

University of Groningen

## Early effects of brain death on kidney injury and outcome after transplantation

Nijboer, Wijmtje Nikeline

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*  
2010

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Nijboer, W. N. (2010). *Early effects of brain death on kidney injury and outcome after transplantation*. s.n.

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

## CHAPTER



### EFFECT OF BRAIN DEATH AND DONOR TREATMENT ON ORGAN INFLAMMATORY RESPONSE AND DONOR ORGAN VIABILITY

Willemijn N. Nijboer; Theo A. Schuurs; Joost A.B. van der Hoeven; Rutger J. Ploeg  
Department of Surgery, Groningen Kidney Transplant Center, University Hospital,  
Groningen, The Netherlands

*Published in Current Opinion of Organ Transplantation, Volume 9(2), June 2004, pp 110-115*

## - ABSTRACT -

### **Purpose of review:**

In the past, brain death in the donor has been recognized as an important risk factor for the success of solid organ transplantation. Brain death-related systemic changes that may influence potential donor organs do not seem to be restricted to hormonal and hemodynamic alterations. This review shows that at present there is convincing evidence for an inflammatory response in the donor organs as a direct result of brain death. This may be caused by systemic factors released from the brain as a result of its damage. Also, new research is reviewed suggesting the presence of other processes that occur at organ level in brain death.

### **Recent findings:**

In most organs, there is currently proof that the endothelium is activated, combined with the expression of adhesion molecules. The amount of leukocyte invasion in the tissue differs between organs. Recently, by using a microarray technique, the authors discovered the upregulation of several genes in brain death, which could be divided into three groups: genes involved in inflammation and coagulation, cell division and fibrosis, and protection and repair processes.

### **Summary:**

These findings in brain death of an inflammatory response at organ level together with a first attempt of the organ to protect itself offer new opportunities in donor treatment and preservation. By focusing attention on the specific blocking or stimulating of these processes, graft survival may be enhanced. The first studies in this direction already show promising results for graft survival.

## - INTRODUCTION -

Over the past few decades, solid organ transplantation has become an accepted therapy in the treatment of end-stage organ failure. Despite all the achievements including standardization of techniques, better understanding and treatment of complications, and the development of a new generation of immunosuppressive drugs in the 1980s, the ultimate goal of a lifelong uncomplicated graft survival has not yet been reached. To date, chronic rejection and organ dysfunction still remain large problems. More recently, it has become clear that the occurrence of these late events is correlated with early injury incurred around the donation and transplantation period. In this respect, during the past few years, more attention has been paid to the condition of the donor in addition to other risk factors such as the surgical procedure or the incompatibility of donor and graft [1].

Most organs used in clinical transplantation have been retrieved from heart-beating, brain-dead patients. For several patient categories –for instance, those waiting for a donor heart– this is inevitable. Because of a persistent shortage of donor organs, however, organs from living related or unrelated donors and, to a lesser extent, from nonheart-beating donors have become more accepted for transplantation over the past few years [2]. This extension of the donor pool has been primarily applied in kidney transplantation, although now both living and nonheart-beating donors are becoming accepted treatment modalities for liver and lung transplantation as well.

Well-matched living donor–recipient combinations were known to have superior transplant results over cadaveric donor kidneys. However, during the past few years, it has become obvious that fully mismatched living unrelated grafts also show better outcomes in survival rates than kidneys retrieved from brain-dead cadaveric donors with a similar matching grade for HLA antigens. Terasaki et al. [3] describe the survival of transplanted kidneys from different donors based on a large database. Cheng et al. [4] show very clearly the advantage of living donation above all other possibilities in liver transplantation for hepatocellular carcinoma. This difference in results could not be fully attributed to prolonged cold ischemia times for grafts procured from brain-dead donors, because no significant effect of cold ischemia time on kidney transplantation outcome was seen with preservation times as long as 24 hours [3–5]. This pointed at the fact that the aspect of living versus dead obviously played a significant role in the quality and viability of donor organs. Thus, the authors and others started to focus on the role and effects of brain death on the function and outcome after transplantation [6,7]. Reporting for the research group of Tilney, Gasser et al. [6] reviewed the detrimental effects of brain death on peripheral organs. Much of the success in organ transplantation is dependent on the ability to recognize, prevent, and possibly repair aspecific inflammatory and specific immune responses triggered during the donor period and intensified during ischemia and reperfusion.

