Each additional hour of cold ischemia time significantly increases the risk of graft failure and mortality following renal transplantation

Agnes Debout, Yohann Foucher, Katy Trebern-Launay, Christophe Legendre, Henri Kreis, Georges Mourad, Valérie Garrigue, Emmanuel Morelon, Fanny Buron, Lionel Rostaing, et al.

Each additional hour of Cold Ischemia Time significantly increases the risk of graft failure and mortality after renal transplantation.

Agnes Debout[1*], Yohann Foucher[1,2*], Katy Trébern-Launay[1,2], Christophe Legendre[3], Henri Kreis[3], Georges Mourad[4], Valérie Garrigue[4], Emmanuel Morelon[5], Fanny Buron[5], Lionel Rostaing[6], Nassim Kamar[6], Michèle Kessler[7], Marc Ladrière[7], Alexandra Poignas[8], Amina Blidi[2], Jean-Paul Soulillou[1], Magali Giral[1,8*]and Etienne Dantan[2*]

[*] Authors with equal contributions

Departments and institutions:

1. Institut de Transplantation Urologie Néphrologie, ITUN, CHU Nantes, RTRS « Centaure », Nantes and Inserm U1064, Labex Transplantex, University of Nantes, France.
2. EA 4275 SPHERE - Biostatistics, Pharmacoepidemiology and Subjective Measures in Health Sciences. University of Nantes, France.
3. Service de Transplantation Rénale et de Soins Intensifs, Hôpital Necker, APHP, Paris, France. University of Paris Descartes, University of Sorbonne Paris Cité, France.
4. Service de Néphrologie, Dialyse et Transplantation, Hôpital Lapeyronie, University of Montpellier, France.
5. Service de Néphrologie, Transplantation et Immunologie Clinique, Hôpital Edouard Herriot, Lyon, France.
6. Département de Néphrologie et de Transplantation d'Organes, CHU Rangueil, Toulouse, France. University Paul Sabatier Toulouse, France.
7. Service de Transplantation Rénale, CHU Brabois, Nancy, France.
8. Centre d'Investigation Clinique biothérapie, 30 bd Jean Monnet, 44093, Nantes, France.

Corresponding author:

Pr. Magali Giral, Institut de Transplantation Urologie Néphrologie, 30 boulevard Jean Monnet, 44093 NANTES CEDEX 01 France

Phone: + 33 2 40 08 74 43 Fax: + 33 2 40 08 74 11

Email: magali.giral@chu-nantes.fr

Running headline:

CIT decreases mid-term kidney graft outcomes

Word count: 2945

Abstract

Although Cold Ischemia Time has been widely studied in renal transplantation area, there is yet no consensus on its precise relationship with the transplantation outcomes. The studied data sample included 3839 adult recipients of a first heart-beating deceased donor kidney transplanted between 2000 and 2011 within the French observational multi-centric prospective DIVAT cohort. The Cox model was used to assess the relationship between Cold Ischemia Time and death-censored graft survival or patient survival by using piecewise log-linear function. We observed a significant proportional increase in the risk of graft failure for each additional hour of Cold Ischemia Time (HR=1.013, p=0.035). As an example, a patient who received a kidney with a Cold Ischemia Time of 30 hours presented a risk of graft failure near 40% higher than a patient with a Cold Ischemia Time of 6 hours. Moreover, we observed that the risk of death also proportionally increased for each additional hour of Cold Ischemia Time (HR=1.018, p=0.026). Our approach supports the etiological role of Cold Ischemia Time in graft failure and patient death. We demonstrated that every additional hour of Cold Ischemia Time must be taken into account in order to increase graft survival and patient survival. These findings are of practical clinical interest since Cold Ischemia Time is among one of the main modifiable pre-transplantation risk factors which can be minimized by an improved management of the peri-transplantation period.

Keywords: Cold Ischemia Time; Kidney transplantation; Graft survival; Patient survival

Introduction

During the past ten years in renal transplantation, the widespread use of Mycophenolic Acid, Tacrolimus and novel induction therapies has played a major role in decreasing the incidence of acute rejection episodes.[1–3] However, mid-term kidney graft and patient survival has not improved as much as expected. This could be due to the increased age of both recipients and donors, and associated with the higher frequency of Expanded Criteria Donors (ECD).[4] Another endeavor should be achieved to reduce Delayed Graft Function (DGF) risk.[5] DGF is well known to influence mid-term graft outcomes, increases hospitalization duration and the frequency of concomitant acute rejection.[6,7] The incidence and severity of DGF has remained stable but varies from 25 to 50% among deceased donor kidneys.[8,9] DGF is the consequence of well described risk factors,[10,11] among which Cold Ischemia Time (CIT) seems to be one of the main explicative variables.[12,13] CIT acts at least in part through patho-physiological pathways that induce ischemia reperfusion injuries.[10,14] To improve mid-term outcomes, it could be preferable to optimize the transplantation organization with the aim of shortening CIT as most as possible and preventing ischemia injury through other strategies like machine perfusion, before treating lesions already established in the graft.

Even though CIT is a well-known risk factor among the renal transplantation community, its precise etiological role on mid-term graft outcomes is still under debate as illustrated by the wide heterogeneity of results observed in the literature. On one hand, some authors have shown that CIT was not significantly associated with graft survival among transplanted patients.[15–18] On the other hand, numerous other studies established that CIT represents a major risk factor of graft survival.[12,14,19–24] Nevertheless, there is no consensus whether CIT should be considered as a continuous risk factor or whether threshold values can be considered to identify subgroups with a relevant excess in the risk of mid-term graft and

patient outcomes.[12,16,18,20] For instance, Salahudeen et al. demonstrated a significantly worse graft survival for patients with a CIT higher than 30 hours,[20] while Opelz et al. described that increasing CIT up to 18 hours was not associated with an increased risk of graft failure.[12] In addition to the heterogeneity of cut-off values used to define high-risk patients, the definition of these values is often arbitrary. Besides, the majority of the previous studies analyzed graft and patient survival and death-censored graft survival, only Johnson et al. studied the association of the CIT with patient survival,[17] and they showed a non-significant association of continuous CIT on patient death. Therefore, taking the opportunity of a large prospective, multicentric and validated cohort, the aim of this study was to revisit the potential relationship between the CIT and either the graft failure (death-censored) or the patient death using an etiological approach.

