

DIPLOMARBEIT

ESTABLISHING A BRAIN DEAD DONOR MODEL IN PIGS

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EIDESSTATTLICHE ERKLÄRUNG

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwende habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

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ZUSAMMENFASSUNG

EINLEITUNG: Die Organtransplantation ist heutzutage die Therapie der Wahl für viele Erkrankungen, die zum Organversagen und somit zum Tode führen würden. Der Großteil der Organe stammt von hirntoten Spendern. Der Hirntod selbst führt allerdings zu einer Reihe von morphologischen und funktionellen Veränderungen in allen Organsystemen, die die Organe stark schädigen können. Es wurde daher eine Reihe von therapeutischen Maßnahmen entwickelt um den negativen Effekten des Hirntodes entgegen zu wirken und die Anzahl der Organe, die transplantiert werden können zu maximieren. Um die Auswirkungen des Hirntodes und die Wirksamkeit der Spenderpflege zu erproben wurde eine Reihe von Hirntodmodellen an Tieren entwickelt.

ZIEL DER STUDIE: In dieser Studie wurde versucht ein neues Hirntodmodell am Schwein zu entwickeln, das eine weitere Erforschung der pathophysiologischen Abläufe während und nach dem Hirntod ermöglicht und genutzt werden kann, um neue Strategien in der Spenderpflege zu erforschen.

METHODEN: An 13 steirische Edelschweine wurde der Hirntod durch Aufblasen eines intrakraniell eingebrachten Katheters herbeigeführt und mittels Apnoe- Test und EEG bestätigt. Während des Hirntodes wurden die Tiere intensivmedizinisch betreut und die Werte von Blutdruck, Puls, Flüssigkeitszu- und ausfuhr notiert. Weiters wurden in regelmäßigen Abständen arterielle Blutproben entnommen und analysiert. Am Ende des Experiments wurde bei 7 Schweinen eine Multiorganentnahme durchgeführt.

ERGEBNISSE: Die Blutdruckwerte wiesen während der Induktion des Hirntodes (HT) eine große Spannweite auf (68/47 bis 216/10 mmHg). Die höchsten Blutdruckwerte fanden sich 5 Stunden nach HT ($115 \pm 15 / 63 \pm 12$ mmHg), die niedrigsten 16 Stunden nach HT ($78 \pm 18 / 41 \pm 1$ mmHg). Der höchste Puls wurde 2 Stunden nach HT (147 ± 21 /Minute), der niedrigste wurde vor dem HT beobachtet (93 ± 18 /Minute). Die größte Urinausscheidung trat 7 Stunden nach HT auf (489 ± 304 ml/Stunde). Die Flüssigkeitsbilanz aller Schweine war positive mit $+2407 \pm 956$ ml. Ebenfalls analysiert wurden die Verläufe von arteriellen Blutgasen, pH-Wert, Bikarbonat, Laktat, Hämoglobin, Hämatokrit, Natrium, Kalium und arterieller Sauerstoffsättigung.

DISKUSSION: Es ist uns gelungen ein neues Hirntodmodell am Schwein zu definieren, das eine längeren Beobachtungszeit aufweist und die Möglichkeit bietet pathophysiologische Vorgänge während und nach dem Hirntod zu beobachten und die Anwendung moderner Maßnahmen der Spenderpflege gestattet.

ABSTRACT

INTRODUCTION: Today organ transplantation is the therapy of choice for many diseases that may lead to fatal end stage organ failure. Most of the transplanted organs are taken from brain dead organ donors. Brain death itself, however, leads to many morphological and functional changes in all organ systems that can severely compromise organ quality. Therefore a number of therapeutic strategies have been developed to diminish the detrimental effects of brain death and to maximize the number of organs available for transplantation. To investigate the effects of brain death and the effectiveness of donor care, several brain death models in animals have been developed.

AIM OF THE STUDY: In this study we tried to introduce a new brain dead donor model in pigs that can be used to further investigate the pathophysiological cascades during and after brain death and to evaluate new strategies in donor care.

METHODS: Brain death was induced in 13 styrian domestic pigs by the means of inflation of the balloon of an intracranially placed catheter. Brain death then was confirmed using the apnea test and EEG. Afterwards intensive care was performed on all pigs and the values of blood pressure, heart rate and fluid intake and urine output were documented. Furthermore arterial blood samples were taken and analyzed at regular intervals. At the end of the experiment multiorgan donation was performed on 7 pigs.

RESULTS: During the induction of brain death (BD) the values of blood pressure ranged widely (from 68/47 to 216/109 mmHg). The highest values of blood pressure were found 5 hours after BD ($115 \pm 15 / 63 \pm 12$ mmHg), the lowest values of blood pressure occurred 16 hours after BD ($78 \pm 18 / 41 \pm 1$ mmHg). The highest value of heart rate was found 2 hours after BD (147 ± 21 beats/minute), the lowest value of heart rate occurred before the induction of BD (93 ± 18 beats/minute). The greatest urine output was noted 7 hours after BD (489 ± 304 ml/hour). Fluid balance was positive in all animals with $+2407 \pm 956$ ml. Furthermore the levels of arterial blood gases, pH, bicarbonate, lactate, hemoglobin, hematocrit, sodium, potassium and arterial oxygen saturation were analyzed.

DISCUSSION: We were able to introduce a new brain dead donor model in pigs that features a longer observation period and can be used to investigate the pathophysiological processes and to apply modern strategies in donor management.

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LIST OF ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
ATN	acute tubular necrosis
ATP	adenosine triphosphate
CP	creatine phosphate
CRH	adrenocorticotrophic releasing hormone
CRP	C-reactive protein
CT	computer tomography
cTnT	cardiac troponinT
ECG	electrocardiography
EEG	electroencephalography
ESS	euthyroid sick syndrome
FiO ₂	fraction of inspired oxygen
FSH	follicle-stimulating hormone
fT ₃	free triiodothyronine
GH	growth hormone
GRF	growth-hormone-releasing factor
HCO ₃	bicarbonate
HIV	human immunodeficiency virus
ICAM-1	intercellular adhesion molecule-1
ICP	intracranial pressure
ICU	intensive care unit
IL-1	interleukin-1
IL-1 β	interleukin-1 β
IL-10	interleukin-10
IL-13	interleukin-13
IL-6	interleukin-6
iNOS	inducible nitric oxide synthase
LH	luteinizing hormone
LH-RH	luteinizing hormone-releasing hormone

LVSP	left ventricular systolic pressure
MAP	mean arterial pressure
MCP-1	monocyte chemotactic protein-1
MHC	major histocompatibility complex
MIP-1 α	macrophage inflammatory protein-1 α
mRNA	messenger ribonucleic acid
NF- κ B	nuclear factor 'kappa-light-chain-enhancer' of activated B-lymphocytes
NT-proBNP	N-terminal pro-brain natriuretic peptide
OCR	oculocephalic reflex
PAC	pulmonary artery catheter
pCO ₂	partial pressure of carbon dioxide
PEEP	positive end-expiratory pressure
PMNs	polymorphonuclear cells
pO ₂	partial pressure of oxygen
PVR	pulmonary vascular resistance
ScvO ₂	central venous oxygen saturation
SD	standart deviation
SO ₂	oxygen saturation
SVES	extrasystoles of supraventricular origin
SvO ₂	venous oxygen saturation
SVR	systemic vascular resistance
T ₃	triiodothyronine
T ₄	thyroxine
THS	thyroid-stimulating hormone
TNF- α	tumor necrosis factor- α
TNFR-1	tumor necrosis factor-receptor type 1
VCAM-1	vascular cell adhesion molecule-1
VES	ventricular extrasystole

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1 INTRODUCTION

Organ transplantation is the lifesaving therapy for many diseases that may lead to end stage organ failure or even death. Therefore, the demand for organs excessively exceeds the number of organs available. The majority of the donors (>90%) are brain dead patients (cadaveric donors) from intensive care units (ICUs). (1) However, organs from living-related or altruistic living-unrelated donors can be used for kidney and split-liver transplantation. Living-donor split-liver transplantation is mainly used for small children, who suffer from liver failure, because only few allografts from cadaveric donors match in size. Most commonly the parents offer themselves as donors. It has to be mentioned though that severe complications such as hemorrhage can occur during organ retrieval, and that not many people are willing to take that risk. (2, 3)

Therefore it is still mandatory to try to maximize the donor pool of brain dead patients and the number of organs harvested from them.

1.1 GENERAL CONSIDERATIONS ON BRAIN DEATH

Brain death is considered to be the irreversible loss of all functions of the brain including the cerebellum and the brain stem and is equal to the death of the individual (4) irrespective of its etiology.

Most frequently brain death is the result of severe head injury, intracerebral hemorrhage or aneurysmal subarachnoid hemorrhage but may also be caused by large ischemic strokes associated with brain swelling, hypoxic-ischemic encephalopathy or fulminant hepatic failure (5) as well as prolonged cardiac arrest and untreated meningitis. (6)

Supra- and infratentorial mass lesions, such as tumor, hemorrhage, edema or abscess with a volume greater than 40-80 ml (depending on the age of the patient) or a diameter of ≥ 8 mm cannot be compensated by a shift of the cerebrospinal fluid from intracranial space into the spinal canal, which results in the rapid rise of intracranial pressure (ICP). In addition to this shift, increase of ICP will cause coma through means of brain- herniation. (7)

Brain herniation can take different forms depending on the location of the mass lesion:

Central herniation occurs when the diencephalon and midbrain are pushed caudally through the tentorial notch by means of diffuse brain swelling (e.g. after trauma) or a centrally located tumor causing diencephalic and midbrain dysfunction leading to a loss of sympathetic output of the hypothalamus. (8)

Lateral mass herniation at first causes the diencephalon to move away from the related mass, causing a compression of the ipsilateral or a distortion of the contralateral cranial nerve III, leading to a corresponding palsy and enlargement of the pupil. Bilateral disturbances may also be related to midbrain ischemia as the brain stem is moving away from the basilar artery. If the process continues, the diencephalon and the ipsilateral temporal lobe herniate through the tentorial notch with the uncus and the hippocampus being the first structures to cross the tentorial edge. This may cause compression of the posterior cerebral artery and ischemia in the temporal and occipital lobe. (8)

In subfalcine herniation medial frontal structures like the cingulate gyrus move beneath the falx. Sometimes this causes ischemia in the area fed by the anterior cerebral arteries. (8)

Cerebellar tonsillar herniation occurs if the tonsils are pushed into or through the foramen magnum. It is mostly caused by posterior fossa masses and may lead to a compression of the medulla followed by a dysfunction of the medullary respiratory centres.(8)

Upward transtentorial herniation may occur if ventriculostomy for relief of hydrocephalus is performed in patients with increased ICP. The contents of the posterior fossa then may herniate into the diencephalic region. (8)

Brain herniation also can cause secondary midbrain hemorrhage, known as Duret hemorrhage. (7)

Elevated ICP and brain herniation can lead to various symptoms depending on the localisation of the mass causing it. The pupils can either be small or dilated and be reactive to light. (7)

The bodily posture can be decorticate, which means adduction at the shoulders, flexion at the elbows and wrists, hyperpronation of the forearms, and extension of the knees and₂

ankles or decerebrate with adduction and internal rotation at the shoulders, extension at the elbows, flexion at wrists and fingers and extension of the legs. Additionally, there might be an overextension of the thoracolumbal spine and neck. Babinski sign also may be present. (7)

The crucial effect on the elevated ICP is the consecutive impairment of the cerebral blood flow.

Cerebral perfusion pressure (defined as mean arterial pressure [MAP] minus ICP) is getting lower when ICP rises. This lack of oxygen supply to the brain will lead to a loss of consciousness and finally to complete brain failure. (7)

1.2 DIAGNOSING BRAIN DEATH

Brain death is mainly a clinical diagnosis which should be expanded by at least one confirmatory test and further examinations if specific components cannot be reliably evaluated in a patient. (5)

First there must be a definite evidence of an acute cerebral damage. Complicating medical factors such as primary hypothermia (body temperature $\leq 32^{\circ}\text{C}$), hypovolemic shock and coma caused by severe endocrine or metabolic disturbances must be ruled out before brain death diagnostic can be started. (5)

If any drugs affecting the central nervous system, or more than two boluses of barbiturates, benzodiazepines, opiates or opioids and/or propofol have been administered during intensive care, there should be an observation period of twelve hours to rule out any interaction with the clinical testing. If there is a reasonable suspicion of any influence, the drug level of the respective substance should be immunologically tested. (4)

1.2.1 THE ROLE OF THE GLASGOW COMA SCORE

According to the ÖBIG guidelines issued by the Austrian Federal Institute of Public Health, the next step should be to evaluate the Glasgow Coma Score. (4) The patient should not have more than three points on the score, which means that he does not open his eyes spontaneously or on command, does not show motor responses of the limbs to painful stimuli, and does not give any verbal responses. (4, 5)

However, spinal cord reflexes may persist after brain death. They can produce the so called Lazarus sign, which can even lead to complex spinal automatisms such as head shaking, movement of the arms like hand clasping and lower extremity stepping activity. They can occur spontaneously or after stimulation. (9)

1.2.2 TESTING BRAINSTEM REFLEXES

Very important is the confirmation of the absence of any brain stem reflexes.

There should be no reaction to bright light in both pupils and they should be in middle position or enlarged. (4) Preexistent abnormalities and the administration of mydriatic agents have to be ruled out before the testing. An irregular shape of the pupils is compatible with brain death. (5)

The oculoccephalic reflex (OCR) should be absent. It is tested by sharp passive head rotation from the middle position to 90° on both sides. In healthy individuals this would result in eye deviation to the opposite side (5) but after brain death the loss of the reflex leads to the failure of adduction of the bulbos, causing the so called doll's-eyes phenomenon. (5) This test should only be performed after fracture or instability of the cervical spine have been ruled out. (4)

The vestibulo- ocular reflex is tested by irrigation of the tympanon on each side with ice water. Basal fractures of the temporal bone or a perforated tympanon should be excluded prior to testing. (5)

The ciliospinal reflex produces a dilation of the pupils in the healthy individual when it is afflicted by a painful stimulus to the neck. In a brain death donor the reaction should not be present. (4)

The absence of the masseter reflex, a short contraction of the masseter muscle following a knock on the patient's chin, has also to be verified. (4)

The corneal reflexes are tested by touching the patient's eyes with a throat swab. There should be no grimacing or closing movements of the eyelids, triggered by that touch.

Grimacing and/ or a rise of systolic blood pressure of more than 40 mmHg should also be absent when pressure is applied to the emersion point of the trigeminal nerve.

The results of all of these tests are difficult to evaluate and interpret if severe facial trauma with a lot of swelling is present. (5)

The pharyngeal reflex is tested by trying to provoke a gag response by touching the posterior pharynx with a tongue blade, the tracheal reflexes by using bronchial suctioning to get a cough response. Both are absent in the brain dead individual, and the gag response may be difficult to observe in the orally intubated patient. (5)

1.2.3 THE APNEA TEST

The apnea test is obligatory for the clinical confirmation of brain death. It is only performed if all of the brainstem reflexes have been found to be negative, and an atonic tetraplegia is observed. (4)

Before the test is performed the following prerequisites have to be reached:

- a core temperature $\geq 36,5^{\circ}\text{C}$
- a systolic blood pressure ≥ 90 mmHg
- euvoemia, or a positive fluid balance in the previous 6 hours
- eucapnia
- normoxemia. (5)

In the next step a pulse oximeter is connected to the patient and arterial blood gases are determined to get baseline values.

Then the ventilator is disconnected and a cannula applying 100% O₂ with a flow of 3-7 l/min is placed at the level of the carina.

After 10 minutes arterial blood gases are evaluated again. If there is a rise of pCO₂ up to a value greater than 60 mmHg, the result of the apnea test is compatible with brain death. (4)

The patient is closely observed for respiratory movements during the test, which are defined as abdominal or chest excursions that provide sufficient tidal volumes. If present they are likely to occur early in the apnea test. Respiratory- like movements - which are compatible with brain death - may include shoulder elevation and adduction, back arching and intercostal expansion. (5)

If the tests described above have to be repeated depends on the localisation of the lesion that is made responsible for brain death. (4)

If the lesion responsible for brain death is primary supratentorial, a second clinical examination is performed after an observation period of 12 hours. If its results are compatible with brain death, the diagnosis is secured.