## - CONCEPT OF BRAIN DEATH -

The concept of brain death did not come into use until the 1960s. This can be explained by the development of mechanical ventilators not much earlier. In 1959, the French neurologists Mollaret and Goulon described a state of irreversible coma without reflexes (the coma dépassée) after massive cerebral injury that required mechanical ventilation [8]. The comatose state would typically end in death after several days or weeks. A few years later, the concept was picked up in the rest of the world. To deal with this upcoming problem of deceased patients with intact circulation, a committee was installed in 1968 at Harvard Medical School, followed by other committees in several countries, which formulated criteria for brain death so that in these cases, ventilators could be turned off [9].

Brain death was thus medically and legally defined as a state of nonfunctioning of the brain, thereby requiring mechanical ventilation to support certain basic functions for a limited time. This development has formed a legal basis for using deceased patients as organ donors. Nowadays, brain death is seen as irreversible nonfunction of the brain as a central integrating organ regulating the body. This disintegration will therefore lead to disruption of several regulatory systems in the body, thereby affecting the potential donor organs.

## - MECHANISMS AND SYSTEMIC CHANGES IN BRAIN DEATH -

Irreversible cerebral injury leading to brain death usually results from intracranial hemorrhage or traumatic brain injury. In both instances, there is an increasing mass effect in the brain caused by intracranial volume expansion. This results in compression of brain tissue and a compromised liquor drainage and venous return, thereby inducing cerebral hypoxia and edema leading to an ongoing increase in intracranial pressure. Then brain stem compression occurs, with herniation of the brain stem into the foramen magnum, thereby causing respiratory arrest. Finally, complete brain ischemia precedes brain death. Mertes [10] provides a nice introduction to the physiologic aspects of brain death and their consequences in organ transplantation.

At first, some amount of viable tissue can still remain in the brain-dead patient. Total ischemia and necrosis of the spinal cord usually follow after destruction of the brain. Similarly, in some patients, the pituitary gland temporarily keeps its function. However, in most cases, the complete and irreversible loss of all brain stem functions can be established by clinical neurologic examination. The unresponsive patient shows an absence of brain stem reflexes and absolute apnea on stopping the mechanical ventilation. In most countries, confirmatory tests besides clinical examination such as electroencephalography or cerebral angiography are required to confirm the diagnosis of brain death.

Increases in intracranial pressure and brain ischemia lead to a series of major pathophysiologic changes usually referred to as the autonomic storm. After a few minutes of excessive parasympathetic activity during the onset of brain death, the body reacts with an initial

attempt to overcome increased intracranial pressure by severe vasoconstriction and, thereby, an increase in blood pressure, known as the Cushing reflex. This vasoconstriction occurs because of the fulminant release of endogenous catecholamines and probably causes a relative hypoperfusion in peripheral organs. Progressive paralysis of the spinal cord sympathetic pathways then leads to sympathetic deactivation with a decline in systemic vascular resistance to levels of less than 50% of baseline values. The period that follows is characterized by hemodynamic instability with hypotension and hypoperfusion of abdominal organs. In this phase, catecholamine levels drop to below baseline levels [10].

