Angiopoietins in renal replacement therapy

Westendorp, Welmoet Hillegonda

CHAPTER 3

Circulatory and renal angiopoietin-2 release in living donor renal transplantation

WH Westendorp
GJ Nieuwenhuijs-Moeke
NJ de Vries
PJ Ottens
H van Goor
RJ Ploeg
MMRF Struys
HGD Leuvenink

ABSTRACT

Background

The Angiopoietin/Tie2-system has been suggested to play an important role in the endothelial activation in renal transplantation. Little is known about baseline angiopoietin-2 (Ang2) levels throughout renal transplantation. Therefore, we aimed to define baseline Ang2 changes in living donor renal transplantation from donation to reperfusion in the recipient.

Methods

In a single center transplantation population, circulatory and arteriovenous Ang2 was measured in 53 matched living kidney donors and recipients.

Results

In the donor, plasma Ang2 increased significantly between preoperative levels and kidney retrieval (p=0.01). During reperfusion in the recipient, Ang2 levels were increased compared to Ang2 levels at time of donation but no arteriovenous differences were found. Plasma Ang2 decreased significantly at 2h postoperative compared to preoperative Ang2 in the recipient (p=0.003).

Conclusion

Circulating Ang2 is affected by renal transplantation, although Ang2 release from the kidney itself is not affected by reperfusion in this single center living kidney transplant population.

INTRODUCTION

The role of the endothelium in renal transplantation is considered increasingly important[1]. The key to long term allograft survival may lie in maintaining the endothelial cells, the inner lining of all blood vessels, in a quiescent state. Various effects during renal transplantation like endotoxemia and inflammation in the deceased brain dead (DBD) donor as well as ischemia/reperfusion injury (IRI) in the living and deceased donor, will activate the endothelium[2-5]. In both donor types, the activated endothelium forms an important mediator in the pathological processes negatively affecting patient and graft survival[6-8].

Endothelial homeostasis is regulated by the Ang/Tie2-system[9]. The endothelial cells are supported by pericytes which produce Angiopoietin-1 (Ang1)[10,11]. Ang1 and Angiopoietin-2 (Ang2) are the most important ligands of the tyroskine kinase receptor Tie2, which is mostly expressed by endothelial cells[12]. It is suggested Ang1 and Ang2 have opposite effects, with Ang1 inducing endothelial survival signals, inhibiting apoptosis and vascular inflammation and suppressing vascular/endothelial leakage[13,14]. By contrast, Ang2 acts as an Ang1 antagonist destabilizing the endothelium[15-17]. After endothelial activation by increased cytokine release, endothelial storage granules, Weibel Palade bodies (WPB), quickly release Ang2[18,19].

Studies on the role of angiopoietins in renal transplantation are limited. A study in a limited number of patients showed an increased Ang2 release after reperfusion in kidneys derived from living as well as deceased donors[20,21]. In rats an increase in Ang2 protein expression in the transplanted kidney after reperfusion in rats was shown[20,21]. Experimental studies on renal endothelial damage report that Ang1 overexpression improved renal function and blood flow after renal IRI in mice, as well as decreased influx of inflammatory cells and renal interstitial fibrosis[22]. In a mouse model of antiglomerular basement membrane glomerulonephritis, glomerular capillary loss was associated with reduced Ang1 and increased Ang2 expression, suggesting a relation between angiopoietin disbalance and endothelial cell loss[23]. In humans, besides the scope of renal transplantation, endothelial activation and dysfunction is a known cause of Ang2 release in chronic kidney disease and hemodialysis[24,25].

Although circulating angiopoietin levels have been studied in renal transplant recipients and small numbers of living and deceased kidney donors[21,26-28], little is known of the Ang2 response during a renal transplantation procedure. Studying plasma Ang2 in living donor kidney transplantation gives the opportunity to observe Ang2 levels in donors and recipients in the absence of profound systemic changes as demonstrated in deceased donors.