If an infratentorial lesion is present or any of the tests is negative, a confirmatory test has to be performed. (4)

1.2.4 CONFIRMATORY TESTS

1.2.4.1 EEG

The EEG is the method of choice because it is best at giving account for cortical activity, is noninvasive and can be performed directly at the patient's bed. (4, 5)

In the brain dead individual at least an 8-channel EEG should show no electrical activity (isoelectrical EEG) above 2 μ V at a sensitivity of 2 μ V/mm. The examination is performed for 30 minutes and in parallel an ECG should be performed. (4)

1.2.4.2 TRANSCRANIAL DOPPLER ULTRASONOGRAPHY

The stop of cerebral perfusion can be tested using transcranial Doppler ultrasonography. There are several approaches to reach the intracranial arteries with ultrasound. Most commonly the bilateral transtemporal approach is used to detect blood flow in the middle cerebral artery and the insonation of the internal carotid artery. To evaluate the posterior circulation the vertebral or basilar arteries are insonated through the suboccipital transcranial window. (5)

In 2002 Lampl et al. have proven that the use of a transorbital approach is extremely valuable to observe the anterior circulation arteries, which improved the percentage of definite diagnoses of brain death from 79% to 88%. (10)

Three different perfusion patterns are associated with brain death. Small systolic spikes during the early systole occur because of the highly increased ICP that hinders cerebral blood flow. Oscillating flow results from a retrograde flow towards the heart during diastole. The complete absence of diastolic flow is also possible and is due to the contractive forces of the arteries. (5)

The lack of transcranial Doppler signals is not confirmatory for brain death, because it might be due to temporal bone thickness. (10)

1.2.4.3 ANGIOGRAPHICAL METHODS

Conventional angiography, CT- angiography or isotope angiography can also be used to show suspended cerebral perfusion. CT- angiography has a shorter duration than the other two methods. (5)

One disadvantage of conventional and CT- angiography is that repeated injections of contrast will increase the effect of nephrotoxicity and can lead to rejection in the organ recipients. (5)

To ensure that the contrast is reaching the cerebral arteries the examiner should also confirm the filling of the external carotid arteries. (4)

2 DETRIMENTAL EFFECTS OF BRAIN DEATH ON THE WHOLE ORGANISM

In many experimental studies of organ transplantation young, healthy living animals are used as donors. In this case brain death is rarely considered a risk factor for both initial and long-term results. (11)

In clinical practice, however, most organs come from cadaveric donors. (11)

This leads to the conclusion that it is important to know about the severe physiological and structural changes that may occur during or after brain death in each organ. (11)

2.1 THE AUTONOMIC STORM

The autonomic storm (also called catecholamine storm or catecholamine burst) is characterised by a transient massive increase of circulating catecholamines, especially epinephrine and norepinephrine, which leads to severe hemodynamic changes in the whole organism. (11)

In contrast to the elevated levels of epinephrine and norepinephrine during the autonomic storm, no significant increase of dopamine levels was found. (11) The severity of the autonomic storm is correlated to the suddenness and the extent of the increase of the ICP. If ICP increases slowly, the autoregulative mechanisms of the brain stem are able to adapt to the elevated ICP and compensate in some extent, and the hemodynamic responses are less severe. (11, 12)

The autonomic storm can be divided into different stages which reflect the development of brain ischemia.

During the first stage a vagal response is triggered when ICP reaches the ischemic threshold and cerebral perfusion ceases. It is marked by a decrease of MAP, heart rate and cardiac output. (12)

The second stage shows a mixed vagal and sympathetic activation and occurs when ischemia approaches the brain stem. This part of the autonomic storm is called the Cushing response. This term describes a triad of bradycardia, hypertension, and an irregular breathing pattern. Cardiac output is still low at this time. (12)

As soon as ischemia reaches the lower end of the medulla oblongata, the vagal cardiomotor nucleus becomes ischemic and only sympathetic stimulation takes effect. At this time a peak in the heart rate can be observed. The MAP is still increased, and now there is an increase in the cardiac output as well. (12)

During this last stage systemic vascular resistance has also increased significantly, which causes a shift of the blood towards the capacity vessels and the lung, which can lead to pulmonary edema. (13)

In the year 2000 Szabó et al. showed in a canine model that there is no Cushing response if ICP is elevated very fast to a very high level (> 300 mmHg). They stated that this was partly because sympathetic nervous discharge and the activation of the sympathoadrenal system exceed the parasympathetical component, and partly because such a rapid and high rise of ICP immediately disrupts the lower brain stem reflexes, including the vagal response. (14)

They also showed that the combination of the humoral pathway by activation of the adrenal gland and the neural pathway by the release of catecholamines from the nerve endings results in a significantly stronger reaction. This might be due to presynaptic α_2 - and β_2 -adrenergic receptors. While the α_2 -receptors limit the neural norepinephrine discharge by a negative feedback loop, the β_2 -receptors activate a positive feedback loop if stimulated by epinephrine from the adrenal glands, which leads to continued norepinephrine release. (14)

All catecholamine levels return to normal values some time after brain death, and in animal studies norepinephrine has shown to decrease significantly below baseline values. (15)

2.2 HEMODYNAMIC CHANGES AFTER BRAIN DEATH

Brain death causes rapid swings in blood pressure and in most cases leads to persistent hypotension in the end although cases where normotension was maintained until organ retrieval have been reported. (11) In most cases, however, blood pressure drops to less than 50% of the baseline level, and even in patients that were hemodynamically stable upon admission MAP was below 80 mmHg at the time of organ retrieval. (12, 16)

During the autonomic storm, however, very high pressure peaks can be achieved with a MAP of up to about 200 mmHg. (17)

The increase of blood pressure depends on the speed of the rise of ICP. (12) The combination of neural and humoral factors during the autonomic storm produces the strongest response of blood pressure. (14)

As systemic vascular resistance (SVR) also rises due to peripheral vasoconstriction, peripheral organ perfusion is significantly reduced despite of the high blood pressure. (11, 15)

This rise in SVR along with low pulmonary vascular resistance (PVR), greater compliance of the pulmonary vessels and the right ventricle as well as increased pulmonary capillary reserve leads to an increased flow in the pulmonary arteries. (15)

As the left atrial pressure exceeds the pulmonary artery pressure, this constellation leads to damage of the pulmonary capillaries and an abnormal permeability resulting in acute pulmonary edema with high protein content and interstitial hemorrhage. (13, 15, 18)

As vascular autoregulation fails, the blood supply to the organs is diminished. (11)

Hemodynamic instability is exacerbated by hypovolemia and neurogenic vasodilatation. (18) It is one of the factors along with fluid, electrolyte and metabolic disturbances that will lead to cardiac arrest in the end. (17)

There are also hemodynamic responses to noxious stimuli not involving the brain stem. They occur on cutaneous surgical stimulation, and if the parietal peritoneum or the splanchnic nerves are stimulated during organ retrieval.

Those reflexes are of spinal origin and will cause hypertension. (19)

2.3 STRUCTURAL AND FUNCTIONAL CHANGES IN THE HEART

Catecholamines, which reach very high levels during the autonomic storm, are known to cause damage in the donor heart. The local exocytic release from sympathetic nerve endings of the heart and the nonexocytic release of norepinephrine after depletion of high-energy phosphates independent of the central activation aggravate this effect. (11)

Catecholamine cardiotoxicity results from relative hypoxia in the tissue, direct stimulation of the myocardium, the coronary microcirculatory effects, and changes on the molecular level. The products of catecholamine oxidation (adrenochromes) themselves are also said to have a cardiotoxic effect. (12)

The voltage gated calcium channels are known to have a keyrole in the excitation-contraction coupling process. The inadequate supply with oxygen, glucose and ATP leads to the opening of calcium channels and leads to cytosolic calcium overload. There is also a marked increase in the calcium uptake into the sarcoplasmatic reticulum. The rise in cytosolic calcium leads to the activation of lipases, proteases, endonucleases and the production of free radicals. (13)

The degradation of adenosine, a byproduct of the ATP catabolism, causes the production of more radicals. (13)

Oxygen free radicals participate in the peroxidation of unsaturated free fatty acids and lipids and damage the cell membrane and the endothelium. (13)

This leads to loss of cellular integrity and intracellular edema. (12)

Some time after the catecholamine exposure during the autonomic storm, an impairment of the β -adrenergic receptor/adenylyl cyclase system and a desensitization of the myofilament for calcium have been noted. (14)

In addition to the changes presented above, there is also strong evidence of a switch from aerobic to anaerobic metabolism after brain death. A reduction in myocardial energy stores, especially creatine phosphate (CP) and glycogen, and a rise in tissue lactate and free fatty acids gives evidence to this. ATP levels are thereby sustained at expense of CP.

It also seems that, as the rates of pyruvate, glucose and palmitate are decreasing, there is a mitochondrial inhibition or damage taking place, because these molecules can only be metabolized in the mitochondrion. (15)

During the autonomic storm there are severe changes in heart rate and great swings in blood pressure.

At first a significant increase in heart rate and left ventricular systolic pressure (LVSP) can be observed. (14)

The left atrial pressure then exceeds the pulmonary artery pressure, which might be due to mitral valve regurgitation induced during cardiac ischemia at the peak of SVR, causing pulmonary capillary damage and acute pulmonary edema.

The temporary decrease of coronary blood flow during the peak of SVR also leads to typical changes in the ECG like ST-segment changes. (12, 15)

Other electrocardiographic changes during the autonomic storm include sinus bradycardia, sinus standstill, complete heart block and junctional escape beats during the early parasympathetic impact, and tachycardia, ventricular extrasystoles (VES) and extrasystoles of supraventricular origin (SVES) in some cases during the sympathetic burst. Later abnormal J waves and T waves can occur. (12, 13)

Functional testing of the heart of brain dead donors revealed a reduction of the cardiac output, stroke volume and left ventricular systolic pressure, and an elevated left ventricular enddiastolic pressure. (13)

After the profound reduction of sympathetic outflow the inotropic and chronotropic state of the heart is impaired, causing diminished blood and oxygen supply in the whole organism. (11)

Characteristic morphologic changes are found in biopsies taken from the hearts of brain dead donors.

Under light microscopic view focal ischemic damage can be found. Petechial hemorrhage, contraction bands and coagulative myocytolysis along with mononuclear cell infiltrate are the result of the cardiotoxic effect of catecholamines. (11)

Similar changes like contraction bands have also been found in the smooth muscle cells of the coronary arteries. They might be caused by severe vasospasm during the autonomic storm. (13, 15)

Higher magnification also shows necrotic clarification of the nuclei, swollen mitochondria and microinfarctions caused by intracellular edema. (11, 12)

Electron microscopy reveals a hypercontractile state of the sarcomere, disruption of the cristae and secondary lysosomes containing parts of damaged mitochondria. (13)

These changes are most severe in the subendocardium of the left ventricle. (11)

It was found that it takes about six hours after brain death for the damage to take place and be visible in preischemic biopsies. (12)

Mild disruption of the contractile elements of the myocardium, however, might be reversible to some degree. (12)

Damage to the conductive tissue of the heart can also be found and is likely to be responsible of donor heart failure in the recipient due to heart block or arrhythmia soon after transplantation. (15)

The rate of injuries to the heart is significantly reduced if brain death is caused by a gradual increase of ICP. (11)

The greatest damage to the heart, however, occurs during the reperfusion of the cells depleted of cellular energy charge. (13)

There is a burst of superoxide, peroxide, hydroxyl radical and peroxynitrite production, which leads to direct injury of the myocytes and causes the production of even more radicals. The primary source for these postischemic radicals is the endothelial xanthine oxidase pathway. Hypoxanthine, which is the substrate of that enzyme, accumulates in the ischemic myocardium. (18)

The radicals cause peroxidation of membrane lipids and denaturation of proteins leading to an increased permeability of the cell membrane and to a damage to the sarcoplasmic reticulum. This will induce a hindered calcium transport to the mitochondria and an impaired oxidative phosphorylation. (18)

Byproducts of arachnoidonic acid oxidation, which accumulate during these events, act as chemotactic factors for leukocytes. There is also an increased production of the cytokines $\text{TNF-}\alpha$, interferon- γ and the chemokines macrophage inflammatory protein-1 α (MIP-1 α) and monocyte chemotactic protein-1 (MCP-1). (18)

Selectins on the endothelium of coronary vessels cause the adhesion of leukocytes, which then block the capillary flow and release cytotoxic molecules. They lead to local vasoconstriction and the activation of platelets, complement and other neutrophils.

This effect is further magnified by the contact of the blood with the synthetic surface of the extracorporeal circulation during transplantation. (18)

All of these processes lead to ischemic contracture and left ventricular dysfunction which contributes to the early failure of some transplants. (12, 18)

2.4 CHANGES AND DAMAGE AFFECTING THE LUNG

The lung is very susceptible to injury caused by rapid hemodynamic changes during the autonomic storm. (15)

As the SVR increases, the pulmonary capillary reserve is increased and the flow in the pulmonary arteries enhanced, causing the pooling of blood in the lung.

Then as the pressure in the left atrium exceeds the pressure in the pulmonary vessels the pulmonary blood flow temporarily ceases entirely.

This leads to structural damage of the lung. (11) The vascular endothelial cells are most sensitive to ischemia and disruption of the capillary endothelial integrity occurs. (13, 15, 18).

The increased permeability of the capillaries can lead to acute pulmonary edema rich in protein and other pulmonary tissue abnormalities like hemorrhage in the alveolar walls and spaces and the interstitium. (13, 15)

This finally can lead to the deterioration of pulmonary function and can make the lung unsuitable for engraftment. (11)

Nevertheless there are also other factors related to brain death and the following supportive intensive care that can be detrimental to the lung. (18)

Environmental contamination, to which the lung is exposed, may impair the functional capabilities. Such agents can be microbiologic pathogens and toxic substances. The exposure to them is accentuated by endotracheal intubation necessary for donor management. Further damage can be caused by aspiration of oropharyngeal and/or gastric contents. (18)

These findings are supported by the fact that about 50% of all intubated comatose patients in ICUs acquire pneumonia within one week. (18)

Damage to the lung tissue is also caused by resuscitation attempts. The injury inflicted by the compression of the thorax leads to a pulmonary inflammatory response. (18)

The lung has an absolute requirement for aerobic metabolism but unlike other organs it can use ambient oxygen for glucose metabolism and can thereby maintain its energy balance even during ischemia. (18)

Reperfusion leads to leukocyte adhesion to endothelial cells of the pulmonary vessels which by the pathway - already described previously - finally leads to the production of oxygen-derived free radicals and peroxides, which can damage the lung tissue. (18)

2.5 CHANGES AFFECTING THE KIDNEYS AND THE ELECTROLYTE BALANCE

The marked vasoconstriction during the autonomic storm causes afferent arteriolar spasm, which in combination with the following prolonged systemic hypotension causes ischemia in the kidneys. This acute prerenal failure and the alterations in the regulatory function of the sympathetic nervous system are possibly the major causes of the damage the kidneys take during brain death. (11, 13, 18)

Systolic blood pressure or MAP below 80 mmHg is correlating with a decline in the autoregulation of renal blood flow and the filtration capacity. This marked hypotension and hemodynamic instability along with high doses of dopamine ($> 10 \mu\text{g/kg/min}$) are also known risk factors for the occurrence of acute tubular necrosis (ATN) after brain death. (11, 16)

The histopathological changes that follow ischemia in the kidney are characterized by immediate onset of extensive hyperemia in the beginning. This decreases some days after brain death. Later glomerulitis, endothelial dysfunction and finally periglomerulitis will occur. Within three days after brain death, vacuolar and acidophilic degeneration, atrophy and necrosis of the tubular cells develop. (11, 18)

Marked reduction of glomerular capillary spaces and arterial intimal proliferation can be observed, too. (11, 13)

The electron microscopy shows scattered necrotic cells and damaged mitochondria in the kidneys of brain dead donors. (13)

In immunohistological examinations infiltrating polymorphonuclear leukocytes (PMNs) as well as the induction of selectine and complement activation were found. (18)

Other signs of early inflammatory changes are the prompt and massive expression of major histocompatibility antigens, adhesion molecules and other acute phase proteins. Upregulation of interleukin-6 (IL-6) and nonimmune cellular and molecular inflammatory changes induced in the kidneys of brain dead donors seem to be connected to the initial nonspecific and later immunological reactivity after engraftment. (18)

Reperfusion also causes the upregulation of interleukin-1 β (IL-1 β) and macrophage inhibitory protein-1 mRNA. (18)

The renal slice function can be impaired due to ischemia, which leads to depletion of cellular energy stores and thereby disrupts the intracellular sodium/potassium ratio, which is regulated by energy consuming mechanisms. (11)

In more than 80% of brain dead donors hyponatremia (> 145 mmol/ml) and in a lower percentage hyper- and hypokalemia were present while creatinine and urea remain quite stable. There is also severe metabolic acidosis in almost all cases. (1, 16)

This electrolyte imbalance as well as hypovolemia, decrease of blood pressure and metabolic disturbances is caused by neurogenic diabetes insipidus, a state of hypothalamic-pituitary dysfunction, which leads to a depletion of antidiuretic hormone (ADH). ADH is a hormone from the posterior pituitary gland and its disappearance is observed in 30-90% of all brain dead donors. (11, 12, 20)

Massive polyuria (4 ml/kg/h or more) of diluted urine (specific gravity < 1005 and urine osmolality < 200 mosm/l) will occur and unless fluid substitution is administered, urine output will cease completely some hours after brain death. (1, 15, 20)

Hyponatremia as well as polyuria have a detrimental effect on the kidney function after engraftment. While polyuria correlates with high serum creatinine levels in the recipient shortly after transplantation, hyponatremia seems to have an influence on the long term results. (20)

2.6 CHANGES AFFECTING THE LIVER

The liver is quite resistant to the decreased blood pressure, which occurs after brain death and has a large physiological reserve. (11) The hepatic energy status especially in regard to ATP is well maintained for a long time. (18)

There are however some deleterious changes that can reduced the organ quality when compared to those grafts from living related donors. (11)

Some days after brain death, central venous congestion can be observed although it is not entirely clear if this is due to brain death itself or the intensive care treatment with vasopressive agents like vasopressin and epinephrine (11, 18)

Intrahepatic cholangitis can be observed some days after brain death, most likely being the result of the medication administered in ICUs and of vagal denervation leading to gallbladder cholestasis and altered hepatic bile flow. (18)

Two weeks after brain death piecemeal necrosis and periportal necrosis have been observed in some cases, but the morphological changes in the liver are not that clearly defined as in other organs like heart or kidney. (11)

Electron microscopy of the liver also reveals scattered mitochondrial injury, cell necrosis and the loss of glycogen. (13)

A study on brain dead rats by Van Der Hoeven et al. in 2003 has shown that brain death induces apoptosis in the donor liver. (21)

It showed that inflammation occurs in the liver with an increased expression of adhesion molecules and an influx of leukocytes. The key mediator of the process leading to programmed cell death is tumor necrosis factor- α (TNF- α), which activates proapoptotic factors and an antiapoptotic survival pathway through the activation of the nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF- κ B). (21)

TNF- α binds to the TNF-receptor type 1 (TNFR-1) and thereby activates procaspase-8 and the NF- κ B survival pathway. (21)

Other ways by which apoptosis can be induced include the Fas death receptor and mediators that can penetrate the cell membrane and directly modulate the expression of proapoptotic factors. (21)

The proapoptotic factors are of the Bcl-2 family and include Bax, Bak and Bid. Bid is needed to relay the signal from the cell receptor mediated pathway to the mitochondrial pathway, which causes the release of cytochrome-c from mitochondria leading to the activation of caspase-9. (21)

Since hepatocytes are type II cells, in which a strong activation of caspase-8 and caspase-3 can be found downstream of the mitochondria, both pathways need to be activated for the induction of apoptosis. (21)

The final common effect of the mitochondrial and the receptor pathway is the activation of the execution caspase-3 by caspase-8 and caspase-9. (21)

NF- κ B regulates the expression of inhibitors of caspases (IAP-family), proteins involved in the protection of the mitochondrial membrane (antiapoptotic Bcl-2 family members, including A1, Bcl-2 and Bcl-xl) and the inducible nitric oxide synthase (iNOS). (21)

An inhibition of NF- κ B hereby causes increased apoptosis, too. (21)

In the end the balance of pro- and antiapoptotic factors will decide whether the cell survives or dies. (21)

In the study of Van Der Hoeven et al. there was an increased number of apoptotic cells found in the livers of brain dead rats, these cells being primarily hepatocytes. (21)

An infiltration of leukocytes occurs some hours after brain death. It is mainly composed of neutrophils and polymorphonuclear granulocytes and at this time does not contain T-lymphocytes. Other studies show that an influx of T-lymphocytes occurs at a later stage. (21)

Several proapoptotic as well as antiapoptotic factors were found to be increased. (21)

Of the death receptor pathway only Fas was significantly increased. Since Fas is predominantly expressed on cytotoxic T-lymphocytes, which were not present at this stage, it could be that an increased Fas could be mediated by the TNF-receptor pathway as well.