Results

Characteristics at the time of transplantation

The mean CIT was 20.6 hours (range from 6 to 58.6 hours; SD=7.8). The CIT duration was between 6 hours and 16 hours for 1274 (33.2%) patients, 16 hours and 24 hours for 1531 (39.9%), 24 hours and 36 hours for 853 (22.2%) and longer than 36 hours for 181 patients (4.7%). Figure 1 displays boxplots of CIT for each year of transplantation. Over the past decade, we observed a global decrease in CIT duration (median from 23.0 hours in 2000 to 16.3 hours in 2011). However, this progress was more important for prolonged CIT (third quartile from 32.7 hours in 2000 to 21.8 hours in 2011) than for short CIT (first quartile from 17.6 hours in 2000 to 13.1 hours in 2011). Demographic and baseline characteristics at the time of transplantation according to the CIT are described in Table 1. Patients with a history

of cardiovascular diseases (p=0.022), hypertension (p=0.001), those undergoing hemodialysis (p=0.047), those with anti-class II PRA (p=0.006), those who received kidneys from old donor (p<0.001), or from cerebrovascular deceased donor (p=0.025) and a depleting induction therapy (p=0.003) displayed the longest CIT. As expected, we observed an increased risk of DGF with CIT (p<0.0001): from 22% for CIT between 6 to 16 hours, to 40% for CIT above 24 hours. Finally, ECD was distributed differently in the 4 CIT-based groups (p<0.001). Only 48 patients (1.3%) received kidneys placed under hypothermic machine perfusion, leading to an unbalanced parameter which was not taking into account in the multivariate modelling.

Follow-up description

Among the 3839 patients, 449 lost their graft and 238 died with a functioning graft. The cumulative follow-up covered 15978 patients-years. Graft failure and patient survival curves and their corresponding 95% confidence intervals are presented in Figure 2. The graft survivals at 1, 5 and 10 years post-transplantation were respectively 95%, 88% and 77%. The patient survivals at 1, 5 and 10 years post-transplantation were respectively 98%, 93% and 87%. Additionally, the graft failure and death risks appeared higher in the first year post-transplantation than afterwards. Indeed, the incidence rates were equal to 54.1 and 20.8 in the first year respectively for graft failures and death per 1000 patients-year while the mean incidence rates were 21.3 and 13.9 after the first year post-transplantation. For patients with less than 16 hours CIT, the corresponding absolute risks were 4%, 10% and 20% for graft failure and 1%, 6% and 11% for death. Patients with CIT between 16 and 24 hours had absolute risk close to those with CIT between 24 and 36 hours (5%, 13% and 24% for graft failure and 3%, 7% and 13% for death) while the risk appears slightly higher for patients with more than 36 hours CIT (8%, 13% and 36% for graft failure and 5%, 15% and 22% for death).

Graft survival analysis

While we considered a large number of non-linear associations, we finally retained a proportional relationship between CIT and the risk of graft failure. Results of the unadjusted analyses and the final multivariate model are presented in Table 2 and illustrated in Figure 3A. For each additional hour of ischemia time, the risk of graft failure was multiplied by 1.013 (p=0.035). For instance, patients with 12 hours CIT had a risk of graft failure 8% (1.013^{12-6}) higher than patients with 6 hours CIT. This relationship is constant regardless of the baseline CIT level, i.e. this excess of risk being similar between patients with 30 hours and those with 24 hours. This association was independent from other possible confounding factors.

Patient Survival analysis

In this model, we also retained a proportional association between CIT and risk of death. Table 3 presents the unadjusted analyses and the final multivariate model. Actually, the risk of death was multiplied by 1.018 (p = 0.026) for each supplementary hour of CIT. Figure 3B illustrates this relationship. As an example, patients with 30 hours CIT will have 53% (1.018^{30-6}) more risk of death than patients with 6 hours CIT. This relationship was constant regardless of the baseline CIT level. This association was independent from the other possible confounding factors.

Exploration of interactions

For both survival analyses, no clinically relevant interaction with CIT appeared statistically significant. Specifically, we assumed that prolonged CIT could be more deleterious among patients receiving kidneys from ECD than from non ECD. However, by testing the interaction between CIT and ECD, we could not demonstrate a significant difference in the relationships

between CIT and both graft and patient survivals in patients who received kidney from Extended or Standard Criteria Donor (p= 0.319 and p=0.408 respectively for graft and patient survivals).

Discussion

From our cohort-based analysis, we describe the negative influence of CIT on mid-term outcomes. The originality of these findings is demonstrated in the proportional relationship between CIT and the risk of graft failure. Moreover, we also demonstrated, for the first time to our knowledge, the proportional relationship between CIT and the risk of patient death. More precisely, and for both outcomes, the results suggest that every additional hour of CIT matters. According the description of CIT trend within the last ten years (Figure 1), we observed an important decrease of prolonged CIT whereas this decrease was not so important for short CIT. However, our study highlights that this effort to minimized CIT has to be considered either for short and long CIT. Nevertheless, such management strategies do not exclude also minimizing and/or treating lesions already established in the graft by using therapeutic strategies,[25–27] or by spreading the use of hypothermic machine perfusion.[28]

Even if this proportionality was assumed in other studies,[14,21,23] it has not been clearly demonstrated that this assumption corresponds to a real relationship. In other words, our analyses highlighted the absence of CIT thresholds, in contrast to numerous manuscripts in which patients are stratified according to CIT intervals.[12,16–18] The proportional increase in risk of graft failure and patient death related to CIT avoids loss of information and loss of power which may explain the non-significant association between the CIT and the mid-term graft outcomes in previous studies.[15,18] For instance, by considering a CIT threshold at 18 hours like Opelz et al.[12], we estimated an increased risk near 21% and 18% higher in patients

with a CIT above 18 hours than below for graft failure and patient death respectively (HR=1.210, p=0.069, HR=1.180, p=0.239). These risk excesses appeared non-significant, may be due to the loss of power induced by CIT categorization.

As usual, there are several limitations for such an observational study. Firstly, we performed two separate analyses as is common in renal transplantation: the graft survival was studied by considering death as censoring while the patient survival was analysed by considering returns to dialysis as censoring. Patients are not further followed up after their return to dialysis in the DIVAT network of transplantation centres. However, censoring patient death by graft failure leads to an underestimate in the post-transplantation mortality, particularly due to the over mortality related to return to dialysis. Nevertheless, since CIT was also identified as a risk factor for graft failure, one could conclude that we underestimated the association between CIT and patient mortality. Secondly, even if we elaborated an adjusted model to consider CIT confounders, we could not exclude the potential bias induced by non-observed confounding factors. For instance, hypothermic machine perfusion represents only 1.3% of the entire sample. Nevertheless, we performed a sensitivity analysis of the retained models from a sub-sample exclusively including patients not under machine perfusion, leading to similar conclusions about CIT association. Thirdly, conclusions essentially concerned patients respecting the defined inclusion and exclusion criteria. In particular, excluding patients with missing data could also induce a selection bias. We did not identify any difference in CIT mean between our studied patients and those excluded for missing data (20.6 hours vs. 20.5 hours, p= 0.913). One can reasonably assume the missing data at random.