The aim of the present study was to define baseline Ang2 changes in living donor renal transplantation from donation to reperfusion in the recipient in a single center transplantation population. Results from the current study will provide crucial knowledge on physiological changes in Ang2 levels helping to interpret changes

found in deceased donation and transplantation. These baseline levels can be used as a best standard in human renal transplantation since living kidney donors are healthy individuals selected on the basis of absence of any disease. Furthermore, these baseline levels will provide a starting point for designing further intervention studies, modulating the Ang/Tie2-axis, as a potential strategy to improve donor organ quality and subsequently, transplantation outcome.

METHODS

Study population

For this study, we analyzed Ang2 levels in stored plasma samples of patients that participated the Volatile Anesthetic Protection of Renal transplants (VAPOR)-1 trial, a prospective randomized control trial on the effects of two different anesthetic regimens on renal outcome in living donor kidney transplantation (LDKT). Inclusion criteria were written informed consent, ≥18 years, and donation of the left kidney. Exclusion criteria were: right kidney donation, generalized central neurological disorder, donor-recipient couples from the ABO-incompatible program and altruistic donors. The Institutional Review Board approved the study protocol (METc 2009/334), which was in adherence to the Declaration of Helsinki. Between September 2010 and September 2012 125 LDKT were performed of which 88 involved the left kidney. Of that 88 couples 60 couples (68.2%) met inclusion criteria and gave written informed consent. Patients were randomly assigned to three groups according to the anesthetic regiment they received during the procedure. PROP if the donor and recipient received propofol based anesthesia, SEVO, if they received a sevoflurane based anesthesia and SERE if the donor received propofol and the recipient a sevoflurane based anesthesia. In the PROP group three patients were lost to follow up, two by violation of the surgical protocol and one patient died nine days post transplantation due to bleeding complications. In the SEVO group one patient was lost to follow up by violation of the immunosuppressive protocol. For this study we focused on Ang2 levels without division in the three groups. Therefore plasma of 53 donor-recipient couples was available for Ang2 and IL-6 measurement.

Operation and sample withdrawal

Kidney donation procedure was performed via the hand assisted laparoscopic (HAL) technique. Left gonadal vein which ends in the left renal vein was also dissected and retrieved. After explantation the kidney was immediately flushed and perfused with cold University of Wisconsin solution (Costorsol, Bridge to Life, USA) and stored on ice. In the recipient kidney transplantation was performed according to local standardized protocol. Prior to implantation a small sampling catheter was inserted in the gonadal vein as described by de Vries et al[29]. Immune

suppressive protocol was according to standard institutional guidelines. Patients received myophenolate mofetil (MMF) and cyclosporine/tacrolimus preoperatively at the ward and basiliximab and (methyl)prednisolon after induction of anesthesia. Post operatively they received an additional dose of basiliximab on day four and a protocol of MMF, methylprednisolon and ciclosporin/tacrolimus.
In donor and recipient pre-, per- and postoperative EDTA and citrate blood samples were withdrawn at standardized time points from an arterial line in the radial artery of the non-dominant/non-shunt arm. In the recipients additional samples were taken after reperfusion of the kidney via the catheter in the gonadal vein. These samples were taken simultaneously with systemic arterial samples at 30 sec, 5, 10 and 30 min after reperfusion. Two open kidney biopsies were performed, a cold biopsy when the kidney prior to implantation and a reperfusion biopsy approximately 45 minutes after reperfusion. Biopsies were performed using a Pro-Mag 2.2 Biopsy Gun with a 16-gauge needle (Manan Medical Products, USA) and subsequently stored in formalin and paraffin fixed until analysis. All samples were immediately placed on ice. Blood samples were centrifuged (1500g, 20 min, 4°C) and stored at -80°C until measurement. Prior to assays, samples were thawed and recentrifuged. Samples were analyzed in one batch to eliminate inter-assay variability.