Of the mitochondrial pathway Bid was significantly increased. (21)

The antiapoptotic factors, whose mRNA was found to be increased, were A1 and Bcl-xl of the Bcl-2 family and cIAP-2 of the IAP-family. (21)

The final effect of these activations was a proapoptotic one, indicated by an almost doubled caspase-3 activity. (21)

Hypernatremia, which is often found in brain dead donors due to diabetes insipidus, has been found to promote the accumulation of idiogenic osmoles within cells of the liver. (20)

This may have a detrimental effect because it can promote water accumulation, cell lysis and cell death during the phase of reperfusion at the time of engraftment. (20)

2.7 CHANGES AFFECTING THE ENDOCRINE PANCREAS AND THE GLUCOSE METABOLISM

There are only a few and very slight morphological changes to the pancreas during brain death. Immunohistochemical analysis shows that the islets of Langerhans had a normal capacity for insulin in beta cells and for glucagon in alpha cells in almost all cases. (22)

However, hyperglycemia is often observed in brain dead donors despite the presence of elevated insulin and C-peptide levels. (11)

And although some studies suggest a reduction of insulin or insulin in a low normal range (13, 15) most authors agree that, as the endocrine pancreatic function remains effective after brain death, there must be other reasons for this hyperglycemic state. (11, 22)

There are several theories trying to explain the obvious insulin resistance in peripheral tissue that occurs after brain death. (22)

One suggests that hyperglycemia might be a consequence of the destruction of a metabolically related area in the central nervous system. (22)

An altered insulin secretion during the early phase of brain death or an impairment of the secretion secondary to acute hyperglycemia during the autonomic storm explain why the insulin concentrations are low at the early stage of brain death. However after a rapid and sustained rise in glycemia the plasma C-peptide concentration does not show the pancreatic response to glycemia at that very moment. (22)

Later after the autonomic storm has passed the catecholamine levels drop and the responsiveness of beta cells is restored. The effect on the peripheral tissue, however, is prolonged. (22)

Glucose intolerance and insulin resistance are included in the metabolic consequences of stress. An increased hepatic gluconeogenesis and reduced glucose uptake and an increased insulin turnover have been noted. (22)

Two main mechanisms are known to cause this insulin resistance: impaired receptor binding causing decreased insulin sensitivity, and alterations in the intracellular postreceptor metabolism leading to a decreased insulin responsiveness. The latter is made responsible for the insulin resistance in brain dead donors. (22)

Another factor that helps to sustain the hyperglycemic state is the elevation of counterregulating hormones by neurally mediated mechanisms. Catecholamines, especially epinephrine, which rise to a very high level during the autonomic storm impair sensitivity to insulin and cause a decrease in glucose clearance. (22)

Glucagon, which is often found elevated in brain dead donors, increases gluconeogenesis and glycogenolysis. (11, 22)

Cortisol, which is administered in intensive care treatment, causes an intracellular postreceptor block of insulin metabolism. (22)

In addition, proinsulin, which is much less potent than insulin in its function, is elevated in stress. (22)

Other special nonhormonal factors also might play a role in posttraumatic insulin resistance. (22)

In the kinin/prostaglandin system, for example, a lack of bradykinin can lead to alterations in glucose metabolism. (22)

Cytokines like interleukin-1 (IL-1) and TNF- α are known to increase neoglycogenesis and peripheral glucose uptake as well as the release of counterregulating hormones. (22)

2.8 CHANGES AFFECTING THE SMALL INTESTINE

The small intestine is known to be extremely sensitive to ischemia. (18)

It has been shown that stress and ischemia lead to extensive inflammation in the jejunum and ileum and that the mRNA of a number of inflammatory molecules and adhesion molecules is upregulated in the intestine of brain dead donors. (23)

Another theory suggests that blood borne factors released from the brain into the bloodstream initiate this inflammatory response. (23)

The levels of E-selectin, which causes leukocyte rolling on the endothelial surface, the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell

adhesion molecule-1 (VCAM-1) as well as the proinflammatory cytokines IL-1 β , IL-6, TNF- α and MCP-1 increase promptly after brain death in the jejunum and ileum. (23)

In the jejunum there is a further increase of E-selectin, VCAM-1 and IL-6 over time while a decrease of ICAM-1 can be observed in the ileum. (23)

The presence of ICAM-1 and VCAM-1 preceded by the upregulation of E-selectin is an indicator for inflammation and in accordance with the classical inflammatory cascade. (23)

There is a marked increase in the level of these proteins in the activated endothelial cells of capillaries and venules in the lamina propria, where they lead to the capture of leukocytes. (23)

The histological examination of the small intestine of brain dead donors has shown a marked infiltration with PMNs and monocytes in the lamina propria. In the jejunum this influx was a progressive one while in the ileum there was no further increase of the inflammatory cell infiltrate over time. (23)

The acute inflammatory response also triggers gene activation and production of the powerful antiinflammatory cytokines interleukin-10 (IL-10) and interleukin-13 (IL-13) in tissue macrophages as a counteraction to the inflammation. (23)

The process of apoptosis occurring in the tips of the villi in the jejunum and ileum is a physiological phenomenon and reflects the renewing rate of the intestine. (23)

In brain dead donors, however, there is a significant increase in the number of caspase-3 positive cells, indicating an increased apoptotic activity. This can lead to a damage in the jejunal structure and barrier function. (23)

All of these inflammatory changes along with the fact that the small intestine is exposed to microorganisms and toxins from the external environment can severely compromise organ quality before transplantation. (18, 23)

During retrieval and storage the mucosa may be further damaged by contact with pancreatic or other proteolytic enzymes. (18)

Reperfusion also causes further tissue injury due to the production of oxygen free radicals.

Especially the villus tip where the most severe ischemic damage has occurred, is a rich source of xanthine oxidase, which is responsible for the production of free radicals. (18)

2.9 CHANGES IN THE ENDOCRINE SYSTEM

A close relationship exists between brain death and a disruption of the hypothalamic-pituitary axis. (11)

In histological examination the pituitary gland was found to show varying degrees of edema, hemorrhage and coagulative necrosis and, since brain death does not necessarily lead to endocrine failure, it has been suggested that cerebral blood flow might persist in this area for some time after brain death. (11)

This theory is supported by the fact that the anterior pituitary hormones - growth hormone (GH), adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH) follicle-stimulating hormone (FSH) and luteinizing hormone (LH) - remain detectable in the blood of brain dead donors. (18)

ADH of the posterior pituitary gland, however, decreases after brain death, causing neurogenic diabetes insipidus as explained above. (11)

Luteinizing hormone-releasing hormone (LH-RH), which is of hypothalamic origin is also still present after brain death. Growth-hormone-releasing factor (GRF) and adrenocorticotropic releasing hormone (CRH) were still detectable in half of the brain dead patients. (18)

Still the hypothalamic function appears markedly suppressed and hypothalamic necrosis is present, which implies a possible extracranial origin of these circulating hormones. (18)

Although ACTH remains detectable in the blood of brain dead patients, cortisol levels show variable changes after brain death. (11)

Some studies showed that plasma cortisol levels first rose and then declined (15), others found them to either decrease or remain relatively stable at a low normal level. (18)

The greatest impact of brain death can be observed in the thyroid hormonal balance. (11, 13, 15)

There is a rapid reduction of the thyroid hormones free triiodothyronine (fT₃) and thyroxine (T₄) to an undetectable level after brain death. This in combination with the²²

unchanged TSH levels is in accordance with the euthyroid sick syndrome (ESS), which is known to occur in patients in acute stress-shock states. (11, 13, 15, 18)

The lack of fT_3 has been found to be the major cause of the impairment of the aerobic metabolism after brain death. It causes diffuse mitochondrial injury and dysfunction and an impaired energy production at the cellular level. This leads to a progressive depletion of high-energy stores. (11, 13, 15)

Thyroid hormone depletion has also been made responsible in large parts for the hemodynamic instability after brain death. (12)

2.10 CHANGES IN THE GENERAL CYTOKINE DISTRIBUTION AND THEIR EFFECT

Tissue damage has been reported to lead to an increase of the soluble cytokines IL-1, IL-6, TNF- α and interferon- γ . (11)

However, the only cytokine that increases markedly after brain death is IL-6. (11, 24)

Cytokines are released by monocytes and macrophages in necrotic areas but are also produced by neurons, microglial cells, pituitary cells and the endothelial and smooth muscle cells of brain blood vessels. (24)

Catecholamines which increase to a very high level during the autonomic storm - explained above - can also trigger the production of IL-6.

This explains the rise in IL-6 levels after brain death and why these levels correlate with the extent of the brain lesion. (24)

IL-6, as well as IL-1 and TNF- α , play an important role in metabolic and immunological responses. They lead to an increase of hypothalamic corticotropin releasing factor but can also directly influence the release of ACTH and glucocorticoids. (24)

Another effect of these cytokines is the activation of the acute-phase response which explains their strong correlation with CRP levels. (24)

IL-6 is also involved in the development of the sick euthyroid syndrome and inversely correlates with the levels of T_3 as well as T_4 . In addition hypothalamic-pituitary damage aggravates these effects of IL-6 on the thyroid metabolism. (24)

Hypothalamic-pituitary dysfunction is also made responsible for the inadequate rise of ACTH and cortisol in such a stressful situation. Although IL-6 is the strongest stimulus for ACTH and cortisol release, the values stay within the normal range of unstressed individuals. (24)

There is a significant inverse correlation of IL-6 and testosterone, which suggest an involvement of the cytokine in the process of inflammation-induced hypogonadism. It inhibits steroidogenesis and at the same time blocks the activation of the hypothalamic-pituitary axis, which explains why gonadotropine levels are not increased. (24)

2.11 HOW BRAIN DEATH CAN INCREASE IMMUNOGENICITY

Organ ischemia before transplantation has two components: warm ischemia during brain death and organ retrieval, and cold ischemia during preservation and storage. This period as well as the time of reperfusion contribute to tissue damage. (11)

Slowing down of the blood flow - caused for example by low blood pressure - and bradycardia cause the so called “rolling effect“, which leads to the slowing of leukocyte traffic along the vascular wall, the adherence of these cells to the endothelium and infiltration into the graft tissue. Early adhesion molecules (E-selectin) and other classes of adhesion molecules like ICAM-1 and VCAM-1 are expressed after injury and the proinflammatory cytokines TNF- α and interferon- γ are released. (11)

This last cytokine is responsible for the up-regulation of the major histocompatibility complex (MHC) classes I and II. These proteins play an important role in the process of T-cell recognition and activation and thereby increase the immunogenicity of the graft. (11)

Reperfusion aggravates the ischemia induced damage through means of prolonged focal ischemia (no-reflow) or because proinflammatory mediators are activated (reflow paradox). (11)

3 DONOR MANAGEMENT – STRATEGIES TO AMELIORATE ORGAN QUALITY AFTER BRAIN DEATH

Only 15-20% of the individuals who satisfy the criteria for becoming organ donors actually donate. (1)

This happens for three main reasons:

- The patient signed in to the registry of objection (e.g. Austria), does not carry a donor card (e.g. USA) or the family refuses to give consent.
- The patient is declared unsuitable because of age, general condition, hepatitis or HIV status or malignancy.
- 5-10% of potential donors are lost secondary to hemodynamic collapse and cardiopulmonary arrest due to inaccurate or insufficient donor maintenance care in the ICU. (1, 25)

The general management of brain dead patients is dependant upon the very complex nature of this situation. (18)

Adverse events, which can occur include cardiovascular changes, endocrine and metabolic disturbances and the disruption of internal homeostasis - including blood coagulation and hydro-electrolytic balance. (18)

The most common of these are hypotension (> 80%), diabetes insipidus (about 65 %), relative hypothermia, plasma electrolyte imbalance, disseminated intravascular coagulation, pulmonary edema, cardiac arrhythmias and metabolic acidosis. (1)

The primary goals of adequate donor care therefore must include the normalisation and maintenance of blood pressure, the restoration of intravascular volume, the optimization of lung function, the correction of acid/base and electrolyte imbalances and the maintenance of normothermia. (1, 18, 26)

Early identification of potential donors and their admission to specialized ICUs with a trained team is essential to create a vantage point for optimal donor care. (26)

3.1 MONITORING DURING DONOR CARE

Continuous invasive monitoring is essential during donor care. (1)

Standard monitoring after brain death should include:

- monitoring of the clinical signs of perfusion.
- Clinical assessment of skin turgor and mucous membrane hydration to guide fluid replacement therapy.
- Urine output. Diuresis should not exceed 150 ml/hour or 3-7 ml/kg/hour.
- Central venous pressure, which should be kept between 8 and 12 mmHg or below 8 for potential lung donors.
- Continuous invasive monitoring and recording of arterial blood pressure with a systolic blood pressure between 90 and 140 mmHg and an MAP not lower than 70 mmHg.
- ECG monitoring to detect arrhythmias and assess heart rate which should be between 60 and 120 per minute.
- A pulmonary artery catheter (PAC or Swan-Ganz catheter) should be used in patients who remain hemodynamically unstable despite fluid replacement and adequate vasopressor therapy or have an ejection fraction below 40%. The target values are 6-10 mmHg for pulmocapillary wedge pressure (PCWP) and 10-15 Wood units (80-120 mPa/s/m³) for SVR.
- Monitoring of the arterial blood gases is necessary and should be performed regularly. pCO₂ should be between 4,7 and 6,0 kPa to prevent acidosis and vasoconstriction and pO₂ should be between 9,3 and 13,3 kPa if the donor is ventilated with FiO₂ below 0,45.
- Central venous oxygen saturation (ScvO₂), measured continuously via an oxymetric central venous catheter or venous oxygen saturation (SvO₂), measured intermittently by blood sampling are reliable indicators of adequate organ perfusion. ScvO₂ should be above 70% and SvO₂ above 65%.
- Venous blood sampling of the brain dead donor should be performed at regular intervals and include hemoglobin, which should stay above 10 g/dl, hematocrit at about 30%, glucose, plasma urea and serum creatinine, which should be no higher than 113 µmol/l, electrolyte concentrations as well as inflammatory markers and a coagulation profile.

- Peripheral temperature should be controlled because brain death can lead to hypothermia, which can cause cardiac instability. (1, 25, 26, 27, 28, 29, 30, 31)
- Cardiac troponinT (cTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) are reliable predictive markers of a decreased left ventricular function in potential heart donors. While elevated cTnT is associated with myocyte injury and myocardial necrosis, higher values are also detected in apparently healthy individuals older than 60 years, in critically ill patients with myocarditis, sepsis, hypotension or renal failure, and in those with subclinical myocyte damage, which will not cause alterations in ventricular function. For this reason false positive test results are possible.

NT-proBNP secretion is induced by increased left ventricular wall stretch caused by an increased cardiac load and observed in regional or global impairment of left ventricular systolic or diastolic function.

Both of these mechanisms are involved in the left ventricular dysfunction that might occur after brain death.

If cTnT and NT-proBNP are measured in combination the accuracy of this biological test is significantly increased.

The cutoff points are 0,1 µg/l for cTnT and 1700 ng/l for NT-proBNP.

If both markers are above these values a severe decrease in left ventricular function is likely and the potential donor should be examined with transesophageal echocardiography. The early diagnosis of left ventricular dysfunction may also help to improve hemodynamic management and organ function.

