCD procedure in The Netherlands started with the withdrawal of treatment in the intensive care unit when end-of-life care is given, and the patient dies. This is known as the agonal phase. The agonal phase was defined as the interval between the start of withdrawal of treatment and cessation of cardiac output. In The Netherlands, the procurement team waits for 2 h in case of kidney donation. If the occurrence of death lasts >2 h, the organ donation procedure will be stopped. From the moment the mean arterial pressure drops to <50 mm Hg until death, perfusion and oxygenation of the organs are suboptimal.[12] Hypoperfusion period was defined as the interval between the moment the mean arterial pressure drops <50 mm Hg until cessation of cardiac output. This is based on the criteria in accordance with the Eurotransplant manual.[15]

In The Netherlands, the WIT starts after the patient has been declared dead. At that moment, the metabolism in the donor will switch to anaerobic metabolism because oxygen shortage. The donor will be transported to the operation theater, and the CIT starts with a cross-clamp of the aorta and perfusion of the organs. First WIT was defined as the interval between circulatory arrest and the start of cold flush in the donor. During the retrieval process, all organs are cooled topically with ice water. Hypoperfusion time and the first WIT combined were defined as the functional WIT.

After removal of the organs, the cooling process continues in the ice box and the kidney will be transported to the allocated transplant center. During the cooling process in the ice box, the organ temperature drops to 4 °C and the metabolism drops to 10%.[16] Upon arrival, the implantation will be done at the transplant center. A time that is necessary to perform the vascular anastomosis is called the anastomosis time (AT). The kidney donor is out of the ice and rewarmed during the AT, another ischemic period with a higher risk of DGF.[17]

Extraction time was defined as the interval between cross-clamp and the start of aortic cold flush in the donor and the end of nephrectomy when the kidney was placed on ice on the back table. It is standard protocol in The Netherlands that all events up to transportation are monitored on-site by the transplant coordinator, and the times are documented. This includes the extraction time. CIT was defined as the interval between the start of aortic cold flush in the donor until the kidney left the ice to be implanted in the recipient. The AT

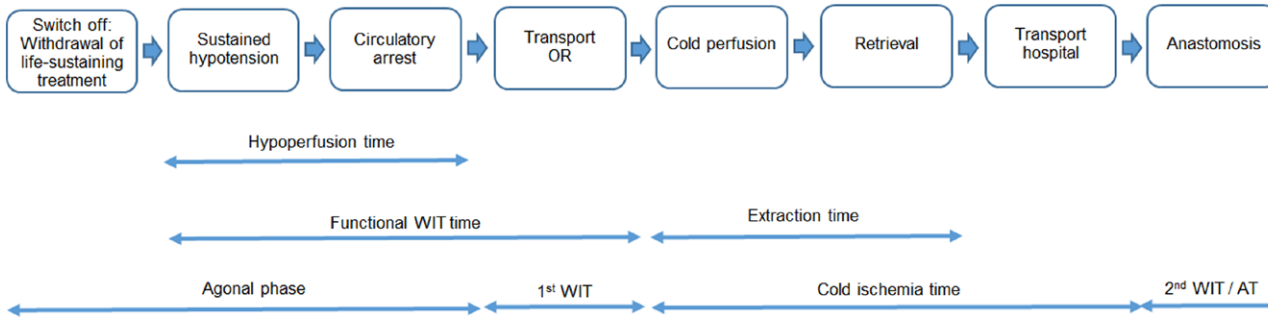


**FIGURE 1.** Different ischemic periods are graphically shown during DCD process in The Netherlands. AT, anastomosis time; DCD, donation after circulatory death; WIT, warm ischemia time.

was the duration of the implantation until the anastomosis was applied and the circulation was recovered in the recipient.

DGF was defined as requiring dialysis in the first 7 d after transplantation, and PNF was defined as a never-functioning graft, which was concluded in retrospect 3 mo after transplantation. Time to graft failure was taken as the interval from transplantation to graft nephrectomy or return to dialysis, whichever came first, and uncensored for the death of a patient with a functioning graft.

The primary outcome parameter was the incidence of DGF.

### Statistical Analysis

For baseline characteristics, continuous variables were analyzed with independent-sampled *t* test and categorical variables with chi-square tests. In our analysis, we used extraction time as a continuous variable. Logistic regression analysis was used to predict the risk of DGF after transplantation. The Kaplan-Meier method with log-rank test and Cox regression analysis were used for time-to-event data. For model development, variables with a *P* value <0.2 or the variable of interest were selected from univariable analysis for the multivariable analysis. A 2-sided *P* value of <0.05 was considered significant. A linear regression analysis with receiver operating characteristic curves was performed to evaluate associations between extraction time and primary and secondary outcomes parameters. In all analyses, missing values in the included cases were not imputed. IBM SPSS Statistics version 25 was used for all statistics analyses.

