Angiopoietins in renal replacement therapy

Westendorp, Welmoet Hillegonda

CHAPTER 7

Brain death induced renal injury

WH Westendorp
HGD Leuvenink
RJ Ploeg

Published in Current Opinion on Organ Transplantation
2011 Apr;16(2):151-6
Digital object identifier: 10.1097/MOT.0b013e328344a5dc

PURPOSE OF THE REVIEW

The considerable demand for donor kidneys against a persisting organ donor shortage has forced most centers to nowadays accept suboptimal donor kidneys. Despite the substantial increase in the past decade in kidney transplantation with grafts retrieved from living donors and after donation from deceased brain dead (DBD) and extended criteria donation (ECD) donors, the supply of donor kidneys still does not meet the actual numbers needed. Moreover, older and more marginal kidney donors following the physiologically abnormal state of brain death do function less and have a shorter graft survival. In this review, we present an overview of the current knowledge of renal injury induced by pathophysiological effects of brain death and its relevance for renal transplant outcome. The better insight in the role of brain death induced renal injury has clearly demonstrated its detrimental effect on outcome but, also, offers new opportunities for donor management and evaluation of new biomarkers to assess kidney graft quality in the brain dead donor. The option to intervene and selectively block or enhance a pathway as well as identify specific parameters for graft quality at time of organ retrieval in the deceased brain dead donor will ultimately benefit early function and long-term survival.

INTRODUCTION

Brain death, in the past also referred to as coma dépassée, was first described in 1959 by the French Mollaret and Goulon[1] as a state of irreversible coma without reflexes after massive cerebral injury that required mechanical ventilation. At that time, no consensus had been reached on the implications of this irreversible coma. In 1968, a committee at Harvard Medical School proposed to add irreversible coma to the death criterion, so that in these cases ventilators could be turned off. Its report established the term of brain death for the first time defining it as a 'permanently nonfunctioning brain', providing diagnostic criteria and establishing brain death legally the equivalent to death[2]. This development has formed since then the legal basis for the possibility to obtain organs for transplantation from deceased patients who are brain dead, however, do still have an intact extracerebral circulation.

To date, the declaration of brain death has been accepted by most societies as a point of no return and has made organ donation possible. From the moment of cerebral injury and certainly after herniation of the brain stem a cascade of events takes place that does affect all potential donor organs. This sequence includes an increase of intracranial pressure following cerebral trauma or cerebrovascular hemorrhage and multiple systemic and hormonal changes. One of the significant brain death related events that affects the quality of grafts-to-be prior to organ retrieval is the profound inflammatory and pro-coagulatory response syndrome.

Brain death injury

To date, the majority of organs is still derived from brain dead donors as the main source used in transplantation. Since the need for organs is significantly larger than the availability of ideal donors, criteria for acceptation of donors have been expanded and led to the use of extended criteria donation (ECD). ECD includes brain dead donors who are older than 60 years, or are aged over 50 years in combination with at least two of the following risk factors: a history of hypertension and a terminal serum creatinine more than 1.5 mg/dl or a cerebrovascular cause of death[3]. The period of donor management after brain death is a dynamic and unphysiological course of events that influences a number of pathophysiological processes in the human body. These processes have been demonstrated both in animal models and during clinical investigations in patients.

Brain death often results from cerebral hemorrhage, trauma, or an anoxic event. When the brain becomes ischemic it begins to swell, which leads to increased intracranial pressure, arterial vasodilatation and further increase of edema and intracranial pressure. When this intracranial pressure exceeds the mean arterial pressure, brain perfusion stops, the pituitary gland is damaged, and its hormone secretion rapidly ceases[4,5].

Ischemia of the hypothalamus activates the sympathetic nervous system and releases a massive amount of catecholamines, which typically causes hypertension. After the 'catecholamine storm', hemodynamics reach a hypotensive phase resulting in hypoperfusion of the body in general and of organs in particular[6,7]. This significant ischemic state is marked by elevated serum lactate levels and should be corrected by fluid resuscitation and, if needed, inotropic support. The initial increase in systemic blood pressure is usually followed by hypoperfusion[8,9]. The autonomic or sympathetic catecholamine storm triggers a cascade of derangements that leads to extensive endothelial cell injury, release of cytokines in the circulation and ischemia in all organs of the body. The endothelial injury in the brain will lead to leakiness of the blood–brain barrier. Dysfunction of the blood–brain barrier then allows cytokines produced in the brain to leak into the serum[10].

