Chapter 1:

General Introduction
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INTRODUCTION

It is well-known that solid organ transplantation has been accepted as the therapy of choice for end-stage organ failure. The main source of donor organs for transplantation has been heart-beating brain dead patients. Due to the persistent shortage of donor organs and growing waiting list, it was encouraged to enlarge the donor pool using marginal donors, to decrease the gap between supply and demand. However, grafts from brain-dead donors have more frequently delayed graft function and higher rejection rates compared to living donor [1]. Thus, brain death is believed to be a main risk factor leading to damage of the potential donor organ before transplantation. This thesis focuses on brain dead donor pre-conditioning to improve the graft function and survival.

Organ transplantation nowadays

Renal transplantation is the optimal treatment for most patients who have end-stage renal failure. The Eurotransplant Statistics reported for 2013, a total of 3183 kidney transplants while living donor kidney transplants were only 1402. Meanwhile the waiting list was still 10757 patients in the European region. In China, 6471 patients received a renal transplant in 2013 according to incomplete data. While 20% of donor kidneys were from living donors, 80% were from cardiac dead donors. Because of traditional culture and law limitations no brain dead donors are used in China. The amount of patients on the waiting list is more than 1 000 000 of which 20% of the patients are treated by blood dialysis and others receive drug management.

The contradiction between donors and patients in waiting list is aggravating. It is inevitable to expand the donor organ pool and face the high risk of marginal grafts-to-be.

Concept of brain death

Brain death, referred to as coma dépassée, was described as early as 1959 by the French Mollaret and Goulon as a state of irreversible coma without reflexes after
massive cerebral injury that required mechanical ventilation [2, 3]. At that moment, no consensus had been reached on the implications of this irreversible coma. In 1968, a committee was installed at Harvard Medical School that proposed to add irreversible coma to the death criterion, so that in these cases ventilators could be turned off. Their reports established the term of brain death for the first time, defined it as a ‘permanently non-functioning brain’, gave diagnostic criteria and redefined brain death (BD) as legally equivalent to death[4]. This development has formed since then the legal basis for using deceased patients who are brain dead however still have an intact extra–cerebral blood circulation to organ donors.

The clinical examination to determine brain death includes documentation of coma, the absence of brain–stem reflexes (Table1) and apnea to be formally tested. Cerebral angiography, electroencephalography, transcranial Doppler ultrasonography and nuclear imaging with technetium are commonly used (Table2). Neurologic examination to determine whether a patient is brain dead can proceed only if the following prerequisites are met: the ruling out of complicated medical conditions that may confound the clinical assessment, particularly severe electrolyte, acid–base, or endocrine disturbances; the absence of severe hypothermia, defined as a core temperature of 32°C or lower; hypotension; and the absence of evidence of drug intoxication, poisoning, or neuromuscular blocking agents[4, 5].

### Table1: CLINICAL CRITERIA FOR BRAIN DEATH IN ADULTS AND CHILDREN

<table>
<thead>
<tr>
<th>Coma</th>
<th>Absence of motor responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of pupillary responses to light and pupils at midposition with respect to dilatation (4–6 mm)</td>
<td>Absence of corneal reflexes</td>
</tr>
<tr>
<td>Absence of corneal reflexes</td>
<td>Absence of caloric responses</td>
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<tr>
<td>Absence of gag reflex</td>
<td>Absence of coughing in response to tracheal suctioning</td>
</tr>
<tr>
<td>Absence of sucking and rooting reflexes</td>
<td>Absence of respiratory drive at a PaCO₂ that is 60 mm Hg or 20 mm Hg above normal base–line values*</td>
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</table>

Interval between two evaluations, according to patient’s age

Term to 2 mo old, 48 hr

>2 mo to 1 yr old, 24 hr

>1 yr to < 18 yr old, 12 hr

"18 yr old, interval optional

Confirmatory tests

Term to 2 mo old, 2 confirmatory tests

>2 mo to 1 yr old, 1 confirmatory test

>1 yr to < 18 yr old, optional

"18 yr old, optional

*PaCO₂ denotes the partial pressure of arterial carbon dioxide.
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 sequence of events take place that affect the potential donor organs. Following cerebral trauma or cerebro-vascular hemorrhage, frequently the intracranial pressure increases and consequently leads to the onset of brain death resulting in profound systemic changes. These changes are summarized in four categories: endocrine, metabolic, immunologic and hemodynamic response to brain death prior to organ retrieval.