The aim of our study was not to assess the mechanism explaining the role of the CIT as a determinant of the mid-term patient or graft survivals, but rather to highlight the precise relationship between CIT and graft or patient survivals. Therefore, we voluntarily designed

etiological models by taking into account only risk factors available at the time of the transplantation.[29] Actually, the delayed graft function or the occurrence of acute rejection episodes was not considered, these post transplantation intermediate events being partially due to prolonged CIT. In contrast, Hernández et al. described that CIT was still associated with graft failure when adjusting on DGF and also on acute rejection (HR=1.04; CI95% 1.01-1.10).[24] By performing such adjustments, the HR was certainly underestimated. To illustrate this, Mikhalski et al. compared two approaches: one considering only pre-transplant risk factors, and one also adjusting for the occurrence of acute rejection episodes and DGF.[14] In contrast to the first approach, CIT was not associated with graft survival in the second one, supporting a causal pathway from CIT to graft failure through DGF and/or acute rejection. Indeed, CIT might not be directly correlated with mid-term outcomes, since its correlation with poor graft or patient survivals would probably go through mainly ischemic injury leading to DGF. Butala et al. also demonstrated that for patients without DGF, CIT was not significantly associated with graft and patient survivals.[30] Even if it does not constitute our principal objective, we also agree with this mechanistic hypothesis since, from our data, the proportional CIT relationship with mid-term outcomes appears to disappear after adjustment for DGF (HR = 1.00, p = 0.448 for graft survival; HR = 1.01, p = 0.433 for patient survival).

In conclusion, we demonstrated for the first time that each hour of CIT may have an impact on both graft and patient survivals. To this end, further efforts have to be established focusing on clinical coordination and organization, to shorten the cold storage of kidneys. Whilst efforts over the past decade have mainly focused on reduction of long CIT, we propose that a similar practise should also be applied to shortening short-term CIT, and may significantly prolong graft and patient survivals.

Methods

Study population

4777 patients were extracted from the prospective DIVAT cohort of transplanted patients (reference for the French Research Ministry: RC12_0452, last agreement N° 13 334, May 16 2013, www.divat.fr, N° CNIL 891735 version 2, August 2004).[31] All patients meeting the following inclusion criteria were studied: adult recipients who received a first renal transplantation performed between January 2000 and December 2011 from heart-beating deceased donors, and maintained under immunosuppressive therapy with Tacrolimus and Mycophenolic Acid. CIT was defined as the duration between the start of cold preservation and the reperfusion after implantation. Nevertheless, several clinical variables may lead to different CIT and these are associated with possible difficulties at the time of kidney allocation. As they also represent well-known risk factors of graft or patient outcomes, these "empirically defined" confounding factors were taken into account in the multivariate analyses. For this reason, our exclusion criteria concerned all patients with missing data concerning these variables: CIT (n=19), as the main explicative variable and donor age (n=23), donor gender (n=36), last donor serum creatinine before the organ removal (n=94), donor cause of death (n=25), technique of dialysis (n=13) and historical anti-class I (n=410) and anti-class II PRA (n=784), possible confounding factors of CIT. Finally, 3839 patients made up the patient sample.

Donor parameters were: age, gender, serum creatinine, cause of death (cerebro-vascular versus others) and ECD criteria. Recipient parameters were: age, gender, Body Mass Index (BMI), history of diabetes, hypertension, dyslipidemia, cardiac and/or vascular diseases, dialysis technique before transplantation (no dialysis, hemodialysis and peritoneal dialysis), HLA A-B-DR incompatibilities, historical anti-HLA immunization (historical peak of anti-

class I and II PRA), induction therapy with Anti-Thymocyte Globulin (ATG) and Anti-Lymphocytes Globulins (ALG), or anti-Il-2 receptor monoclonal antibodies and DGF defined by the need for dialysis in the first week post transplantation.

Statistical analysis

Quantitative characteristics at the time of transplantation were expressed as mean and standard deviation (SD) for continuous variables, or as count and percentage for categorical characteristics. For a more convenient description of patients according to the CIT level, we first arbitrarily defined 4 CIT-based groups: 6 – 16 hours, 16 – 24 hours, 24 – 36 hours, and more than 36 hours. Comparisons of characteristics regarding the CIT-based groups were performed using Analysis Of Variance, or Chi-square test respectively for quantitative or categorical variables. Our principal aim was to construct an etiological analysis, i.e. to investigate the relationship between CIT and mid-term graft or patient survivals. In order to avoid confounding results, all variables differentially distributed between CIT-based groups and that were not consequences of CIT were included in multivariate models (Figure 4A).[29,32] DGF and acute rejection are post-transplant parameters that may occur partly due to CIT since CIT is one of their well-known risk factors.[11,13,14] For this reason, we voluntarily did not adjust our models on DGF while it is probably on the same causal pathway (Figure 4B).[30] Likewise, we did not consider acute rejection for the same reason.

Graft survival analyses were based on the time between transplantation and the graft failure (censoring of death) and patient survival analyses on the time between transplantation and death with a functioning kidney (censoring of returns in dialysis). Analyses were performed in two steps. Firstly, possible risk factors associated with time-to-event were selected by univariate analyses (Log-Rank test, $p<0.20$). As well as confounding factors empirically defined, these variables were further analysed in a multivariate approach by using the Cox

proportional hazards frailty model.[33] Frailty term was introduced to consider the time-to-event correlation within transplantation centres. Non-significant variables were removed respecting a descending procedure ($p<0.05$). In order to avoid the log-linearity assumption and to facilitate the interpretation of the results, only categorical adjustment variables were included using thresholds traditionally used in the literature. Proportional hazards assumption was evaluated by plotting log-minus-log survival curves and by analysing the scaled Schoenfeld residuals.[34] In respect to the main objective of our study, during the descending procedure, CIT was the only variable kept in continuous form and maintained in the model whatever the associated p-value. In addition, to identify a potentially different effect of CIT given its duration, different piecewise log-linear functions for the relationship between CIT and the time-to-failure were compared: 1) the first one without any CIT threshold assuming a homogeneous increase in the risk given CIT, 2) the second one assuming a continuous increase in the risk given CIT until one CIT threshold and an accentuated risk increase beyond this threshold, and 3) the third one with two CIT thresholds assuming two changes in the continuous increase in the risk. The retained function was chosen by minimizing the Bayesian Information Criterion. In order to verify the retained relationship between CIT and failure risk, we graphically assessed the Martingale residuals given the CIT.[35] All analyses were performed using the 2.15.0. version of the R software.[36]

Disclosure

The authors have nothing to disclose.