If both markers are normal, echocardiography may be delayed to assess the quality of the cardiac graft later. (29, 32)

3.2 CONTROLLING AND MAINTAINING BLOOD PRESSURE

3.2.1 HYPERTENSION

Hypertension occurs primarily during the autonomic storm. Short acting β-blocker drugs and nitroprusside should be administered in that case to prevent myocardial injury and dysfunction. (1)

3.2.2 HYPOTENSION

Severe relative intravascular hypovolemia due to increased fluid loss caused by the administration of mannitol to reduce ICP, by diuretic therapy, by diabetes insipidus and venular blood pooling is a common finding after brain death. (1)

The reduction of SVR, decreased myocardial contractility leading to left ventricular dysfunction and neurogenic shock aggravate hypotension. (28, 30)

To maintain hemodynamic stability, it therefore is necessary to achieve normovolemia by volume expansion and to reach a sufficient perfusion pressure and blood flow gradient, which promote organ function by the use of vasoactive drugs. (1)

3.2.2.1 FLUID RESUSCITATION

In an adequate fluid replacement therapy a balance has to be found between the antagonistic competing organ interests. The kidneys for example need adequate volume to maintain their function, while for the lung minimal volume load is best. (30)

Fluid replacement therapy should be guided by the following parameters:

- Fluid intake and loss calculated for the previous period of intensive care.
- CVP and pulmonary artery pressure
- Urine output and urine electrolytes and osmolality
- Serum electrolytes and glucose
- Core to peripheral temperature gradient (18, 27, 28, 33)

Initial volume replacement should consist of crystalloids like Ringer's lactate solution. They can be given in quite large amounts as long as the osmotic gradient is high enough to prevent tissue edema for which liver and lung are especially sensitive. (28, 33)

Colloidal fluids containing albumin should be added if osmotic pressure drops and additional volume expansion is needed. They have to be titrated to preserve kidney function and the target value to prevent tissue edema is serum albumin above at least 25 g/l. (28, 33)

Once adequate volume expansion is achieved maintenance fluids containing dextrose (for example 5% dextrose or 5% dextrose in 45% saline solutions) should be given to preserve the intrahepatic glucose stores. (28, 33)

The inappropriate use of 0,9% sodium chloride solutions can cause progressive hyponatremia and therefore should be avoided. (27, 28)

Aggressive volemic expansion can help to rapidly achieve an ScvO₂ of > 85% and results in shorter hypotension time, lower doses of vasopressors and better organ retrieval. (1)

3.2.2.2 THE ROLE OF VASOACTIVE DRUGS

If hypotension persists despite adequate fluid resuscitation, which happens in more than 80% of all cadaveric donors, vasoactive and inotropic agents should be administered. (1, 27, 28, 30, 33)

Vasopressor administration should be guided by the data obtained from invasive monitoring. (30)

The criterium for optimal circulation should be optimized organ perfusion and not just optimal systolic blood pressure. (27, 28)

After brain death cardiac activity is influenced by the sympathetic system only. (28)

Dopamine has been the traditional inotropic agent of choice after brain death. At low rates (< 5 µg/kg/min) it causes vasodilatation in the renal arteries, which can minimize the ischemic damage. It also directly stimulates diuresis and seems to be a better inotropic agent than dobutamine. (28, 29, 30)

At a dosage greater than 5 µg/kg/min systolic blood pressure further increases but organ perfusion diminishes due to vasoconstriction caused by the activation of α-receptors. (28)

At dopamine rates above 15 µg/kg/min an impairment of hepatosplanchnic and renal perfusion occurs with a higher incidence of acute tubular necrosis in the kidneys and a deleterious effect on the liver energy metabolism of these donors. (18, 28, 29)

Dopamine might also suppress the function of the anterior pituitary gland. (29)

Recipients of cardiac grafts from donors who received high doses of dopamine have difficulties in attaining hemodynamical stability, which might be explained by the change of β-receptor density in these grafts. (28)

β-agonists like dobutamine, dopexamine or isoproterenol have minimal to no peripheral vasoconstrictive effect. (28, 33)

Dobutamine can be very useful to detect transient regional wall motion abnormalities like systolic inward motion or thickening in dobutamine stress echocardiography. These dobutamine-responsive wall motion abnormalities are characteristic of a neurologically stunned myocardium and potentially reversible after engraftment. (1, 29)

However, dobutamine's vasodilator effect can also aggravate hypotension and cause tachycardia. In addition, high doses of β -agonists lead to a depletion of the myocardial ATP stores and to a down regulation of β -receptors. (1)

Recently the early use of vasopressin in its vasopressor dosage (1-8 IU/hour) has been recommended.

Its use has a positive effect on organ perfusion because it is less likely to cause metabolic acidosis and pulmonary hypotension. (1, 28, 29, 30, 33)

α -adrenergic vasoconstrictors like norepinephrine, epinephrine and phenylephrine should only be used in the lowest possible dosage if a short term stimulation of the α -adrenergic receptors is needed, for example in spinal shock or a state of vasoplegic shock if the measures mentioned above fail. They can cause massive vasoconstriction, pulmonary hypertension and metabolic acidosis. (1, 28, 33)

3.3 TREATING DIABETES INSIPIDUS

The failure of the posterior pituitary gland after brain death causes a depletion of ADH leading to massive polyuria. In 75-90% of all brain dead donors ADH has dropped to undetectable levels. (1, 27, 30)

The sudden onset of polyuria can cause dehydration, hypotension, hypernatremia, hypokalemia and cardiac arrhythmias. Therapy of this condition therefore should be based on volume status, serum sodium levels and serum and urine osmolality. (1, 28, 33)

If diuresis exceeds 150 ml/hour, the early use of vasopressin at an optimal dosage of a continuous infusion of 0,1-0,4 IU/hour can simplify fluid management and help to lower inotropic and vasopressor support. (27, 28, 33)

At rates greater than 0,04 IU/minute, however, vasopressin may also cause severe coronary, renal and splanchnic vasoconstriction especially in combination with other vasopressor drugs. (29, 30, 33)

There are three different receptors for vasopressin. While V_1 is located in the blood vessels mediating the vasopressor effect and V_3 is stimulating the secretion of ACTH in the anterior pituitary gland, V_2 can be found on renal collecting duct epithelia and is responsible for the antidiuretic effect of ADH or vasopressin. (29)

The synthetic vasopressin analogon desmopressin (1-desamino-8-D-arginine vasopressin) is highly selective for the V_2 -receptor and therefore has a significantly diminished vasopressor activity and is less likely to cause severe peripheral vasoconstriction. (28, 29, 33)

Because of its longer duration, the typical doses of desmopressin of 2 to 6 mg are given intravenously every 6 to 8 hours, depending on urine output. The infusion should be stopped about 6 hours before organ retrieval. (33)

Desmopressin will not provide hemodynamical support, but it can be very useful in treating diabetes insipidus, especially in those donors who prove resistant to vasopressin treatment. (1, 28, 29, 30, 33)

If urine output drops and remains below 1 ml/kg/hour after optimal fluid replacement, diuretics should be used. (30)

3.4 HORMONAL REPLACEMENT THERAPY

The striking hormonal depletion after brain death, especially of the thyroid hormones T_3 and T_4 is made responsible for the depletion of myocardial energy stores and a shift from aerobic to anaerobic metabolism, manifested by a rise in serum lactate and free fatty acids as well as marked acidosis represented by an increasing base deficit. (1, 33)

So the lack of T_3 and T_4 is likely to cause hemodynamic instability and deterioration of cardiac function. (1, 33)

Because of these findings it was postulated that the replacement of thyroid hormones T_3 as liothyronine and T_4 as L-thyroxine will lead to a complete reversal of anaerobic metabolism, to a reduction in inotropic and vasopressor requirements and will cause a drop in the number of donors lost to cardiac instability. It also should have a beneficial effect on the hemodynamically unstable donors and will significantly improve the one-month survival rate of the recipients of cardiac allografts. (26, 29)

T_3 is regarded a prohormone of T_4 , and more potent. It shows a faster onset of action but has a shorter half-life. Since brain death might impair the conversion from T_3 to T_4 , the former should be used as the primary agent. (33)

There are, however, limitations of the therapy with T_3 . In most cases the pretreatment levels of thyroid hormones are unknown, and it remains unclear if the administration of thyroid hormones causes hyperthyroidism in the donor. (26)

It is known that T_3 leads to non-DNA-dependant effects, like the normalisation of ionic calcium concentration and the activation of key enzymes of aerobic metabolism. Still little is known of the actual acute hemodynamic response to thyroid hormone therapy. (15, 26)

In contrast to those studies promoting the beneficial effect of thyroid hormone replacement therapy and suggesting that the use of T_3 can reactivate mitochondrial function and improve hemodynamic conditions, there are also studies that could not prove a favourable effect on cell metabolism. (15, 18, 28)

This inconsistency leads to the combination of thyroid with other hormones forming a 3-drug hormonal resuscitation package. It consists of:

- Either a single intravenous methylprednisolone bolus of 15 mg/kg or 2 g every 24 hours or low dose hydrocortison administered at a rate of 50 mg over 6 hours.
- Liothyronine (T_3) as an intravenous bolus of 4 μ g followed by an infusion of 3 μ g/hour or an intravenous bolus of 20 μ g L-thyroxine.
- Vasopressin administered as a 1 IU bolus infusion at a rate of 0,5-4 IU/hour titrated to an SVR of 10-15 Wood units (80-120 mPa/s/m³). (1, 26, 33)

This combination is strongly recommended if ejection fraction falls below 40% or hemodynamic instability persists despite full vasoactive support with the combined vasopressor need of dopamine and epinephrine exceeding 10 μ g/kg/minute. (1, 26, 33)

After this therapy a marked rise in MAP, CVP and cardiac output can be observed. This results in less cardio-vascular and metabolic problems, reduces the need for inotropic and vasoactive support, can convert the “unsuitable” donor and leads to a reduced incidence of early graft dysfunction. (26, 29, 33)

The shift from anaerobic back to aerobic metabolism is reflected by the reduced need for bicarbonate, and an increase in the arterial blood keton body ratio shows the reactivated reduction oxidation potential of hepatic mitochondria. (1, 18, 27, 29, 30, 33)

The replacement of glucocorticoids also has some other beneficial effects on the brain dead donor.

Brain death can lead to relative adrenal insufficiency and the significantly lower serum cortisol levels of donors who received dexamethasone during the intensive care period before declaration of brain death suggests a further suppression of the adrenal cortex. (ph)

Cortisol administration, however, can reduce the production and release of proinflammatory cytokines after brain death and can also alternate the immunological response of the recipient and prevent recipient leukocyte immigration after engraftment. (33)

Glucocorticoids should therefore be given as a part of the hormonal replacement therapy and because of its modulatory effect on the inflammatory processes after brain death. (30)

Another beneficial effect of the early use of high dose glucocorticoids like methylprednisolone at a dosage of 14,5 mg/kg every 12 to 24 hours is the reduction of pulmonary edema, the significant improvement of oxygenation and the increase of lungs recovered from these donors. (1, 29, 30, 33)

Hyperglycemia is present to some degree in all brain dead donors due to insulin resistance, the great increase of catecholamine levels during the autonomic storm and the infusion of hypotonic dextrose-containing fluids. (29)

If blood glucose is kept between 6,1 and 10 mmol/l, a beneficial effect on the hemodynamic stability of the donor and the survival of recipients can be observed. (29)

These findings suggested the inclusion of insulin into the hormonal resuscitation package. Regular insulin should be given at a minimum rate of 1 IU/hour titrated to blood glucose levels. In hemodynamically unstable donors with normal glucose values rapid intravenous boluses of one ampule of 50% dextrose and 20 IU of regular insulin is recommended.

During insulin treatment serum potassium levels should be closely monitored because the administration of insulin can aggravate hypokalemia. (26, 29, 30, 33)

3.5 VENTILATORY STRATEGIES AND PROTECTION OF THE LUNG AFTER BRAIN DEATH

Mechanical ventilation is required in all brain dead donors.

More than 30% of the lungs of brain dead donors that are primarily suitable for transplantation develop severe hypoxemia and an abnormal chest X-ray during the intensive care period. (1)

Acute respiratory distress syndrome (ARDS) is present in 15-20% of all cases along with neurogenic pulmonary edema, aspiration, atelectasis, hemo-pneumothorax and pneumonia. (1, 27, 29, 34)

One ventilatory strategy, which is similar to the one used in patients with severe brain injury consists of the use of high tidal volumes and low positive end-expiratory pressure (PEEP). This however may further exacerbate the pulmonary and systemic inflammatory response. (1, 34)

A shift from this “cerebral protective“ strategy towards a strategy using low tidal volumes and high PEEP or pressure-controlled ventilation for a short duration along with physiotherapy can prevent derecruitment and hyperinflation of the lung and convert unsuitable lung donors. (1, 29)

However, since the ventilatory pattern used should not only preserve lung function but also non pulmonary organ systems and the acid-base balance, the negative effects of high PEEP have to be taken into consideration. (1, 27, 28, 29)

PEEP of more than 5 cmH₂O leads to a decrease in thoracic venous return and a decrease in cardiac output. It should therefore be avoided in hemodynamically unstable donors. (1, 27, 28, 34)

Prolonged ventilation in the supine position can cause microatelectasis, a reduction of pulmonary compliance and functional capacity, despite the use of PEEP. (29)

Supplemental oxygen should be administered if necessary to maintain an arterial oxygen saturation of at least 95%. Hyperoxia with an unnecessary high concentration (>60%)³⁴

should be avoided because of its pulmonary toxicity and the development of atelectasis. (27, 34)

Arterial blood gases should be measured frequently, and minute ventilation and tidal volume should be adjusted to maintain an arterial pH of at least 7.4. If severe metabolic acidosis is present, for example, a higher minute ventilation is required to maintain pH goals. This can cause an increase of intrathoracic pressure and consecutively a drop in cardiac output. (27, 34)

CVP should be measured and fluid administration adjusted to achieve a CVP of 4-6 mmHg, which can help to minimize pulmonary edema. Higher values of CVP can increase the alveolar-arterial oxygen gradient. (29, 30)

Since bronchial colonisation with bacteria and/or yeast can be observed in up to 80% of all donors, every donor should undergo bronchoscopy for therapeutic bronchial toilet and to isolate potential pathogens to guide antibiotic therapy in the donor and the recipient. (1, 29, 30)

Prophylactic antibiotic treatment of the donor without identification of a pathogen is not recommended. (1, 29, 30)

As mentioned above, the use of high dose glucocorticoids also has a beneficial effect on potential lung donors. (1, 29, 30, 33)

3.6 OTHER THERAPEUTIC CONSIDERATIONS

3.6.1 PREVENTING HYPOTHERMIA

After brain death hypothermia, which is caused by a lack of hypothalamic regulation is often observed. Without passive warming, the donor's body temperature will fall to that of the environment, causing vasoconstriction, bradycardia and cardiac instability. (27, 28)

To maintain a temperature of at least 35°C, a warming mattress, or blanket should be applied. If severe hypothermia is observed, intravenous fluids should be warmed and

oxygen administered through the respirator should be humified and heated to 40-46°C. (26, 27, 28, 34)

3.6.2 TREATING BLOOD LOSS AND COAGULOPATHY

Blood loss should be replaced with blood transfusions to maintain a hematocrit of 30% and a hemoglobin level of 10 g/dl. This ensures oxygen supply while preventing changes in blood flow due to increased viscosity. (27, 29)

Disseminated intravascular coagulation is present in up to 88% of patients with lethal head injuries but dilutional coagulopathy from blood loss, preexisting coagulation abnormalities or the use of anticoagulants should be taken into consideration as well. (26, 31)

Severe brain injuries can cause the release of tissue thromboplastin and lead to abnormalities in fibrinolysis. These adverse effects can ultimately cause cardiac arrest.

Usually coagulopathies are corrected with the use of plasma or vitamin K but in some cases that might not be successful. (31)

In those cases recombinant factor VIIa can help to stabilize the patient.

Although thrombembolic events have been observed after this treatment, no increase in the risk of thrombosis in the transplanted organs of donors that received recombinant factor VIIa has been found. (31)

3.6.3 CORONARY ANGIOGRAPHY

Coronary angiography should be performed in all potential heart donors who are 40 years or older, have other risk factors for coronary artery disease or require high inotropic support. (29)

To prevent the development of contrast nephropathy after angiography, acetylcystein and bicarbonate can be administered. (29)

3.6.4 PROTECTIVE STRATEGIES FOR THE KIDNEYS

Protection of the kidney function requires the maintenance of adequate systolic blood pressure above 80-90 mmHg and adequate cardiac output as well as the avoidance of vasoconstriction. (29)

The administration of naloxone also improves kidney function because of its ability to antagonize endorphine activation and inhibit neutrophil superoxide radical release. This helps to reverse cellular hypoxia, improves the transmembrane potential and protects the sodium-potassium pump. (15)

High doses of dopamine (above 10 $\mu\text{g/kg/min}$) should be avoided because they increase the incidence of acute tubular necrosis and allograft failure. (29)

Low doses of dopamine (2 $\mu\text{g/kg/min}$) in combination with an infusion of 20 g of mannitol over 30 minutes and an intravenous bolus of furosemide 10 mg immediately before organ retrieval, however, can improve allograft function in the recipient. (18, 27, 29, 30)

3.6.5 PROTECTIVE STRATEGIES FOR THE LIVER

Several liver function tests exist, but it remains unclear in which extent they should be used in the brain dead donor. (18)

Hypernatremia with serum sodium values above 155 mmol/l should be avoided to prevent the accumulation of idiogenic osmoles and the consecutive intracellular water accumulation after engraftment. (29, 30)

A CVP between 8 and 10 mmHg and the usage of low PEEP help to prevent hepatic congestion. (30)

Adequate nutrition of the donor during intensive care can restore liver glycogen stores and reduce the incidence of liver allograft loss. (18, 30)

3.6.6 STRATEGIES TO MINIMIZE REPERFUSION INJURY

A calcium antagonist like verapamil can decrease intracellular calcium and thereby reduce the production of oxygen radicals and the damage after reperfusion.

Allopurinol, catalase, superoxide dismutase, chlorpromazine and lidocaine given prior to ischemia also have proven to decrease the incidence of reperfusion injury, especially in the kidney and the heart. (28)

4 EXISTING BRAIN DEATH ANIMAL STUDIES

Various animal studies have been designed to further examine the effects of brain death on the function of peripheral organs and to evaluate strategies in donor management, which might contribute to a better organ quality and ameliorate allograft function.

The earliest experiments have been performed on baboons by Novitzky et al. in 1984.

