Original Paper

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Initial hepatosplanchnic blood flow distribution and oxygen metabolism in experimental model of hypotensive brain death

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Summary

Background: Organs from the so-called marginal donors have been used with a significant higher risk of primary non function than organs retrieved from the optimal donors. We investigated the early metabolic changes and blood flow redistribution in splanchnic territory in an experimental model that mimics marginal brain-dead (BD) donor.

Material/Methods: Ten dogs (21.3±0.9 kg), were subjected to a brain death protocol induced by subdural balloon inflation and observed for 30 min thereafter without any additional interventions. Mean arterial and intracranial pressures, heart rate, cardiac output (CO), portal vein and hepatic artery blood flows (PVBF and HABF, ultrasonic flowprobe), and O_2-derived variables were evaluated.

Results: An increase in arterial pressure, CO, PVBF and HABF was observed after BD induction. At the end, an intense hypotension with normalization in CO (3.0±0.2 *vs.* 2.8±2.8 L/min) and PVBF (687±114 *vs.* 623±130 ml/min) was observed, whereas HABF (277±33 *vs.* 134±28 ml/min, $p<0.005$) remained lower than baseline values.

Conclusions: Despite severe hypotension induced by sudden increase of intracranial pressure, the systemic and splanchnic blood flows were partially preserved without signs of severe hypoperfusion (i.e. hyperlactatemia). Additionally, the HABF was mostly negatively affected in this model of marginal BD donor. Our data suggest that not only the cardiac output, but the intrinsic hepatic microcirculatory mechanism plays a role in the hepatic blood flow control after BD.

Key words **brain death • hemodynamic • splachnic perfusion • metabolic effects • organ function**

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BACKGROUND

The shortage of donor organs is still one of the central problems in transplantation. Despite an increase in the use of organs from living and after cardiac death donors, the majority of the organs used for transplantation are still derived from heart-beating brain dead donors [1–4].

Accumulating evidence has revealed that brain death (BD) is associated with hemodynamic, metabolic, and hormonal changes that may lead to altered cardiovascular physiology and metabolism with serious impact on organ quality and graft outcome [5–8]. This assumption has led various investigators to recommend hormonal replacement and inotropic and/or vasoactive therapy in potential organ donors in order to preserve organ function and improve the graft and patient survival rates [9–11].

Recently, the increase of patients requiring an organ graft has resulted in the acceptance of less than optimal organs from hemodinamically unstable patient. Organs from the so-called marginal donors have been used with a significant higher risk of primary non function than organs retrieved from the optimal donors [12–15].

In spite of a variety of brain dead animal models previously studied [16–20], the initial splanchnic hemodynamic and metabolic alterations following brain death has not been yet sufficiently investigated in a large animal model. Nevertheless, hepatosplanchnic anatomy and physiology are highly complex and the lack of an accessible and noninvasive tool to monitor the perfusion of this vascular bed limited the evaluation of this territory in the clinical setting.

Therefore, we designed an experimental protocol in order to investigate the early hemodynamic and metabolic changes in splanchnic bed in an experimental model that mimics marginal brain-dead donor.

MATERIAL AND METHODS

This study was approved by the Animal Care and Use Committee of the University of Sao Paulo Medical School and conducted in compliance with the guidelines of the National Regulations for the Care and Use of Laboratory Animals.

Animal preparation

Ten adult mongrel dogs, weighing 21.3±0.9 kg were fasted for 12 hours before the study, with free access to water.

Anesthesia was induced with an intramuscular injection of 50mg/kg of ketamine and was maintained with halothane at 1.5% by pressurized and warmed vaporizer Ohmeda Tec-6 (Ohmeda, Madison, WI). A cuffed endotracheal tube was placed into the trachea to allow mechanical ventilation with fractional inspired oxygen (FiO_2) of 1.0 and a tidal volume of 10 ml/kg (670 Takaoka ventilator, Sao Paulo, Brazil). The respiratory rate was adjusted to maintain an initial arterial pCO_2 at 40±5 mmHg.

A urinary bladder catheter was placed for urinary drainage and a heating pad was used to maintain normothermia. To compensate fluid losses, isotonic saline solution was maintained during surgical preparation, at 10 ml/kg/h infusion rate.