Hormonal changes during brain death result in a transient and massive increase in circulating catecholamines as well as a decrease in arginine vasopressin (AVP) due to hypothalamic–pituitary dysfunction leading to diabetes insipidus. In addition, Novitzky *et al*.[9] reported a marked decrease in levels of thyroid hormones and cortisol in human brain death donors. They suggested that hormonal changes are the major cause of mitochondrial dysfunction with impaired energy production at the cellular level. This inhibition of mitochondrial function results in diminished organ function from the loss of energy stores from a rapid loss of circulating Fe^{3+} [11].

Pathophysiological effects of brain death on the kidneys

As a result of cerebral injury and brain death the elevated levels of circulating cytokines induce a local inflammatory response in the kidney[10,12]. Microarray studies have demonstrated upregulated expression of genes related to inflammatory response and reparative mechanisms in kidneys after brain death[8,13]. In addition, NF-kB-activation in kidneys and subsequent expression of genes involved in inflammation as a consequence of brain death has been reported as well. Serum levels of interleukin-6, interleukin-8, interleukin-10, and monocyte chemoattractant protein-1 (MCP-1) are significantly increased after brain death. Mitogenactivated protein kinases (MAP kinases) probably play a major role in the transition from the systemic inflammatory signal to the local inflammatory reaction in the kidney[10].

Since the discovery in 1996, the angiopoietin-Tie ligandreceptor system has been studied extensively in critical illness like sepsis[14-17]. The Ang-Tie ligand-receptor system is crucial in regulating vascular integrity and quiescence. Angiopoietin-1 (Ang1) has anti-inflammatory effects while angiopoietin-2 (Ang2) triggers an inflammatory response by activating the endothelium and inducing permeability. Vascular quiescence is maintained by signaling with an Ang1/Ang2 ratio in favor of endogenous Ang1 when Ang2 post transplantation downregulates[18].

In a small cohort of donation after brain death and living kidney donors, we recently found higher Ang1 and Ang2 levels in brain death donors compared to living donors.

During the brain death period the Ang1/Ang2 ratio was decreased. We also noted that elevated Ang2 levels in the donor were associated with an increased risk of post transplantation rejection and that the decrease in Ang1/Ang2 ratio was associated with an increased risk of delayed graft function (DGF) (unpublished).

The effects of adhesion molecules, leukocyte infiltration, gene expression and stress-related heat shock proteins have been extensively studied in human brain dead kidney donors[19-21]. The results showed an increased presence of the early adhesion molecule E-selectin after brain death, but not of the subsequent adhesion molecules ICAM-1 and VCAM-1. A significant difference between brain death and living donor kidneys in the presence of leukocytes in the interstitium was found as well.

Gene expression of two protective heat shock proteins, heme oxygenase-1 (HO-1) and Hsp70, and the growth factor TGF-β, were measured to assess the kidney's response to stress. Both HO-1 and Hsp70 showed an upregulation during brain death, but TGF-β was not significant activated. In the group of living donor kidney recipients, elevated HO-1 expression in the transplanted kidney had a strongly positive effect on low 1-year and 3-year serum creatinine levels. This finding suggests that brain death causes severe renal damage to such an extent that the protective effect of HO-1 is probably insufficient. Further upregulation of protective heat shock proteins may improve outcome of kidneys retrieved from brain dead donors after transplantation[21].

Because of renal vasoconstriction due to excessive secretion of catecholamines and volume depletion, the kidney is exposed to hypoperfusion and ischemic damage during brain death. Renal inflammatory and degenerative lesions appear on histological examination, including glomerulitis, periglomerulitis, vacuolization, atrophy, and necrosis of proximal and distal tubules, as well as proliferation of arterial intima and glomerular endothelium. Upregulation of circulating cytokines and chemokines, increased endothelial cell expression of adhesion molecules and major histocompatibility classes I and II, as well as greater infiltration of T cells, macrophages, and polymorphonuclear leukocytes into renal parenchyma, result in increased renal innate immunogenicity and subsequent host alloresponsiveness[22].