References

1. Gonwa T 1, Johnson C, Ahsan N *et al.* Randomized trial of tacrolimus + mycophenolate mofetil or azathioprine versus cyclosporine + mycophenolate mofetil after cadaveric kidney transplantation: results at three years. [Miscellaneous Article]. *Transplant. June 27 2003* 2003; **75**: 2048–2053.

2. Knight SR, Russell NK, Barcena L *et al.* Mycophenolate mofetil decreases acute rejection and may improve graft survival in renal transplant recipients when compared with azathioprine: a systematic review. *Transplantation* 2009; **87**: 785–794.

3. Ekberg H, Bernasconi C, Tedesco-Silva H *et al.* Calcineurin inhibitor minimization in the Symphony study: observational results 3 years after transplantation. *Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg.* 2009; **9**: 1876–1885.

4. Metzger RA, Delmonico FL, Feng S *et al.* Expanded criteria donors for kidney transplantation. *Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg.* 2003; **3 Suppl 4**: 114–125.

5. Yarlagadda SG, Klein CL, Jani A. Long-term renal outcomes after delayed graft function. *Adv. Chronic Kidney Dis.* 2008; **15**: 248–256.

6. Jayaram D, Kommareddi M, Sung RS *et al.* Delayed graft function requiring more than one-time dialysis treatment is associated with inferior clinical outcomes. *Clin. Transplant.* 2012; **26**: E536–E543.

7. Giral-Classe M, Hourmant M, Cantarovich D *et al.* Delayed graft function of more than six days strongly decreases long-term survival of transplanted kidneys. *Kidney Int.* 1998; **54**: 972–8.

8. Tapiawala SN, Tinckam KJ, Cardella CJ *et al.* Delayed Graft Function and the Risk for Death with a Functioning Graft. *J. Am. Soc. Nephrol.* 2010; **21**: 153–161.

9. Yarlagadda SG, Coca SG, Formica RN *et al.* Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrol. Dial. Transplant.* 2009; **24**: 1039–1047.

10. Perico N, Cattaneo D, Sayegh MH *et al.* Delayed graft function in kidney transplantation. *Lancet* 2004; **364**: 1814–1827.

11. Irish WD, Ilsley JN, Schnitzler MA *et al.* A risk prediction model for delayed graft function in the current era of deceased donor renal transplantation. *Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg.* 2010; **10**: 2279–2286.

12. Opelz G, Döhler B. Multicenter analysis of kidney preservation. *Transplantation* 2007; **83**: 247–253.

13. Chapal M, Le Borgne F, Legendre C *et al.* The DGFS: A useful scoring system for the prediction and management of delayed graft function following kidney transplantation from heart beating deceased donors. *Revis. Kidney Int.*

14. Mikhalski D, Wissing KM, Ghisdal L *et al.* Cold ischemia is a major determinant of acute rejection and renal graft survival in the modern era of immunosuppression. *Transplantation* 2008; **85**: S3–9.

15. Kayler LK, Srinivas TR, Schold JD. Influence of CIT-Induced DGF on Kidney Transplant Outcomes. *Am. J. Transplant.* 2011; **11**: 2657–2664.

16. Kayler LK, Magliocca J, Zendejas I *et al.* Impact of Cold Ischemia Time on Graft Survival Among ECD Transplant Recipients: A Paired Kidney Analysis. *Am. J. Transplant.* 2011; **11**: 2647–2656.

17. Johnson RJ, Fuggle SV, O'Neill J *et al.* Factors influencing outcome after deceased heart beating donor kidney transplantation in the United Kingdom: an evidence base for a new national kidney allocation policy. *Transplantation* 2010; **89**: 379–386.

18. Summers DM, Johnson RJ, Hudson A *et al.* 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**: 727–734.

19. Ojo AO, Wolfe RA, Held PJ *et al.* Delayed graft function: risk factors and implications for renal allograft survival. *Transplant. April 15 1997* 1997; **63**: 968–974.

20. Salahudeen AK, Haider N, May W. Cold ischemia and the reduced long-term survival of cadaveric renal allografts. *Kidney Int.* 2004; **65**: 713–718.

21. Quiroga I, McShane P, Koo DDH *et al.* Major effects of delayed graft function and cold ischaemia time on renal allograft survival. *Nephrol. Dial. Transplant.* 2006; **21**: 1689–1696.

22. Morris PJ, Johnson RJ, Fuggle SV *et al.* Analysis of factors that affect outcome of primary cadaveric renal transplantation in the UK. *The Lancet* 1999; **354**: 1147–1152.

23. Van der Vliet JA, Warlé MC, Cheung CLS *et al.* Influence of prolonged cold ischemia in renal transplantation. *Clin. Transplant.* 2011; **25**: E612–E616.

24. Hernández D, Estupiñán S, Pérez G *et al.* Impact of cold ischemia time on renal allograft outcome using kidneys from young donors. *Transpl. Int.* 2008; **21**: 955–962.

25. Neumayer HH, Kunzendorf U, Schreiber M. Protective effects of calcium antagonists in human renal transplantation. *Kidney Int. Suppl.* 1992; **36**: S87–93.

26. Dawidson IJ, Sandor ZF, Coorpender L *et al.* Intraoperative albumin administration affects the outcome of cadaver renal transplantation. *Transplantation* 1992; **53**: 774–782.

27. Powell JT, Tsapepas DS, Martin ST *et al.* Managing renal transplant ischemia reperfusion injury: novel therapies in the pipeline. *Clin. Transplant.* 2013; **27**: 484–491.

28. Moers C, Kornmann NS, Leuvenink HG *et al.* The influence of deceased donor age and old-for-old allocation on kidney transplant outcome. *Transplantation* 2009; **88**: 542–52.

29. Jager KJ, Zoccali C, Macleod A *et al.* Confounding: what it is and how to deal with it. *Kidney Int.* 2008; **73**: 256–260.

30. Butala NM, Reese PP, Doshi MD *et al.* Is delayed graft function causally associated with long-term outcomes after kidney transplantation? Instrumental variable analysis. *Transplantation* 2013; **95**: 1008–1014.

31. Ladrière M, Foucher Y, Legendre C *et al.* The Western Europe Cohort Of Kidney Transplant Recipients - The DIVAT Network. *Clin. Transpl.* 2010: 460–461.