The right femoral artery was dissected and cannulated with a polyethylene catheter (PE240) and its tip placed at the level of the abdominal aorta to measure mean arterial pressure (MAP) as well as to collect arterial blood samples. Through the right common femoral vein, a polyethylene catheter (PE240) was introduced into the inferior vena cava for fluid administration.

A balloon-tipped catheter 93A-13IH-7F (Baxter Edwards Critical Care, Irvine, CA, USA) with thermal filament was inserted into the pulmonary artery through the right external jugular vein under guidance of pressure waves. This catheter was then connected to a cardiac computer (Vigilance, Baxter Edwards Critical Care), to measure cardiac output, mean pulmonary arterial pressure (MPAP), and pulmonary capillary wedge pressure (PCWP). This catheter was also used to collect mixed venous samples for blood gas analysis.

The catheters, placed into the abdominal aorta and pulmonary artery, were connected to disposable pressure transducers (Transpac Disposable Transducer, Abbott, Chicago, IL) and to a computerized multichannel system for biological data acquisition (Acknowledge® III MP 100 WSW, Biopac Systems, Inc. Goleta, CA).

Another catheter (a modified Fogarty 5F) was inserted through the right external jugular vein

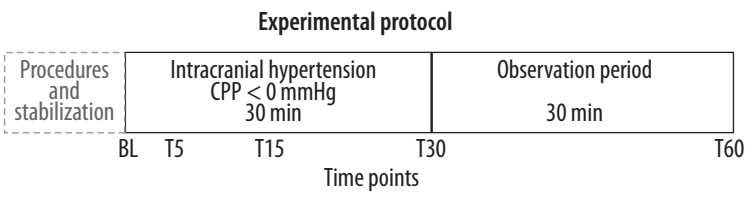

Figure 1. Schematic presentation of the experimental protocol. At each timepoint (BL, T5, T15, T30 and T60) hemodynamic variables were measured. Blood samples were collected at BL and T60.

and its tip placed in the right suprahepatic vein in order to collect blood samples.

Through a midline laparotomy, the spleen was removed and a fluid-filled polyethylene catheter (PE240) was placed into the portal vein through the splenic vein to draw blood samples. A transit time ultrasonic flowprobes (Transonic System Inc., Ithaca, NY, USA) were placed around the portal vein and hepatic artery and connected to a flowmeter (PVBF and HABF; T206 Transonic Volume Flowmeter, Transonic Systems, Inc, Ithaca, NY, USA).

Brain death model

The brain death was induced by consecutive insufflations of a balloon catheter in the epidural space with a controlled and standardized herniation of the brain stem described as explosive brain death [8,20–22].

For intracranial catheter placement, the skin covering the skull was locally removed and two drill holes (left and right temporal) were created (Ø 8 mm) using an electric drilling machine (Black & Decker, mod S 2A and a drill Mackenzie). A measurement probe, for detection of intracranial pressure (ICP) (mod. 110-4G, Camino Laboratories, San Diego, California, USA) was inserted through the left temporal hole and connected to a monitor (Camino Laboratories, San Diego, California model V 420). The balloon catheter (8 French, Foley catheter, Kendall Co., Germany) was inserted into the epidural space through the right temporal hole. ICP was then elevated in a standardized fashion by a stepwise filling of the balloon with 6 to 15 ml of saline.

After a 30 min period with constant ICP elevation above systolic arterial pressure (cerebral pressure perfusion <0 mmHg, T0–T30), BD was confirmed by evaluation of neurological reflexes (corneal-palpebral, papillary, and oculomotor) atropine test, and the absence of respiratory drive. No other drugs or interventions were employed thereafter. The animals were observed for an additional 30 min (observation period, T30 to T60) and then killed with an overdose of pentobarbital and potassium chloride (Figure 1).

Measured variables

Mean systemic and pulmonary arterial pressures, heart rate, ICP, hepatic artery, and portal vein blood flows were continuously recorded. Cardiac output was determined using 3-ml bolus injections of isotonic saline at 20°C. Each determination was the arithmetic mean of three consecutive measurements when their differences did not exceed 10%. Central venous blood temperature was recorded from the thermistor in the pulmonary artery catheter.