Other endocrinologic disturbances have also been widely reported. In most brain-dead patients, the function of the posterior pituitary gland is completely affected, as reflected by the onset of diabetes insipidus. However, in some patients, parts of the posterior lobe of the pituitary gland have been proven to be preserved. Also, patients have been described in whom circulating anterior pituitary hormones such as growth hormone, adrenocorticotropic hormone, and thyroid-stimulating hormone were detected until 1 week after brain death. Even so, most patients have a nonfunctioning hypothalamic-pituitary-thyroid axis. Whether substitution with triiodothyronine, cortisol, and insulin has beneficial effects on donor condition still remains contradictory. Novitzky [11] reported a reversal of anaerobic to aerobic metabolism and an improved hemodynamic status, but Gifford [12] failed to find any correlation between hormone levels and outcome parameters. Finally, levels of cortisol, insulin, atrial natriuretic peptide, and the renin-angiotensin-aldosterone axis do not seem to alter dramatically with onset of brain death.

During these systemic changes, many substances circulate through the body that are also seen during inflammatory responses. Shortly after brain death, high levels of cytokines such as interleukin-1  $\beta$  and interleukin-6, soluble cytokine receptors like sIL-2 receptor and sTNF receptor II, and vasoactive substances like endothelin-1 can be detected in the peripheral blood of the patient [13,14]. Lopau et al. [14] measured the full hormonal state of the brain-dead human donor. These substances may well originate from the brain as in less severe forms of brain damage such as acute ischemic stroke. A rise in both systemic soluble proinflammatory cytokines and soluble adhesion molecules could also be detected [15,16], whereas subarachnoid hemorrhage is known to cause a release of endothelin-1 in serum [17,18]. Likewise, in traumatic brain injury not followed by brain death, high interleukin-6 levels were detected in serum [19].

## - PATHOPHYSIOLOGIC EFFECTS ON ORGANS DURING BRAIN DEATH -

As stated before, peripheral organs suffer from the effects of brain death. The specific nature of the stress stimulus or stimuli in brain death to which organs respond remains to be investigated. Systemic hemodynamics—that is, hypertension at the onset of brain death and subsequent hypotension and hypoperfusion—may be held responsible for the upregulation of several detrimental factors and the induction of an inflammatory endothelial reaction.

These hemodynamic changes probably cause an amount of endothelial shear stress followed by a period of vasoconstriction leading to temporary ischemia of the abdominal organs. These same factors, shear stress and ischemia, are known to be able to activate the endothelium and cause an inflammatory response [20,21].

On the other hand, brain damage itself may cause a release of known or as yet unknown factors that trigger a response on the level of the organ itself. Possible candidates for these factors are cytokines, soluble adhesion molecules, or vasoactive substances such as endothelin-1. Indeed, molecules involved in inflammation could well be responsible for a part of the detrimental effects on organs, because the beginning of an inflammatory response on the organ level has now been reported for several organs.

### *Heart*

During the onset of brain death, catecholamines are released not only in the systemic circulation but also locally in the heart. These local concentrations, derived from sympathetic nerve endings, are far higher than plasma norepinephrine levels and are well capable of inducing various types of myocyte necrosis, such as contraction band necrosis, coagulation necrosis, and myocytolysis. Administration of similar high doses of exogenous catecholamines to experimental animals showed the same effects of necrosis [10]. This damage is further increased by massive calcium uptake of myocytes, stimulated through their  $\beta$ -receptor. Sympathectomy and calcium antagonists have been shown to reduce cardiac damage substantially. Wilhelm et al. [22] described the systemic changes to the heart induced by brain death. Also, as in other organs, endothelial cells are injured by brain death, which is a beginning for cardiac allograft vasculopathy, the limiting factor in prolonged cardiac survival [23]. This process happens during a diffuse inflammatory reaction with expression of cytokines and adhesion molecules in the heart [24].