Further studies used rats, pigs, dogs, cats and rabbits. (35)

4.1 PIGS

In animal brain dead studies domestic pigs have been used because of their availability and resemblance to the human cardiovascular system.

The pigs were 4-6 months old and weighted between 25 and 60 kg, the experiments usually lasted for 6 hours and after that period laparotomy was performed and the organs were harvested and analysed.

The aims of existing brain death studies on pigs included the examination of physiological changes after brain death and the impact of hormonal replacement. (15, 36, 37)

One study by Mertes et al. in 1994 was designed to monitor changes in hemodynamic and metabolic parameters in the absence of inotropic or fluid support. Plasma vasopressin and atrial natriuretic peptide concentrations were measured to assess the suppression of pituitary function and evaluate the endocrine role of the heart. (36)

A volume expansion protocol using 20 ml/kg of dextran infused over 15 minutes was performed as well to study the dynamic responses of hemodynamic parameters. (36)

Other studies examined the effect of hormonal resuscitation and compare them with a norphenephrine- and fluid-based protocol. (15, 37)

The pigs received a combination of triiodothyronine, corticosteroids, insulin and vasopressin. (37)

The impact on cardiac function, hemodynamics and need for vasopressors were recorded and analysed. (15, 37)

4.1.1 METHODS

The pigs were anesthetized, intubated and placed into the supine position to catheterized the bladder under direct vision through a suprapubic incision. (36, 37)

Continuous monitoring in brain death studies on pigs included ECG, pulse oxymetry, end tidal CO₂, core temperature, invasive arterial blood pressure, CVP, left ventricular pressure, pulmonary artery pressure, pulmocapillary wedge pressure, venous oxygen saturation and cardiac output. (36, 37)

In two experiments by Mertes et al. and by Hing et al. the pigs were monitored for a duration of 6 hours. (36, 37)

Different kinds of invasive monitoring like a PAC inserted through the external jugular vein or an ultrasonic dimension transducer affixed to the epicardium have been used. (36, 37)

4.1.2 INDUCTION AND CONFIRMATION OF BRAIN DEATH

Two different methods have been used to induce brain death in the pig. (36, 37)

The first method consisted of drilling a burr hole in the right frontoparietal region of the skull and inserting a Foley catheter into the subdural space. The balloon of the catheter was inflated with saline solution at a rate of 20±10 ml over 15 seconds or with water at a rate of 3 ml every 30 seconds to a total of 24 ml. (36, 37)

The second method included clamping or ligation of the two main arteries, which arise of the arch of the aorta, so that both, carotid and vertebral arterial flow, were interrupted causing sudden ischemia of the brain. (15)

Brain death was confirmed by the absence of response to painful stimuli and the absence of pupillary and corneal reflexes as well as complete disappearance of electroencephalic activity. (36, 37)

4.1.3 DONOR MANAGEMENT IN PIGS

Donor management was not necessarily performed in brain death studies using pigs. (36)

If it was applied, it consisted of fluid replacement with intravenous saline 0,9% solution infusion at 10 ml/kg in the first hour, followed by 5 ml/kg/hour titrated to a CVP of 0-5 mmHg.

If MAP fell below 60 mmHg despite this, intravenous norepinephrine infusions of 20µg/ml were given to reach an MAP of 60-70 mmHg. (37)

At hourly intervals arterial and venous blood gases and pH, glucose, blood lactate and hemoglobin were analysed. (36, 37)

4.2 OTHER ANIMALS

Brain death studies on animals are similar in many aspects, irrespective of the kind of animal used.

Still there are some differences among the species.

4.2.1 RATS

Rats are often used in brain death animal studies. (38, 39, 40)

The aims of studies on rats were for example the development of a reproducible rat model that does not require inotropic support, and the investigation of blood flow redistribution after brain death, using coloured microspheres. (38, 39)

Anesthesia is induced either with inhaled isoflurane or with intraperitoneal injection of various anesthetics. (38, 39)

The rats were either intubated under direct vision, or tracheostomy is performed because of the small size of the anatomical structures. (38, 39, 40)

Hemodynamic monitoring consisted of catheters placed into the aorta through the left femoral artery to monitor arterial blood pressure and blood gases, and into the right jugular vein to monitor CVP. (38, 39, 40)

Brain death was induced by inserting a catheter into the subdural or extradural space and inflating the balloon of this catheter.

Brain death was confirmed by a positive apnea test and the absence of brain stem reflexes. (38, 39)

A different approach was used to investigate the impact of diffuse brain injury associated with subdural hemorrhage. A burr hole was drilled into the skull but the dura was left intact. A steel disc was then fixed to the skull and a brass weight of 450 g was dropped from 1 or 2 meters onto this disk. After that 300-400 µl of non-heparinized autologous blood were infused into the subdural space for 6 minutes. (40)

Donor care consisted of adjustments in ventilation and the administration of colloidal fluids. (38, 39)

One interesting finding in one of these studies was that rats react differently to brain death than dogs in regard to changes to SVR. While in the rats SVR increased only for a very short time after induction of brain death and then dropped below the control value, dogs showed a continuous significant increase in SVR. (39)

An explanation for this difference could be that in living rats there is a dominance of the sympathetic tone over the parasympathetic one, while in dogs the parasympathetic tone is dominant. Thereby the removal of the dominant part of the autonomic tone after brain death causes a substantial vasodilatation in rats and a rise in heart rate and arterial blood pressure combined with less vasodilatation in dogs. (39)

4.2.2 DOGS

Dogs were used in animal brain death studies to investigate the changes in hormone concentration and catecholamines, to compare the effects of gradual and explosive increase of ICP and to evaluate the impact of neural and humoral changes caused by brain death on the heart. (12, 14, 41)

Anesthesia was induced with fentanyl, pentobarbital and piritramide and the dogs were paralyzed with pancuronium bromide.

They were endotracheally intubated and ventilated. (12, 14, 41)

Monitoring included the invasive measurement of peripheral arterial blood pressure, left ventricular pressure and cardiac output as well as continuous ECG, EEG, urine output, rectal temperature and ICP monitoring. (12, 14, 41)

Brain death was induced either by ligation of both internal carotid and vertebral arteries close to their origin from the subclavian artery, or by the inflation of the balloon of an epidurally or subdurally placed catheter. (12, 14, 41)

To study the effects of gradual increase of ICP an infusion pump was used to infuse saline solution at a rate of 4 ml/hour. The explosive increase of ICP was simulated by injecting a bolus of 4 ml every hour until brain death. (12)

Brain death was confirmed by neurological examination, an isoelectric EEG and a cerebral perfusion pressure below 0 mmHg. (12, 14, 41)

Donor care included fluid replacement with Ringer's solution and 5% dextrose in normal saline as well as substitution of potassium and bicarbonate if necessary. (14, 41)

Blood samples were taken before the induction of brain death and at regular intervals to control arterial blood gases, pH, electrolytes, circulating catecholamines, lactic dehydrogenase and creatine kinase as well as the levels of cortisol, vasopressin, insulin, glucagon, T₃ and T₄. (14, 41)

To differentiate between neural and humoral effects on the heart after brain death, a cross-circulated heart model was used. (14)

To test neural effects, the heart of a brain dead dog was isolated from its own blood flow and perfused with the blood of a healthy support dog. (14)

The hearts of healthy support dogs were explanted and parabiotically perfused with the blood of brain dead dogs to evaluate humoral factors. (14)

The combination of both factors was tested by leaving the hearts of brain dead dogs *in situ* but controlling coronary blood flow and perfusion pressure with an extracorporeal roller pump system to keep loading conditions stable. (14)

4.2.3 CATS AND BABOONS

Cats and baboons are used less often in animal brain death studies.

The aims of studies on these two species were the observation of hemodynamical changes after brain death, the effects of hormonal replacement therapy and the impact on the myocardium. (15, 42)

Induction of brain death, monitoring and donor care were performed in the ways described above. (15, 42)

5 AIM OF THE STUDY

Currently organ transplantation is the method of choice in treating irreversible, terminal organ failure. The recipients of transplanted organs gain a very good quality of life and experience only a mild modification to their everyday life. In established transplantation centres one-year survival rates after transplantation reach 98%.

Apart from the development of better surgical techniques as well as improved concepts of post-operative treatment, it is mostly due to the discovery of potent immunosuppressant drugs that organ transplantation could finally become part of clinical routine.

First and foremost the organs for transplantation are retrieved from cadaveric donors, i.e. brain dead donors.

Because of a shortage in donors and organs available the number of transplantations stayed at the same level or even slightly decreased in the previous years. This discrepancy between organs available and the need for transplantations leads to an increasing mortality of people on the waiting list. This discrepancy grows as the indications for transplantation are assessed more generously and the acceptance of transplantation grows among the population while the willingness for organ donation stays unchanged at a low level.

Irrespective of the immunological compatibility, allografts from unrelated living donors still show a better short- and long-term function than those from cadaveric donors. This fact points out the importance of antigen-independent factors and relativizes the role of HLA-differences.

The assumption that the difference in organ survival of allografts from cadaveric and from living donors is based more strongly on pathophysiological than on genetic factors leads to research in the field of functional and structural changes after brain death and their connection with allograft function after transplantation.

Although some pathophysiological changes after brain death like hormonal and metabolical alterations have already been investigated, several factors influencing organ quality after multiorgan donation are still unknown and are difficult to investigate in humans.

Therefore the need for a reproducible and reliable animal model arises – a model that imitates human conditions concerning the development of brain death and donor care. Such a model might not only be useful to monitor pathomechanisms of brain death and

biochemical cascades in the organism after brain death, but could also help to investigate new strategies for the improvement of organ quality and functionality after multiorgan donation.

Hence the aim of this study was to establish a new brain dead donor model in pigs that can be used for such research. Primary endpoint in our study was the induction of brain death. Secondary endpoints were collecting and analysing data about blood pressure, heart rate and fluid balance, taking and analysing arterial blood samples and to perform multiorgan donation and consecutive analysis of the organ quality.

6 MATERIALS AND METHODS

6.1 MATERIALS

The materials used in this study were the following:

Aquillion® One, Toshiba Medical Systems GmbH, Wiener-Neudorf, Austria.

Alpha-trace digitalEEG®, B.E.S.T. medical systems - Dr. Grosseegger & Drbal GmbH, Vienna, Austria.

Dräger Sulla® 808V, Drägerwerk AG & Co. KGaA, Lübeck, Germany.

Braun Perfusor sucra®, B. Braun Melsungen AG, Melsungen, Germany.

SEVOrane®, Abbott GMBH, Vienna, Austria.

Stresnil®, Janssen Pharmaceutica, Beerse, Belgium.

Ketasol®, Dr. E. Gräub AG, Bern, Switzerland.

Gewacalm®, Nycomed Austria, Linz, Austria.

Diprivan®, Astrazeneca Österreich, Vienna, Austria.

Nimbex®, GlaxoSmithKline Pharma GmbH, Vienna, Austria.

ELOMEL® semiton, Fresenius Kabi AG, Graz, Austria.

Voluven®, Fresenius Kabi AG, Graz, Austria.

Arterenol®, Sanofi-aventis, Vienna, Austria.

Brevibloc®, BAXTER Vertriebs GmbH, Vienna, Austria.

Lasix®, Sanofi-aventis, Vienna, Austria.

Minirin®, Csl Behring, Vienna, Austria.

ARGYLE® THI Aortic Perfusion Cannulas, Tyco Healthcare Austria GmbH, Brunn am Gebirge, Austria.

Herztransplant Set®, HMT Medizintechnik GmbH, Maisach, Germany.

ViaSpan®, Bristol-Myers Squibb, Vienna, Austria.

Celsior®, Genzyme Austria GmbH, Vienna, Austria.

6.2 METHODS

6.2.1 ANIMALS

13 styrian domestic pigs, *suus domesticus*, were chosen for this experiment. They weighed about 35 kg and their age corresponded to the human age of 25 years. The animals were housed and treated in accordance to the Austrian Laboratory Animal Law and the study was approved by the ethical committee. The experiment was performed three times on a group of 4 pigs and once on a group of 5 pigs

6.2.2 PREMEDICATION AND ANESTHESIA

The animals were fasted overnight and premedication was administered about 20 minutes before the first peripheral intravenous catheter was inserted.

It consisted of intramuscular injection of 10 mg diazepam (Gewacalm®), 360 mg azaperone (Stresnil®) and 1500-2500 mg ketamine (Ketasol®).

After sufficient disinfection of the skin a small peripheral intravenous catheter was placed into the vein of the pig's ear.

After sufficient preoxygenation anesthesia was induced with 2-3 µg/kg of fentanyl and 3 mg/kg of propofol (Diprivan®). The pigs were intubated with an endotracheal cuffed spiral tube and after successful and verified intubation 0,15 mg/kg of cisatracurium (Nimbex®) were administered.

Anesthesia was maintained using sevoflurane (SEVOrane®) 2% inhaled gas and the pigs were volume-controlled ventilated at a 1:2 ratio with a stroke volume of 8 ml/kg, using a Dräger Sulla® 808V anesthetic machine. Ventilation was adapted to CO₂ values to prevent hyper- or hypoventilation.

6.2.3 MONITORING

After induction of anesthesia the pigs were placed into supine position and a catheter was inserted into the internal jugular vein on both sides to be able to monitor CVP and administer fluids as well as catecholamines if necessary.

The right or left femoral artery was cannulated in order to monitor arterial blood pressure invasively.

Further monitoring included pulse oxymetry at the pig's tail, capnometry, a rectal temperature probe and continuous ECG.

The bladder was catheterised under direct vision through a suprapubic incision to monitor urine output.

Systolic and diastolic blood pressure, heart rate and urine output as well as the amount of fluids and drugs administered were noted down on a standard intensive care sheet.

6.2.4 INDUCTION AND CONFIRMATION OF BRAIN DEATH

After trepanation of the frontal skull of the pig, a bladder catheter (CH 16, 50 ml) was inserted into the epidural space along with an intracranial pressure probe to monitor ICP.

The balloon of the catheter was then expanded with 50 ml of saline solution at a rate of 99 ml/hour, using a Braun Perfusor sucra® perfusion pump.

Brain death diagnostic was performed after anesthesia was stopped 60 minutes after complete inflation of the balloon. It consisted of a clinical neurological examination, which included the testing of brain stem reflexes and reaction to painful stimuli, CT-scan of the brain, apnea test and 8-channel needle EEG monitoring for 30 minutes.

The EEG was recorded as described in a study by Drenthen et al. in 2003. (43) The first pair of electrodes was inserted 1 cm behind a reference line that connected both medial eye borders and 3 cm to the left and right of the midline. The second and third pair of electrodes was positioned 4 cm to the left and right of the midline in a distance of 2,5 and 4 cm to the reference line. The last two electrodes were inserted 2 cm in front of the reference line and 1 cm to the left and right of the midline. The reference electrode was inserted in the midline.

The EEG was evaluated by a neurologist and only if the EEG was isoelectric, the apnea test showed CO₂ values of above 60 mmHg and all reflexes were absent, brain death was confirmed.

6.2.5 DONOR CARE

After the pig's brain death was confirmed donor care according to the ÖBIG guideline was performed. This included the following measures:

The rate of fluid replacement was calculated, using the 4/2/1 rule, which means 4 ml/kg/hour for the first 10 kg, 2 ml/kg/hour for the second 10 kg, and 1 ml/kg/hour for any weight over 20 kg. For a pig weighing 35 kg this was about 75 ml/hour of cristalloid solution (ELOMEL® semiton with glucose, Ringer's lactate or saline solution 0,9%) for normotensive periods and colloidal solutions (Vluven®) for hypotensive periods. The need for fluid substitution was adjusted to the amount of urine output during donor care.

If necessary hypotension was also treated with norepinephrine (Arterenol®) 25 mg in 50 ml of saline solution infused at a rate of about 0,7 ml/hour and dopamine 5 µg/kg/hour.

Tachycardia was treated with boluses of esmolol (Brevibloc®).

Diabetes insipidus was controlled with desmopressin and if urine output dropped below 1 ml/kg/hour, furosemide (Lasix®) was given as bolus.

Aspirin was given to all animals at the beginning of the experiment to prevent unwanted coagulation.

If strong spinal automatisms occurred, cisatracurium (Nimbex®) was given as a bolus of 2-10 mg.

Diabetes insipidus was treated with desmopressin (Minirin®) 1 µg.

Hypothermia was treated with a heating lamp.

Arterial blood samples were drawn before brain death and about every three hours after brain death, depending on the pig's condition. They included hemoglobin and hematocrit, the levels of sodium, potassium, chloride, free calcium, glucose and lactate, blood gases, oxygen saturation, bicarbonate and base excess.

At the beginning of the experiment a venous blood sample was drawn and analysed for complete blood count (leukocytes, erythrocytes, thrombocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean platelet volume, eosinophiles, basophiles, monocytes, nucleated red blood cells), sodium, potassium, chloride and calcium levels, creatinine, urea and uric acid levels, pancreatic amylase and glucose levels as well as NT-proBNP.

Urine analysis was performed simultaneously and both tests were repeated once seven hours after brain death.

6.2.6 ORGAN RETRIEVAL AT THE END OF THE EXPERIMENT

Similar to organ harvesting in humans all organs were dissected at the same time but in this case the heart was explanted prior to the liver, pancreas and kidneys.

First median sternotomy was performed and the pericardium was opened. The aorta and the superior and inferior vena cava were prepared before the cannula for cardioplegy from the HTM set for heart transplantation was inserted. 10000 IU of heparin were administered after the abdominal perfusion cannula had been inserted and after another two minutes the aorta was clamped and cardioplegy using Celsior® and ice water was started. The superior vena cava was ligated, the inferior vena cava was cut through and the left main trunk of the pulmonary artery was cut open. After ten minutes of cardioplegy the remaining structures, i.e. superior vena cava, aorta, pulmonary artery and left atrium, were cut through and the heart was placed into preservation solution and packed for storage.

