Received Date : 10-Jun-2016

Revised Date : 01-Aug-2016

Accepted Date : 22-Aug-2016

Article type : Original Article

The impact of anastomosis time during kidney transplantation on graft loss: A Eurotransplant cohort study

Line Heylen^{1,3}, Jacques Pirenne^{2,4}, Undine Samuel⁵, Ineke Tieken⁵, Maarten Naesens^{1,3}, Ben Sprangers^{1,3*}, Ina Jochmans^{2,4*}

*authors contributed equally

¹Department of Nephrology and Kidney Transplantation, and ²Department of Abdominal Transplantation Surgery, University Hospitals Leuven, Belgium; ³Laboratory of Nephrology and ⁴Laboratory of Abdominal Transplant Surgery, Department of Microbiology and Immunology, KU Leuven, Belgium; ⁵Eurotransplant International Foundation, Leiden, The Netherlands.

Corresponding author: Ina Jochmans, ina.jochmans@uzleuven.be

Running title: Anastomosis time and kidney transplant loss

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ajt.14031

This article is protected by copyright. All rights reserved.

Abbreviations: CI, confidence interval; DBD, donation after brain death; DCD, donation after circulatory death; ECD, extended criteria donation; HR, hazard ratio; HTK, Histidine-tryptophan-ketoglutarate; IQR, interquartile range; KDRI, Kidney Donor Risk Index; SCD, standard criteria donation; UNOS, United Network for Organ Sharing; UW, University of Wisconsin solution.

Abstract:

Recent studies raise the concern that warm ischemia during the time to complete the vascular anastomoses in kidney implantation harms the transplant, but its precise impact on outcome and its interaction with other risk factors remain to be established. We investigated the relationship between anastomosis time and graft survival at 5 years after transplantation in 13964 recipients of solitary deceased-donor kidney transplants in the Eurotransplant region. Anastomosis time was independently associated with graft loss after adjusting for other risk factors (adjusted hazard rate 1.10 for every ten-minute increase, 95% confidence interval 1.06-1.14; $P < 0.0001$), while it did not influence recipient survival (hazard rate 1.00, 95% confidence interval 0.97-1.02). Circulatory-dead donor (DCD) kidneys were less tolerant to prolonged anastomosis time than brain-dead donor kidneys (P for interaction=0.02). The additive effect of anastomosis time with donor warm ischemia time explains this observation, since DCD status was no longer associated with graft survival when adjusted for this sum warm ischemia time and there was no interaction between DCD status and sum warm ischemia time. Thus, the time to create the vascular anastomoses in kidney transplantation is associated with inferior transplant outcome, especially in recipients of DCD kidneys.

Introduction

Kidney transplantation is the treatment of choice for patients with end-stage renal failure, improving survival and quality of life compared to renal replacement therapy. Still, 3 to 5 % of grafts are lost annually after transplantation (1, 2). One of the major obstacles to kidney transplant success is

ischemia-reperfusion injury. Extensive research on this matter is ongoing and large multicenter trials are currently investigating whether strategies like machine perfusion preservation can diminish this injury (3, 4).

Most research to date focuses on cold ischemia-induced injury during storage prior to transplantation (5, 6), and on warm ischemia prior to organ retrieval (7). In contrast, the exposure of the organ to warm ischemia during transplantation, when the kidney is removed from the ice but not yet revascularized, has received remarkably little attention. However, being a period of warm ischemia, this implantation time could represent an important risk factor, potentially providing new strategies to improve kidney transplant outcome.

The few studies that did evaluate the effect of longer anastomosis time suggested an increased risk of delayed graft function as well as poorer long-term graft function and more fibrosis on biopsy (8, 9).

Recently, a study on the data registry of the United Network for Organ Sharing (UNOS) demonstrated that this warm ischemia time is associated with worse patient and graft survival (10). Still, the effect on both graft as well as patient survival raises concerns on the existence of confounding factors that influence both anastomosis time as well as outcome (11). Further validation of these findings is therefore warranted. In addition, the UNOS study did not evaluate any interaction effects with other risk factors, nor were factors determining warm ischemia time explored.

In this article, we aimed to define the effect of anastomosis time on kidney transplant outcome and explore its potential interactions and determinants in the registry of Eurotransplant, the deceased donor organ allocation organism of 8 European countries.

