The impact of cerebral injury in donation and transplantation

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Chapter 1 **Introduction and Aim**

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Introduction

Organ transplantation is a life saving therapy for patients with end stage organ failure. In general, patients who receive a solid organ transplantation live longer and have a better quality of life compared to those on organ replacement therapies (1;2).

Nowadays, due to improved organ preservation methods, better surgical techniques and new immunosuppressant drugs and regimens, severe rejection is less frequent and outcome after transplantation improved (3). Infectious complications are recognised earlier and treated better.

When corrected for recipient characteristics, such as increased age, diabetes, vascular nephropathy, re-transplantation and duration of prior replacement therapy, a significant improvement in recipient survival has been shown (4). Due to these major achievements, the number of patients eligible for transplantation has increased steadily, but because of the inclusion of patients with more co-morbidities, over the last years transplant survival has stabilised.

The worldwide increasing demand for donor organs has resulted in a gradual shift towards acceptance of suboptimal donor organs from older brain dead donors and even donors after cardiac death. In kidney transplantation, grafts donated by a living kidney donor have the highest function and survival rate (5;6). This is caused by selection since obviously, only healthy people are allowed to donate a kidney to a renal patient in need. In addition, the better performance of a living donor transplant can be explained by the elective setting of the donor- and recipient operation, as well as a shorter cold storage period.

To date, however, the majority of organs are still recovered from deceased donors. In deceased donors, after approval, and depending on donor characteristics one or more organs are donated at the same time. In the 1960s and before most organs for transplantation were retrieved after cardiac death (7). The introduction of the brain death criteria in 1968 enabled the use of brain dead donors, resulting in a substantial increase in available donor organs (8). Brain death is defined as irreversible full destruction of the brain, including the brain stem, during ventilatory support. This means death with intact circulation. The absence of warm ischemia that is present in cardiac death donors and the fact that the typical donor was considered to be a young donor that died from cerebral trauma as a result of a traffic accident can explain the term ‘ideal donors’ that is often used for brain death donors. Brain death does not necessary only result from a head trauma but also from cerebral haemorrhage. In 1994, in the Netherlands, the cause of brain death due to trauma occurred in 37% of deceased donors, this decreased in 2008 to 21%. Also, the average donor age increased: In 1994 18% of the donors were aged 55 years or more, versus 39% in 2008 (9;10). The organ shortage has forced many transplant centers to widen their acceptance of donors. Over the past 15 years organ donation after cardiac death has become an accepted medical

practice. According to the Maastricht criteria four types of non heart beating donors are recognized: type I: dead on arrival; type II: unsuccessful resuscitation; type III: awaiting heart arrest and type IV: heart arrest in brain dead donor. In 2007, in Europe, only the Netherlands, Belgium, Austria and Spain, recovered organs from non heart beating donors, also called donation after cardiac death (DCD) donors (11). Kidneys recovered from DCD donors show a higher rate of delayed graft function (DGF) and early graft failure compared to deceased brain dead (DBD) donors indicating the detrimental effect of cardiac arrest with a period of inevitable warm ischemia (12;13). Fortunately, in long term studies albeit in small cohorts, similar or almost similar clinical outcome of DCD compared to heart beating donation is seen. A disadvantage of DCD donation compared to heart beating donation is that fewer organs can be used for transplantation. While from DBD donors heart, lungs, pancreas, liver, intestine and kidneys can be used, is the number of eligible organs in a DCD donor restricted to predominantly kidneys and less frequent liver and lungs (11;14;15).

INJURY IN DECEASED BRAIN DEAD DONORS

As previously mentioned, organs recovered from living donors have a better outcome than organs recovered from deceased donors (5). Many donor characteristics in deceased donors will have an influence on transplant outcome of the recipient, including the pre-existing state of the donor (history of hypertension, diabetes mellitus), cause of death, type of donor (DCD vs. DBD), age, sex, race, warm and cold ischemia time and method of preservation (16;17). The inferior survival of deceased donation cannot be attributed to differences in immunogenicity alone (6). In 1994, Matzinger introduced the so called danger model, which concerned a novel insight on injury and innate immune system. This model can also be applied in donation and transplantation (18;19).

With her danger model Matzinger proposes that the immune response is primary concerned with entities that cause damage, rather than distinguish between self and non-self. Matzinger explained in her essay, that antigen-presenting cells respond to "danger/alarm signals" - from injured cells, such as those exposed to pathogens, toxins and mechanical damage (18). Recently Matzinger added to her danger model, the importance of the regulatory function of the tissue itself (20). This hypothesis could also explain the better survival outcome after transplantation with living donor organs compared to deceased donor organs (18).

We hypothesized that in deceased donors the onset and progression of changes during brain death take place on several levels. These changes can be seen as danger signals which force the donor on a systemic and local organ level, to respond and react. Thus, both dangers signals as well the regulatory function of the tissue may have an effect on the donor organ and transplantation outcome.