Arterial, portal, hepatic, and mixed venous base deficit, pH, pCO_2, oxygen tension, oxygen saturation, hemoglobin, hematocrit, bicarbonate levels, were all obtained at baseline (BL) and 60 minutes after BD induction (T60). All blood samples were analyzed immediately after their collection by a Stat Profile Ultra Analyzer (Nova Biomedical, Waltham, MA). Sodium, potassium, glucose plasma concentration and lactate level were obtained from arterial sample at the same timepoints (Figure 1).

Blood samples were also frozen at 20°C for further analysis of albumin, creatinine, creatine phosphokinase (CPK), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by Cobas Mira Plus CC (Roche CH 6343, Rotkreuz-Switzerland).

Systemic, splanchnic, and hepatic oxygen delivery, consumption and extraction (DO_2syst, VO_2syst, O_2ERsyst, DO_2splanch, VO_2splanch, O_2ERsplanch, DO_2hepatic, VO_2hepatic and O_2ERhepatic, respectively) were calculated using standard formulae. Cerebral perfusion pressure (CPP) was calculated by the difference between intracranial and mean arterial pressures.

Experimental protocol

After surgical preparation, the animals were allowed to stabilize for 45 minutes. The hemody-

Table 1. Intracranial pressure, heart rate, mean pulmonary arterial and pulmonary capillary wedge pressure (MPAP and PCWP), central temperature, and portal vein and hepatic artery blood flows/cardiac output ratio during experimental protocol (n=10).

	BL	T5	T15	T30	T60
Intracranial pressure, mmHg	19±4	274±16.6*	194±25.2*	137±12.3*	37±3.3*
Heart rate, bpm	142.9±8.1	104.1±16.4*	131.9±9.5	142.6±9.1	127,6±8.7
PCWP, mmHg	5±1.1	16.8±3.7*	8.6±2.1*	6.9±1.6	5.8±1.3
MPAP, mmHg	15.6±1.4	25.9±2.4*	21.7±3.2*	17.9±2.6	16.5±1.5
Central temperature, °C	36.3±0.7	36.3±0.8	36.4±0.7	36.5±0.7	36.5±0.7
PVBF/CO%	22.9±3.5	24.1±6.8	27.1±3.0	24.9±2.9	22.4±2.7
HABF/CO%	9.5±1.3	14.4±1.7*	8.8±2.0	5.4±0.8*	5.4±1.1*

Baseline (BL), 5, 15 and 30 minutes after brain dead induction by intracranial hypertension (T5, T15 and T30) and 30 minutes after brain dead (T60). Data are presented as mean ± standard error of the mean. *p <0.05 *vs* baseline.

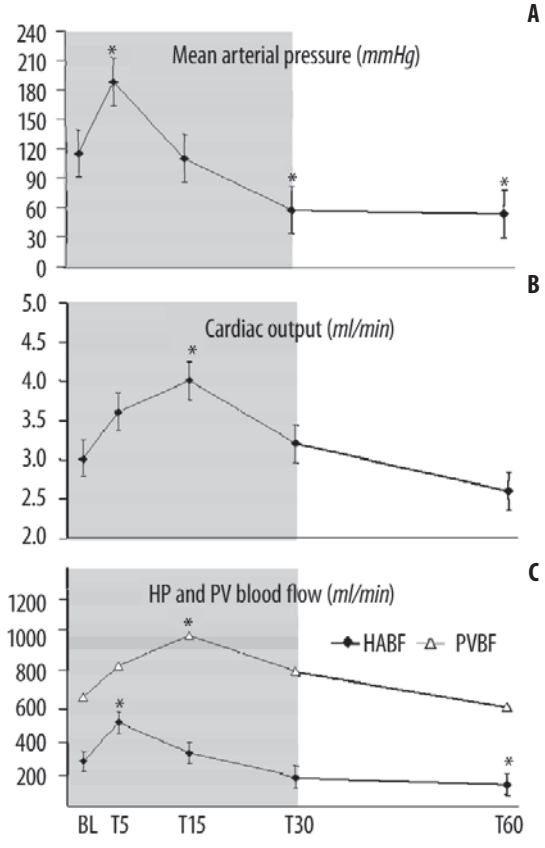

Figure 2. Hemodynamics variables measured during the experiment (n=10): (**A**) mean arterial pressure (mmHg), (**B**) cardiac output (ml/min) and (**C**) hepatic arterial and portal vein blood flows (HABF, PVBF; ml/min). Data are shown as mean ±SEM; *p<0.05 vs. baseline.