Methods:

Study population

Eurotransplant is an international non-profit organization that manages patient-oriented allocation and cross-border exchange of deceased donor organs to achieve the best possible match between available donor organs and patients on the transplant waiting list in 8 different countries: Austria, Belgium,

Croatia, Germany, Hungary, Luxembourg, the Netherlands, and Slovenia. The Eurotransplant registry records data for all kidney transplants performed in 72 kidney transplant centers in its region (12).

Data is collected on a voluntary basis to develop best practice recommendations and policies to improve organ allocation and transplant outcomes. We analyzed data submitted to this registry from all recipients of solitary kidney transplants from deceased-donors undertaken between Jan 1, 2004 and Dec 31, 2013. Anastomosis time was defined as the time between the graft leaving the ice and restoration of blood flow by opening of the vascular clamps in the recipient. Donor warm ischemia time in circulatory-dead donor (DCD) kidneys was defined as the time between therapy withdrawal in the potential donor and cold flush of the kidney. These data are collected prospectively in the Eurotransplant registry.

Graft failure was taken as time from transplantation to graft nephrectomy or return to dialysis, whichever was earlier, and censored for death of the patient with a functioning graft. Survival of the patient was defined as time from transplantation until death.

Extended criteria donation (ECD) was defined as any kidney procured from a donor aged ≥ 60 years or any donor aged 50-59 years with two of the following: cerebrovascular accident as the cause of death, medical history of arterial hypertension, and terminal creatinine > 1.5 mg/dl (or > 133 μmol), in contrast to standard criteria donation (SCD)(13). We calculated the Kidney Donor Risk Index (KDRI) for all transplants(14). Data on donor ethnicity and hepatitis C status were not available. We therefore considered all donors as false for the African American criterion and excluded hepatitis C from the KDRI calculation.

Statistical analysis

Follow-up analysis of the study population included all data submitted to Eurotransplant by Nov 5, 2015. We excluded recipients of double or en bloc kidney transplants. Only recipients for whom complete data for both anastomosis time and graft survival were available were included in the study.

Kidney transplant outcomes were graft failure (death-censored graft survival) and patient survival, censored at 5 years. We used unadjusted Cox proportional hazards regression and Kaplan-Meier curves to show graft survival. Associated p-values in Kaplan-Meier curves were derived from the univariable log-rank test. We used adjusted Cox proportional hazards regression to evaluate the independent effect of anastomosis time on transplant outcome. Transplant variables for inclusion in the multivariable models were identified based on scientific literature of factors shown to affect transplant outcome, dependent of their availability in the Eurotransplant registry. In addition, factors that could confound the association between anastomosis time and outcome were considered. The adjusted analyses included only transplants for which complete data for all covariates were available. Donor-related variables considered for inclusion in the final model were: age, sex, type (donation after brain death (DBD) versus DCD), terminal serum creatinine, body mass index and medical history (diabetes and hypertension). Recipient related factors were: age, sex, body mass index and retransplant versus first transplant. Other variables considered were: total number of HLA mismatches, cold ischemia time, anastomosis time, type of preservation fluid and transplant year. In addition, we included transplant center in the multivariable model as a random effect. Only factors shown to be significant with a stepwise variable deletion approach were included in the multivariable model.

There was no violation of non-proportionality of hazards of anastomosis time in the multivariate model. We performed interaction analyses to determine whether anastomosis time had more effect on graft survival in recipients of DCD kidneys than in recipients of DBD kidneys, and whether the effect of anastomosis time was modified by donor age, donor body mass index, preservation fluid and cold ischemia time. In the subgroup of paediatric recipients (age < 18y at the time of transplantation), we included only variables in the multivariable Cox regression analysis that were significantly associated with graft survival in the univariable Cox proportional hazards analysis, adjusted for transplant center as random effect, as this was a small cohort.

Anastomosis time of transplants with different characteristics was compared with Wilcoxon tests. The associations of anastomosis time with continuous data were assessed with Spearman correlation. We used multivariate linear regression to determine the independent determinants of anastomosis time, adjusted for transplant center as random effect. Variables significantly associated with anastomosis time were considered for inclusion in this multivariate model.

All tests were two sided and p values of <0.05 were considered significant. RStudio (R version 3.1.3) was used for all statistical analyses.