Table 2. CONFIRMATORY TESTING FOR DETERMINATION OF BRAIN DEATH.
Cerebral angiography
The contrast medium should be injected under high pressure in both anterior and posterior circulation.
No intracerebral filling should be detected at the level of entry of the carotid or vertebral artery to the skull.
The external carotid circulation should be patent.
The filling of the superior longitudinal sinus may be delayed.
Electroencephalography
A minimum of eight scalp electrodes should be used.
Interelectrode impedance should be between 100 and 10,000Ω.
The integrity of the entire recording system should be tested.
The distance between electrodes should be at least 10 cm.
The sensitivity should be increased to at least 2 μV for 30 minutes with inclusion of appropriate calibrations.
The high-frequency filter setting should not be set below 30 Hz, and the low-frequency setting should not be above 1 Hz.
Electroencephalography should demonstrate a lack of reactivity to intense somatosensory or audiovisual stimuli.
Transcranial Doppler ultrasonography
There should be bilateral insonation. The probe should be placed at the temporal bone above the zygomatic arch or the vertebrobasilar arteries through the suboccipital transcranial window.
The abnormalities should include a lack of diastolic or reverberating flow and documentation of small systolic peaks in early systole. A finding of a complete absence of flow may not be reliable owing to inadequate transtemporal windows for insonation.
Cerebral scintigraphy (technetium Tc 99m hexametazine)
The isotope should be injected within 30 minutes after its reconstitution.
A static image of 500,000 counts should be obtained at several time points: immediately, between 30 and 60 minutes later, and at 2 hours.
A correct intravenous injection may be confirmed with additional images of the liver demonstrating uptake (optional).


**Brain death injury**

**Hemodynamic response**
Increasing intracranial pressure (ICP) and brain tissue ischemia lead to a series of major patho–physiologic changes usually referred to as the autonomic storm. In the first few minutes during the onset of brain death, the excessive parasympathetic activity is triggered resulting in a decreased systemic blood pressure (BP) Cushing response; and then the body reacts with an initial attempt to overcome the increased ICP by severe vasoconstriction and subsequently increased BP occurs because of the fulminant release of endogenous catecholamines. It is likely a relative hypoperfusion in peripheral organs[6–9]. After the "catecholamines storm", the hemodynamic state reaches a hypotensive phase resulting in hypo–perfusion of peripheral organs. In this phase, catecholamine levels drop to below baseline level because of its depletion[10].

**Hormone response**
In addition to the changes in sympathetic activity and catecholeamine plasma levels, brain death may also cause significant endocrine changes reflecting anterior and posterior pituitary failure. Induction of brain death in animals is followed by a rapid decrease in triiodothyronine(T3), cortisol, and insulin[11, 12]. The pattern in brain–dead patients seems to be somewhat different. Cortisol and insulin levels remain within normal levels in most cases, and the decline in T3 plasma levels is not uniform [13–15]. Anterior pituitary function seems to be well preserved in most donors with normal values of thyroid–stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), and human growth hormone, indicating some residual function and thus perfusion of the hypothalamic–pituitary neuro–endocrine system. Thyroid hormone and TSH levels are typical for the ‘sick euthyroid syndrome’ that often accompanies severe brain injury, rather than a result of TSH deficiency. Although plasma cortisol values remain within normal levels in most brain–dead donors[13, 14], the capacity to increase secretion upon ACTH stimulation seems to be reduced[15]. Dimopoulou reported that after brain death plasma levels of cortisol were significantly lower, and the brain–dead patients did much less respond to ACTH stimulation. Thus, the prevalence of an attenuated adrenocortical response to physiologic concentrations of ACTH seems to be high in brain–dead patients, but its clinical implications are not settled. Corticoid treatment of brain–dead donors is
routinely used by many centers, not to substitute adrenocortical failure, but to attenuate immune responses. The rationale for this treatment will be discussed later.

In contrast to anterior pituitary function, posterior pituitary function is clinically lost in as much as 80% of brain–dead organ donors, and diabetes insipidus with electrolyte disturbances, hypovolemia, and circulatory instability may cause major problems in donor management[13, 14]. These observations are in accordance with histological findings in which the pituitary gland shows varying degrees of damage, suggest persistence of partial cerebral blood flow in some part in brain dead patients[16].

