

## Influence of brain death and associated trauma on solid organ histological characteristics<sup>1</sup>

### Influência da morte encefálica e do trauma associado nas características histológicas de órgãos sólidos

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#### ABSTRACT

**PURPOSE:** To evaluate histopathological alterations triggered by brain death and associated trauma on different solid organs in rats.

**METHODS:** Male Wistar rats (n=37) were anesthetized with isoflurane, intubated and mechanically ventilated. A trepanation was performed and a balloon catheter inserted into intracranial cavity and rapidly inflated with saline to induce brain death. After induction, rats were monitored for 30, 180, and 360 min for hemodynamic parameters and exsanguinated from abdominal aorta. Heart, lung, liver, and kidney were removed and fixed in paraffin to evaluation of histological alterations (H&E). Sham-operated rats were trepanned only and used as control group.

**RESULTS:** Brain dead rats showed a hemodynamic instability with hypertensive episode in the first minute after the induction followed by hypotension for approximately 1 h. Histological analyses showed that brain death induces vascular congestion in heart (p<0.05), and lung (p<0.05); lung alveolar edema (p=0.001), kidney tubular edema (p<0.05); and leukocyte infiltration in liver (p<0.05).

**CONCLUSIONS:** Brain death induces hemodynamic instability associated with vascular changes in solid organs and compromises most severely the lungs. However, brain death associated trauma triggers important pathophysiological alterations in these organs.

**Key words:** Brain Death. Craniocerebral Trauma. Pathology. Rats.

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#### RESUMO

**OBJETIVO:** Avaliar as alterações histopatológicas desencadeadas pela morte encefálica e pelo trauma associado em diferentes órgãos sólidos em ratos.

**MÉTODOS:** Ratos Wistar machos (n=37) foram anestesiados com isoflurano, entubados e mecanicamente ventilados. Foi realizada trepanação e um cateter foi inserido na cavidade intracraniana e insuflado rapidamente para induzir morte encefálica. Após a indução, os ratos foram monitorados por 30, 180 e 360 min para parâmetros hemodinâmicos e exsanguinados pela aorta abdominal. Coração, pulmão, fígado e rim foram removidos e fixados em parafina para avaliação de alterações histológicas (H&E). Ratos falso-operados foram apenas trepanados e usados como grupo controle.

**RESULTADOS:** Ratos com morte encefálica apresentaram instabilidade hemodinâmica com episódio hipertensivo no primeiro minuto após a indução seguido de hipotensão por aproximadamente 1 hora. Análises histológicas demonstraram que a morte encefálica induz congestão vascular no coração (p<0,05) e pulmão (p<0,05); edema alveolar (p=0,001); edema tubular (p<0,05); e infiltrado leucocitário no fígado (p<0,05).

**CONCLUSÕES:** A morte encefálica induz instabilidade hemodinâmica associada com mudanças vasculares em órgãos sólidos e compromete mais severamente os pulmões. Contudo, o trauma associado à morte encefálica desencadeia importantes alterações fisiopatológicas naqueles órgãos.

**Descritores:** Morte Encefálica. Traumatismos Craniocerebrais. Patologia. Ratos.

## Introduction

Brain death (BD) means cessation of all metabolic pathways in central nervous system, but paradoxically, since it installs, a lot of events are triggered on the whole organism and many pathophysiological changes may be seen in different organs<sup>1</sup>. As BD patients are the most important source of allografts for transplantation and the scarcity of donors is an increasing problem in the whole world<sup>2</sup>, understanding these alterations will contribute to improving management, optimizing outcomes and providing organs that, without treatment, would be unsuitable for transplantation.

The pathophysiological changes that occur during and after BD include hemodynamic, hormonal and inflammatory events. Moreover, vasoconstriction triggered by catecholamine storm leads to peripheral ischemia impairing the organs perfusion. However, many factors associated with BD which occur before it, such as multi-trauma, hemorrhage and hypoxia can trigger different pathophysiological responses in the organs<sup>3-5</sup>.

The present study aims to differentiate the features triggered by BD itself on the principal solid organs commonly used for transplantation in a rodent model, comparing them with sham-operated animals submitted to the same time points of observation.

## Methods

Male Wistar rats weighing 250-350g (n=37) were used and randomized into 6 groups: sham-operated rats monitored for 30 (SH30, n=7), 180 (SH180, n=6), and 360 min (SH360, n=5); brain-dead rats monitored for 30 (BD30, n=7), 180 (BD180, n=7), and 360 min (BD360, n=5). The animals were maintained at 23°C ± 2°C under a cycle of 12 h light/12h darkness and allowed access to food and water *ad libitum* before the experimental procedure. The experimental protocols were approved by the Animal Subject Committee of the Heart Institute (InCor) of the São Paulo University Medical School.