Results

Characteristics of the study population

32 040 deceased-donor kidney transplants were performed in the Eurotransplant region between Jan 1, 2004 and Dec 31, 2013. 276 recipients of double kidney transplants were excluded. From the 31 764 solitary kidney transplants, we excluded 17 770 transplants with missing data of anastomosis time or outcome, as well as 30 transplants in which anastomosis times were reported to be extremely short (<10 minutes) or long (>200 minutes) (Figure 1). Transplant characteristics were comparable between the 13 964 transplants included and the 17 800 solitary transplants excluded in this study (Table S1).

Median follow-up after transplantation was 4.35 years (interquartile range (IQR)=2.01–7.52). Table 1 shows the characteristics of the donors and recipients at the time of transplantation. 503 recipients (4%) were paediatric (age <18 y at the time of transplantation). Median cold ischemia time was 13.7 hours (IQR=10.2-17.4), and median anastomosis time was 36 minutes (IQR=29-45).

Anastomosis time and graft survival

The results of the Cox proportional hazards models, censored at 5 years after transplant, are displayed in Table 2. When analyzed as a continuous variable, anastomosis time increased graft failure rate at 5 years after transplantation for all deceased-donor kidneys (unadjusted hazard ratio (HR) 1.08 for every 10-minutes increase in anastomosis time, 95% confidence interval (95% CI) 1.06-1.11; $p<0.0001$). In contrast, anastomosis time had no effect on recipient survival (HR 1.00, 95% CI 0.97-1.02, $p=0.87$). Figure 2 illustrates the Kaplan-Meier curve showing death-censored graft survival stratified by anastomosis time in four 10-minutes intervals based on the median and upper quartile, from below 35 minutes to 55 minutes or more. Here, the most striking effect on graft loss was observed in recipients with anastomosis times of 55 minutes or more, of whom 25% (459/1 823) had lost their graft at 5 years after transplantation. 22% (446/2 050) of recipients in whom anastomosis times ranged between 45 and 54 minutes had lost their graft, compared to 19% (775/4 008) of recipients with anastomosis times between 35 and 44 minutes and 18% (1 113/6 083) of recipients with anastomosis times below 35 minutes (Table 2).

In a multivariable Cox regression hazards model with a stepwise variable elimination approach, anastomosis time remained significantly associated with graft failure (adjusted HR 1.10 for every 10 minutes increase in anastomosis time, 95% CI 1.06-1.14; $p<0.0001$), adjusted for donor age, DCD versus DBD, donor hypertension, donor diabetes, donor pre-procurement serum creatinine concentration, recipient age, number of HLA mismatches, cold ischemia time and transplant center (Table 2, Table S2). With every 10-minute increase in anastomosis time, the risk of graft failure increased more than did every hour increase in cold ischemia time (adjusted HR 1.02, 95% CI 1.01-1.03, $p=0.0004$).

Body mass index of neither donor nor recipient associated with graft failure in the multivariable model. In addition, there was no interaction-effect between donor body mass index and anastomosis time. Type of preservation fluid had no independent effect on graft survival and there was no interaction-effect between type of preservation fluid and anastomosis time on graft loss. Also, donor

and recipient gender, transplant year, and whether transplant was a first transplant or retransplant were not independently associated with graft loss. These variables were therefore omitted from the final multivariable model.

Sensitivity analyses

To determine whether the detrimental effect of anastomosis on graft outcome was also evident in the most prevalent anastomosis times, we excluded all recipients with anastomosis times above one hour. In this subgroup, anastomosis time remained significantly and independently associated with graft loss (adjusted HR 1.08 per 10-minute increase, 95% CI 1.05-1.11, $p=0.02$, Table S3).

Both in recipients of DBD kidneys as well as in recipients of DCD kidneys, anastomosis time increased the risk of graft loss (Figure 3, Tables S4 and S5). To determine whether anastomosis time had different relative effects on graft survival in recipients of kidneys from DBDs and DCDs, we examined the interaction effect between DCD kidneys and anastomosis time in the multivariable model. The effect of anastomosis time on graft loss was more pronounced in recipients of DCD kidneys than in recipients of DBD kidneys (adjusted HR 1.11, 95% CI 1.02-1.20, p for interaction = 0.02).

With anastomosis times above the highest quartile (≥ 45 minutes), 33% (81/244) of recipients of DCD kidneys lost their graft at 5 years after transplantation, compared to 23% (842/3 629) of recipients of DBD kidneys. The latter is comparable to that of recipients of DCD kidneys with anastomosis times of less than 45 minutes (24%, 215/914). Recipients of DBD kidneys with anastomosis times of less than 45 minutes had the lowest risk of graft loss at 5 years after transplantation (18%, 1 673/9 177) (Figure 4). Thus, recipients of DCD kidneys with prolonged anastomosis times have a twofold increased risk of graft loss compared to recipients of DBD kidneys with short anastomosis times (adjusted HR 2.06, 95% CI 1.79-2.33, $p<0.0001$) (Table 3).