32. Tripepi G, Jager KJ, Dekker FW *et al.* Testing for causality and prognosis: etiological and prognostic models. *Kidney Int.* 2008; **74**: 1512–1515.

33. Hougaard P. Shared frailty models. In: *Analysis of Multivariate Survival Data*. Springer New York, 2000, pp.215–262.

34. Grambsch P, Therneau T. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* 1994; **81**: 515–26.

35. Therneau TM, Grambsch PM, Fleming TR. Martingale-based residuals for survival models. *Biometrika* 1990; **77**: 147–160.

36. R Development Core Team . *R: A Language and Environment for Statistical Computing*. (Computing RF for S, ed.). Vienna, Austria; 2010.

Acknowledgments

We wish to thank members of the clinical research assistant team (S. Le Floch, A. Petit, J. Posson, C. Scellier, V. Eschbach, K. Zurbonsen, C. Dagot, F. M'Raiagh, V. Godel, X. Longy, P. Przednowed). The DIVAT cohort is partially supported by Roche Laboratory since 1994.

Title and legends

Figure 1: Boxplots representing the minimum, the maximum, the first, second and third quartiles of Cold Ischemia Time duration for each year of transplantation.

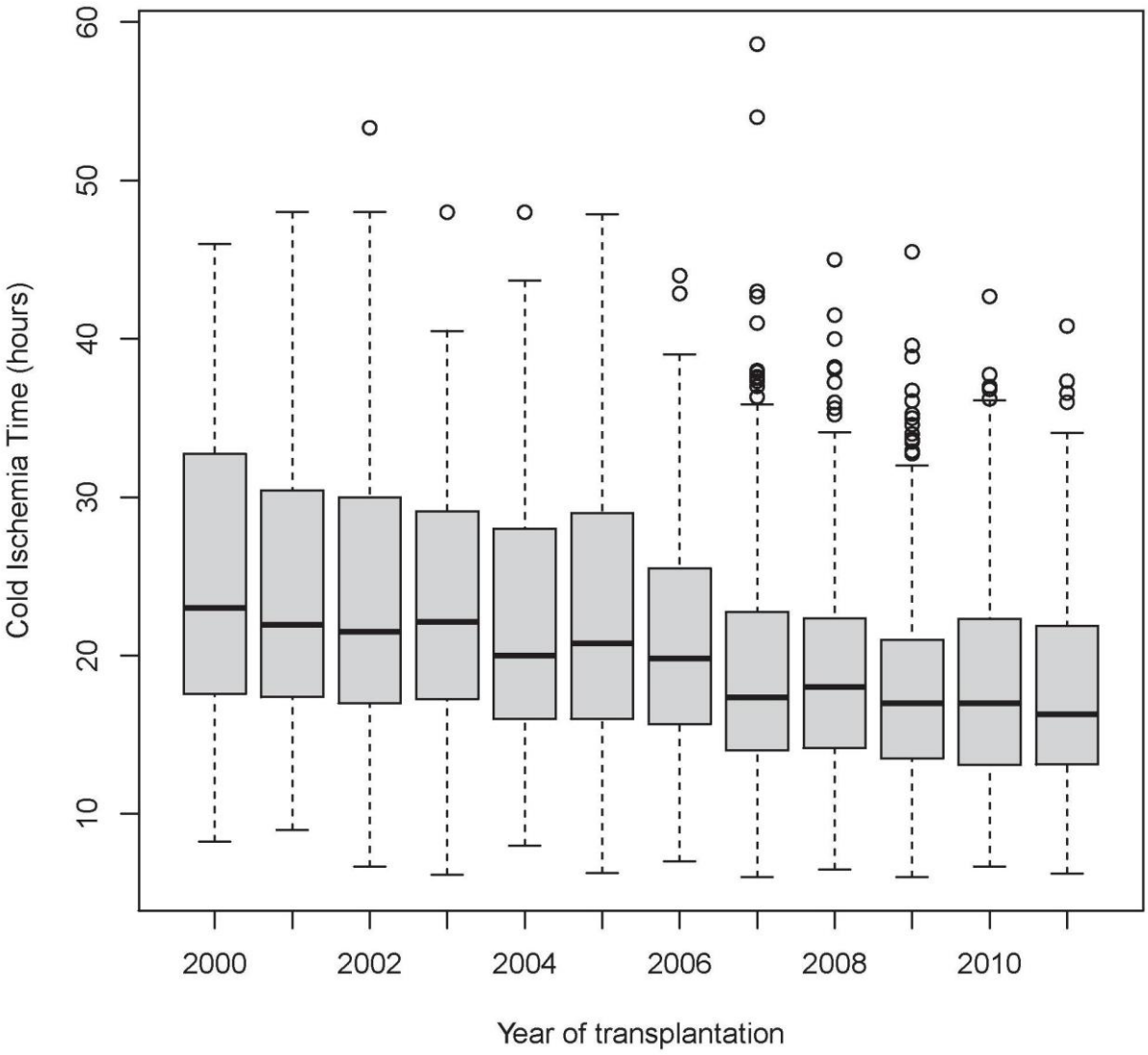
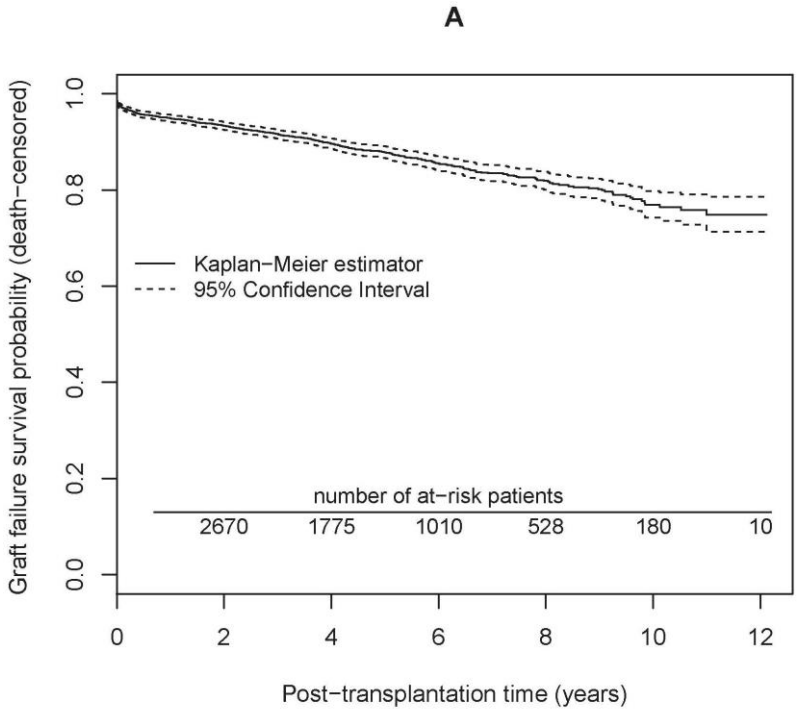


Figure 2: Graft failure (with death-censored) survival probability (A) and Patient survival probability (B) in function of the time since the transplantation from Kaplan-Meier estimator, and their corresponding 95% confidence intervals.