For the retrieval of the abdominal organs median laparotomy was performed. The ascending colon and the duodenum were mobilized and the aorta and its bifurcation into the common iliac arteries as well as the inferior vena cava were dissected. After the administration of heparin, the aorta was cannulated with a straight ARGYLE® THI aortic perfusion cannula and the inferior vena cava was cut open. The aorta then was clamped below the diaphragm and the perfusion with 3000 ml of ViaSpan®-solution was started. Simultaneously the cooling with ice and ice water was performed. The organs were explanted in the following order: liver, pancreas, kidneys.

First the inferior vena cava was dissected as far as the liver and the left and right renal veins were cut at their confluences into the vena cava. Then the vena cava was infrahepatically cut through and the aorta was cut open and its lumen inspected. The renal arteries were cut out along with an aortic patch. For the explantation of the liver the hepatoduodenal ligament was dissected and the hepatic artery and vein as well as the bile duct were cut through. After the suprahepatic veins had been transected and the adhesions to the diaphragm had been divided, the liver could be explanted.

The pancreas was harvested using the no-touch technique. It was mobilized from the lateral to the medial side and the superior mesenteric artery and celiac trunk were cut out along with a big aortic patch. The duodenum was severed postpylorically and above Treitz's arch with a stapler.

The right kidney was dissected from the medial to the lateral-caudal side. The ureter was dissected well caudally sparing the periuretral tissue and transected. The left kidney was explanted the same way.

All organs then were placed into UW-solution and packed for storage.

6.2.7 STATISTICAL ANALYSIS

Analysis of the collected data and construction of the charts was performed using the SPSS® statistical software. The paired-sample t-test was used for normally distributed variables and the Wilcoxon signed-rank test was used for the not normally distributed variables. Statistical significance occurs if p is $<0,05$.

7 RESULTS

The data of 11 animals were used for statistical analysis.

We were able to successfully induce brain death in 10 animals. Brain stem reflexes were absent and the apnea test was positive with a $p\text{CO}_2$ above 60 mmHg in all pigs. These findings correlated with an isoelectric EEG in all 10 cases (Fig. 1).

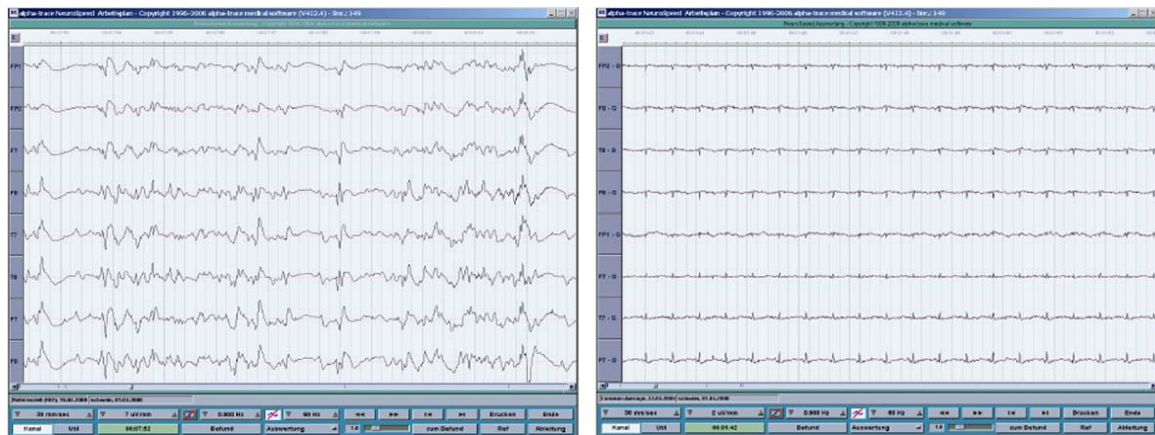


FIG. 1 The left side of the picture shows an example of a physiological 8-channel EEG of a pig. The right side shows the isoelectric EEG after induction of brain death.

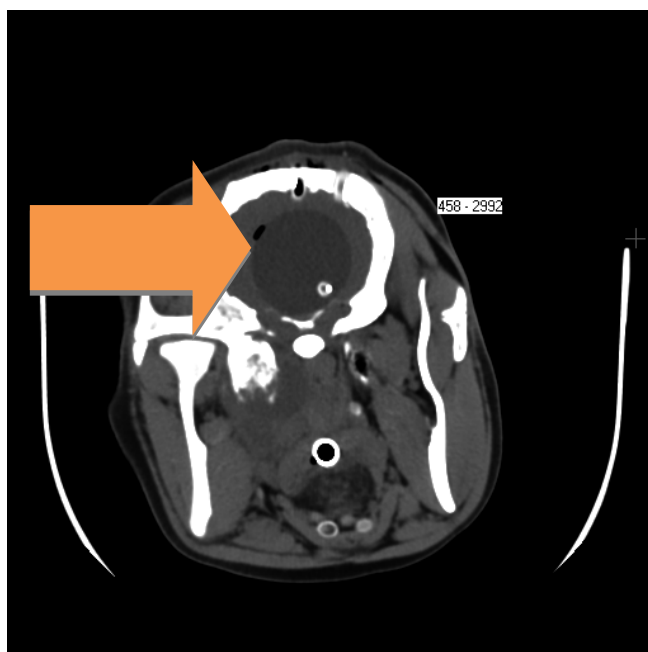


FIG. 2 A CT-scan of a pig's head which was performed after complete inflation of the balloon of the intracranially placed catheter. The arrow shows the displacement of the brain, flattening of the sulci and midline shift.

The CT-scan, which was performed after complete inflation of the balloon of the intracranially placed catheter, showed massive displacement of the brain, flattening of the sulci and midline shift in all animals. (Fig. 2)

Multiorgan donation could be performed in 7 pigs. The organs were harvested as soon as there were signs that indicated that the pig's condition would become unstable in regard to blood pressure and heart rate.

Table 1 shows how long the pigs survived. More than half of the animals (7 out of 11 pigs) survived 14 hours after brain death had been confirmed. 3 pigs were observed for 15 hours after confirmation of brain death but one of them died before multiorgan donation could be performed. Another 2 pigs were stable until the interval between the 16th and the 18th hour. The organs of one pig were retrieved 18 hours after brain death and one pig could be observed for 19 hours after brain death.

4 pigs died before multiorgan donation could be performed.

One pig died immediately after induction of brain death so that no more data could be retrieved from this animal.

Another pig died 30 minutes after brain death of blood loss after surgical insertion of the urine catheter.

One more pig was lost because of a malfunction of the anesthetic machine 7 hours after brain death had been confirmed.

The fourth pig died in the 15th hour after brain death had been confirmed. Prior to the animal's death, 9 hours after brain death, successful cardio-pulmonal resuscitation had been performed on this pig, after blood pressure had dropped to undetectable values. 30 minutes before the animal was lost to uncontrollable hypotension, severe cramps were observed in this animal.

	+ 5 hours	+ 7 hours	+ 14 hours	+ 16 hours	+18 hours	+ 19 hours
Number of pigs alive	9	8	7	4	2	1

TABLE 1 This table shows the number of pigs alive at 5, 7, 14, 16, 18 and 19 hours after brain death had been confirmed.

Great swings in blood pressure could be observed during donor care. The summarized values can be found in Fig. 3 and the individual values of all pigs are shown in Fig. 4.

At the time of brain death the values of systolic blood pressure differed greatly between the pigs. This is followed by a relatively stable period until 13 hours after brain death when some pigs became hypotonic while in others blood pressure rose to very high levels. Nevertheless, none of these changes reached statistical significance compared to the baseline value.

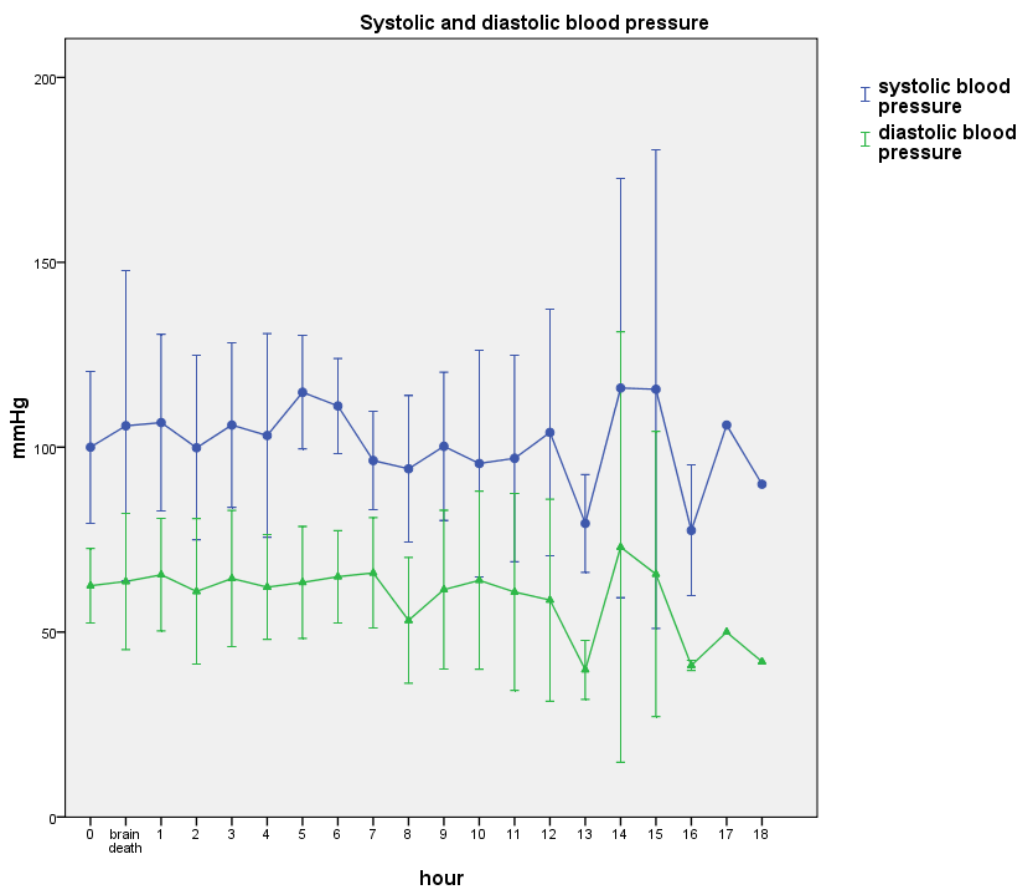


FIG. 3 Changes in systolic and diastolic blood pressure during the observation of the pigs. All values are expressed as mean $\pm$ standard deviation (SD). The number of pigs whose data were available for analysis (n) changed over time. At the time of brain death, the values of systolic blood pressure differed greatly between the pigs. This is followed by a relatively stable period until 13 hours after brain death when some pigs became hypotonic while in others blood pressure rose to very high levels. None of these changes reached statistical significance ($p < 0,05$).

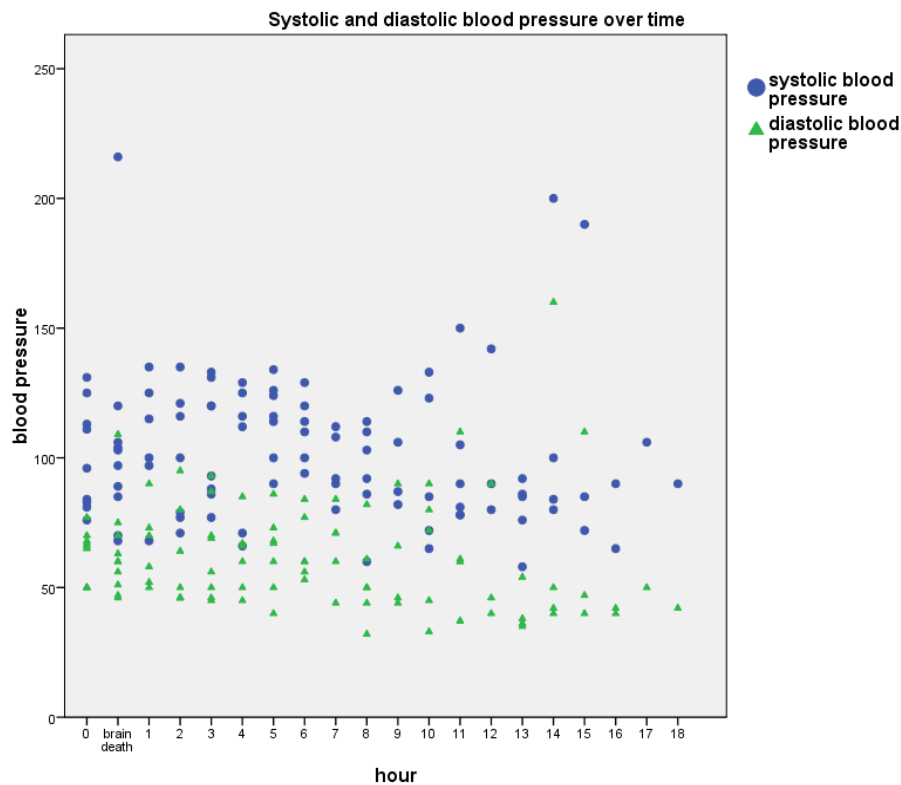


FIG. 4 This figure shows the systolic and diastolic blood pressure of all pigs over time. The baseline values already show great differences in systolic blood pressure. At the time of brain death one pig's systolic blood pressure rose to 216 mmHg, while another pig became hypertonic with a blood pressure of 200/160 and 190/110 mmHg 14 and 15 hours after brain death.

The baseline value of blood pressure before the induction of brain death (n=9) was $100 \pm 21 / 63 \pm 10$ mmHg. During the induction of brain death values differed greatly, from 68/47 to 216/109 mmHg, with a mean of $106 \pm 42 / 64 \pm 18$ (n=10).

The highest mean of blood pressure was found 5 hours after brain death, $115 \pm 15 / 63 \pm 12$ (n=7). The lowest mean of blood pressure was found 16 hours after brain death, $78 \pm 18 / 41 \pm 1$ mmHg (n=2).

The highest individual values of blood pressure were noticed during brain death, 216/109 mmHg and 14 hours after brain death had been confirmed, 200/160 mmHg.

The lowest individual values of blood pressure were noticed 8 hours, 60/32 mmHg and 13 hours after brain death 58/36 mmHg.

The changes of heart rate over the observation period is depicted in Fig. 5.

Heart rate rose during brain death and further increased until 2 hours after. This was followed by a decrease until the 11th hour after brain death. A slight increase between the 11th and the 12th hour after brain death was followed by another decrease towards the end of the observation period.

The baseline value of heart rate before induction of brain death (n=8) was 93 ± 18 beats/minute. During the induction of brain death similar to blood pressure values differed greatly from 74 to 151 beats/minute with a mean of 114 ± 32 beats/min (n=10). Significant changes ($p < 0,05$) in heart rate compared to the baseline value could be found 2 hours (147 ± 21 beats/min), 3 hours (141 ± 27 beats/min), 4 hours (146 ± 25 beats/min), 5 hours (137 ± 33 beats/min), 6 hours (130 ± 24 beats/min), 8 hours (125 ± 21 beats/min) and 9 hours (124 ± 18 beats/min) after brain death.

The highest mean of heart rate was found 2 hours after brain death, 147 ± 21 beats/min (n=7). The lowest mean of heart rate was found before the induction of brain death, 93 ± 18 beats/min (n=8).

The highest individual values of heart rate were observed 1 hour, 187 beats/minute and 4 hours after brain death, 180 beats/min.

The lowest individual values of heart rate were noticed during the induction of brain death, 74 beats/min and before the induction of brain death 77 beats/minute.

The changes of fluid intake and urine output are shown in Fig. 6.

The highest mean of fluid intake was found 8 hours after brain death, 571 ± 450 ml (n=7), while the lowest mean of fluid intake was found 14 hours after brain death, 275 ± 35 ml (n=2).

The highest mean of urine output was found 7 hours after brain death, 489 ± 304 ml (n=8), while the lowest mean of urine output was found 16 hours after brain death, 112 ± 53 (n=2).

The highest individual amount of fluids received in one hour was observed 8 hours after brain death and was 1500 ml. The lowest amount of fluids received in one hour was observed 13 hours after brain death and was 150 ml.

The highest individual amount of urine output in one hour was observed 2 hours after brain death and was 980 ml. The lowest individual amount of urine output was observed 13 hours after brain death and was 20 ml.