DANGER SIGNAL AND TISSUE RESPONSES

In this paragraph, we address the local and systemic hemodynamic, endocrine and inflammatory changes. Following cerebral injury and during the development

towards brain death, when the pontine part of the brain stem becomes ischemic, the so called Cushing reflex takes place. The Cushing reflex is an attempt to maintain perfusion to the brain in response to the elevated intracranial pressure, by increasing the arterial blood pressure (21). Catecholamines play a dominant role in the attempt to increase blood pressure. The “catecholamine storm” which takes place during development of brain stem herniation, is characterised by significantly elevated levels of the catecholamines epinephrine, nor-epiniphrine and dopamine (22). After a period of prolonged brain death catecholamine levels decrease to below baseline levels. Hypotension and changes in regional perfusion are then induced by this systemic drop in vasopressor levels (22;23).

In addition to the catecholamine storm, changes take place on the endocrine level. Several studies provide evidence that changes in thyroid hormones will occur during brain death. Triiodothyronine (T3) gradually decreases after cerebral injury (24;25). However, what exactly happens with other thyroid hormone components, such as thyroxin and thyroid stimulation hormone (TSH) has not been unravelled yet (26). Furthermore, any acute stress will provoke the condition known as diabetes of injury (27;28). Strict glycaemic control by intensive insulin therapy has shown to be effective in renal protection and reducing mortality in intensive care units (29;30). Prediction of organ function after transplantation would be a most valuable tool to improve transplant outcome or adjust posttransplant treatment. In an attempt to predict the quality of the deceased donor graft, it is common in the United States of America, to evaluate graft quality with pre-transplant donor biopsies. However, the prognostic value of these biopsies remains uncertain (31), although it has now been shown that the presence of moderate arteriosclerosis and/or moderate arteriolosclerosis was a significant predictor of graft outcome (32). Recently, a report was published, in which an increased plasma interleukin-6 level in donors is associated with longer hospital stay after transplantation (33). Unfortunately, to date no donor biomarkers are available that have a relevant and independent predictive value for kidney transplant outcome.

Beside the hemodynamic and endocrine changes, our group and others have demonstrated a pro-inflammatory state in deceased brain dead donors. Ischemia and hormonal imbalance are in part responsible for this inflammatory state. As demonstrated by J. van der Hoeven, the systemic inflammatory state is characterized by circulating cytokines including interleukin-6, interleuking-10, tumor necrosis factor-alpha and tumor growth factor-beta. Furthermore, enhanced immune activation in kidneys and livers recovered from brain dead donors was reflected by a deteriorated I/R injury as proven by elevated alanin-amino-transferase (ALT), aspartat-amino-transferase (AST) and bilirubin levels, increased rates of acute rejection and primary non function (34). In accordance with the liver, the kidney recovered from a brain dead donor shows interstitial leukocytes and upregulation of the adhesion molecule E-selectin (35). As demonstrated by Morariu, the endothelium is activated, characterised by higher plasms levels of von Willebrand factor (36). Furthermore, in experimental studies, multiple research groups showed that brain death induces an inflammatory response (37-41). This

inflammatory state is characterized by influx of inflammatory cells in the kidney, liver and lung, coinciding with the presence of proteins, such as intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) and E-Selectin and apoptosis (37;42;43).

THE EFFECT OF BRAIN DEATH ON THE INTESTINE

Despite the fact that intestinal transplantation is almost exclusively depending on DBD donors as sources of donor intestine the effect of BD on the intestine is a largely unexplored field of investigation. In addition, the intestine is regarded as an important player in the pathophysiology of severe acute illness, including burns, acute pancreatitis, trauma and hemorrhagic shock (44;45). The intestine is more vulnerable to ischemia/ reperfusion injury, compared to other organs (46). The intestine has a complex tissue architecture composed of the mucosa, submucosa, and an external smooth muscle layer. In experimental small bowel transplantation without cold ischemia, the villi show damage to the tips of the villi with no further exacerbation at reperfusion and complete healing 24 hours thereafter. Moderate villous injury was demonstrated following 5 h of cold ischemia following reperfusion but was almost completely healed 24 h later (47). An ideal marker of intestinal viability should be able to reflect this complexity, thus allowing the distinction between damage limited to the mucosa, and full-thickness intestinal infarction (48). Traditional markers of intestinal ischemia include lactate, amylase, lactate dehydrogenase, is suboptimal for routine clinical use (48). Recently, a number of new markers have been introduced, e.g. intestinal fatty acid binding protein (I-FABP). I-FABP is primarily limited to the mature enterocytes of the small intestine, with only trace amounts identified in the stomach and large intestine. It is suggested that I-FABP may be a useful marker of the extended inflammatory process (49;50). LPS binding protein is an acute phase protein that binds to bacterial lipopolysaccharide to elicit immune responses by presenting the LPS to important cell surface pattern recognition receptors. It is regarded as a potential marker to quantify endotoxemia (51;52).