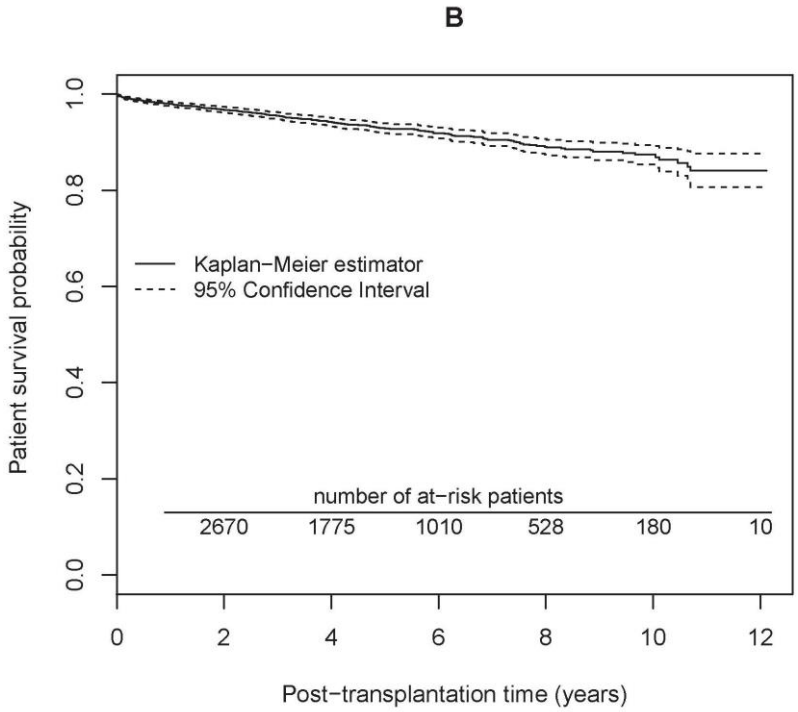


Figure 3: Evolution of Hazard Ratio (HR) relative to graft failure (with death-censored) risk (A) and patient death risk (B) in function of Cold Ischemia Time duration in hours (Subjects with 6 hours of CIT chosen as references) and their corresponding 95% confidence intervals, from multivariate Cox models.

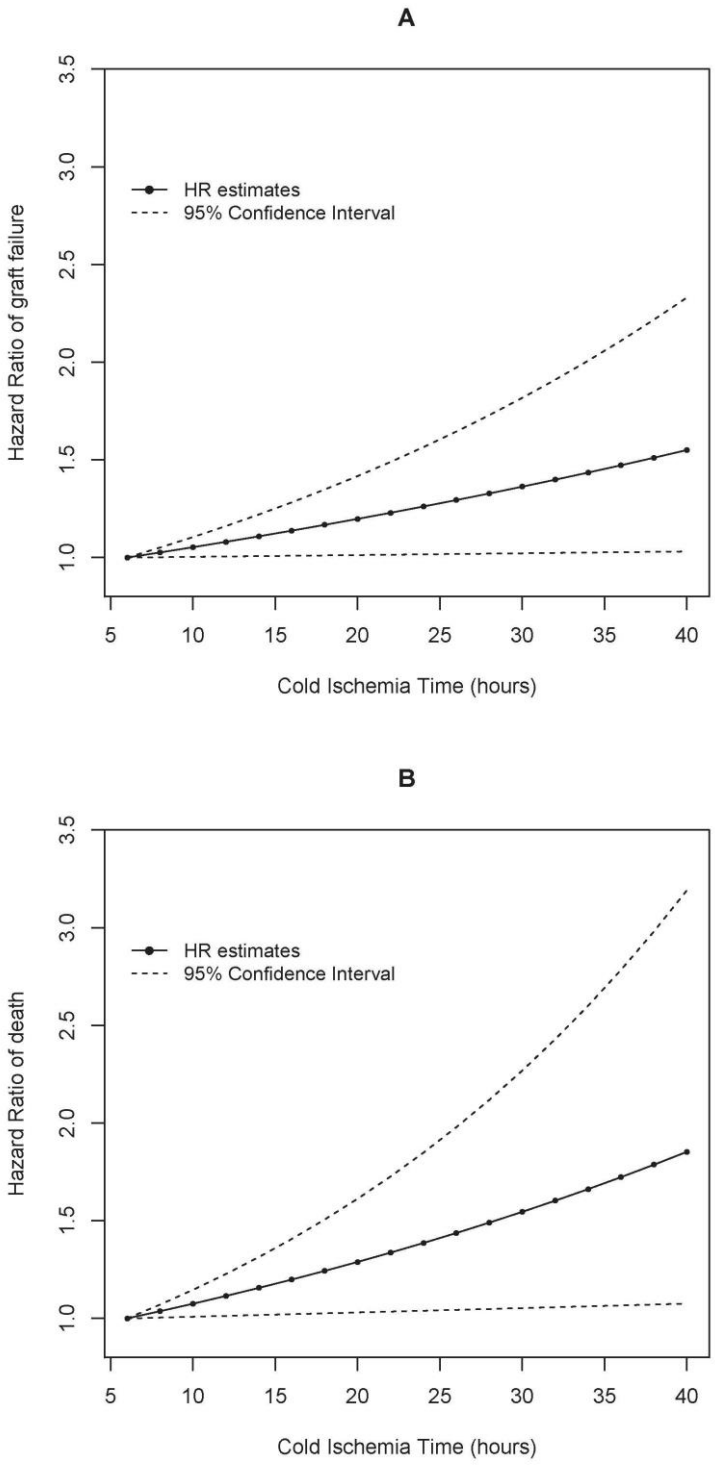


Figure 4: Concepts of confounding factor and causal pathway in evaluating the relationship between Cold Ischemia Time (CIT) and graft outcomes (graft survival with death-censored or patient survival), adapted from Jager et al.[29] - Confounding factor associated to CIT and also a risk factor of graft outcomes (A); Causal pathway through Delayed Graft Function (DGF): DGF a consequence of CIT and also a risk factor of graft outcomes (B).