Plasma measurements

Plasma levels of Ang2 and IL-6 were determined by enzyme linked immonusorbent assays (ELISA) according to manufacturers' instructions (R&D Systems, Minneapolis, USA). All samples were analyzed in duplicate and read at 450 nm using a microplate spectrophotometer (Victor3, 1420 multi-label counter, Perkin Elmer, USA). Serum creatinine was determined using an enzymatic essay on a Roche Modular chemistry analyzer (Roche Diagnostics, USA).

Clinical parameters

Data on donor and recipients' health status, medical history, renal function and medication were noted in a case record file as well as data about the procedure and postoperative period. Body weight and height were measured with participants wearing indoor clothing without shoes. BMI was calculated as weight divided by height squared (kg/m^2). Information on graft function (delayed graft function (DGF) and rejection) was retrieved from local digital patient records. DGF was defined as need for dialysis in the first week after transplantation other than immediately postoperative. Rejection was biopsy proven with decline in kidney function and the need of treatment.

Statistical analyses

Differences between arterial and venous samples were tested using the paired, nonparametric Wilcoxon test. Unpaired differences of Ang2 between two time

points were tested using the Mann-Whitney test. Graph error bars represent the SEM, unless otherwise stated. Normal distribution was tested using normal probability plots. Correlations were tested using Spearman's rho or lineair regression. Associations with graft function were assessed using Cox regression analysis. P<0.05 was considered significant. Statistical analyses were performed with SPSS version 20 (SPSS Inc. Chicago, USA).

RESULTS

Demographics of the adult patients undergoing donor nephrectomy and their matched recipients for living donor kidney transplantation are shown in table 1. Donor mean age was 53 ± 11 years and recipient mean age was 50 ± 13 years. As anticipated, the rate of DGF and rejection was low in this study population (<2%). Three patients developed DGF, nine patients showed an acute rejection episode during the two year follow up. As shown in table 2, no correlations between preoperative donor plasma Ang2 and clinical parameters were found.

Table 1. Demographics of 53 living kidney donor-recipient couples

Donor	
Living unrelated donor, n (%)	26 (49.1)
Age (years)	53 ± 11
Male sex, n (%)	24 (45.3)
BMI (kg/m^2)	26.9 ± 3.2
Duration of hospital stay (days)	6 [5-7]
IL-6 (pg/ml) after start anesthesia	2.3 [1.7-4]
Transplant demographics	
HLA mismatches (% of 0 mismatches)	7 (13.2)
Cold ischemia time (min)	177.4 ± 29.7
Recipient	
Age (years)	50 ± 13
Male sex, n (%)	24 (45.3)
BMI (kg/m^2)	25.1 ± 3.5
Duration of hospital stay (days)	17 [17-18]
Post-transplant parameters	
Serum creatinine 3 months after Tx (µmol/L)	131.1 ± 39.3
Delayed graft function (%)	3 (5.7)
Biopsy proven rejection in first 2 years after Tx (%)	9 (17)

Data are presented as mean ± SD in case of normal distribution and as median [interquartile range] in case of skewed distribution. BMI: body mass index, Tx: transplantation. Delayed graft function: the need for dialysis within <1 week after transplantation.

Table 2. Univariate analysis between systemic preoperative donor Ang2 and clinical parameters

Clinical parameter	ρ	P
Age (years)	0.07	0.60
BMI (kg/m^2)	0.23	0.10
Gender	-0.04	0.78
Cold ischemia time (min)	0.22	0.11
Duration of hospital stay (days)	0.02	0.90

ρ: Spearman's correlation coefficient, BMI: body mass index.

Table 3. Univariate analysis between systemic plasma Ang2 one day after transplantation and clinical parameters in the recipient

Clinical parameter	ρ	P
Age (years)	0.28	0.04
BMI (kg/m^2)	0.06	0.67
Gender	-0.05	0.72
Duration of hospital stay (days)	0.36	0.01
IL-6 1st day after Tx (pg/ml)	0.34	0.02

ρ: Spearman's correlation coefficient, BMI: body mass index.