In the 1960s, the idea of gut-origin infection was born, after the observation that in patients with severe burns no detectable microorganisms were found after repeated wound cultures, but blood cultures were usually positive for gut flora (53). Several factors, including intestinal inflammation, gut barrier failure, and sepsis, have been implicated in the development of multiple organ failure (53-55). However, the exact pathogenesis is not fully elucidated, the presence of (parts of) bacteria is probably not the complete explanation. Recent literature suggests that not only enhanced permeability and bacterial translocation, but also non-bacterial factors in lymph play an important role in distant organ injury. These factors in the lymph activate neutrophils and induce injury to the endothelium (56). Organ injury observed in DBD donors resembles distant injury to the lung and heart seen after severe burns (43;56).

Summarizing, in deceased brain dead donors, we hypothesize that intestinal inflammation leads to enhanced intestinal permeability, which causes bacterial

translocation, provoking cytokine release (Figure 1). This vicious circle could enhance the inflammatory state of potential donor organs.

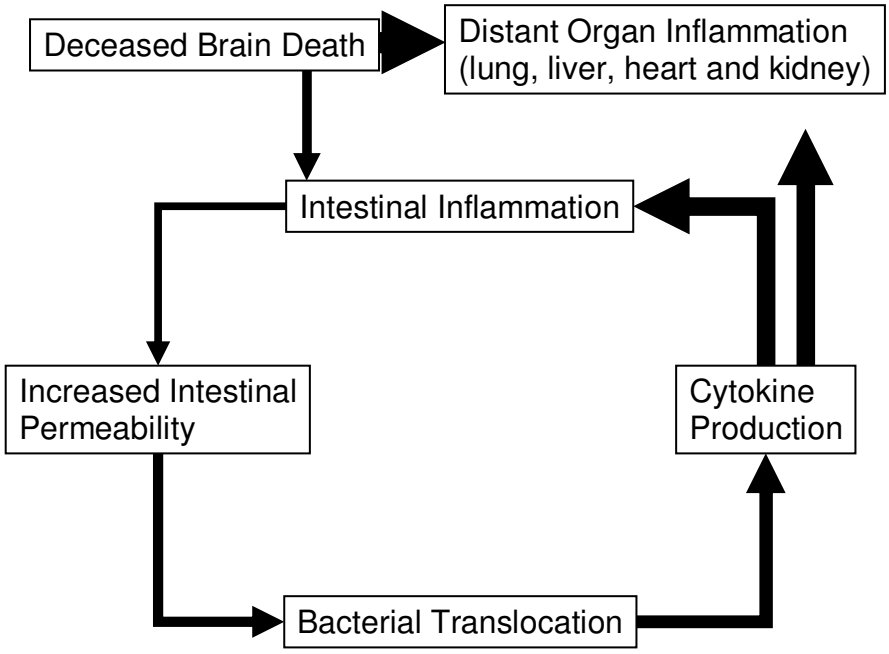

Figure 1 Schematical representation of the hypothesis that intestinal inflammation leads to enhanced intestinal permeability, which causes bacterial translocation, provoking cytokine release.

Aim

The outcome after deceased donation is inferior to living donation. The proposed "danger" in DBD donor organs is the systemic pro-inflammatory state. Moreover, in several conditions such as severe burns, brain injury and Acute Respiratory Distress Syndrome (ARDS), the intestine is considered as a crucial player in the development of (distant) organ injury. However, in brain death induced injury, the role of the intestine is not investigated yet. The aim of this thesis is to study the role of the intestine in brain death induced injury. Studies are focussed on inflammation in relation with intestinal permeability. Furthermore, we aimed at understanding the condition of the blood-brain barrier in brain dead donors and the potential role of novel biomarkers.

In **chapter 1** an introduction is given on transplantation in general, including the various types of donors and the pro-inflammatory state in organs derived from deceased donors. Since brain dead induces various inflammatory changes in donor organs we studied changes in the intestine after brain dead in **chapter 2**. Based on the inflammatory changes in the brain dead donor intestine, we then studied intestinal permeability in brain dead rats (**chapter 3**), assuming that increased intestinal permeability, induced during brain death contributes to the systemic inflammatory state, this may result in further distant organ failure and subsequent inferior transplant outcome. With this chapter, we provide a basis to further explore this hypothesis in future research.

Based on evidence that immunomodulatory molecules produced by the injured brain are secreted into the circulation through a defect blood-brain barrier, we investigated whether the inflammatory response present in deceased brain dead donors could be explained by the leakage of pro-inflammatory proteins from the injured brain into the circulation. Therefore we measured glial fibrillary acidic protein (GFAP) as a marker of blood-brain barrier dysfunction, and interleukin-6 (IL-6) as a key pro-inflammatory cytokine, at the beginning and the end of the brain death period (**chapter 4**). Recently it was shown that in endotoxic patients the angiopoietin levels are influenced. Because bacterial translocation and endotoxemia as well as inflammation are frequent in DBD donors, we studied the inflammatory proteins angiopoietin-1 and angiopoietin-2 in plasma of these donors in **chapter 5**. Since angiopoietin-2 is a prognostic survival marker in critically ill patients, we investigated whether donor angiopoietins have a predictive value in recipients of a kidney transplant in **chapter 6**. Finally, the results of this thesis are summarised and future perspectives are given in **chapter 7**.

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