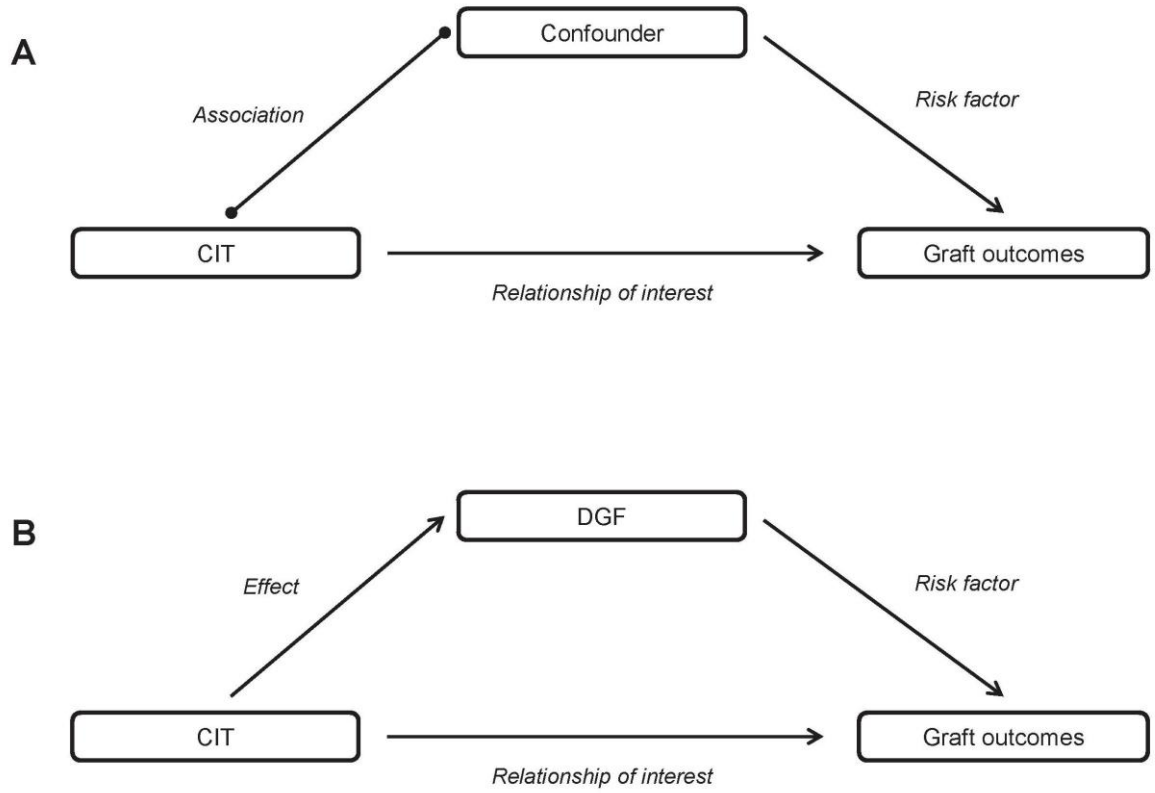


Table 1: Description of recipient, donor and transplantation characteristics of the global study population and according CIT-based groups (6 – 16 hours, 16 – 24 hours, 24 – 36 hours and > 36 hours). Quantitative characteristics expressed as Mean and Standard Deviation; Categorical characteristics expressed as Number (%).

	Missing data	Global N=3839	CIT from 6 to 16 hours N=1274	CIT from 16 to 24 hours N=1531	CIT from 24 to 36 hours N=853	CIT above 36 hours N=181	p-value
Quantitative characteristics : Mean ± SD							
Recipient age (years)	0	51.6±13.2	51.0±13.4	52.3±13.3	51.3±12.7	51.4±13.4	0.051
Recipient BMI (kg/m²)	38	24.4±4.3	24.3±4.3	24.4±4.2	24.5±4.5	24.3±4.4	0.894
Donor age (years)	0	50.5±16.2	48.9±16.4	52.1±16.1	50.0±15.5	50.3±16.6	< 0.001
Donor serum creatinine (mg/ml)	0	93.9±56.1	90.7±47.9	96.0±65.4	95.6±51.3	91.6±43.7	0.061
HLA incompatibilities ABDR	87	3.3±1.3	3.4±1.2	3.2±1.3	3.3±1.4	3.3±1.3	< 0.001
Categorical characteristics : N (%)							
Recipient men	0	2365 (61.6)	794 (62.3)	958 (62.6)	504 (59.1)	109 (60.2)	0.345
Dialysis technique							0.047
Pre-emptive transplantation	0	304 (7.9)	120 (9.4)	123 (8.0)	54 (6.3)	7 (3.9)	
Hemodialysis	0	3251 (84.7)	1053 (82.7)	1300 (84.9)	735 (86.2)	163 (90.0)	
Peritoneal dialysis	0	284 (7.4)	101 (7.9)	108 (7.1)	64 (7.5)	11 (6.1)	
Detectable anti-class I PRA	0	750 (19.5)	228 (17.9)	313 (20.4)	165 (19.3)	44 (24.3)	0.131
Detectable anti-class II PRA	0	608 (15.8)	172 (13.5)	243 (15.9)	155 (18.2)	38 (21.0)	0.006
History of cardiovascular diseases	0	1394 (36.3)	427 (33.5)	557 (36.4)	335 (39.3)	75 (41.4)	0.022
History of hypertension	0	3036 (79.1)	981 (77.0)	1197 (78.2)	700 (82.1)	158 (87.3)	0.001
History of dyslipidemia	0	1138 (29.6)	380 (29.8)	450 (29.4)	250 (29.3)	58 (32.0)	0.894
History of diabetes	0	466 (12.1)	144 (11.3)	202 (13.2)	97 (11.4)	23 (12.7)	0.398
Donor men	0	2277 (59.3)	772 (60.6)	894 (58.4)	502 (58.9)	109 (60.2)	0.672
Expanded Criteria Donor	0	1295 (33.7)	379 (29.8)	589 (38.5)	264 (31.0)	63 (34.8)	< 0.001

Cerebro-vascular donor death	0	2169 (56.5)	686 (53.9)	904 (59.1)	470 (55.1)	109 (60.2)	0.025
Depleting induction	17	1521 (39.6)	500 (39.3)	567 (37.0)	366 (42.9)	88 (48.6)	0.003
Delayed Graft Function	110	1204 (31.4)	280 (22.0)	474 (31.0)	342 (40.1)	108 (59.7)	< 0.001
Machine perfusion	0	48 (1.3)	24 (1.9)	14 (0.9)	10 (1.2)	0 (0)	0.0579

Table 2: Cox unadjusted analyses and Cox multivariate analysis (n=3701, 138 observations deleted due to missingness concerning covariates) of graft failure risk (with death-censored). Each additional hour of Cold Ischemia Time increases significantly the risk of graft failure (HR = 1.013, p = 0.024), independently of possible confounding factors.