namic measures such as heart rate, cardiac output, pressures (MAP, MPAP, PCWP, ICP), as well as cerebral perfusion pressure (CPP), central temperature, and regional blood flows (portal vein and hepatic artery blood flows) were recorded at

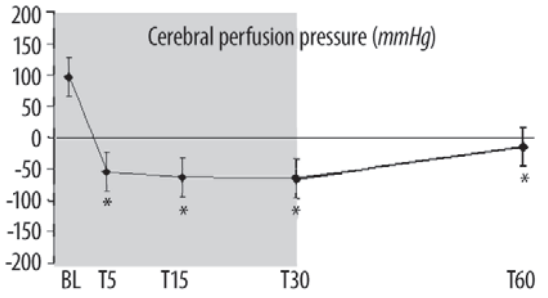

Figure 3. Cerebral perfusion pressure (mmHg) during the experimental protocol (n=10). Data are shown as mean ±SEM, *p<0.05 vs. baseline.

baseline (BL), during the period of intracranial hypertension (T5, T15, T30), and 30 minutes after balloon deflation (T60) (Figure 1).

Statistical methodology

Data were expressed as mean ± standard error of the mean. We used the t-student test which was controlled by the Levine test objectifying no differences among the variables in comparison with the averages values of the variables observed. Differences were considered statistically significant when p<0.05.

Results

All animals were hemodynamically stable at baseline (Table 1, Figure 2). The increase and maintained intracranial pressure above systolic arterial pressure resulted in a negative cerebral perfusion pressure throughout BD induction protocol (Figure 3). The acute intracranial hypertension induced an immediate and short period of intense bradycardia (142±8.1 to 36±5.1,

Table 2. Hemoglobin and lactate levels, arterial pH, HCO_3 and pCO_2 at baseline and at end of the experimental protocol (n=10).

	BL	T60
Arterial Hb, g/dL	13±0.8	12.3±0.8
Arterial lactate, mmol/L	0.6±0.3	0.9±0.4
Arterial pH	7.38±0.03	7.24±0.03*
Arterial HCO_3, mEq/L	24.5±0.8	24.5±0.7
Arterial pCO_2, mmHg	42.4±3	60.5±5.7*

Baseline (BL) and 30 minutes after brain dead (T60). Data are presented as mean ± standard error of the mean. *p <0.05 *vs* baseline.

Table 3. Systemic and regional calculated oxygen-derived variables at baseline and at end of the experimental protocol (n=10).

		BL	T60
Systemic	DO_2syst, ml/min	520.1±51.8	411.4±34.6*
	VO_2syst, ml/min	98.2±10.2	86.9±5.8
	O_2ERsyst,%	17.8±1.6	20.5±1.6
Splanchnic	DO_2splanch, ml/min	130.8±9.6	108.7±8.2*
	VO_2splanch, ml/min	16.9±2.7	15.9±2.1
	O_2ERsplanch,%	15.2±1.8	18.1±2.6
Hepatic	DO_2hepat, ml/min	160.4±16	113.8±18.7*
	VO_2hepat, ml/min	25.6±4.8	24.2±9
	O_2ERhepat,%	17.6±2.8	27.4±5.7*

DO_2syst – systemic oxygen delivery; VO_2syst – systemic oxygen consumption; O_2ERsyst – systemic oxygen extraction rate; DO_2splanch – splanchnic oxygen delivery; VO_2 splanch – splanchnic oxygen consumption; O_2ERsplanch – splanchnic oxygen extraction rate; DO_2hepat – hepatic oxygen delivery; VO_2hepat – hepatic oxygen consumption; O_2ERhepat – hepatic oxygen extraction rate. BL – baseline; T60 – 30 minutes after brain dead. Data are presented as mean ± standard error of the mean. * p <0.05 *vs* baseline.

$p<0.05$ / first 2 min after induction of BD), accompanied by a transient hyperdynamic state in all animals, characterized by a significant increase in mean arterial pressure, cardiac output, mean pulmonary arterial and cardiac filling pressures (Table 1 and Figure 2). At the end of brain death induction, an intense hypotension with normalization in cardiac output and heart rate was observed (Table 1, Figure 2).