### *Lungs*

The rapid hemodynamic changes that occur during brain death have detrimental effects on the lungs as well. As the heart receives an increased venous return of blood during the period of extreme vasoconstriction, the left atrial pressure exceeds the pulmonary artery pressure for a few seconds, causing a temporary circulatory stop and pulmonary hypertension in the capillaries of the lung. The lung tissue that is exposed to this type of pressure becomes severely injured, and the capillary integrity within the lungs is disrupted, causing pulmonary edema and interstitial hemorrhage [25]. Obviously, the prerequisite of mechanical ventilation in brain death has an additional adverse effect on the condition of the lungs.

The detrimental effects of brain death can also be found at a cellular level. Fisher et al. [26] found increased gene expression levels of interleukin-8 and growth-related gene- $\alpha$  after brain death compared with control ventilated patients. These interleukin-8 levels strongly correlated with the extent of neutrophil infiltration in the lungs.

## *Liver*

Although quite difficult to establish, evidence is mounting that the phase of brain death has similar negative effects on liver viability to those observed in other organs [27,28]. Histologic changes to liver tissue induced by brain death are described in some studies. Nagareda et al. [29] described the results of sequential liver biopsies in humans as long as 48 days after onset of brain death. Because of the Japanese system, which until recently did not allow brain-dead patients to be used as organ donors, this unique study shows histologic features of brain-dead livers after a prolonged period of brain death [29]. Central venous congestion during the first days after onset of brain death is reported. This is probably caused by brain death-induced circulatory failure. No significant central fibrosis, fatty metamorphosis, piecemeal necrosis, or periportal fibrosis was observed. Sometimes intrahepatic cholangitis was observed, generally more than 5 days after onset of brain death. Cholangitis was most likely caused by the known effect of medication or vagal denervation leading to gallbladder cholestasis and altered hepatic bile flow [30].

The onset of brain death does not seem to have an effect on the metabolic function of the liver [31,32]. Brain death did, however, have a negative effect on the preservation rate of ATP production after heat loading, also a viability assay for mitochondrial function. Although such an impairment might not affect graft function in uncomplicated transplantation situations, under unfavorable effects on posttransplant liver function are likely [33]. Effects of the use of vasoactive drugs to overcome brain death-induced hypotension on liver metabolism have also been subject of several studies. Although consensus still has not been reached, certain vasoactive drugs seem to have an effect on liver metabolism. Dopamine use in the donor has been shown to increase the incidence of primary graft failure, probably by reducing the redox state of liver mitochondria [32]. Vasopressin alone or in combination with epinephrine has no negative effects on the metabolic function of the liver [34]. To unravel the detrimental effects of brain death on the potential donor organ, the authors developed an animal model simulating brain death after intracranial trauma. In this model, brain death is induced by inflation of an intracranial balloon catheter in the anesthetized rat. In the rat brain-death model, tissue damage, as reflected by standard serum parameters, was profound: a threefold rise in serum creatinine and AST levels of more than 150 IU were detected after 6 hours of brain death [35]. These livers were used in a liver transplant model. Even without cold preservation, graft survival already decreased with 25% after transplantation of livers derived from brain-dead donors [36]. The decreased organ survival after brain death is shown very clearly in the study by Van der Hoeven et al. [36] using a rat liver transplant model. In an isolated perfused liver model, livers from brain-dead donors showed far more decrease in function (lower bile production and active transport mechanisms) and increase in injury parameters (higher transaminases and LDH; Unpublished data). Histologically, an infiltration of leukocytes, particularly of PMNs and macrophages but also a small increase in T cells, is seen after brain death. Also, expression of cell adhesion molecules intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) is increased [35]. The detrimental effects of brain death on the liver are also demonstrated



by the occurrence of apoptosis of hepatocytes, as assessed by caspase 3 activity and TUNEL assay after only 6 hours of brain death [37].

### *Kidney*

Epidemiologic studies were the first to show the detrimental effects of brain death on organ quality in kidneys. A good comparison can be made between recipients of cadaveric kidneys and the large number of recipients of living related and unrelated donor kidneys [3].