In the recipients, plasma Ang2 measured at the first day after transplantation was associated with the length of hospital stay and IL-6 level (ρ 0.36, p=0.01 and ρ 0.34, p=0.02 respectively, table 3). In univariate lineair regression analysis, the first and second day after transplantation, plasma Ang2 correlated with plasma IL-6 (St. beta 0.33, p=0.02 and St. beta 0.43, p=0.002, respectively). No associations between plasma Ang2 and graft function were found.

Ang2 levels in the living kidney donor

Donor plasma Ang2 levels are shown in figure 1. In the living kidney donor, preoperative plasma Ang2 levels decreased significantly from 956 [510-768] pg/ml to 752 [461-661] pg/ml at organ retrieval (p=0.01). At 2 hours postoperatively, Ang2 levels increased significantly to 916 [535-844] pg/ml (p=0.002).

Ang2 levels during reperfusion

Arteriovenous Ang2 measurements over the reperfused kidney are shown in figure 2. Compared to the pretransplant Ang2 levels in the living kidney donor, an elevated trend in Ang2 levels was observed. No significant differences between arterial and venous plasma Ang2 were found.

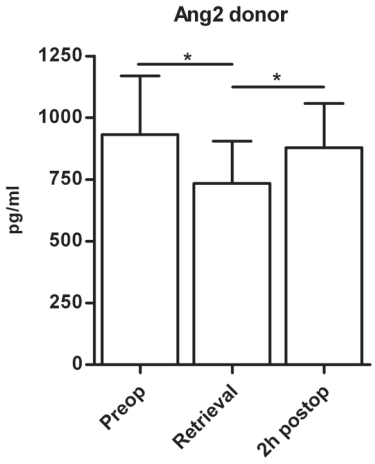

Figure 1. Preoperative, intra- and post transplantation systemic Ang2 plasma levels in living kidney donors. Error bars indicate mean±SEM of 53 living kidney donors. Preoperative plasma Ang2 levels decreased from 956 [510-768] pg/ml to 752 [461-661] pg/ml at organ retrieval (p=0.01). At 2 hours postoperatively, Ang2 levels increased to 916 [535-844] pg/ml (p=0.002).

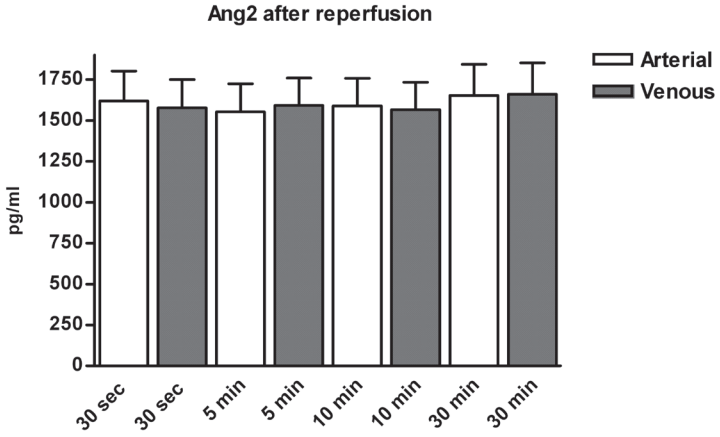

Figure 2. Arteriovenous Ang2 levels over the reperfused kidney. Error bars indicate mean±SEM of 53 arteriovenous Ang2 kidney measurements. No significant differences between arterial and venous plasma Ang2 were found.

Recipient Ang2 levels

Plasma Ang2 levels in the recipient are shown in figure 3. Ang2 levels decreased significantly from 2243 [723-3289] pg/ml preoperative to 1044 [556-1309] pg/ml 2 hours postoperative (p=0.003). No differences between plasma Ang2 one day after transplantation and Ang2 levels 2 hours after transplantation were determined.