At regional level, in the hyperdynamic phase, an initial and transient increase in portal vein and hepatic artery blood flows was observed (Figure 2). Afterwards, the portal vein blood flow returned to baseline values, whereas the hepatic artery blood flow remained lower then baseline value (Figure 2).

Table 4. Laboratorial and electrolytic variables at baseline and at end of the experimental protocol (n=10).

	BL	T60
CPK, UI	581.6±95.7	876.3±146*
AST, U/L	51.1±7.4	54.1±7
ALT, U/L	48.3±11.4	44.9±8.9
Creatinine, mg/dL	0.8±0.1	0.9±0.1
sodium, mEq/L	148.3±0.9	148.9±0.8
potassium, mEq/L	3.5±0.1	3.7±0.2
glucose, mg/dL	102.3±3.4	122.6±10.9

CPK – creatine phosphokinase; AST – aspartate aminotransferase; ALT – alanine aminotransferase. Baseline (BL) and 30 minutes after brain dead (T60). Data are presented as mean ± standard error of the mean. *p <0.05 *vs* baseline.

In spite of the reduction in systemic and regional oxygen delivery at the end of experimental protocol, no significant changes in oxygen consumption where observed. A significant increase in hepatic oxygen extraction was observed 30 minutes after balloon deflation. Lactate level remained unchanged at the end of experiment (Tables 2 and 3).

No significant changes in hemoglobin level, creatinine, ALT, AST, sodium, potassium and glucose were observed at T60 (Tables 2 and 4). A significant increase in arterial pCO_2 leading to a respiratory acidosis accompanied the hemodynamic changes during the experimental protocol. No variation in bicarbonate level was detected (Table 2). CPK levels show a significant increase after induction of brain death (Table 4).

Discussion

There have been few studies evaluating the initial hepatosplanchnic hemodynamic and metabolic alterations following brain death in a large animal model. In this study we evaluate the systemic and regional changes in a model that mimics marginal brain-dead donor (hypotensive). We were able to demonstrate that despite severe hypotension the systemic (i.e. cardiac output) and hepatosplanchnic flows were partially preserved. Additionally, we observed at regional level that

hepatic arterial blood flow is mostly negatively affected by hemodynamic changes induced by the brain death when compared with venous (i.e. portal) blood flow.

The model presented simulates the clinical findings in most patients with brain death due to a sudden increase in intracranial pressure. As described by others, in BD the cardio-pulmonary alterations normally occur in two phases: an initial systemic hyperdynamic phase related to the brainstem herniation and secondly, hypodynamic phase associated with poor regional perfusion [15,22,23].

The hyperdynamic state is associated with a significant increase of mean arterial pressure (62.8%), cardiac output (33.3%), and regional flows (PVBF 56.7% and HABF 88%). These changes are related with a brainstem herniation and significant increase in plasmatic concentration of catecholamines. Chen et al. have demonstrated in a canine model of brain death a sudden increase in circulating concentrations of dopamine, epinephrine, and norepinephrine (800%, 700% and 100%, respectively) 15 minutes after the induction of intracranial hypertension [8].

The hyperdynamic state induced by the sudden increase in ICP can be associated with different heart injuries, including myocardial transient ischemia, infarction, and arrythmias that could lead in some instances, to hemodynamic deterioration and death [24].

Different models can be used to address the impact of brain death in the graft function and survival rates. Experimentally, the extent of catecholamine release, as well as the systemic hemodynamic changes, depends on the rate of rise of intracranial pressure; i.e. the technique used for the production of brain death [25,26]. Kolkert et al. described a gradual onset of brain death models in rats with a progressive inflation (16 ml/minute) of intracranial balloon. In studies which the intracranial hypertension is induced gradually the systemic hemodynamic changes are not as sudden and intense [26].

In the present study we induce a rapid and progressive increase in intracranial pressure (19±4 to 274±16.6 mmHg) in order to obtain a model that could reproduce the hemodynamic changes observed in marginal brain-dead donors. In clinical setting a sudden increase of ICP in the donors (as observed in patients with acute intracerebral hemorrhage) has also been associated with poor graft survival, probably associated with regional hypoperfusion and/or uncontrolled activation of inflammatory mediators in splanchnic territory [27].