From the onset of brain death through the catecholamine storm until brain death induced hypotension, kidney perfusion can be significantly compromised in potential organ donors. Because decreased kidney perfusion is regarded as a major cause of kidney damage [38], the effect of brain death on kidney viability has been studied. Viability has been verified by histologic examination. Morphologic changes in potential donor kidneys before organ procurement have been found. A limited degeneration of tubular lining cells can be seen from the day of onset of brain death, increasing with the duration of the agonal phase. These degenerative changes are more extensive in the distal than in the proximal tubuli. Also, progression of arterial intimal proliferation, periglomerular fibrosis, and periglomerulitis are found after the onset of brain death [39]. In a study investigating effects of brain death on rabbit kidneys, however, only slight tubular and glomerular changes were observed.

Although increases of inflammatory cytokines and invading neutrophils could already be found in animal models and human studies after brain death, a massive invasion of recipient macrophages and T cells and an upregulation of adhesion molecules (both selectins and Ig superfamily members) and HLA-DR antigens could be found after reperfusion compared with reperfused nonbrain-dead controls in human and rat kidney grafts [40,41].

In our rat brain death model, the authors not only detected rises in serum creatinine, indicating decreased kidney function, but also demonstrated a progressive inflammatory response in the donor kidney. Leukocyte influx increased twofold in the kidney after 6 hours of brain death. Adhesion molecules VCAM-1 and ICAM-1 were clearly more expressed in brain-dead donor organs. The immediate early gene FOS was found in kidney biopsies from brain-dead animals only [42].

Similar to the results of the rat studies, the authors' findings were increased levels of cell adhesion molecules E-selectin, ICAM-1, and VCAM-1 in human cadaveric kidneys compared with control biopsies from living related kidneys [43]. Recently, an isolated perfused kidney model was developed as well. Using this model, a dramatic decrease in function (almost no urine production, sodium retention) in kidneys from brain-dead donors and, even more, in kidneys from non heart-beating donors was [44].

Recently, the authors isolated mRNA from fine-needle kidney biopsies from human brain-dead and living donors. With reverse-transcription polymerase chain reaction, the authors studied expression of several genes possibly involved in brain death. Despite the heterogeneous background of the donors, the authors found elevated levels of HO-1 and hsp70 in brain-dead donors. HO-1 and hsp70 encode proteins with several cytoprotective properties against ischemia, heat, and toxins. Furthermore, after correlating donor variables with gene

expression, a strong association was found between elevated AST and ALT serum levels and increased HO-1 gene expression. This also indicates a damage response on brain death, although it is not entirely clear whether HO-1 expression in the kidney and serum AST/ALT are both manifestations of systemic stress and organ damage or whether elevated AST/ALT levels are produced by the kidney itself [43].

## - SYSTEMATIC ASSESSMENT OF PATHWAYS INVOLVED IN THE REACTION ON BRAIN DEATH -

Although knowledge regarding brain death has broadened during the past few years, the undoubtedly complex mechanisms by which brain death leads to these processes remain unclear. To unravel these regulatory pathways further, the authors recently performed microarray studies with RNA derived from brain-dead rats [45]. DNA microarrays enable the expression analyses of multiple genes simultaneously. In this technique, isolated RNA is labeled and used as a probe on an array containing several thousands of spotted genes involved in a wide range of biologic pathways. By using oligonucleotide microarrays containing 5000 unique rat sequences, 72 genes were identified as being differentially expressed, which was confirmed by reverse-transcription polymerase chain reaction for a selected number of genes. Further analyses of the data enabled the authors to categorize a substantial number of these genes in different functional groups: (1) upregulation of inflammation and coagulation (with adhesion molecules like e-selectin, cytokines like interleukin-1 $\alpha$  and interleukin-6, chemokines (eg, MCP-1), and  $\alpha$ -fibrinogen and  $\beta$ -fibrinogen involved in coagulation), (2) upregulation of cell division and fibrosis (with cell cycle arrest gene KIM-1 putatively involved in regeneration of proximal tubular cells and fibrosis genes collagen  $\alpha$ 1 and 2), (3) upregulation of genes involved in protection and repair (heat shock proteins, antiapoptotic genes, and the oxygen-radical scavenger MnSOD), and (4) downregulation of several genes involved in metabolism and transport of the kidney. Also, genes encoding transcription factors and proteins involved in signal transduction were identified, of which an extended number have been shown to be regulated by key transcription factors as NF- $\kappa$ B and Egr-1. These results indicate not only that deleterious processes such as inflammation and fibrosis occur in brain-dead donor organs but also that genes involved in protection and repair processes are activated. More research about the mutual interaction of these processes must be conducted in the future.