	Unadjusted Cox models			Multivariate Cox frailty model[†]		
	uHR[*]	95% CI	p-value	aHR[**]	95% CI	p-value
Cold Ischemia Time (hours)	1.018	1.007 – 1.030	0.001	1.013	1.001 – 1.025	0.035
Donor age			< 0.001			< 0.001
51-60 years vs. ≤50 years	1.362	1.072 – 1.730	0.011	1.228	0.943 – 1.598	0.128
61 years and more vs. ≤50 years	2.080	1.675 – 2.581	< 0.001	2.036	1.535 – 2.699	< 0.001
Donor gender (men vs. women)	0.851	0.706 – 1.026	0.091	0.843	0.692 – 1.028	0.091
Donor serum creatinine (≥15mg/ml vs. <15mg/ml)	1.248	0.974 – 1.599	0.080	1.391	1.069 – 1.809	0.014
Donor cause of death (vascular vs. others)	1.271	1.052 – 1.537	0.013	1.048	0.852 – 1.289	0.659
Recipient age (≥55 years vs. <55 years)	1.342	1.114 – 1.615	0.002	0.878	0.697 – 1.119	0.280
Recipient BMI (≥30 kg/m² vs. <30 kg/m²)	1.984	1.544 – 2.549	<0.001	1.822	1.408 – 2.358	<0.001
Dialysis technique			0.201			0.052
Peritoneal dialysis vs. pre-emptive transplantation	1.232	0.714 – 2.126	0.453	1.028	0.585 – 1.806	0.924
Hemodialysis vs. pre-emptive transplantation	1.405	0.930 – 2.121	0.106	1.316	0.865 – 2.002	0.199
History of cardiovascular diseases (yes vs. no)	1.436	1.192 – 1.730	<0.001	1.255	1.030 – 1.529	0.024
PRA anti-class I (detectable vs. undetectable)	1.398	1.113 – 1.755	0.004	1.429	1.094 – 1.867	0.009
PRA anti-class II (detectable vs. undetectable)	1.115	0.865 – 1.438	0.399	0.939	0.697 – 1.264	0.677
HLA incompatibility ABDR (>4 vs. ≤4)	1.276	0.995 – 1.636	0.055	1.300	1.003 – 1.684	0.047
Induction therapy (depleting vs. non depleting)	1.159	0.959 – 1.400	0.126	1.077	0.875 – 1.326	0.482
Recipient gender (men vs. women)	0.865	0.716 – 1.044	0.129	-	-	-
History of hypertension (yes vs. no)	1.051	0.829 – 1.331	0.682	-	-	-
History of dyslipidemia (yes vs. no)	1.278	1.051 – 1.553	0.014	-	-	-
History of diabetes (yes vs. no)	1.419	1.088 – 1.850	0.010	-	-	-
Expanded Criteria Donor (yes vs. no)	1.860	1.542 – 2.244	<0.001	-	-	-

[*] uHR: unadjusted hazard ratio; [**] aHR: adjusted hazard ratio; [†] random effect variance=0.025

Table 3: Cox unadjusted analyses and Cox multivariate analysis (n=3738, 101 observations deleted due to missingness concerning covariates) of patient death risk (with a functioning graft). Each additional hour of Cold Ischemia Time increases significantly the risk of death (HR = 1.019, p = 0.023), independently of possible confounding factors.

	Unadjusted Cox models			Multivariate Cox frailty model[†]		
	uHR[*]	95% CI	p-value	aHR[**]	95% CI	p-value
Cold Ischemia Time (hours)	1.016	1.000 – 1.031	0.044	1.018	1.002 – 1.035	0.026
Donor age			< 0.001			0.006
51-60 years vs. ≤50 years	2.345	1.664 – 3.304	< 0.001	1.663	1.145 – 2.415	0.008
61 years and more vs. ≤50 years	3.735	2.718 – 5.133	< 0.001	2.043	1.381 – 3.021	<0.001
Donor gender (men vs. women)	0.847	0.655 – 1.094	0.203	0.934	0.713 – 1.223	0.619
Donor serum creatinine (≥15mg/ml vs. <15mg/ml)	0.758	0.504 – 1.139	0.183	0.854	0.555 – 1.313	0.471
Donor cause of death (vascular vs. other)	1.496	1.147 – 1.952	0.003	1.009	0.758 – 1.342	0.953
Recipient age (≥55 years vs. <55 years)	3.202	2.421 – 4.235	< 0.001	1.754	1.253 – 2.457	0.001
Dialysis technique			0.043			0.118
Peritoneal dialysis vs. preemptive transplantation	1.155	0.490 – 2.719	0.742	1.024	0.434 – 2.417	0.956
Hemodialysis vs. preemptive transplantation	1.825	0.968 – 3.441	0.063	1.586	0.838 – 3.003	0.157
PRA anti-class I (detectable vs. undetectable)	1.298	0.939 – 1.793	0.114	1.501	1.035 – 2.179	0.032
PRA anti-class II (detectable vs. undetectable)	0.976	0.675 – 1.410	0.896	0.768	0.499 – 1.182	0.230
History of cardiovascular diseases (yes vs. no)	2.795	2.154 – 3.627	< 0.001	2.028	1.538 – 2.672	<0.001
History of diabetes (yes vs. no)	2.892	2.153 – 3.886	< 0.001	1.942	1.423 – 2.651	<0.001
HLA incompatibility ABDR (>4 vs. ≤4)	1.142	0.800 – 1.630	0.464	1.081	0.751 – 1.560	0.670
Induction therapy (depleting vs. non depleting)	0.769	0.584 – 1.012	0.061	0.774	0.582 – 1.029	0.077
Recipient BMI (≥30 kg/m² vs. <30 kg/m²)	1.990	1.410 – 2.809	< 0.001	-	-	-
Recipient gender (men vs. women)	1.400	1.025 – 1.778	0.032	-	-	-
History of hypertension (yes vs. no)	1.162	0.830 – 1.627	0.382	-	-	-

History of dyslipidemia (yes vs. no)	1.486	1.143 – 1.932	0.003	-	-	-
Expanded Criteria Donor (yes vs. no)	2.644	2.047 – 3.415	< 0.001	-	-	-

[*] uHR: unadjusted hazard ratio; [**] aHR: adjusted hazard ratio; [†] random effect variance=0.002