We also identify a significant elevation in arterial pCO_2 with respiratory acidosis at the end of the experiment probably due to an increase in left and right ventricle post-load by systemic and pulmonary hypertension, secondary to the autonomic storm. The sudden increase of mean pulmonary arterial and the pulmonary capillary wedge pressure by 66 and 136% respectively, can support the hypothesis of congestion and neurogenic pulmonary edema. Previous studies have demonstrated an increase of 10% in pulmonary extravascular fluid in dogs subjected to a brain death protocol [15].

The physiopathological mechanisms implicated in the "hypodynamic" phase are caused by the imbalance between the sympathetic and vagal stimulation of rostral ventrolateral medulla with consequent loss of vascular tonus and hypotension [26,28]. The sudden onset brain death models frequently required inotropic support. Organ donors who required catecholamine support have been shown to be deficient in vasopressin [29]. Therefore, vasopressin has been recommended by American College of Cardiology for hemodynamic support and the treatment of diabetes insipidus in cadaveric donors [30,31].

We did not perform a hemodynamic resuscitation neither using fluids nor vasoactive drugs. However, our goal was to address the acute impact of brain dead on hepatosplanchnic circulation, allowing us to detect the physiologic blood flow redistribution before any pharmacological intervention.

In the present study, we demonstrated that the hyperdynamic phase was characterized by an increase of the regional perfusion followed by a reduction and maintenance of the hepatosplanchnic blood flow close to the baseline values during the hypodynamic period. These changes on splanchnic blood flow were not able to promote any significant alteration on oxygen consumption, in spite of serious systemic hypotension.

The severe hypotension observed in our protocol (115.3±6.3 mmHg to 53.8±3.1 mmHg; BL *vs.* T60) presented little repercussion in splanchnic blood flow, as could be demonstrated by no significative change in portal vein blood flow. It

should be considered that the splanchnic blood flow is regulated especially, but not only, by cardiac output which was maintained after intracranial balloon deflation. This phenomenon could explain why the regional blood flow suffers only a slight reduction despite the severe hypotension. Additionally, the "exclusion" of cerebral circulation could have led to blood flow redistribution preferable to the hepatosplanchnic territory. This is an interesting hypothesis, since this phenomenon is different from other shock states (septic or hemorrhagic shock) in which cerebral blood flow needs to be maintained in detriment of perfusion in other territories [32–35].

Despite preservation of global oxygen metabolism, it should be highlighted that PVBF was better preserved than the hepatic artery blood flow. A significant reduction of HABF/CO ratio (9.5±1.3% *vs.* 5.4±1.1%) and preservation of PVBF/CO ratio (22.9±3.5% *vs.* 22.4±2.7%) was observed after BD induction. These findings suggest that not only the cardiac output but the intrinsic hepatic microcirculatory mechanism play a role in the hepatic blood flow control after brain-death. In a recent study Okamoto et al have shown that BD induces a deleterious change in liver microcirculation and function independent of the systemic blood pressure [36].

We observed a significant increase on O_2ER hepatic that can be related to a reduction of the arterial blood flow to the liver. The intrinsic autoregulatory mechanism is able to maintain hepatic oxygen delivery through compensatory increases in hepatic artery blood flow (i.e. hepatic artery buffer response) was not observed in this study [37–39].

The main reason for these findings was the maintenance of portal vein blood flow close to the baseline values throughout the experiment.

Previous studies in animals have demonstrated that the liver metabolism is tolerant to marked hypotension after BD induction [40]. However, none of these studies evaluated precisely the splanchnic blood flow redistribution and oxygen metabolism in a model of hypotensive brain death.

There are some limitations in our experimental model and protocol. The animals are not genetically selected; otherwise they can reproduce the diversity of potential patients with BD. Additionally, the short observation time makes it impossible to analyze the later impact of the brain dead on the development of organ failure. Prolonged observation period, inotropic agents and other interventions to improve the organ preservation are been evaluated in specifically designed experimental studies by our group.

Conclusions

In summary, we were able to demonstrate that despite severe hypotension induced by brain death the systemic (i.e. cardiac output) and hepatosplanchnic flows were partially preserved. Additionally, we observed at regional level the hepatic arterial blood flow is mostly negatively affected by the hemodynamic changes induced by brain death. Further investigations are required, including longer follow-up periods to appraise the real impact of brain death on the development of multiple organ dysfunction.

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