## - CONCLUSION -

These exciting results of microarray studies outline further studies needed to explain the process of brain injury and brain death in a potential donor in detail. The exact mechanisms and pathways responsible for the reduced graft quality are still largely unknown. No detailed models and hypotheses have been published to date. Also, there have been few brain death intervention studies published.

The authors have shown that brain death in the rat has definite effects on three important groups of genes: genes involved in inflammation and coagulation, cell division and fibrosis, and protection and repair. From a general idea of preventing injury, artificial upregulation of the heat shock protein heme oxygenase-1 has been correlated to an improvement in both kidney graft function and survival after brain death [46]. However, in this study, HO-1 was induced with the toxic substance cobalt-protoporphyrin, thereby rendering this approach unsuitable for a translation to the human situation. Other, less toxic upregulators of heat shock proteins like HO-1 may thus prove to be a great advance in treatment.

Another promising approach is to block or suppress the proven inflammatory processes and to test its effect on transplantation outcome. Until now, very few intervention studies have been performed to test whether inflammation could be suppressed. This is not surprising, because not many groups have used a functional brain death animal model. Until now, only the administration of soluble P-selectin glycoprotein ligand during brain death in a rat kidney transplant model has been tested [47,48]. Recipients of treated kidneys showed survival times comparable with living donor controls and significantly better than the untreated allografts. This approach shows promising results, even while focusing only on a very small aspect of the process of brain death.

The authors speculate that the activation of T cells later during brain death will also lead to injury and result in an increased rate of rejection. To block the specific immune system with the potential of decreased immunogenicity, improved function and less rejection may also be a starting point of further study.