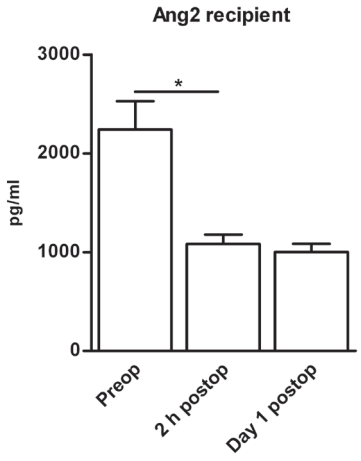

Figure 3. Systemic Ang2 plasma levels of 53 recipients after living kidney donor transplantation. Error bars indicate mean±SEM plasma Ang2 of 53 kidney recipients. Ang2 levels decreased from 2243 [723-3289] pg/ml preoperative to 1044 [556-1309] pg/ml 2 hours postoperative (p=0.003). No differences between plasma Ang2 one day after transplantation and Ang2 levels 2 hours after transplantation were found.

DISCUSSION

The current study is the first demonstrating systemic and local renal venous Ang2 levels throughout living donor renal transplantation. In both donor and recipient systemic Ang2 changes were observed while renal Ang2 release did not differ at different time points during the first 30 minutes of reperfusion.

In renal transplant recipients, Ang2 levels have been associated with renal function and all-cause mortality[26]. Although we did not find an association between systemic and/or renal Ang2 levels and transplantation outcome, these results are inconclusive due to the small study population and low incidence of events in our cohort.

The renal reperfusion Ang2 levels we found are not lower and show less variability than the study of de Vries and colleagues. Using the same technique they showed in six living and six deceased kidney donors, an increased Ang2 release shortly after reperfusion[21]. This is remarkable because of the relatively small study population compared to our study population. Furthermore, despite utilization of the same Ang2 ELISA the renal venous Ang2 levels we measured were in general considerably lower than the reperfusion levels determined by de Vries et al. Possibly, the lower Ang2 levels we measured during the donor and recipient procedure were caused by dilution. During both procedures patients received between 3000 and 5000 ml of crystalloids. Another explanation may be that less endothelial activation was already present and developed during nephrectomy in our healthy living donors, resulting in less WPB exocytosis of Ang2 during reperfusion. It has been well-known

that living donor kidney grafts are retrieved from healthy individuals selected on the absence of any disease, suffering from limited IRI and showing good function and high survival rate[30-33]. Therefore, the finding that in patients with normal kidney function after successful renal transplantation activation of the endothelial layer is indistinguishable from controls, underlines our speculation that endothelial activation in the living donors we studied may have been limited.

Without further studying endothelial activation of our donor-recipient couples, possibly via immunohistochemistry or real-time PCR of the retrieved renal biopsies, no definite conclusion in comparing the two living donor study populations can be made. This would be interesting since this previous study on local Ang2 release reports an increased local renal Ang2 plasma release in both living and deceased kidney donors and interestingly, no difference in Ang2 mRNA expression between living and deceased donor kidneys was observed[21]. This is remarkable as much evidence demonstrate a profound activation of the endothelium and inflammation in deceased donors, especially deceased brain dead donors, compared to living donors[3,34-36].

Since venous measurements of the reperfused graft during transplantation are an elegant method to study Ang2 release from the kidney itself and angiopoietins may reflect the immunogenic state and quality of the donor organ, future studies using this method are needed to draw more definite conclusions on the role of the Ang/Tie2-system in renal transplantation. That is, the Ang/Tie2-system has been shown to play a critical role in maintaining vascular stability while activated endothelium triggers an inflammatory response, affecting donor organ quality and function[37-41]. Ideally, circulatory and renal plasma Ang2 release together with the expression of Ang1 and Tie2 and the intensity of endothelial activation will be determined by immunohistochemistry and protein quantification in baseline living and deceased renal transplantation. This will possibly pave the way for performance enhancing intervention studies, targeting endothelial activation via the Ang/Tie2-system, possibly improving donor organ quality and subsequently, transplantation outcome.