## RESULTS

### Baseline Characteristics

A total of 1524 DCD kidneys were transplanted in The Netherlands between January 1, 2012, and December 31, 2018. Six recipients and 52 transplants from donors aged younger than 18 y were excluded. Additionally, 83 transplants were excluded because extraction time or DGF was not available. In 2 cases, the extraction time was <10 min and 7 transplant kidneys were included in the normothermic machine perfusion pilot trial. This resulted in a final cohort of 1374 adult kidney recipients from adult DCD donors in The Netherlands. Baseline characteristics stratified according to the occurrence of DGF are shown (Table 1).

**TABLE 1.**

**Donor and recipient demographics**

| | | DGF | nDGF | |
|---|---|---|---|---|
| | N = 1374 | N = 764 | N = 610 | |
| **Donor parameters** | **Mean (SD)/n (%)** | **Mean (SD)/n (%)** | **Mean (SD)/n (%)** | ***P*** |
| Age, y | 54 (13) | 55 (12) | 53 (14) | 0.003 |
| Body mass index, kg/m$^2$ | 26 (5) | 26 (4) | 25 (5) | 0.002 |
| Cause of death | | | | |
| CVA | 462 (34) | 263 (34) | 199 (33) | 0.003 |
| Trauma | 314 (23) | 188 (25) | 126 (21) | |
| Ischemia | 383 (28) | 217 (28) | 165 (27) | |
| Other | 216 (15) | 96 (13) | 120 (20) | |
| History of hypertension | 329 (24) | 215 (28) | 114 (19) | <0.001 |
| **Donation process parameters** | | | | |
| Multiple organ donation | 916 (67) | 504 (66) | 412 (68) | 0.539 |
| Agonal phase, min | 23 (19) | 22 (18) | 23 (20) | 0.521 |
| Hypoperfusion time, min | 8 (9) | 8 (9) | 9 (9) | 0.574 |
| First WIT, min | 16 (11) | 17 (14) | 15 (8) | 0.001 |
| Functional WIT, min | 85 (34) | 88 (36) | 81 (30) | <0.001 |
| Extraction time, min | 62 (32) | 65 (34) | 59 (29) | 0.002 |
| Total relatively WIT, min | 84 (34) | 88 (36) | 80 (31) | <0.001 |
| CIT, min | 809 (279) | 840 (287) | 772 (264) | <0.001 |
| AT, min | 33 (14) | 33 (15) | 33 (13) | 0.648 |
| **Recipient parameters** | | | | |
| Age, y | 57 (13) | 57 (13) | 57 (13) | 0.399 |
| Gender, male | 873 (64) | 497 (65) | 376 (62) | 0.192 |
| Body mass index, kg/m$^2$ | 27 (8) | 27 (10) | 26 (5) | 0.117 |
| Cause of renal failure | | | | |
| Diabetes | 153 (11) | 86 (11) | 67 (11) | 0.774 |
| Hypertension | 187 (14) | 105 (14) | 82 (13) | |
| Others | 696 (51) | 378 (49) | 318 (52) | |
| Cause unknown | 338 (24) | 196 (26) | 143 (23) | |
| **Outcome parameters** | | | | |
| PNF | 36 (2.6) | | | |
| Graft failure | 288 (21) | 210 (27) | 78 (13) | <0.001 |
| Duration graft survival, mo | 33 (24) | 34 (25) | 31 (22) | 0.084 |
| Death | 190 (14) | 125 (16) | 65 (11) | 0.002 |
| Duration patient survival, mo | 33 (24) | 34 (24) | 32 (22) | 0.026 |

AT, anastomosis time; CIT, cold ischemia time; CVA, cerebrovascular accident; DGF, delayed graft function; nDGF, no delayed graft function; PNF, primary nonfunction; WIT, warm ischemia time.

The mean donor age was 54 y (SD 13) with a mean body mass index of 26 kg/m$^2$ (SD 5). The mean recipient age was 57 y (SD 13) with a body mass index of 27 kg/m$^2$ (SD 8). Overall extraction time ranged from 14 to 237 min (Figure 2), with a mean of 62 min (SD 32). There were 458 kidney-only donors and 916 multiple abdominal organ donors. Extraction time was significantly shorter in kidney-only procurements compared with multiple abdominal organ donors (45 min [SD 23] versus 70 min [SD 33], $P < 0.001$).