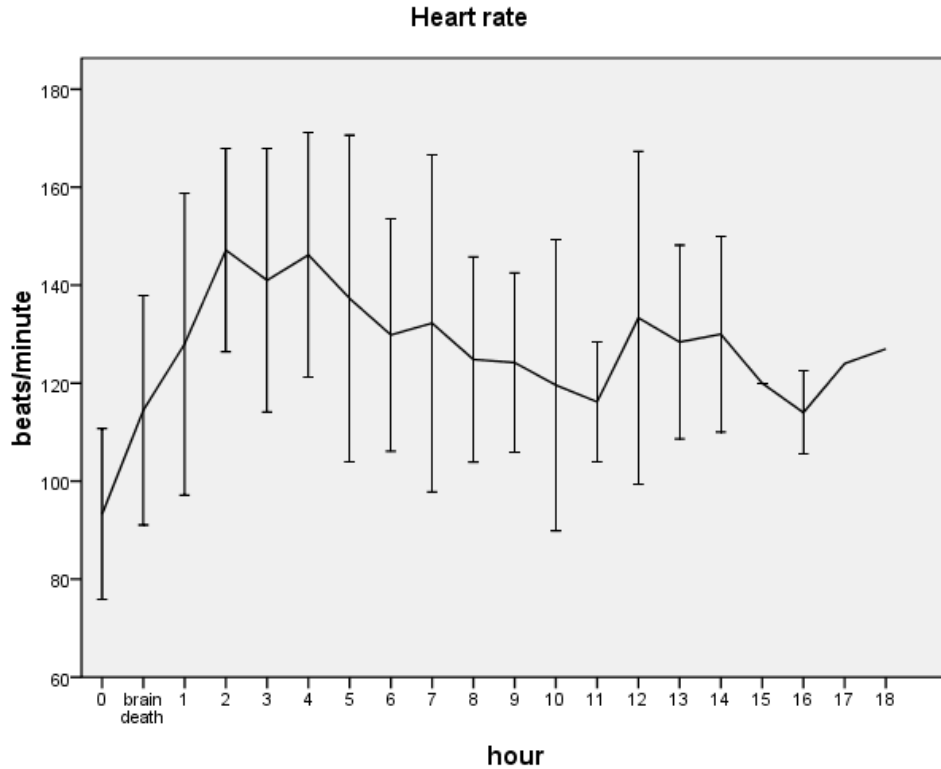


FIG. 5 Changes in heart rate during the observation of the pigs. All values are expressed as mean $\pm$ SD. The number of pigs whose data were available for analysis (n) changed over time. Heart rate rose during brain death and further increased until 2 hours after. This was followed by a decrease until the 11th hour after brain death. Towards the end of the observation period heart rate first slightly increased again and then fell until the 16 hour after brain death. Significance in the change of heart rate ($p < 0,05$) compared to the baseline value was reached 2, 3, 4, 5, 6, 8 and 9 hours after brain death.

The mean of fluid balance at the time of organ harvesting or the pig's death was $+2407 \pm 956$ ml. The highest individual fluid balance was +4000 ml, observed in a pig whose organs were harvested 17 hours after brain death. The lowest individual fluid balance was +560 ml, observed in a pig that died 7 hours after brain death due to a malfunction of the anesthetic machine.

The mean amounts of fluids received was greater than the amount of urine output during the whole experiment, except 7 hours after brain death, when mean urine output exceeded mean fluid intake by 95 ml, and 12 hours after brain death, when mean urine output exceeded mean fluid intake by 48 ml.

The changes in fluid requirements and urine output did not reach statistical significance compared to the baseline values.

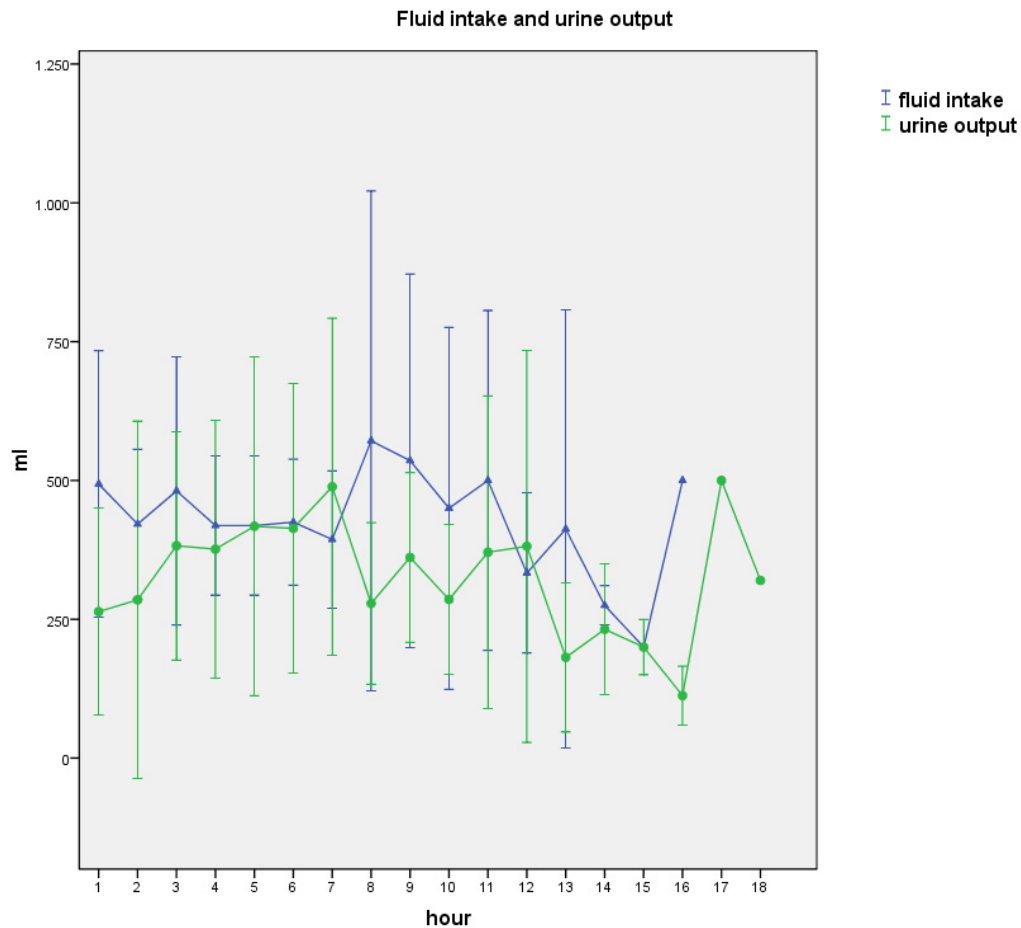


FIG. 6 Changes in fluid intake and urine output during the observation of the pigs. All values are expressed as mean $\pm$ SD. The number of pigs whose data were available for analysis (n) changed over time. Urine output increased until the 7th hour, followed by a decrease until the 16th hour after brain death. Fluid balance was positive during the whole observation period except during the 7th and the 12th hour after brain death. Fluid intake increased during the 8th hour and then fell until the 15th hour after brain death. None of these changes was statistically significant.

The normal values of health juvenile and adult pigs of the parameters assessed from arterial blood sampling in this experiment are shown in Table 2. (44, 45)

pO ₂	98 mmHg
pCO ₂	50 mmHg
pH	7,420
HCO ₃	20-30 mmol/l
Lactate	0-11 mmol/l
Hemoglobin	10,8-15,0 g/l
Hematocrit	33-45%
Sodium	135-170 mmol/l
Potassium	4,5-6,5 mmol/l

TABLE 2

This table shows the normal values of pO₂, pCO₂, pH, HCO₃, lactate, hemoglobin, hematocrit, sodium and potassium of an adult or juvenile pig. (44, 45)

The changes of arterial blood gases are shown in Fig. 7 and Table 3.

pO₂ decreased 10 hours after brain death. pCO₂ increased between the 6th and the 15th hour after brain death.

The highest mean of pO₂ was found before the induction of brain death, 185±57,49 mmHg (n=9). The lowest mean of pO₂ was found 10 hours after brain death, 108,34±40,25 mmHg (n=7). At this time the changes in pO₂ and pCO₂ were statistically significant (p<0,05) compared to the baseline value. The highest mean of pCO₂ was found 5 hours after brain death, 85,36±81,99 mmHg (n=5). The lowest mean of pCO₂ was found before the induction of brain death, 39,29±5,91 mmHg (n=9)

The highest individual value of pO₂ was observed 3 hours after brain death and was 337 mmHg. The lowest individual value of pO₂ was observed 10 hours after brain death and was 50,50 mmHg.

The highest individual value of pCO₂ was observed 15 hours after brain death and was 232 mmHg. The lowest individual value of pCO₂ was observed before the induction of brain death and was 31,80 mmHg.

	before brain death (n=9)	+ 3 hours (n=9)	+ 6 hours (n=6)	+ 10 hours (n=7)	+ 15 hours (n=5)	+ 18 hours (n=2)
pO ₂ (mmHg)	185±57	175±70	180±48	108±40*	124±25	116±48
pCO ₂ (mmHg)	39±6	44±8	40±4	50±10*	85±82	50±3
pH	7,44±0,04	7,41±0,08	7,48±0,05	7,39±0,09	7,29±0,25	7,38±0,10
HCO ₃ (mmol/l)	27,5±3,3	27,0±1,4	29,2±2,0	29,7±3,6	31,4±4,4*	29,4±5,2
Lactate (mmol/l)	2,2±1,5	1,6±1,2	1,1±0,9	1,5±1,6	0,8±0,5	0,6±0,2
Hemoglobin (g/dl)	9,1±2,0	8,6±1,7	9,5±0,9	9,2±1,5	9,6±1,6	8,0±1,1
Hematocrit (%)	28,3±6,2	26,6±5,2	29,3±2,8	28,4±4,5	29,8±4,8	24,8±3,2
Sodium (mmol/l)	138±2	138±2	142±3*	143±2*	145±6	153±6
Potassium (mmol/l)	4,0±0,5	3,7±0,4	3,7±0,2	4,1±0,4*	4,6±0,6	4,2±0,4
SO ₂ (%)	99,8±0,4	99,5±0,8	99,7±0,8	95,3±7,9*	96,0±8,4	99,0±1,4

TABLE 3 This table shows the changes of pH, pO₂, pCO₂, HCO₃, lactate, hemoglobin, hematocrit, sodium, potassium and SO₂ assessed from arterial blood samples over time. All values are expressed as mean ± SD.

* p<0,05, statistical significance.

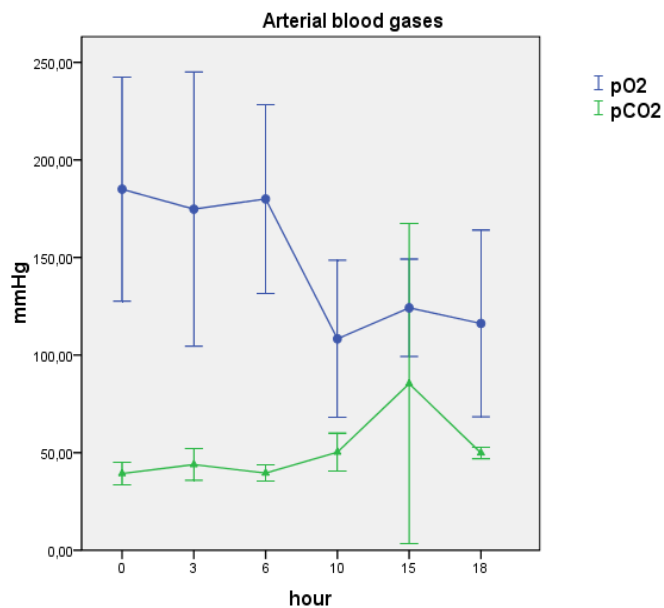


FIG. 7 Changes in arterial blood gases during the observation of the pigs. All values are expressed as mean ± SD. The number of pigs whose data were available for analysis (n) changed over time. pO₂ decreased 10 hours after brain death. pCO₂ increased between the 6th and the 15th hour after brain death. The changes of pO₂ and pCO₂ 10 hours after brain death were significant (p<0,05).

The changes of arterial blood pH are depicted in Fig. 8 and Table 3.

pH decreased between the 6th and the 15th hour after brain death. This decrease did not reach statistical significance compared to the baseline value.

The highest mean of pH was found 6 hours after brain death, $7,482 \pm 0,046$ (n=6). The lowest mean of pH was found 15 hours after brain death, $7,291 \pm 0,251$ (n=5).

The highest individual value of pH was observed 6 hours after brain death and was 7,560.

The lowest individual value of pH was observed 15 hours after brain death and was 6,845.

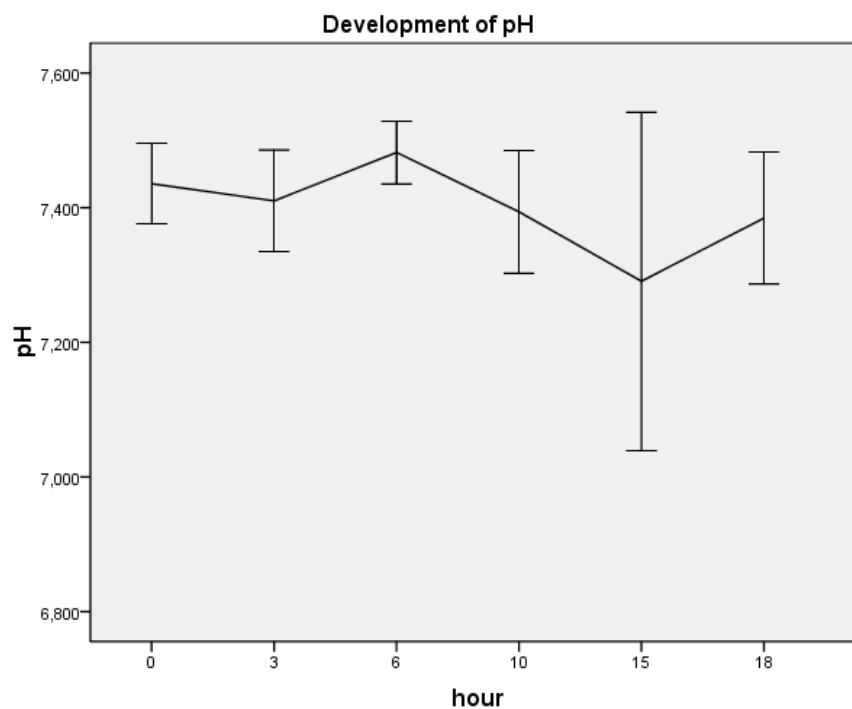


FIG. 8 Changes in arterial blood pH during the observation of the pigs. All values are expressed as mean $\pm$ SD. The number of pigs whose data were available for analysis (n) changed over time. pH decreased between the 6th and the 15th hour after brain death. Changes in arterial pH were not statistically significant compared to the baseline value.

The changes of arterial bicarbonate (HCO_3) are shown in Fig. 9 and Table 3.

HCO_3 increased steadily from brain death until the 15 hour after, when this increase reached statistical significance ($p < 0,05$). This was followed by slight decrease towards the end of the experiment.

The highest mean of HCO_3 was found 15 hours after brain death, $31,4 \pm 4,4$ mmol/l (n=5). The lowest mean of HCO_3 was found 3 hours after brain death, $27,0 \pm 1,4$ mmol/l (n=9). The highest individual value of HCO_3 was observed 15 hours after brain death and was 37,9 mmol/l. The lowest individual value of HCO_3 was observed before induction of brain death and was 22,9 mmol/l.

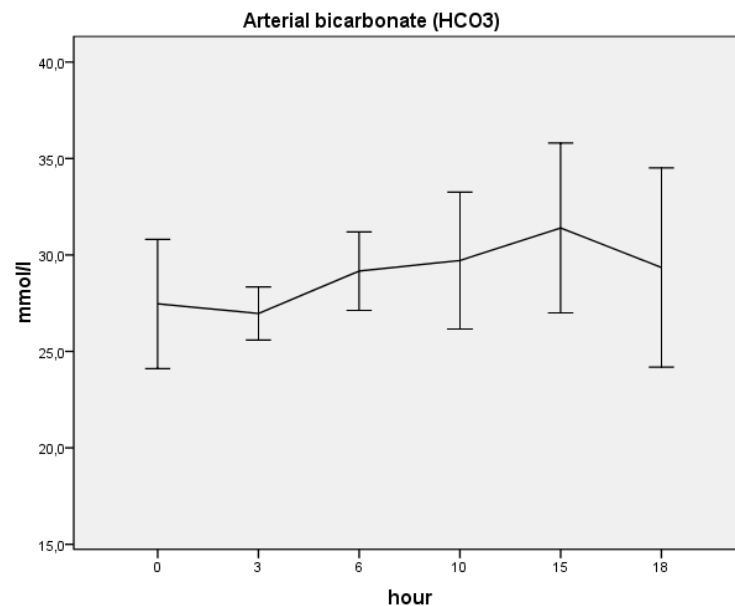


FIG. 9 Changes in arterial bicarbonate (HCO_3) during the observation of the pigs. All values are expressed as mean $\pm$ SD. The number of pigs whose data were available for analysis (n) changed over time. HCO_3 increased steadily from brain death until the 15 hour after, which was statistically significant ($p < 0,05$), followed by slight decrease.

The changes of arterial lactate values are depicted in Fig. 10 and Table 3.

Lactate values decreased steadily until the 6th hour after brain death, followed by an increase until the 10th hour after brain death and another decrease towards the end of the experiment. None of these changes was statistically significant compared to the baseline value.

The highest mean of lactate was found before brain death, $2,17 \pm 1,46$ mmol/l (n=9). The lowest mean of lactate was found 18 hours after brain death, $0,55 \pm 0,21$ mmol/l (n=2).

The highest individual value of lactate was 5,10 mmol/l, observed before induction of brain death and 10 hours after brain death in 2 different pigs.

The lowest individual value of lactate was 0,30 mmol/l, observed 15 hours after brain death.

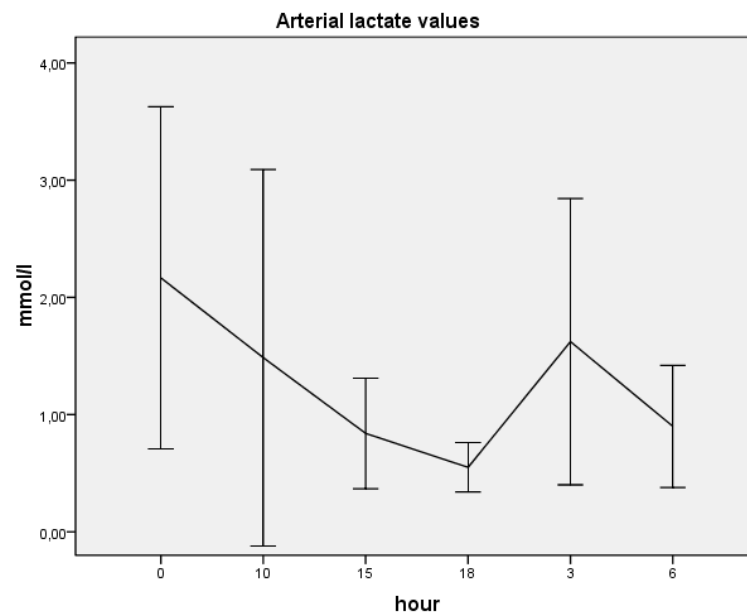


FIG. 10 Changes in arterial lactate values during the observation of the pigs. All values are expressed as mean $\pm$ SD. The number of pigs whose data were available for analysis (n) changed over time. Lactate values decreased until the 6th hour after brain death, followed by an increase until the 10th hour after brain death and another decrease towards the end of the experiment. Statistical significance was not reached.