The increased vulnerability of DCD kidneys might be explained by the additive effect of warm ischemia time in the donor with anastomosis time in the recipient, leading to a longer total warm ischemia time in these kidneys. Therefore, we also considered donor warm ischemia time that occurs before cold flush in DCD donors. When adjusting for donor warm ischemia time in the final multivariable Cox regression, anastomosis time remained significantly associated with graft survival (HR 1.15, 95% CI 1.06-1.24, $p=0.003$). There was no interaction effect between donor warm ischemia time and anastomosis time ($p=0.13$). When donor warm ischemia time and anastomosis time were summed in the total cohort, this sum warm ischemia time also associated with graft survival in the adjusted Cox regression analysis (HR 1.10, 95% CI 1.06-1.14, $p<0.0001$). Interestingly, DCD status was no longer significant when adjusted for this sum warm ischemia time (HR 1.11, 95% CI 0.92-1.30, $p=0.30$). There was also no significant interaction between DCD status and sum warm ischemia time ($p=0.13$). Thus, the increased risk of anastomosis time in DCD kidneys is explained by their longer total warm ischemia time.

We next evaluated whether advanced donor age or prolonged cold ischemia time might affect the susceptibility of the graft to increased anastomosis time. Interaction effects between anastomosis time and donor age, and anastomosis time and cold ischemia time were separately investigated in the multivariate model. Both donor age (adjusted HR 1.02, 95% CI 1.01-1.02, $p<0.0001$) and cold ischemia time (adjusted HR 1.02, 95% CI 1.01-1.03, $p=0.0004$) were independently associated with graft survival. However, the unfavorable effect of prolonged anastomosis time on graft survival was not influenced by older donor age (p for interaction = 0.48) or longer cold ischemia time (p for interaction = 0.32).

In addition, we evaluated whether organ quality (as defined by ECD and SCD, and as calculated by the KDRI score) modified the effect of anastomosis time. With donor criteria in the final multivariable Cox regression, anastomosis time remained significantly associated with graft survival

(adjusted HR 1.09, 95% CI 1.07-1.12, $p<0.0001$). There was no interaction between ECD status and anastomosis time ($p=0.48$). Similarly, anastomosis time remained associated with graft survival with KDRI in the final model (adjusted HR 1.10, 95% CI 1.06-1.14; $p<0.0001$), but there was no interaction between the two ($p=0.43$).

The association between anastomosis time and graft failure was evident both in pediatric as well as adult recipients (Figure 2), but in pediatric recipients the effect was only significant in the unadjusted model (unadjusted HR 1.22, 95% CI 1.07-1.40, $p=0.004$; adjusted HR 1.16, 95% CI 0.99-1.33, $p=0.09$).

Determinants of anastomosis time

Finally, we investigated which donor and recipient characteristics determine the length of anastomosis time. Unadjusted comparisons are available in the Supplementary data (Table S6). In the multivariable linear regression adjusted for transplant center as random effect, a graft having more than one renal artery or vein, right versus left donor kidney, donor history of hypertension, and donor age were independently associated with increased anastomosis time (Table S7). Anastomosis time remained independently associated with graft survival, also when adjusting for presence of multiple arteries, presence of multiple veins and right versus left kidney.

Discussion

By using the Eurotransplant registry covering 13 964 solitary deceased donor kidney transplants, our analysis confirmed that prolonged anastomosis time increases the risk of graft loss. Every ten-minute increase in anastomosis time had a more detrimental effect on graft outcome than every hour increase

in cold ischemia time. Also, when excluding transplants with anastomosis times above one hour to evaluate more common anastomosis times, the effect remained.

Based on our results, an anastomosis time of less than 45 minutes seems to be crucial to reduce the risk of graft loss. This time span is in line with previously reported effects of anastomosis time on immediate and medium term graft function (8). Similar warm ischemia times impair postoperative renal function after clamping during partial nephrectomy in solitary native kidneys (15-17). In contrast, in a study on native kidney biopsies after surgical renal clamping during 15 to 53 minutes, only mild structural changes and no acute functional loss was observed (the effect on long-term outcome was not investigated) (18). Why warm ischemia times, tolerated by native kidneys, have an effect on transplant outcome might be explained by the combination of cold and warm ischemia.