Our group has demonstrated an immediate procoagulatory and pro-inflammatory activation of vascular endothelium after brain death in kidney donor rats, which is proportional with the duration of brain death. E-selectin and P-selectin, Aa/Bb-fibrinogen mRNA were abruptly progressively upregulated already half an hour after brain death and their levels of expression increased progressively over time. Plasma von Willebrand factor (vWF) was significantly higher after 2 and 4 h brain death. Urine heart-fatty-acid-binding-protein (H-FABP) and N-acetyl-

glucosaminidase (NAG) were used as specific markers of proximal and distal tubular damage and found to be significantly increased after half an hour; with a maximum at 4 h. Oxidative stress was detectable, but only very late, after the establishment of tubular injury[23].

To clarify subclinical pathological changes in the grafted kidney, Kotsch *et al.*[24] investigated messenger RNA (mRNA) gene expression profiles in renal zero-hour biopsies from deceased and living donors. A significant induction was observed of the chemokine receptor seven ligands 19/21 [C–C motif] in the deceased donor group. In addition, in parallel with the induction of the activation marker CD69, significant elevated mRNA levels of the subunits PSMB8, PSMB9, and PSMB10 were detected. Although multiple studies have broadened the insight in brain death induced renal injury during the past years, it still remains unknown yet which exact signal transduction pathways are involved inducing the inflammatory response in the kidney. Further analysis of the expression of multiple genes encoding transcription factors and proteins involved in signal transduction, protection and repair is needed for proper identification.

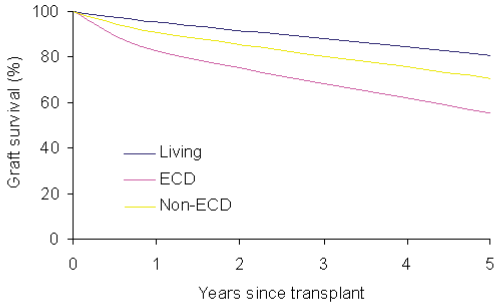
Living donor grafts are associated with lower rates of delayed graft function and better graft survival than kidneys retrieved from brain death donors (figure 1, table 1)[25]. The difference may in part be attributed to the intense release of inflammatory mediators that follows brain death. As a matter of fact, brain death in the donor has been reported as a probable risk factor for developing vascular rejection[26].

Perspectives on donor management and pretreatment

Strategies to reduce the pro-inflammatory status of the graft in the donor are becoming more attractive and may help to improve organ quality and graft function before and after transplantation[27]. The use of pharmacological interventions to counteract the decline of renal function appears to be an elegant and promising approach during brain death.

Due to hypotension and hypoperfusion in the donor during the period of brain death in the ICU, vasopressors such as norepinephrine, dopamine, and vasopressin are normally required[28]. Recently, a randomized controlled trial by Schnuelle *et al.*[29] demonstrated that low-dose dopamine (4mg/kg/min) is beneficial as pretreatment of brain dead kidney donors. Administration of dopamine reduced the need for dialysis from 35.4% to 24.7% within the first week after kidney transplantation.

Furthermore, we have also investigated the impact of brain death on renal function in a rat model of isolated perfused kidneys. Animal groups were pretreated before inducing brain death with erythropoietin (EPO) or an EPO derivate, the carbamylated EPO (cEPO) versus controls. Kidney function was significantly reduced in vehicle-treated rats with brain death but not in the experimental groups and in sham-operated rats. It was shown that cEPO can inhibit the inflammatory response and endothelial activation caused by brain death more effectively than EPO, whereas



Source: UNOS

Figure 1. Graft survival for living-, ECD, and non-ECD-derived renal grafts. Graft survival of kidney transplants performed in the United States over a period of 5 years between 2005 and 2010. Data were obtained from the United Network for Organ Sharing (UNOS).

Table 1. One- and five-year graft survival for living and deceased (ECD/non-ECD) donors following renal transplantation

		One-year survival (%)	Five-year survival (%)
Kidney donor type	Number of transplants	2000-2009	2000-2009
Living	59 627	95.3	80.89
ECD	14 632	82.94	55.39
Non-ECD	75 989	90.99	70.70

Source: UNOS. ECD: extended criteria donation.

both substances do restore kidney function after brain death. Therefore, EPO analogs are interesting candidates for renal protection and clinical intervention[30]. Intervening in the Ang1/Ang2 balance may also be of therapeutical value to improve outcome after brain death renal transplantation. Increasing the Ang1 concentration to restore the balance was found to have a significant anti-inflammatory potential in animal models[31]. Moreover, Ang2 neutralizing reagents have been developed as antiangiogenic tumor drugs and could also be used to decrease the pro-inflammatory status of the donor[32].