Rats were anesthetized in a chamber with 5% isoflurane, intubated and ventilated with a rodent ventilator (Harvard Apparatus, model 683, USA) with tidal volume of 10 mL/kg, and frequency of 70 breaths/min. The animals were maintained with continuous inhalation of 2% isoflurane. The carotid artery was cannulated for continuous blood pressure monitoring and blood sampling. The jugular vein was cannulated for infusion of saline solution.

## Brain death model

Brain death was induced by rapid inflation of a catheter Fogarty-4F (Baxter Health Care Co., USA) with 500 µL of saline solution, through a drilled parietal burr hole. BD was confirmed by maximal pupil dilatation, apnea, absence of reflex, and drop of mean arterial pressure (MAP). After BD induction the anesthesia was stopped. Sham-operated animals were trepanned only, with no catheter insertion, and the anesthesia was maintained throughout the experiment. BD rats received an infusion of saline solution (2mL/h) through the jugular vein to minimize dehydration.

## Histopathological analyses

After 30, 180, or 360 min, animals were exsanguinated from the abdominal aorta, and heart, lung, liver and kidneys were removed, fixed in formalin, and paraffin embedded. Samples were cut into 4µm sections, and stained with hematoxylin and eosin (H&E). The following features were evaluated: vascular congestion, leukocyte infiltration, edema, and alveolar collapse. Analyses were performed by two researchers and the score used to measure the intensity of tissue alterations was 0, 1, 2, 3 (absent, slight, moderate, and intense, respectively).

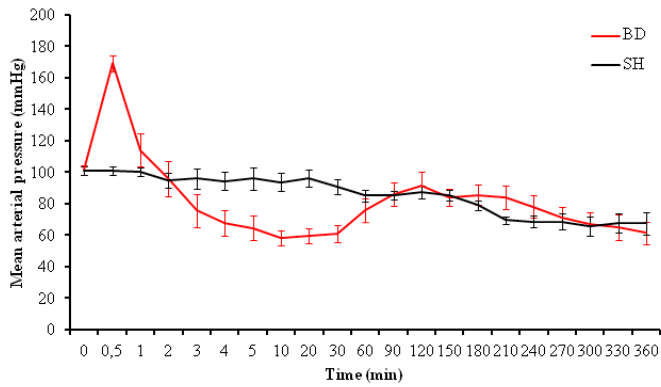
## Statistical analyses

All data are presented as median and upper/lower limits. The overall group differences were compared using a multivariate general linear model with group and time as the factors with a post hoc Bonferroni test. A p-value of less than 0.05 was considered to be significant. All statistical analyses were performed using SPSS for Windows, version 17.0 (SPSS, Chicago, IL, USA).

## Results

### Hemodynamic parameters

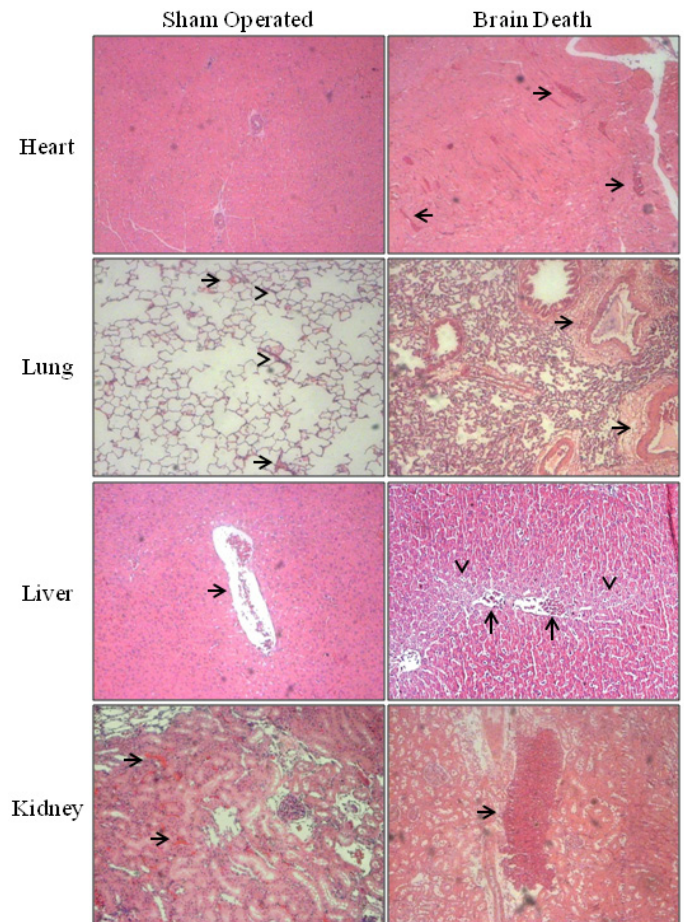
All rats submitted to BD induction showed an immediate increase of the mean arterial blood pressure (MAP) in the first minute after catheter inflation, followed by a decrease in that parameter below baseline for approximately 1h (Figure 1). In sham-operated rats no differences were observed in the MAP. There were no significant differences in heart rate between groups. Moreover, no differences were observed in the arterial blood gases, electrolytes, lactate and hematocrit values in among groups (data not shown).