## References

1. Vazquez MA, Jeyarajah DR, Kielar ML, et al.: Long-term outcomes of renal transplantation: a result of the original endowment of the donor kidney and the inflammatory response to both alloantigens and injury. *Curr Opin Nephrol Hypertens* 2000, 9:643–648.
2. Koffman G, Gambaro G: Renal transplantation from non-heart-beating donors: a review of the European experience. *J Nephrol* 2003, 16:334–341.
3. Terasaki PI, Cecka JM, Gjertson DW, et al.: High survival rates of kidney transplants from spousal and living unrelated donors. *N Engl J Med* 1995, 333:333–336.
4. Cheng SJ, Pratt DS, Freeman RB Jr, et al.: Living-donor versus cadaveric liver transplantation for non-resectable small hepatocellular carcinoma and compensated cirrhosis: a decision analysis. *Transplantation* 2001, 72:861–868.
5. Gupta P, Hart J, Cronin D, et al.: Risk factors for chronic rejection after pediatric liver transplantation. *Transplantation* 2001, 72:1098–1102.
6. Gasser M, Waaga AM, Laskowski IA, et al.: The influence of donor brain death on short and long-term outcome of solid organ allografts. *Ann Transplant* 2000, 5:61–67.
7. 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, 64:1874–1882.
8. Mollaret P, Goulon M: [The depassed coma (preliminary memoir)]. *Rev Neurol (Paris)* 1959, 101:3–15.
9. 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, 205:337–340.
10. Mertes PM: Physiology of brain death. In: Tilney NL, Strom TB, Paul LC, editors. *Transplantation Biology: Cellular and Molecular Aspects*. Philadelphia: Lippincott-Raven Publishers; 1996:275–289.
11. Novitzky D: Detrimental effects of brain death on the potential organ donor. *Transplant Proc* 1997, 29:3770–3772.
12. Gifford RR, Weaver AS, Burg JE, et al.: Thyroid hormone levels in heart and kidney cadaver donors. *J Heart Transplant* 1986, 5:249–253.
13. Stangl M, Zerkaulen T, Theodorakis J, et al.: Influence of brain death on cytokine release in organ donors and renal transplants. *Transplant Proc* 2001, 33:1284–1285.
14. Lopau K, Mark J, Schramm L, et al.: Hormonal changes in brain death and immune activation in the donor. *Transpl Int* 2000, 13(suppl 1):S282–S285.
15. Fassbender K, Rossol S, Kammer T, et al.: Proinflammatory cytokines in serum of patients with acute cerebral ischemia: kinetics of secretion and relation to the extent of brain damage and outcome of disease. *J Neurol Sci* 1994, 122:135–139.
16. Fassbender K, Mossner R, Motsch L, et al.: Circulating selectin- and immunoglobulin-type adhesion molecules in acute ischemic stroke. *Stroke* 1995, 26:1361–1364.
17. Juvela S: Plasma endothelin concentrations after aneurysmal subarachnoid hemorrhage. *J Neurosurg* 2000, 92:390–400.
18. Fassbender K, Hodapp B, Rossol S, et al.: Endothelin-1 in subarachnoid hemorrhage: an acute-phase reactant produced by cerebrospinal fluid leukocytes. *Stroke* 2000, 31:2971–2975.
19. McKeating EG, Andrews PJ, Signorini DF, et al.: Transcranial cytokine gradients in patients requiring intensive care after acute brain injury. *Br J Anaesth* 1997, 78:520–523.

20. Wang DL, Wung BS, Shyy YJ, et al.: Mechanical strain induces monocyte chemotactic protein-1 gene expression in endothelial cells: effects of mechanical strain on monocyte adhesion to endothelial cells. *Circ Res* 1995, 77:294–302.
21. Topper JN, Gimbrone MA Jr: Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype. *Mol Med Today* 1999, 5:40–46.
22. Wilhelm MJ, Pratschke J, Laskowski IA, et al.: Brain death and its impact on the donor heart—lessons from animal models. *J Heart Lung Transplant* 2000, 19:414–418.
23. Valantine HA: Cardiac allograft vasculopathy: central role of endothelial injury leading to transplant “atheroma.” *Transplantation* 2003, 76:891–899.
24. Wilhelm MJ, Pratschke J, Beato F, et al.: Activation of the heart by donor brain death accelerates acute rejection after transplantation. *Circulation* 2000, 102:2426–2433.
25. Cooper DK, Novitzky D, Wicomb WN: The pathophysiological effects of brain death on potential donor organs, with particular reference to the heart. *Ann R Coll Surg Engl* 1989, 71:261–266.
26. Fisher AJ, Donnelly SC, Hirani N, et al.: Enhanced pulmonary inflammation in organ donors following fatal non-traumatic brain injury. *Lancet* 1999, 353:1412–1413.
27. Cheng SJ, Pratt DS, Freeman RB Jr, et al.: Living-donor versus cadaveric liver transplantation for non-resectable small hepatocellular carcinoma and compensated cirrhosis: a decision analysis. *Transplantation* 2001, 72:861–868.
28. Gupta P, Hart J, Cronin D, et al.: Risk factors for chronic rejection after pediatric liver transplantation. *Transplantation* 2001, 72:1098–1102.
29. Nagareda T, Kinoshita Y, Tanaka A, et al.: Clinicopathological study of livers from brain-dead patients treated with a combination of vasopressin and epinephrine. *Transplantation* 1989, 47:792–797.
30. Baldwin J, Heer FW, Albo R, et al.: Effect of vagus nerve stimulation on hepatic secretion of bile in human subject. *Am J Surg* 1966, 111:66–69.
31. Imai K, Sato K, Takahashi T, et al.: Experimental evaluation of hepatic circulation, energy metabolism, and morphologic changes in brain death. *Transplant Proc* 1998, 30:3282–3283.
32. Manaka D, Okamoto R, Yokoyama T, et al.: Maintenance of liver graft viability in the state of brain death: synergistic effects of vasopressin and epinephrine on hepatic energy metabolism in brain-dead dogs. *Transplantation* 1992, 53:545–550.
33. Sato T, Asanuma Y, Yasui O, et al.: Impaired hepatic mitochondrial function reserve in brain-dead pigs. *Transplant Proc* 1998, 30:4351–4355.
34. Yamaoka Y, Taki Y, Gubernatis G, et al.: Evaluation of the liver graft before procurement: significance of arterial ketone body ratio in brain-dead patients. *Transpl Int* 1990, 3:78–81.
35. Van der Hoeven JA, Ter Horst GJ, Molema G, et al.: Effects of brain death and hemodynamic status on function and immunologic activation of the potential donor liver in the rat. *Ann Surg* 2000, 232:804–813. The additional detrimental effect of brain death besides hemodynamic instability on donor livers is proven in this elegant article.
36. Van der Hoeven JA, Lindell S, van Schilfgaarde R, et al.: Donor brain death reduces survival after transplantation in rat livers preserved for 20 hr. *Transplantation* 2001, 72:1632–1636.
37. Van der Hoeven JA, Moshage H, Schuurts T, et al.: Brain death induces apoptosis in donor liver of the rat. *Transplantation* 2003, 76:1150–1154.
38. Heyman SN, Lieberthal W, Rogiers P, et al.: Animal models of acute tubular necrosis. *Curr Opin Crit Care* 2002, 8:526–534.