REFERENCES

1. Contreras AG, Briscoe DM. Every allograft needs a silver lining. J Clin Invest. 2007;117(12):3645-3648.
2. van Goor H, Rosman C, Grond J, Kooi K, Wubbels GH, Bleichrodt RP. Translocation of bacteria and endotoxin in organ donors. Arch Surg. 1994;129(10):1063-1066.
3. de Vries DK, Lindeman JH, Ringers J, Reinders ME, Rabelink TJ, Schaapherder AF. Donor brain death predisposes human kidney grafts to a proinflammatory reaction after transplantation. Am J Transplant. 2011;11(5):1064-1070.
4. Reinders ME, Rabelink TJ, Briscoe DM. Angiogenesis and endothelial cell repair in renal disease and allograft rejection. J Am Soc Nephrol. 2006;17(4):932-942.
5. Ponticelli C. Ischaemia-reperfusion injury: A major protagonist in kidney transplantation. Nephrol Dial Transplant. 2013.
6. Boros P, Bromberg JS. New cellular and molecular immune pathways in ischemia/reperfusion injury. Am J Transplant. 2006;6(4):652-658.
7. Menke J, Sollinger D, Schamberger B, Heemann U, Lutz J. The effect of ischemia/reperfusion on the kidney graft. Curr Opin Organ Transplant. 2014;19(4):395-400.
8. 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.
9. Fiedler U, Augustin HG. Angiopoietins: A link between angiogenesis and inflammation. Trends Immunol. 2006;27(12):552-558.
10. Smith SW, Chand S, Savage CO. Biology of the renal pericyte. Nephrol Dial Transplant. 2012;27(6):2149-2155.
11. Thurston G, Rudge JS, Ioffe E, et al. Angiopoietin-1 protects the adult vasculature against plasma leakage. Nat Med. 2000;6(4):460-463.
12. Wong AL, Haroon ZA, Werner S, Dewhirst MW, Greenberg CS, Peters KG. Tie2 expression and phosphorylation in angiogenic and quiescent adult tissues. Circ Res. 1997;81(4):567-574.
13. Papapetropoulos A, Fulton D, Mahboubi K, et al. Angiopoietin-1 inhibits endothelial cell apoptosis via the Akt/survivin pathway. J Biol Chem. 2000;275(13):9102-9105.
14. Maisonpierre PC, Suri C, Jones PF, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science. 1997;277(5322):55-60.
15. Benest AV, Kruse K, Savant S, et al. Angiopoietin-2 is critical for cytokine-induced vascular leakage. PLoS One. 2013;8(8):e70459.
16. Fiedler U, Reiss Y, Scharpfenecker M, et al. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat Med. 2006;12(2):235-239.
17. Parikh SM, Mammoto T, Schultz A, et al. Excess circulating angiopoietin-2 may contribute to pulmonary vascular leak in sepsis in humans. PLoS Med. 2006;3(3):e46.
18. Weibel ER, Palade GE. New cytoplasmic components in arterial endothelia. J Cell Biol. 1964;23:101-112.
19. Ju R, Zhuang ZW, Zhang J, et al. Angiopoietin-2 secretion by endothelial cell exosomes: Regulation by the phosphatidylinositol 3-kinase (PI3K)/Akt/endothelial nitric oxide synthase (eNOS) and syndecan-4/syntenin pathways. J Biol Chem. 2014;289(1):510-519.
20. Khairoun M, van der Pol P, de Vries DK, et al. Renal ischemia-reperfusion induces a dysbalance of angiopoietins, accompanied by proliferation of

pericytes and fibrosis. Am J Physiol Renal Physiol. 2013;305(6):F901-10.