Another way to improve organ quality from DBD donors is to alter the activation state of MAP kinases by inhibiting transcription genes involved in transduction pathways[10].

A different and more recent approach to reduce renal injury and enhance repair was advocated by Elkins[33] using inhalational anesthesia in brain dead donors and benefitting the recipient. Peri-operative management and administration of inhalational anesthetics could be beneficial as they will the catecholamine release

that occurs during surgical stimulation at the time of the organ retrieval procedure. Further research is needed to determine how anesthetic preconditioning could be advantageous to preserve the viability of donor kidneys.

To determine the optimal timing of intervention during donor management and induce repair mechanisms, we have studied whether the length of the period of brain death is a risk factor for outcome after kidney transplantation. In a large database analysis, results revealed that longer duration of brain death in the donor after cerebral injury is not detrimental. Longer brain death duration even appears to have a modest beneficial effect on the odds for immediate graft function and 1-year and 3-year grafts survival after kidney transplantation. The duration of brain death had no influence on acute rejection in the first year after transplantation. Thus, instead of 'to rush and retrieve', the attitude 'to relax and repair' with an unhurried and high-quality ICU donor management during brain death prior to organ retrieval is recommended[34].

A different approach to reduce inflammation in the brain dead donor is to induce repair mechanisms using HO-1 induction [31]. HO-1, the enzyme that converts heme to Fe^{2+}, carbon monoxide and biliverdin, has been extensively studied. HO-1 is overexpressed in organs during brain death and the elimination of the excess heme suppresses generation of oxidative radicals and thereby limits the damage associated with those radicals. Therefore, Kotsch *et al.*[35] examined the impact of donor treatment with cobalt protoporphyrin (CoPP) as the selective inducer of HO-1 on organ quality and transplant outcome in a standardized brain death transplant rat model. Recipients of organs from brain dead donors treated with CoPP survived significantly better than those from untreated brain dead donors and intra-graft analysis showed improved histology. Blockade of HO-1 with zinc protoporphyrin (ZnPP) decreased the survival rates comparable with untreated brain dead donors. These results show that HO-1 induction by one single treatment of CoPP in brain dead donors leads to enhanced allograft survival.

Overall, HO-1 and its products of degradation are strong antioxidants that will inhibit cell death, limit apoptosis, and halt aberrant proliferation[36].

Indicators for brain death induced (renal) injury

The fact that nowadays many older and more marginal deceased donor organs are accepted for transplantation determines graft outcome. To assess the chance of immediate graft function in the context of choice of immunosuppressants more specific parameters of the (expected) kidney graft quality at time of organ procurement in the brain death donor are needed. If transplantation success could be better predicted the appropriate matching of organ and recipient characteristics could be significantly improved.

The type 1 transmembrane protein kidney injury molecule-1 (KIM-1 in humans or Kim-1 in rodents) is known to be very specific and upregulated in renal tubular cells

in a variety of injury models[37]. We have analyzed the effect of brain death on the expression of KIM-1 and evaluated its use as a new biomarker in organ donation. In this study KIM-1 distinctly rose as well as gene expressions within 4 h of brain death, while serum creatinine levels remained within the normal range. In addition, patient data showed that KIM-1 is also upregulated in human brain death donors compared to living kidney donors[38]. Therefore, we concluded that KIM-1 measured at the time of brain death diagnosis is an independent predictive factor for short time kidney function after transplantation.

Other candidate markers such as the molecules CCL19/21 and PSMB8/9/10, were found by Kotsch *et al.*[24] and tested for posttransplantation clinical outcomes showing the potential to predict the development of DGF, acute rejection, and renal function after 6 months.

In addition, our group was able to demonstrate that elevated Ang2 levels in the brain death donor were associated with an increased risk of rejection and the decrease in the Ang1/Ang2 ratio with an increased risk of delayed graft function (unpublished). Further study is now required to confirm the clinical validity of serum Ang2 and to assess if angiopoietins are promising markers indeed to predict the quality of the renal graft.