Indeed, in porcine models of liver transplantation this combination was particularly detrimental (19, 20).

Also, warm ischemia time should logically last longer to evoke graft loss than to reversibly cause graft dysfunction. This might explain why the increased risk for delayed graft function is apparent at shorter warm ischemia times (9, 21). Furthermore, it might also explain why circulatory-dead donor kidneys – exposed to additional donor warm ischemia time between therapy withdrawal and start of cold preservation - have a higher rate of delayed graft function (7), whereas donor warm ischemia time (less than 30 minutes in the latter study) did not seem to affect outcome in this study(7).

Our finding that the duration of anastomosis time affect kidney transplant outcome is consistent with a recent UNOS analysis (10). Interestingly, our results also demonstrate that circulatory-dead donor kidneys are less tolerant to anastomosis time than brain-dead donor kidneys. Their increased vulnerability is explained by the additive effect of warm ischemia time after therapy withdrawal in the donor and during transplantation, leading to a longer total warm ischemia time in these kidneys. DCD kidneys will thus have a similar outcome as DBD kidneys when their sum of donor warm ischemia

This article is protected by copyright. All rights reserved.

time and anastomosis times are comparable to the anastomosis times of a DBD kidney. In contrast, additional quality measures such as ECD vs. SCD or the KDRI did not modify the effect of anastomosis time on graft survival. Given the rise in the use of DCD kidneys (22), raising the awareness that these kidneys are not only more prone to cold ischemic injury (5) but also to prolonged anastomosis times is of major importance to maintain or even improve transplant outcome.

Our finding that increasing anastomosis time is associated with inferior transplant outcome is of particular clinical importance, since this risk factor is potentially modifiable. This might be accomplished by reducing the duration of anastomosis time if technically feasible, by keeping the graft cold during implantation, and/or by developing strategies to reduce the impact of warm ischemia on downstream injury processes. Our findings thus support the set-up of new trials that target warm ischemia during transplantation to improve kidney transplant graft survival. In robot-assisted transplantation, a collaboration of surgeons already proposed to cool the kidney graft with sterile ice during implantation (23). In addition, a reduced rate of delayed graft failure after cooling the kidney during transplantation has been reported (24). Further studies are needed to determine whether cooling of the graft during the anastomosis process improves transplant outcome.

We also evaluated potential determinants of the duration of anastomosis time during kidney transplantation. Since right donor kidneys are associated with longer anastomosis time, this might explain the inferior outcome for recipients of right versus left donor kidneys (25).

The strength of our analysis is the use of a large cohort of transplant recipients in the Eurotransplant region. A limitation inherent to every registry study based on data from many different centers and countries is the lack of detailed information regarding donor- and recipient characteristics and incomplete data registration. In contrast to the USA and UK transplant registries, data submission to

the Eurotransplant registry is not compulsory, explaining the high frequency of missing data in this registry. However, even with mandatory data submission, the final cohort of the recent UNOS study represented only 57.7% of the eligible cohort(10). Also, as transplants that were excluded due to missing data were comparable to the transplants included, we do not suspect that this confounded our results importantly. Furthermore, this large cohort study enabled us to perform survival analyses, but no in depth analyses on different transplant outcome variables. Our previous single-center study did enable this in depth analysis, and demonstrated an increased risk of delayed graft function, impaired graft function and more fibrosis in biopsies, up to three years after transplantation (8). Similarly, unidentified confounders cannot be excluded with certainty based on this large registry. Indeed, it is possible that poor vasculature quality (e.g. atherosclerosis) of donor and/or recipient might hamper the process of anastomosing the vessels as well as impair transplant outcome. Although anastomosis time was associated with donor age and donor hypertension in this cohort, its effect on outcome was independent of these variables. Moreover, we observed no association of anastomosis time with the presence and severity of aortic calcifications and hemodynamic parameters of vascular stiffness of the recipient in our previous study (8). Also, in contrast to previous studies (10, 21), we did not observe an effect of anastomosis time on patient survival. This further strengthens that our study does not entail the possible bias for which these previous studies have been criticized (11).

In summary, anastomosis time is a risk factor for kidney transplant outcome, especially in recipients of kidneys from circulatory-dead donors. To improve outcome after kidney transplantation, efforts should be made by transplant surgeons to limit perioperative warm ischemic injury.