### Primary Outcome

DGF was found in 56% of all recipients. Results from univariable and multivariate logistic regression analyses for DGF are shown (Table 2). In multivariate logistic regression analysis, extraction time was an independent risk factor for the incidence of DGF (odds ratio, 1.008; 95% confidence interval [CI], 1.003-1.013; $P = 0.001$). Age, hypertension of the donor, and CIT were also found to be independent risk factors for the incidents of DGF. In this cohort, the agonal phase, hypoperfusion time, WIT, and AT were not independent risk factors for the incidence of DGF. Receiver operating characteristic analysis showed that extraction time alone could not discriminate between DGF and no DGF (area under the curve 0.537; Figure 3). Subgroup analysis among single and multiple organ donors did not change our results (area under the curve 0.547 and 0.545, respectively).

### Secondary Outcome

The Kaplan-Meier curve showed no difference in patient and graft survival when stratified according to an extraction time over 60 min ($P = 0.429$ versus $P = 0.256$; Figure 4A and B). After DGF, significantly more graft failure (27% versus 13%, $P < 0.001$) and less patient survival (84% versus 89%, $P = 0.002$) were seen. There was a mean follow-up of 33 mo; in the case of DGF, a mean follow-up of 34 mo (SD 25) and in no DGF (nDGF), a mean follow-up of 31 mo (SD 22). Multivariable Cox regression analysis showed donor age, CIT, AT, and recipient age to be independent risk factors for graft failure (Table 3). Extraction time was not an independent risk factor for graft failure (hazard ratio = 1.003; 95% CI, 0.999-1.008; $P = 0.177$).

## DISCUSSION

In our cohort of Dutch DCD kidney donors and recipients, extraction time is an independent risk factor for the incidence of DGF. However, no association was found between extraction time and graft survival. Other ischemic periods like first WIT and CIT were also independently associated with DGF.

Two retrospective cohort studies have also shown that extraction time is an independent risk factor for the incidence of DGF.[5,9] Osband et al[5] described an increased risk of DGF beyond 60 min of extraction time (odds ratio = 1.19 per 5 min beyond 60 min, $P = 0.03$). In the cohort of Heylen et al[9] with nearly 14 000 recipients, extraction time was also independently associated with graft loss (hazard ratio = 1.05 per 10 min increase, $P = 0.026$). Extraction time expands linearly to the number of donated organs.[5] Goldsmith et al[18] demonstrated that multiorgan donors are younger compared with single-organ donors with comparable long-term outcomes. The potential association of extraction time with graft survival may be confounded by these criteria, which could be a reason why an association between extraction time and graft survival could not be established.[18]

In addition to published studies on extraction time, the agonal phase and hypoperfusion period have been included in the analysis because it is known to contribute to impaired