CONCLUSION

To date, there is substantial evidence that brain death is associated with a cascade of hemodynamic, inflammatory, and immunologic events that affect the outcome of transplanted kidneys. Time has come to now focus not only on the victim of injury, for example the kidney graft in its recipient, but also on the source of the syndrome: the brain dead donor himself. Exact mechanisms and pathways responsible for the brain death induced renal injury are still largely unknown. So, although there is no doubt that further research is required to elucidate the mechanisms, we are now already in the position to further therapeutic interventions and introduce efficient biomarkers for brain death induced renal injury in a clinical setting. As the deceased donor shortage will prevail and more suboptimal donor organs accepted, we ought to focus on innovative methods to repair those organs that were consented by donors to benefit transplant recipients and we have in our custody for only a short while.

REFERENCES

1. Mollaret P, Goulon M. The depassed coma (preliminary memoir). Rev Neurol (Paris). 1959;101:3-15.
2. A definition of irreversible coma. report of the ad hoc committee of the harvard medical school to examine the definition of brain death. JAMA. 1968;205(6):337-340.
3. Bos EM, Leuvenink HG, van Goor H, Ploeg RJ. Kidney grafts from brain dead donors: Inferior quality or opportunity for improvement? Kidney Int. 2007;72(7):797-805.
4. Chen EP, Bittner HB, Kendall SW, Van Trigt P. Hormonal and hemodynamic changes in a validated animal model of brain death. Crit Care Med. 1996;24(8):1352-1359.
5. Powner DJ, Boccalandro C, Alp MS, Vollmer DG. Endocrine failure after traumatic brain injury in adults. Neurocrit Care. 2006;5(1):61-70.
6. Perez Lopez S, Otero Hernandez J, Vazquez Moreno N, Escudero Augusto D, Alvarez Menendez F, Astudillo Gonzalez A. Brain death effects on catecholamine levels and subsequent cardiac damage assessed in organ donors. J Heart Lung Transplant. 2009;28(8):815-820.
7. Martikainen TJ, Kurola J, Karja V, Parviainen I, Ruokonen E. Vasopressor agents after experimental brain death: Effects of dopamine and vasopressin on vitality of the small gut. Transplant Proc. 2010;42(7):2449-2456.
8. Schuurs TA, Gerbens F, van der Hoeven JA, et al. Distinct transcriptional changes in donor kidneys upon brain death induction in rats: Insights in the processes of brain death. Am J Transplant. 2004;4(12):1972-1981.
9. Novitzky D, Cooper DK, Morrell D, Isaacs S. Change from aerobic to anaerobic metabolism after brain death, and reversal following triiodothyronine therapy. Transplantation. 1988;45(1):32-36.
10. Bouma HR, Ploeg RJ, Schuurs TA. Signal transduction pathways involved in brain death-induced renal injury. Am J Transplant. 2009;9(5):989-997.
11. Cooper DK, Novitzky D, Wicomb WN, Basker M, Rosendale JD, Myron Kauffman H. A review of studies relating to thyroid hormone therapy in brain-dead organ donors. Front Biosci. 2009;14:3750-3770.
12. Takada M, Nadeau KC, Hancock WW, et al. Effects of explosive brain death on cytokine activation of peripheral organs in the rat. Transplantation. 1998;65(12):1533-1542.
13. Kusaka M, Kuroyanagi Y, Kowa H, et al. Genomewide expression profiles of rat model renal isografts from brain dead donors. Transplantation. 2007;83(1):62-70.
14. Kumpers P, Lukasz A, David S, et al. Excess circulating angiopoietin-2 is a strong predictor of mortality in critically ill medical patients. Crit Care. 2008;12(6):R147.
15. Mofarrahi M, Nouh T, Qureshi S, Guillot L, Mayaki D, Hussain SN. Regulation of angiopoietin expression by bacterial lipopolysaccharide. Am J Physiol Lung Cell Mol Physiol. 2008;294(5):L955-63.
16. van der Heijden M, van Nieuw Amerongen GP, Chedamni S, van Hinsbergh VW, Johan Groeneveld AB. The angiopoietin-Tie2 system as a therapeutic target in sepsis and acute lung injury. Expert Opin Ther Targets. 2009;13(1):39-53.
17. Kumpers P, van Meurs M, David S, et al. Time course of angiopoietin-2 release during experimental human endotoxemia and sepsis. Crit Care. 2009;13(3):R64.
18. Fiedler U, Augustin HG. Angiopoietins: A link between angiogenesis and inflammation. Trends Immunol. 2006;27(12):552-558.
19. van der Hoeven JA, Molema G, Ter Horst GJ, et al. Relationship between duration of brain death and hemodynamic (in)stability on progressive dysfunction and increased