**Inflammatory response**

Recent studies investigating the relation between brain death and activation of peripheral organs, have demonstrated that an explosive increase in intracranial pressure in rats up–regulates various lymphocyte –and macrophage–derived cytokines on somatic organs. Rapid activation of leukocyte populations and their associated products have been demonstrated in heart and kidney allografts from brain dead donors before and during the first days after transplantation. Increased cellular infiltrates have been proven to be present in all other organs from brain dead donors suitable for transplantation[4]. Our previous studies and others reported that the systemic inflammatory state is characterized by circulating cytokines including interleukin (IL) family such as IL–1, IL–6, and IL–8; tumor necrosis factor–alpha, kidney injury molecule(KIM)–1, complement, monocyte chemotatic protein (MCP)–1, et al [8, 17–19]. Interleukin 6 (IL–6) is the central cytokine which correlates with the onset and severity of inflammation. These studies emphasize the pathogenic role of IL–6 in transplantation–related injury and indicate that strategies targeting IL–6 induction during the transplantation process could attenuate IRI and improve graft survival. In this review, we will discuss three moments of IL–6 activation during the transplant process: brain death, IRI and as a result of donor condition. Beside these pro–inflammatory proteins, BD also increased the induction of cytoprotection like HO–1, HSP–70 [20–22]. In addition to the presence of chemokines, cytokines and adhesion molecules, major histocompatibility complex (MHC) class II antigen expression is increased, triggering a more rapid and intense host alloimmune response than that mounted against the more inert grafts from living donors.

Although the acute inflammatory response resulted from increased systemic circulating cytokines after BD, the origin of the inflammatory mediators, the links
between BD and the inflammation, and the interaction among these cytokines and chemokines still remain unclear. Several cytokines had been found in traumatic brain tissue and cerebrospinal fluid after head injury; through a deficient blood–brain barrier, they can leak into the blood circulation to activate target cells and somatic organs [23]. Both hemodynamic instability and the sympathetic storm following BD may lead to hypo–perfusion and ischemia in various and consequently activate the cytokine cascade [24 –27]. The interaction among inflammatory cytokines themselves, and chemokines, the adhesion molecules and leukocyte infiltration could amplify the inflammatory cascade further. Possibly the metabolic derangement caused by BD–induced ischemia is another modulation of the inflammatory response.

As we pointed out above, brain death is related with the release of pro–inflammatory substances before engraftment, resulting in histological damage, decreased function, and lower graft survival compared to organs from living donors [19, 28–33].

**Brain dead donor management**

To counteract the hypotension and hypoperfusion in the donor during brain death, the first strategy to keep normal intravascular volume, colloids and crystalloids could be used to maintain mean arterial pressure above 70mmHg [34]. However, there is a lot of information that crystalloid–based fluid treatment could be correlated with neurogenic pulmonary edema; the use of colloids, like HAES, is correlated with kidney toxicity, although the third generation of this products could be less deleterious. Considering to loss of the sympathetic tone, the use of catecholamines is reasonable. Norepinephrine in donors has been related with an improvement of kidney graft survival and non–change in liver graft survival. Schnuelle et al demonstrated that low–dose dopamine is beneficial as pretreatment of brain dead kidney donor. Administration of dopamine reduced the need for dialysis within first week after kidney transplantation [35].

Brain death is believed to result in a neurohormonal dysregulation of hypothalamic–pituitary–adrenal (HPA) axis. The concept of administrating a hormonal cocktail using thyroid hormones, vasopressin, steroids and insulin may improve the function of the potential organ donor [36]. Insulin therapy is a part of the ordinary attention in ICU [37], as well as vasopressin in hemodynamic stability. But thyroid hormone therapy of the brain–dead potential organ donors remained controversial for many years and is still not universally administered [38, 39]. Early data show a benefit with the administration of other hormones, like erythropoietin which could
improve the outcomes in kidney and liver transplant, but there is not enough evidence available.

An approach to reduce inflammation in the brain dead donor is becoming one focus to improve the function of grafts-to-be. The administration of glucocorticoids, like prednisolone, in brain dead donors prior to retrieval was shown to improve the function of donor kidney[40], which was also confirmed by our experiment. But the effect of pretreatment with prednisolone on donor liver, lung, and heart is still controversial, as well as optimal timing and dosage of intervention during donor management. Another approach to reduce the injury resulted from brain death is to induce repair mechanisms. Kotsch et al [21] reported that recipients of organs from brain dead donors treated with cobalt protoporphyrin (CoPP) as the selective inducer of HO–1 survived significantly better than those from untreated brain dead donors. The opposite results were obtained by blockade of HO–1 with zinc protoporphyrin (ZnPP). However, the high expression of protective cytokines and the low expression of deleterious cytokines are changed synchronously after pretreatment on brain dead donor organ, and timing and dosing of administrating drugs may be crucial.
REFERENCES


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33. Nijboer WN, Schuurs TA, van der Hoeven JA, Fekken S, Wiersema–Buist J,
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