**FIGURE 1** - Mean arterial pressure of brain-dead (BD) and sham-operated (SH) rats 360 min after surgical procedures. The animals were monitored over time. The data are presented as the mean  $\pm$  SEM.

*Histopathological evaluation*

All histological parameters evaluated are presented in Table 1. BD rats presented higher level of myocardial vascular congestion at 30, 180, and 360 min compared with sham-operated rats. However, no more significant alterations were observed in this organ after BD induction (Figure 2).



**FIGURE 2** - Microphotographs of heart, lung, liver, and kidney from brain-dead (BD) and sham-operated (SH) rats. Arrows indicate vascular congestion in heart (BD), liver (BD and SH), and kidney (BD and SH). Lungs from SH rats presented vascular congestion (arrows) and alveolar thickening (arrowheads). Arrows in BD lungs indicate vascular congestion with leukocyte infiltration. In liver arrowheads indicate leukocyte infiltration (original magnification x400).

**TABLE 1** - Histological injury scores.

		Brain Death			Sham-operated			Group p value
		30 min	180 min	360 min	30 min	180 min	360 min	
Heart	Vascular Congestion	1 (2-0)	1 (2-0)	1 (2-1)	0 (1-0)	0 (2-0)	0.5 (2-0)	0.020
	Vascular Congestion	2 (2-1)	1 (2-0)	2 (2-1)	0 (1-0)	1 (2-0)	1 (2-1)	0.017
Lung	Alveolar Edema	1 (3-0)	1 (3-0)	0.5 (2-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.001
	Leukocyte Infiltration	1 (2-0)	1.5 (3-0)	1 (2-1)	1 (2-0)	1 (2-0)	1 (2-0)	0.134
	Alveolar Collapse	0 (2-0)	0 (2-0)	0 (3-0)	0 (1-0)	0 (0-0)	0 (0-0)	0.056
Liver	Vascular Congestion	2 (2-1)	2 (2-1)	1 (2-1)	1 (2-1)	1 (2-1)	1 (2-1)	0.111
	Intracellular Edema	1.5 (3-0)	1.5 (2-1)	1 (2-1)	1 (2-0)	0.5 (2-0)	1 (2-1)	0.067
	Leukocyte Infiltration	1 (1-0)	0.5 (1-0)	1 (2-0)	0 (0-0)	0 (1-0)	1 (1-0)	0.011
Kidney	Vascular Congestion	2 (2-1)	2 (2-1)	2 (2-1)	1 (2-1)	1 (2-1)	2 (2-1)	0.059
	Tubular Edema	0.5 (1-0)	1 (2-0)	1 (2-0)	0 (0-0)	0 (0-0)	1 (2-0)	0.039

The score used to measure the intensity of tissue alterations was 0=absente; 1=slight; 2=moderate; 3=intense. Values are presented as median and upper/lower limits for 5-7 rats/group.

In the lungs, vascular congestion was observed from the first 30 min in all groups. This observation was more expressive in BD rats compared with sham-operated rats (Figure 2). Although it was observed a slight to moderate leukocyte infiltration in BD rats, this feature was not statistically different when compared with sham-operated animals (Table 1). Alveolar edema was documented only in BD rats at different time points of observation. Nevertheless, BD rats demonstrated a wide variation in the assessment of alveolar collapse, resulting in no difference when compared with sham groups (Table 1).

Animals submitted to BD show a moderate, but persistent, leukocyte infiltration in the liver, while sham-operated rats did not show this feature (Table 1). Interestingly, intracellular edema and vascular congestion were observed in BD and sham rats with no differences among groups (Figure 2).