Acknowledgments

LH was supported by a Ph.D. grant from the Research Foundation – Flanders. JP is holder of Centrale Afdeling Voor Fractionering (CAF) and an Institute Georges Lopez (IGL) chair. The authors thank all centers within Eurotransplant for providing the data and Erwin de Vries from Eurotransplant for

This article is protected by copyright. All rights reserved.

Accepted Article
compilation of the data. The authors also thank Steffen Fieuws for his statistical advice and Pieter Busschaert for his advice on modeling in R.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

Figure Legends

Figure 1: Flow diagram demonstrating cohort selection

Figure 2: Kaplan-Meier estimates of graft survival censored at 5 years for all recipients of kidneys from deceased donors, stratified by anastomosis time in four 10-minute intervals.

Figure 3: Kaplan Meier estimates of graft survival for recipients of kidneys from brain-dead donors (A), recipients of kidneys from circulatory-dead donors (B), adult recipients (C) and pediatric recipients (D), stratified by anastomosis time.

Figure 4: Kaplan-Meier estimates of graft survival for recipients of kidneys from brain-dead donors and cardiac-dead donors, stratified by anastomosis time.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1: Demographic and clinical characteristics of transplants for whom complete data collection for anastomosis time and follow-up was available, therefore being included in this study, and of transplants for whom these data were not complete, therefore being excluded in this study.

Table S2: Demographic and clinical characteristics of transplants according to different anastomosis time groups.

Table S3: Demographic and clinical characteristics of DBD and DCD transplants according to anastomosis time lower or higher than 45 minutes. DBD, donation after brain death; DCD, donation after circulatory death.

Table S4: Cox proportional hazards model for graft failure at 5 years post-transplant in recipients of kidneys from deceased donors.

Table S5: Cox proportional hazards model for graft failure at 5 years post-transplant in recipients of kidneys from deceased donors with anastomosis times of one hour or less.

Table S6: Cox proportional hazards model for graft failure at 5 years post-transplant in recipients of kidneys from brain-dead donors.

Table S7: Cox proportional hazards model for graft failure at 5 years post-transplant in recipients of kidneys from circulatory-dead donors.

Table S8: Variables associated with anastomosis time by univariate comparisons.

Table S9: Variables independently associated with anastomosis time by multivariate linear regression with transplant center adjusted for as a random effect.

References

1. Pascual M, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB. Strategies to Improve Long-Term Outcomes after Renal Transplantation. *New England Journal of Medicine* 2002;346(8):580-590.
2. Stegall MD, Gaston RS, Cosio FG, Matas A. Through a glass darkly: seeking clarity in preventing late kidney transplant failure. *Journal of the American Society of Nephrology : JASN* 2015;26(1):20-29.
3. Moers C, Smits JM, Maathuis M-HJ, Treckmann J, van Gelder F, Napieralski BP et al. Machine Perfusion or Cold Storage in Deceased-Donor Kidney Transplantation. *New England Journal of Medicine* 2009;360(1):7-19.
4. Jochmans I, Akhtar MZ, Nasralla D, Kocabayoglu P, Boffa C, Kaiser M et al. Past, Present, and Future of Dynamic Kidney and Liver Preservation and Resuscitation. *American Journal of Transplantation* 2016.

5. Summers DM, Johnson RJ, Hudson A, Collett D, Watson CJ, Bradley JA. Effect of donor age and cold storage time on outcome in recipients of kidneys donated after circulatory death in the UK: a cohort study. *The Lancet* 2013;381(9868):727-734.
6. Debout A, Foucher Y, Trebern-Launay K, Legendre C, Kreis H, Mourad G et al. Each additional hour of cold ischemia time significantly increases the risk of graft failure and mortality following renal transplantation. *Kidney international* 2015;87(2):343-349.
7. Summers DM, Johnson RJ, Allen J, Fuggle SV, Collett D, Watson CJ et al. Analysis of factors that affect outcome after transplantation of kidneys donated after cardiac death in the UK: a cohort study. *The Lancet* 2010;376(9749):1303-1311.
8. Heylen L, Naesens M, Jochmans I, Monbaliu D, Lerut E, Claes K et al. The Effect of Anastomosis Time on Outcome in Recipients of Kidneys Donated After Brain Death: A Cohort Study. *American Journal of Transplantation* 2015;15(11):2900-2907.
9. Marzouk K, Lawen J, Alwayn I, Kiberd BA. The impact of vascular anastomosis time on early kidney transplant outcomes. *Transplantation Research* 2013;2(8).
10. Tennankore KK, Kim SJ, Alwayn IP, Kiberd BA. Prolonged warm ischemia time is associated with graft failure and mortality after kidney transplantation. *Kidney international* 2016;89(3):648-658.
11. O'Neill S, Oniscu GC, Harrison EM. Faster may be better for anastomosis time, but does it really affect survival? *Transplant International* 2015;28(6):764-764.
12. <https://www.eurotransplant.org/>.
13. Port FK, Bragg-Gresham JL, Metzger RA, Dykstra DM, Gillespie BW, Young EW et al. Donor characteristics associated with reduced graft survival: an approach to expanding the pool of kidney donors. *Transplantation* 2002;74(9):1281-1286.