39. Nagareda T, Kinoshita Y, Tanaka A, et al.: Clinicopathology of kidneys from brain-dead patients treated with vasopressin and epinephrine. *Kidney Int* 1993, 43:1363–1370.
40. Takada M, Nadeau KC, Hancock WW, et al.: Effects of explosive brain death on cytokine activation of peripheral organs in the rat. *Transplantation* 1998, 65:1533–1542. A well-controlled animal model is used here to show the peripheral organ activation caused by brain death.
41. Pratschke J, Wilhelm MJ, Kusaka M, et al.: Accelerated rejection of renal allografts from brain-dead donors. *Ann Surg* 2000, 232:263–271.
42. 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, 64:1874–1882.
43. Nijboer WN, Schuur TA, Hoeven JAB van der, et al.: Effect of brain death on gene expression and tissue activation in human donor kidneys. *Transplantation*. 2004 Okt 15;78(7):978-86.
44. van den Eijnden MM, Leuvenink HG, Ottens PJ, 't Hart NA, van OW, Morariu AM et al. Effect of brain death and non-heart-beating kidney donation on renal function and injury: an assessment in the isolated perfused rat kidney. *Exp Clin Transplant* 2003; 1(2):85-95.
45. Schuur 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 Dec;4(12):1972- 81.
46. Pratschke J, Francuski M, Kotsch K, et al.: Risk factor brain death—influence of induction of protective genes on graft function. 11th Congress of the European Society for Organ Transplantation, September 22, 2003.
47. Pratschke J, Koffa G, Wilhelm MJ, et al.: Improvements in early behavior of rat kidney allografts after treatment of the brain-dead donor. *Ann Surg* 2001, 234:732–740.
48. Gasser M, Waaga AM, Kist-Van Holthe JE, et al.: Normalization of brain death-induced injury to rat renal allografts by recombinant soluble P-selectin glycoprotein ligand. *J Am Soc Nephrol* 2002, 13:1937–1945.