In the kidney, BD rats presented more expressive tubular edema than sham-operated rats (Table 1). Kidneys from BD rats presented moderate vascular congestion, whereas sham-operated animals showed slight to moderate levels of this parameter, resulting in no statistical difference among groups (Figure 2).

## Discussion

Many studies have been performed to evaluate the role of BD in graft outcomes. However, it remains unclear what is the role of BD-associated trauma in the organs suitable to transplantation.

Data presented herein indicate that BD induces a hypertensive episode with MAP values rising twice compared with baseline. We also observed that vascular alterations were a common issue presented in all experimental groups. However, premature and more pronounced histological alterations were observed in BD rats when compared to sham-operated rats, including heart and lung vascular congestion, lung alveolar edema, kidney tubular edema, and liver leukocyte infiltration.

Recently, our group described alterations in the mesenteric perfusion of BD rats associated with higher expression of intercellular adhesion molecule (ICAM)-1, and augmentation of leukocyte migration. These findings suggest that the hypoperfusion triggered by BD, as well as leukocyte infiltration, can impair the viability of remote organs. However, the BD-associated trauma was responsible for increases in serum cytokine levels and neutrophil/lymphocyte ratio which suggest activation of the immunologic system, while these parameters were not significantly influenced by BD itself<sup>6</sup>.

Our findings indicate that histological alterations in the heart, although not so pronounced, seem to be associated with BD induction, as documented by a slight, but significant difference on vascular congestion. In a similar model, but using rabbits, Yeh *et al.*<sup>7</sup> found many histological manifestations in the heart, including cytoplasmic clearing, loss of myofibrillar striations and contraction banding. In a model of gradual onset of brain death in rats, Wilhelm *et al.*<sup>8</sup> found no morphological alterations in hearts after 6h. Comparing those both publications to our findings, some

degree of discrepancies may have occurred due to methodological differences and species particularities.

Brain death leads to damage in the lung function through hemodynamic, inflammatory, and neurohumoral mechanisms<sup>9</sup>. Pulmonary dysfunctions may be associated with other events not related to BD such as aspiration, pneumonia, contusion, and ventilator-induced injury. However, it is known that BD induces a neurogenic pulmonary edema, which can occur immediately after neurological insult<sup>3</sup>. In this study, BD rats showed an important alveolar edema, which was observed from 30 min after BD induction, while sham operated animals did not present edema up to three hours of mechanical ventilation. In a different model to evaluate edema, Rostron *et al.*<sup>10</sup> found differences in the lung wet/dry weight ratio two and five hours after BD induction versus sham operated rats. On the other hand, leukocyte infiltration was similar in both conditions at different time points as demonstrated in the present study. Sham-operated rats also presented slight but persistent vascular congestion after 3 hours, although lower than that observed in BD rats. These features indicate that the trauma and the artificial conditions in which patients and animals were maintained can also compromise the lungs, worsening the quality of the organ.

Although kidney and liver transplantation may be performed from living donors, the large patient queues waiting for organ transplantation lead to acceptance of organs from BD donors<sup>11</sup>. In our study, the liver of BD rats showed higher leukocyte infiltration compared with sham-operated rats. The augmentation of leukocyte migration is in accordance with van der Hoven *et al.*<sup>12</sup>. In that study, rats submitted to BD and observed after one and six hours present an increase in adhesion molecules expression, such as VCAM-1 and ICAM-1, and leukocyte migration to the perivascular tissue. Interestingly, our data additionally showed slight to moderate vascular congestion and intracellular edema in sham-operated rats, indicating that the surgical procedures also induced impairment of the hepatic circulation and cellular damage.

In another study on kidney histology, van der Hoven *et al.*<sup>13</sup> found similar results to those observed previously on liver, suggesting that BD induces a progressive immune activation with higher expression of adhesion molecules and influx of leukocytes to perivascular tissue in the kidney. However, in the present study there were no differences in kidney leukocyte migration under BD induction in comparison to sham-operated rats. Nevertheless, moderate tubular edema was observed only in the kidneys of BD groups. Interestingly, BD associated trauma induced an important vascular congestion, similarly to BD itself, also indicating that the associated trauma induces damage on the kidneys.

## Conclusions

The brain death leads to hemodynamic instability and vascular changes in all solid organs. The lungs are most severely compromised under this situation due to the development of significant alveolar edema. On the other hand, brain death associated trauma seems to be also responsible for triggering important pathophysiological changes in solid organs.

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