21. de Vries DK, Khairoun M, Lindeman JH, et al. Renal ischemia-reperfusion induces release of angiopoietin-2 from human grafts of living and deceased donors. Transplantation. 2013;96(3):282-289.
22. Jung YJ, Kim DH, Lee AS, et al. Peritubular capillary preservation with COMP-angiopoietin-1 decreases ischemia-reperfusion-induced acute kidney injury. Am J Physiol Renal Physiol. 2009;297(4):F952-60.
23. Yuan HT, Tipping PG, Li XZ, Long DA, Woolf AS. Angiopoietin correlates with glomerular capillary loss in anti-glomerular basement membrane glomerulonephritis. Kidney Int. 2002;61(6):2078-2089.
24. Padberg JS, Wiesinger A, di Marco GS, et al. Damage of the endothelial glycocalyx in chronic kidney disease. Atherosclerosis. 2014;234(2):335-343.
25. Vlahu CA, Lemkes BA, Struijk DG, Koopman MG, Krediet RT, Vink H. Damage of the endothelial glycocalyx in dialysis patients. J Am Soc Nephrol. 2012;23(11):1900-1908.
26. Molnar MZ, Kumpers P, Kielstein JT, et al. Circulating angiopoietin-2 levels predict mortality in kidney transplant recipients: A 4-year prospective case-cohort study. Transpl Int. 2014.
27. David S, Kumpers P, Hellpap J, et al. Angiopoietin 2 and cardiovascular disease in dialysis and kidney transplantation. Am J Kidney Dis. 2009;53(5):770-778.
28. Dane MJ, Khairoun M, Lee DH, et al. Association of kidney function with changes in the endothelial surface layer. Clin J Am Soc Nephrol. 2014;9(4):698-704.
29. de Vries DK, Kortekaas KA, Tsikas D, et al. Oxidative damage in clinical ischemia/reperfusion injury: A reappraisal. Antioxid Redox Signal. 2013;19(6):535-545.
30. Schnitzler MA, Lentine KL, Gheorghian A, Axelrod D, Trivedi D, L'Italien G. Renal function following living, standard criteria deceased and expanded criteria deceased donor kidney transplantation: Impact on graft failure and death. Transpl Int. 2012;25(2):179-191.
31. Roodnat JI, van Riemsdijk IC, Mulder PG, et al. The superior results of living-donor renal transplantation are not completely caused by selection or short cold ischemia time: A single-center, multivariate analysis. Transplantation. 2003;75(12):2014-2018.
32. 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.
33. 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.
34. 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.
35. van Werkhoven MB, Damman J, van Dijk MC, et al. Complement mediated renal inflammation induced by donor brain death: Role of renal C5a-C5aR interaction. Am J Transplant. 2013;13(4):875-882.
36. 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.
37. Chang FC, Chiang WC, Tsai MH, et al. Angiopoietin-2-induced arterial stiffness in CKD. J Am Soc Nephrol. 2014;25(6):1198-1209.
38. Whitcomb DC, Muddana V, Langmead CJ, et al. Angiopoietin-2, a regulator of vascular permeability in inflammation, is

associated with persistent organ failure in patients with acute pancreatitis from the united states and germany. Am J Gastroenterol. 2010;105(10):2287-2292.

39. Woolf AS, Gnudi L, Long DA. Roles of angiopoietins in kidney development and disease. J Am Soc Nephrol. 2009;20(2):239-244.
40. Pratschke J, Wilhelm MJ, Kusaka M, et al. Brain death and its influence on donor organ quality and outcome after transplantation. Transplantation. 1999;67(3):343-348.
41. Bouma HR, Ploeg RJ, Schuurs TA. Signal transduction pathways involved in brain death-induced renal injury. Am J Transplant. 2009;9(5):989-997.