immunologic activation of donor kidneys. Kidney Int. 2003;64(5):1874-1882.

20. Nijboer WN, Schuurs TA, van der Hoeven JA, et al. Effect of brain death on gene expression and tissue activation in human donor kidneys. Transplantation. 2004;78(7):978-986.
21. Nijboer WN, Schuurs TA, van der Hoeven JA, et al. Effects of brain death on stress and inflammatory response in the human donor kidney. Transplant Proc. 2005;37(1):367-369.
22. Ledinh H, Bonvoisin C, Weekers L, et al. Results of kidney transplantation from donors after cardiac death. Transplant Proc. 2010;42(7):2407-2414.
23. Morariu AM, Schuurs TA, Leuvenink HG, van Oeveren W, Rakhorst G, Ploeg RJ. Early events in kidney donation: Progression of endothelial activation, oxidative stress and tubular injury after brain death. Am J Transplant. 2008;8(5):933-941.
24. Kotsch K, Kunert K, Merk V, et al. Novel markers in zero-hour kidney biopsies indicate graft quality and clinical outcome. Transplantation. 2010.
25. Terasaki PI, Cecka JM, Gjertson DW, Takemoto S. High survival rates of kidney transplants from spousal and living unrelated donors. N Engl J Med. 1995;333(6):333-336.
26. Sanchez-Fructuoso AI, Prats D, Marques M, et al. Does donor brain death influence acute vascular rejection in the kidney transplant? Transplantation. 2004;78(1):142-146.
27. Robert R, Guilhot J, Pinsard M, et al. A pair analysis of the delayed graft function in kidney recipient: The critical role of the donor. J Crit Care. 2010.
28. Mascia L, Mastromauro I, Viberti S, Vincenzi M, Zanello M. Management to optimize organ procurement in brain dead donors. Minerva Anestesiol. 2009;75(3):125-133.
29. Schnuelle P, Gottmann U, Hoeger S, et al. Effects of donor pretreatment with dopamine on graft function after kidney transplantation: A randomized controlled trial. JAMA. 2009;302(10):1067-1075.
30. Nijboer WN, Ottens PJ, van Dijk A, van Goor H, Ploeg RJ, Leuvenink HG. Donor pretreatment with carbamylated erythropoietin in a brain death model reduces inflammation more effectively than erythropoietin while preserving renal function. Crit Care Med. 2010;38(4):1155-1161.
31. Nykanen AI, Krebs R, Saaristo A, et al. Angiopoietin-1 protects against the development of cardiac allograft arteriosclerosis. Circulation. 2003;107(9):1308-1314.
32. Oliner J, Min H, Leal J, et al. Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2. Cancer Cell. 2004;6(5):507-516.
33. Elkins LJ. Inhalational anesthesia for organ procurement: Potential indications for administering inhalational anesthesia in the brain-dead organ donor. AANA J. 2010;78(4):293-299.
34. Nijboer WN, Moers C, Leuvenink HG, Ploeg RJ. How important is the duration of the brain death period for the outcome in kidney transplantation? Transpl Int. 2010.
35. Kotsch K, Francuski M, Pascher A, et al. Improved long-term graft survival after HO-1 induction in brain-dead donors. Am J Transplant. 2006;6(3):477-486.
36. Ollinger R, Pratschke J. Role of heme oxygenase-1 in transplantation. Transpl Int. 2010.
37. Ichimura T, Bonventre JV, Bailly V, et al. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem. 1998;273(7):4135-4142.
38. Nijboer WN, Schuurs TA, Damman J, et al. Kidney injury molecule-1 is an early noninvasive indicator for donor brain death-induced injury prior to kidney transplantation. Am J Transplant. 2009;9(8):1752-1759.