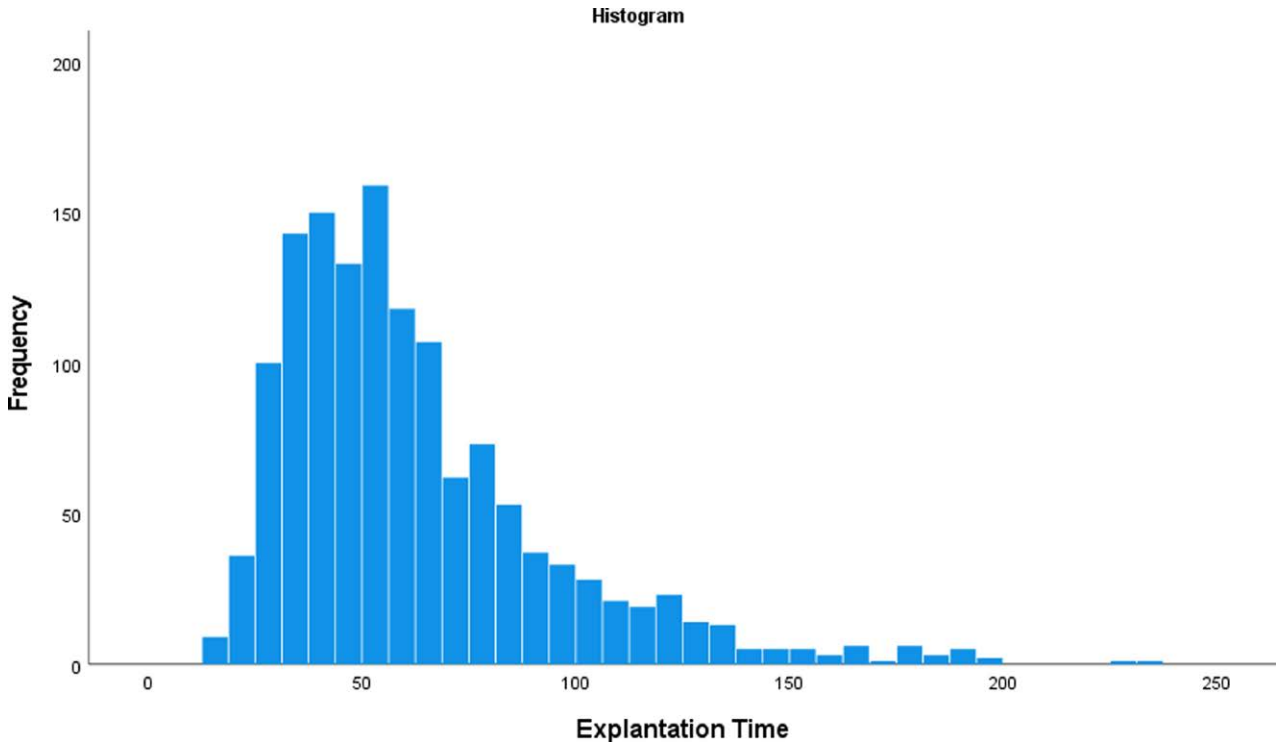


**FIGURE 2.** Histogram of kidney extraction time from donation after circulatory death (DCD).

**TABLE 2.**

**Univariable and multivariable logistic regression analyses on the primary outcome parameter: incidence of DGF**

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| **Donor parameters** | **Odds ratio (95% CI)** | ***P*** | **Odds ratio (95% CI)** | ***P*** |
| Age, y | 1.012 (1.004-1.020) | 0.003 | 1.017 (1.005-1.029) | 0.004 |
| Body mass index, kg/m$^2$ | 1.038 (1.013-1.063) | 0.002 | 1.005 (0.975-1.036) | 0.733 |
| Cause of death | | | | |
| CVA | | 0.004 | | |
| Trauma | 1.129 (0.844-1.511) | 0.414 | | |
| Ischemia | 0.972 (0.757-1.308) | 0.972 | | |
| Other | 0.605 (0.437-0.838) | 0.003 | | |
| History of hypertension | 1.704 (1.317-2.204) | 0.000 | 1.879 (1.334-2.648) | 0.000 |
| **Donation process parameters** | | | | |
| Kidney-only retrieval | 1.073 (0.856-1.346) | 0.539 | | |
| Agonal phase, min | 0.998 (0.992-1.004) | 0.521 | | |
| Hypoperfusion ≤11 min/>11 min | 1.332 (1.010-1.758) | 0.043 | 1.277 (0.913-1.787) | 0.154 |
| First WIT, min | 1.028 (1.009-1.048) | 0.004 | 1.014 (0.997-1.030) | 0.099 |
| Functional WIT, min | 1.007 (1.003-1.010) | 0.000 | | |
| Extraction time, min | 1.005 (1.002- 1.009) | 0.002 | 1.008 (1.003-1.013) | 0.001 |
| CIT, min | 1.001 (1.000-1.001) | 0.000 | 1.001 (1.000-1.002) | 0.000 |
| AT, min | 1.002 (0.993-1.011) | 0.648 | | |
| **Recipient parameters** | | | | |
| Age, y | 0.996 (0.988-1.005) | 0.399 | | |
| Gender, male | 1.158 (0.929-1.445) | 0.192 | | |
| Body mass index, kg/m$^2$ | 1.017 (0.995-1.039) | 0.125 | 1.010 (0.992-1.028) | 0.281 |
| Cause of renal failure | | | | |
| Diabetes | | 0.774 | | |
| Hypertension | 0.988 (0.649-1.535) | 0.991 | | |
| Others | 0.926 (0.651-1.317) | 0.669 | | |
| Cause unknown | 1.062 (0.723-1.562) | 0.758 | | |

AT, anastomosis time; CI, confidence interval; CIT, cold ischemia time; CVA, cerebrovascular accident; DGF, delayed graft function; WIT, warm ischemia time.

outcomes in DCD kidneys.[11,12] Still, both did not appear to be independent risk factors for DGF in our cohort.