14. Rao PS, Schaubel DE, Guidinger MK, Andreoni KA, Wolfe RA, Merion RM et al. A comprehensive risk quantification score for deceased donor kidneys: the kidney donor risk index. *Transplantation* 2009;88(2):231-236.
15. Becker F, Van Poppel H, Hakenberg OW, Stief C, Gill I, Guazzoni G et al. Assessing the Impact of Ischaemia Time During Partial Nephrectomy. *European Urology* 2009;56(4):625-635.
16. Thompson RH, Lane BR, Lohse CM, Leibovich BC, Fergany A, Frank I et al. Every minute counts when the renal hilum is clamped during partial nephrectomy. *Eur Urol* 2010;58(3):340-345.
17. Pouliot F, Pantuck A, Imbeault A, Shuch B, Calimlim B, Audet J-F et al. Multivariate analysis of the factors involved in loss of renal differential function after laparoscopic partial nephrectomy: a role for warm ischemia time. *Canadian Urological Association Journal* 2011;5(2):89.
18. Parekh DJ, Weinberg JM, Ercole B, Torkko KC, Hilton W, Bennett M et al. Tolerance of the Human Kidney to Isolated Controlled Ischemia. *Journal of the American Society of Nephrology* 2013;24(3):506-517.
19. Reddy SP, Bhattacharjya S, Maniakin N, Greenwood J, Guerreiro D, Hughes D et al. Preservation of porcine non-heart-beating donor livers by sequential cold storage and warm perfusion. *Transplantation* 2004;77(9):1328-1332.
20. Monbaliu D, Liu Q, Vekemans K, Roskams T, Pirenne J. Potentiation of adverse effects of cold by warm ischemia in circulatory death donors for porcine liver transplantation. In: *Transplantation proceedings*; 2012: Elsevier; 2012. p. 2874-2879.
21. Weissenbacher A, Oberhuber R, Cardini B, Weiss S, Ulmer H, Bösmüller C et al. The Faster the Better: Anastomosis Time Influences Patient Survival after Deceased Donor Kidney Transplantation. *Transplant International* 2015.
22. Steinbrook R. Organ Donation after Cardiac Death. *New England Journal of Medicine* 2007;357(3):209-213.

23. Menon M, Sood A, Bhandari M, Kher V, Ghosh P, Abaza R et al. Robotic kidney transplantation with regional hypothermia: a step-by-step description of the Vattikuti Urology Institute-Medanta technique (IDEAL phase 2a). *Eur Urol* 2014;65(5):991-1000.
24. Kamińska D, Kościelska-Kasprzak K, Chudoba P, Klinger M. Kidney injury due to warm ischemia during transplantation can be reduced. *American Journal of Transplantation* 2015.
25. Vacher-Coponat H, McDonald S, Clayton P, Loundou A, Allen RD, Chadban SJ. Inferior early posttransplant outcomes for recipients of right versus left deceased donor kidneys: an ANZDATA registry analysis. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2013;13(2):399-405.