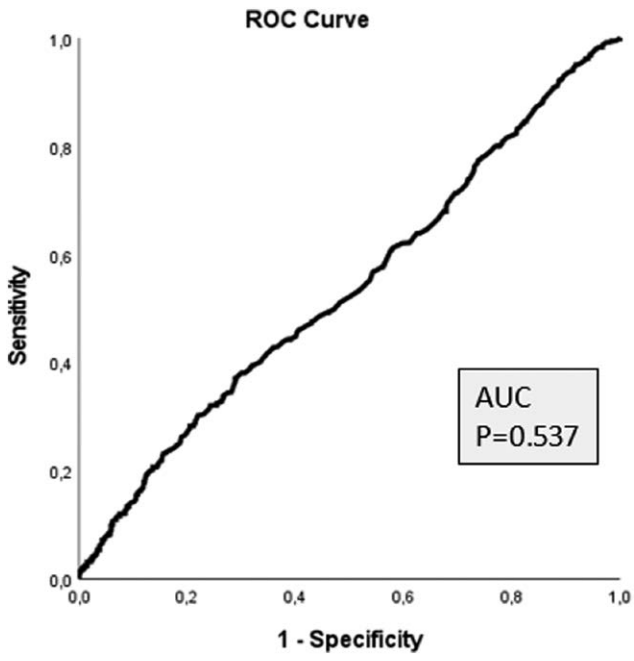


**FIGURE 3.** ROC analysis for association of extraction time on DGF. AUC, area under the curve; DGF, delayed graft function; ROC, receiver operating characteristics.

Extended extraction time is not a contraindication for transplantation. However, it is advisable to keep this period as short as possible because this is an independent risk factor for DGF. DGF posttransplantation is a risk factor for acute rejection, poorer graft function, and graft failure.[19] Furthermore, it provides unnecessary uncertainty for the recipient, and dialysis posttransplantation entails additional costs and medical care.[20] In The Netherlands, organ procurement procedures are performed by certified surgeons. Extraction time should be an important topic during the training period of surgeons for multiorgan procurements. Attention must also be paid to optimum cooling during the removal of the organs. Especially when, for example, there is an unusual anatomy and there is a chance that extended extraction time is unavoidable.

WIT and CIT are both known risk factors for DGF and inferior allograft survival. During the total CIT, extraction time is relatively short. The impact of this period points out the severity of relatively warm ischemia with anaerobic metabolism. After the cold flush through the aorta, organs slowly rewarm until placed on ice. It may be worthwhile to highlight such crucial moments in the donation process. Another interesting time in transplantation is benching of the kidney during procurement and before transplantation. Organs are assessed by transplant surgeons and reconstruction is made if necessary. Different bench practices in donor and transplant hospitals could influence kidney outcomes. Ideally, during benching, the donor kidney should be submerged in cold preservation fluid.[21] However, with deviations in preservation methods in the Eurotransplant region, the kidney could be exposed

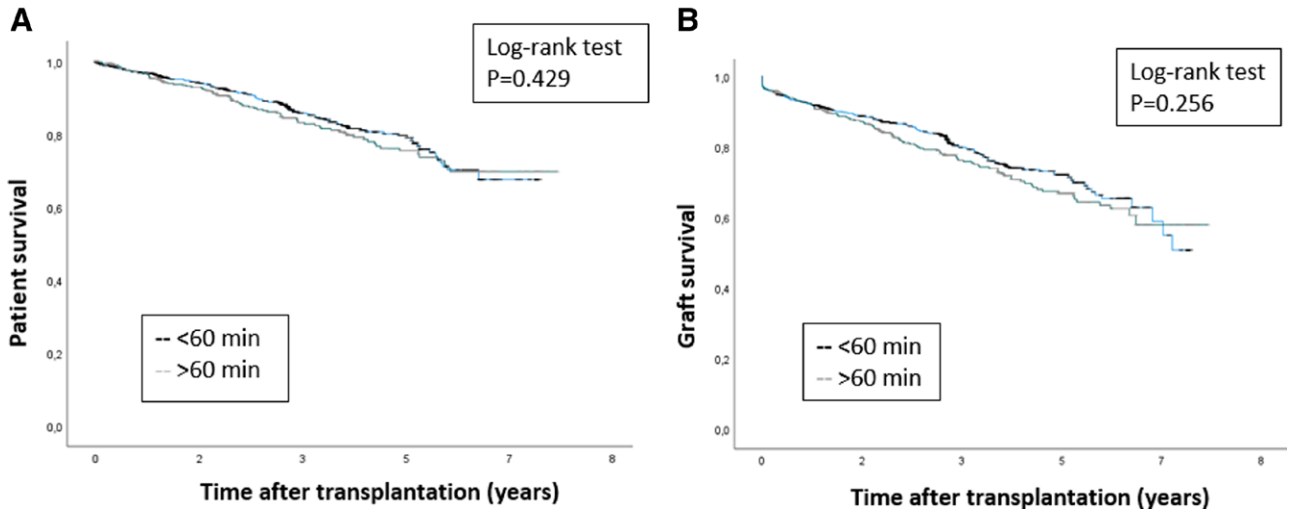


**FIGURE 4.** Kaplan-Meier survival curves for patient survival (A) and graft survival (B), stratified by extraction time.

**TABLE 3.**

**Univariable and multivariable Cox regression analyses on the secondary outcome parameter: graft survival**

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| **Donor parameters** | **Hazard ratio (95% CI)** | ***P*** | **Hazard ratio (95% CI)** | ***P*** |
| Age, y | 1.026 (1.015-1.037) | 0.000 | 1.018 (1.003-1.033) | 0.017 |
| Body mass index, kg/m$^2$ | 1.003 (0.975-1.032) | 0.824 | | |
| Cause of death | | | | |
| CVA | | 0.357 | | |
| Trauma | 0.739 (0.516-1.058) | 0.099 | | |
| Ischemia | 0.824 (0.592-1.148) | 0.252 | | |
| Other | 0.948 (0.644-1.396) | 0.786 | | |
| History of hypertension | 1.412 (1.055-1.889) | 0.020 | 1.063 (0.742-1.523) | 0.739 |
| **Donation process parameters** | | | | |
| Kidney-only retrieval | 1.101 (0.838-1.447) | 0.488 | | |
| Agonal phase, min | 0.998 (0.991-1.006) | 0.673 | | |
| Hypoperfusion ≤11 min/>11min | 0.986 (0.704- 1.380) | 0.934 | | |
| First WIT, min | 1.006 (0.996-1.016) | 0.212 | | |
| Functional WIT, min | 1.001 (0.997-1.005) | 0.596 | | |
| Extraction time, min | 1.001 (0.997-1.005) | 0.728 | 1.003 (0.999-1.008) | 0.177 |
| CIT, min | 1.001 (1.001-1.002) | 0.000 | 1.001 (1.001-1.002) | 0.002 |
| AT, min | 1.008 (0.998-1.018) | 0.130 | | |
| **Recipient parameters** | | | | |
| Age, y | 1.025 (1.014-1.037) | 0.000 | 1.022 (1.008-1.037) | 0.002 |
| Gender, male | 0.965 (0.737-1.262) | 0.793 | | |
| Body mass index, kg/m$^2$ | 1.002 (0.988-1.016) | 0.768 | | |
| Cause of renal failure | | | | |
| Diabetes | | 0.099 | | |
| Hypertension | 1.009 (0.619-1.647) | 0.970 | | |
| Others | 0.678 (0.450-1.022) | 0.063 | | |
| Etiology unknown | 0.766 (0.489-1.200) | 0.245 | | |

AT, anastomosis time; CI, confidence interval; CIT, cold ischemia time; CVA, cerebrovascular accident; WIT, warm ischemia time.

to additional WIT. The same applies to vascular reconstruction in the donor kidney. The kidney will be handled in warm hands and consistently warm up. Surprisingly, there is almost no literature on kidney temperature during benching. A recent study monitoring the temperature of donor livers observed an increase in temperature between the end of procurement and packing of the organ and from the back table to reperfusion, with a maximum of 20 °C.[16] This crucial information, which is lacking in the literature, will help understand and determine the impact every time frame within the donation and transplantation process has on kidney outcome.

The strength of our present study is the full use of the available ischemia data in the recent donation and transplantation process. There was a minimized percentage of missing data.

The limitation of our study is the retrospective character of our research, introducing bias. Selection is based on extraction time because it occurred at the time. The cause of this is not verifiable and may have an impact on the outcome.

In conclusion, extraction time is an independent risk factor for DGF. All known ischemia periods have been included in the analysis. In this cohort, extraction time could not be associated with the incidence of graft failure. However, given the risk of DGF, it remains advisable to keep the extraction time as short as possible to optimize improved outcomes.

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