Table 1: Demographic and clinical characteristics of transplant donors and recipients

Donor		
Age (y)		53 (43-64)
Sex		
	Men	7 398 (53%)
	Women	6 566 (47%)
Type		
	DBD	12 806 (92%)
	DCD	1 158 (8%)
Diabetes ¹		
	No diabetes	5 971 (85%)

	History of diabetes	1 029 (15%)
Hypertension ¹	No hypertension	4 298 (48%)
	History of hypertension	4 576 (52%)
Criteria ¹		
	SCD	7 053 (54%)
	ECD	6 039 (46%)
KDRI ²		1.33 (1.04-1.75)
Body mass index ³ (kg/m ²)		25 (23-28)
Terminal creatinine (mg/dl) ⁴		0.85 (0.65-1.10)
Donor warm ischemia time (min) ⁵	In DCD transplants	17 (13-21)
Recipient		
Age (y)		55 (44-65)
Sex		
	Men	8 765 (63%)
	Women	5 199 (37%)
Retransplant		
	First kidney transplant	11 363 (81%)
	Retransplant	2 601 (19%)
Body mass index ⁶ (kg/m ²)		25 (22-28)

Number of HLA mismatches ⁷	3 (2-4)
Process	
Cold ischemia time (h) ⁸	13.7 (10.2-17.4)
Anastomosis time (min)	36 (29-45)
Preservation fluid ¹	
HTK	9 508 (69%)
UW	4 190 (30%)
other	139 (1%)

Data are median (IQR) or n (%). ¹Variable contains missing data. ²7463 missing values, ³three missing values, ⁴four missing values, ⁵214 missing values, ⁶2 867 missing values, ⁷158 missing values, ⁸560 missing values. DBD, Donation after brain death; DCD, Donation after circulatory death; ECD, extended criteria donation; SCD, standard criteria donation; KDRI, Kidney Donor Risk Index; HTK, Histidine-tryptophan-ketoglutarate; UW, University of Wisconsin solution.

Table 2: Cox proportional hazards regression model for graft failure censored at 5 years after transplantation, unadjusted and adjusted for circulatory-dead versus brain-dead donor, donor age, donor diabetes, donor hypertension, donor last serum creatinine, recipient age, number of HLA mismatches, and cold ischemia time.

	Unadjusted			Adjusted*		
	n	Hazard ratio (95% CI)	P value	n	Hazard ratio (95% CI)	P value
Anastomosis time (per 10 minutes)	13 964	1.08 (1.06-1.11)	<0.0001	5 989	1.10 (1.06-1.14)	<0.0001
Anastomosis time (in 4 categories)						
<35 min	6 083	1.00		2 708	1.00	
≥35 and <45 min	4 008	1.04 (0.95-1.14)	0.38	1 724	1.09 (0.94-1.23)	0.26
≥45 and <55 min	2 050	1.16 (1.04-1.30)	0.008	898	1.20 (1.03-1.37)	0.03
≥55 min	1 823	1.38 (1.24-1.54)	<0.0001	659	1.51 (1.33-1.52)	<0.0001

*Hazard rates and P-values of the other significant covariates are provided in Table S2.

Table 3: Cox proportional hazards regression model for graft failure censored at 5 years after transplantation, unadjusted and adjusted for donor age, donor diabetes, donor hypertension, donor last serum creatinine, recipient age, number of HLA mismatches, and cold ischemia time.

	Unadjusted			Adjusted		
	n	Hazard ratio (95% CI)	P value	n	Hazard ratio (95% CI)	P value
Donation after brain death with anastomosis time <45 min	9 177	1.00		3 701	1.00	
Donation after brain death with anastomosis time ≥45 min	3 629	1.23 (1.15-1.31)	<0.0001	1 353	1.19 (1.05-1.34)	0.03
Donation after circulatory death with anastomosis time <45 min	914	1.25 (1.11-1.39)	0.002	731	1.23 (1.04-1.42)	0.02
Donation after circulatory death with anastomosis time ≥45 min	244	2.03 (1.81-2.25)	<0.0001	204	2.06 (1.79-2.33)	<0.0001

Figure 1: Flow diagram demonstrating cohort selection

Figure 2: Kaplan Meier estimates of graft survival, censored at 5 years, for all recipients of kidneys from deceased donors, stratified by anastomosis time in 10-minute intervals. Note y-axis scale begins at 0.40. P-values were calculated with the use of the log-rank test.

Figure 3: Kaplan Meier estimates of graft survival for recipients of kidneys from brain-dead donors (A), recipients of kidneys from circulatory-dead donors (B), adult recipients (C) and pediatric recipients (D) stratified by anastomosis time. Note y-axis scale begins at 0.40. P-values were calculated with the use of the log-rank test.

Figure 4: Kaplan Meier estimates of death-censored graft survival for recipients of kidneys from brain-dead donors and circulatory-dead donors, stratified by anastomosis time. Note y-axis scale begins at 0.4. P-values were calculated with the use of the log-rank test. DBD, Donation after brain death; DCD, donation after cardiac death.