The changes of arterial hemoglobin values are shown in Fig. 11 and Table 3.

Mean hemoglobin was low throughout the whole observation time. Further decreases of mean hemoglobin were found 3 and 18 hours after brain death.

The highest mean of hemoglobin was found 15 hours after brain death, $9,64 \pm 1,63$ g/dl (n=5). The lowest means of hemoglobin were found 3 hours, $8,56 \pm 1,73$ g/dl (n=9) and 18 hours, $7,95 \pm 1,06$ g/dl (n=2), after brain death.

The highest individual value of hemoglobin was observed before the induction of brain death and was 12,90 g/dl. The lowest individual value of hemoglobin, observed 3 hours after brain death was 5,60 g/dl.

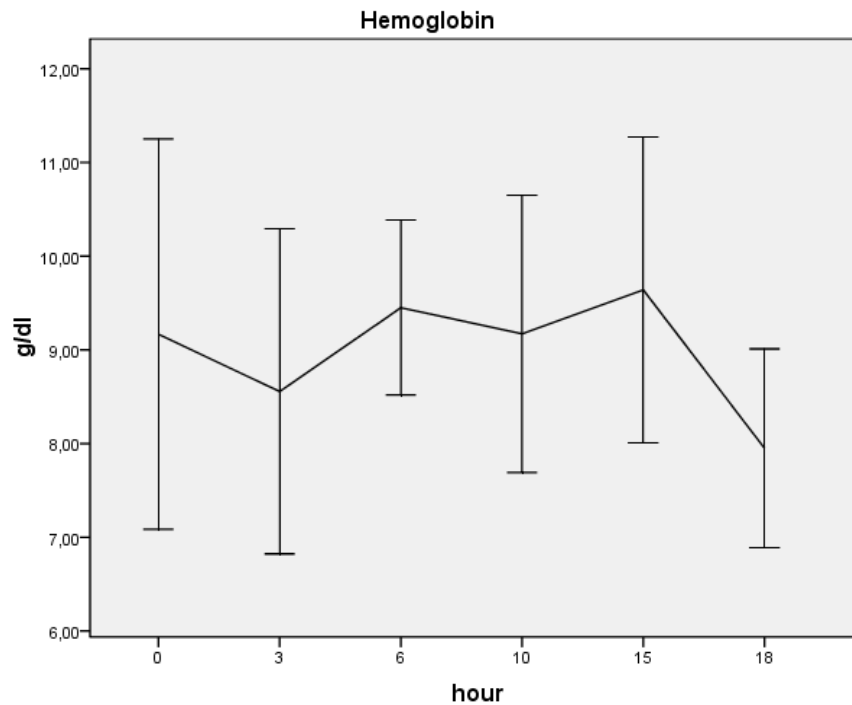


FIG. 11 Changes in arterial hemoglobin levels during the observation of the pigs. All values are expressed as mean $\pm$ SD. The number of pigs whose data were available for analysis (n) changed over time. Mean hemoglobin was low throughout the whole observation time. Further decreases of mean hemoglobin were found 3 and 18 hours after brain death.

The changes of arterial hematocrit are depicted in Fig. 12 and Table 3.

Mean hematocrit remained stable during the observation period. Slight decreases of mean hematocrit were noticed 3 and 18 hours after the induction of brain death.

The highest mean of hematocrit was found 15 hours after brain death, $29,80 \pm 4,83$ % (n=5).

The lowest mean of hematocrit was found 18 hours after brain death, $24,75 \pm 3,18$ % (n=2).

The highest individual hematocrit was observed before the induction of brain death and was 39,30%. The lowest individual hematocrit was 17,70%, observed 3 hours after brain death.

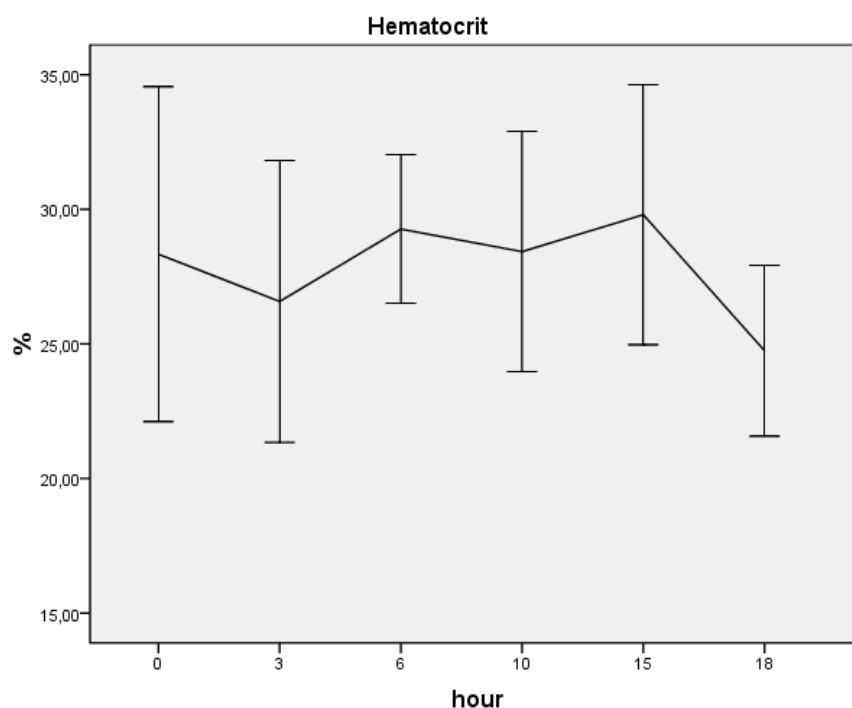


FIG. 12 Changes in arterial hematocrit during the observation of the pigs. All values are expressed as mean $\pm$ SD. The number of pigs whose data were available for analysis (n) changed over time. Mean hematocrit remained stable during the observation period. Decreases of mean hematocrit were noticed 3 and 18 hours after brain death.

The changes of sodium levels are shown in Fig. 13 and Table 3.

Mean sodium concentration remained completely stable for the first 3 hours after the induction of brain death and then rose steadily towards the end of the experiment. The rise was significant ($p < 0,05$) compared to the baseline value 6 and 10 hours after brain death.

The highest mean of sodium levels was found 18 hours after brain death, 153 ± 6 mmol/l ($n=2$). The lowest mean of sodium levels was found before and 3 hours after brain death, 128 ± 2 mmol/l ($n=9$).

The highest individual sodium concentration was observed 18 hours after brain death and was 157 mmol/l. The lowest individual sodium concentration was 134 mmol/l, observed before the induction of brain death.

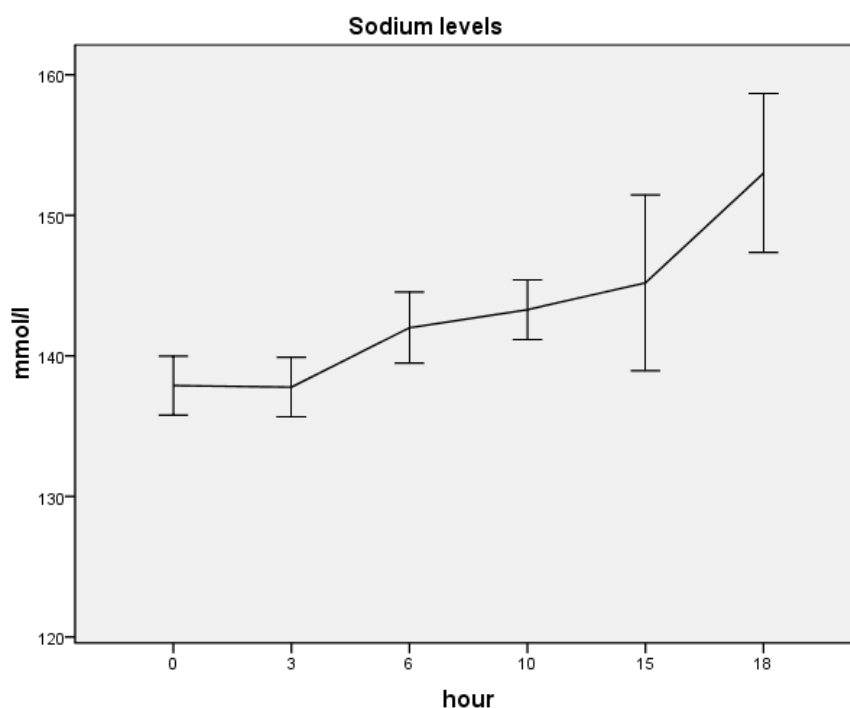


FIG. 13 Changes in sodium levels during the observation of the pigs. All values are expressed as mean $\pm$ SD. The number of pigs whose data were available for analysis (n) changed over time. Mean sodium concentration remained completely stable for the first 3 hours after the induction of brain death and then rose steadily towards the end of the experiment. This rise was significant 6 and 10 hours after brain death ($p < 0,05$) compared to the baseline value.

The changes of potassium levels are depicted in Fig. 14 and Table 3.

Mean potassium concentration fell slightly until the 6th hour and then increased until the 15th hour after brain death. Significance in this increase compared to the baseline value ($p < 0,05$) occurred 10 hours after brain death. This was followed by a slight decrease towards the end of the experiment.

The highest mean of potassium levels was found 15 hours after brain death, $4,6 \pm 0,6$ mmol/l (n=5). The lowest mean of potassium levels was found 3 hours, $3,7 \pm 0,4$ mmol/l (n=9) and 6 hours, $3,7 \pm 0,2$ mmol/l (n=6), after brain death.

The highest individual potassium concentration was 5,4 mmol/l, observed 15 hours after brain death. The lowest individual potassium concentration was noticed 3 hours after brain death and was 3,0 mmol/l.

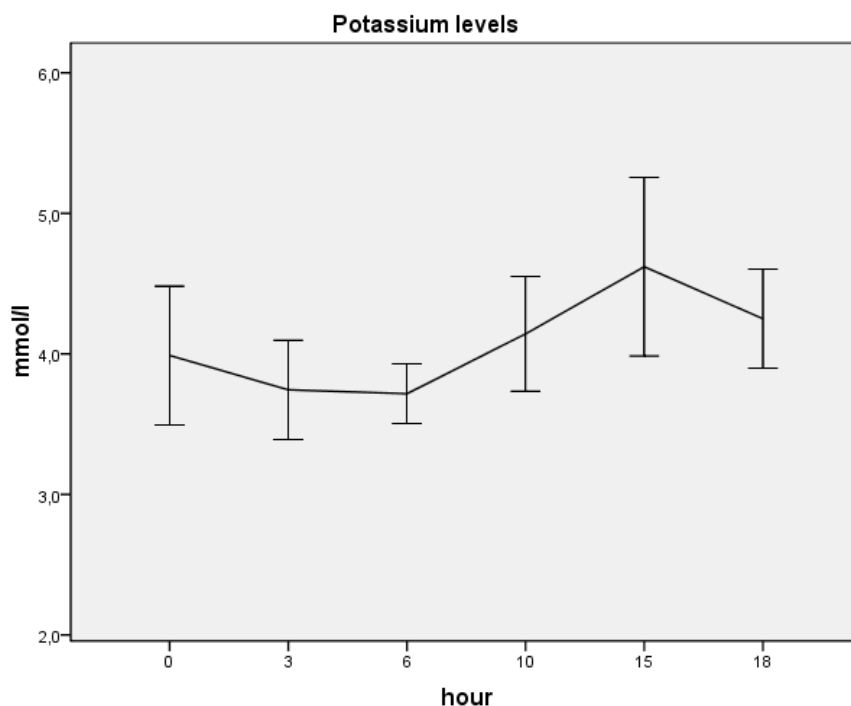


FIG. 14 Changes in potassium levels during the observation of the pigs. All values are expressed as mean $\pm$ SD. The number of pigs whose data were available for analysis (n) changed over time. Mean potassium concentration fell slightly until the 6th hour and then increased until the 15th hour after brain death. The rise was significant 10 hours after brain death ($p < 0,05$) compared to the baseline value.

The changes of arterial oxygen saturation (SO_2) are shown in Fig. 13 and Table 3.

SO_2 was very high before and during the first 6 hours after the induction of brain death. It decreased between the 6th and the 10th hour after brain death. This decrease was statistically significant ($p < 0,05$) compared to the baseline value.

The highest mean of SO_2 was found before the induction of brain death, $99,8 \pm 0,4$ % (n=9).

The lowest mean of SO_2 was found 10 hours after brain death, $95,3 \pm 7,9$ % (n=7).

An SO_2 of 100% was observed in 7 out of 9 pigs before the induction of brain death, in 6 out of 9 pigs 3 hours after brain death, in 5 out of 6 pigs 6 hours after brain death, in 2 out of 7 pigs 10 hours after brain death, in 3 out of 5 pigs 15 hours after brain death and in 1 out of 2 pigs 18 hours after brain death.

The lowest individual value of SO_2 was 78,0%, observed 10 hours after brain death.

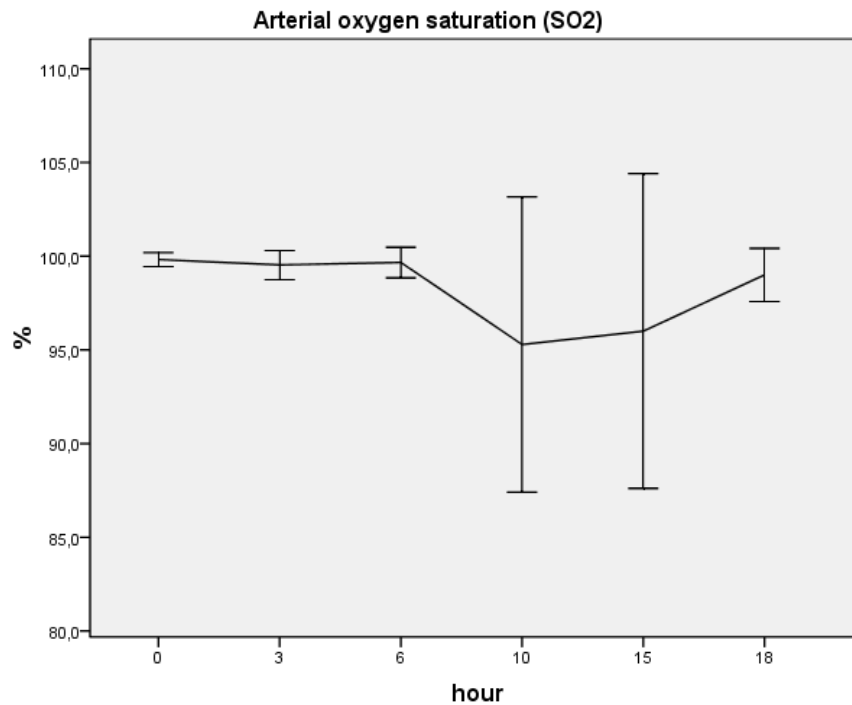


FIG. 15 Changes in arterial oxygen saturation (SO₂) during the observation of the pigs. All values are expressed as mean $\pm$ SD. The number of pigs whose data were available for analysis (n) changed over time. SO₂ was high during the first 6 hours after brain death. It decreased between the 6th and the 10th hour after brain death and then returned to high values. The decrease 10 hours after brain death was statistically significant ($p < 0,05$) compared to the baseline value.

Table 4 and Fig. 14 provide information on the drugs used during donor care.

7 pigs required treatment of tachycardia with esmolol (Brevibloc®). 3 pigs were treated with esmolol only before the 10th hour after brain death. 4 pigs were treated with esmolol between the 10th and the 16th hour after brain death. One pig was treated 4 times with esmolol during the observation period, one pig was treated 3 times, one pig was treated twice and 3 pigs were treated once. The mean amount of esmolol each pig received was 30 ± 59 mg.

6 pigs required treatment of low urine output with furosemide (Lasix®). 5 pigs received furosemide only between the 2nd and the 5th hour after brain death. One pig required treatment with furosemide 12 and 14 hours after brain death. Two pigs were treated twice

and 4 pigs were treated once with furosemide during observation time. The mean amount of furosemide each pig received was 40 ± 21 mg.

	Number of pigs treated	Mean amount received
Esmolol (Brevibloc®)	7	30 ± 59 mg
Furosemide (Lasix®)	6	43 ± 21 mg
Cisatracurium (Nimbex®)	7	57 ± 58 mg
Norepinephrine (Arterenol®)	3	4 ± 0 mg
Desmopressin (Minirin®)	2	1 ± 0 µg
Dopamine	1	5 ± 0 mg

TABLE 4 This table provides information on the drugs used during donor care and shows the number of pigs treated with each specific drug as well as the amount applied, expressed as mean $\pm$ SD.

7 pigs required treatment of severe spinal automatisms with cisatracurium (Nimbex®). 4 pigs were treated with cisatracurium only before the 10th hour after brain death and 3 pigs required this treatment also between the 10th and the 15th hour after brain death. One pig was treated 6 times, one pig was treated 5 times, 2 pigs were treated 4 times, one pig was treated 3 times, one pig was treated twice and one pig was treated once with cisatracurium during the observation period. The mean amount of cisatracurium each pig received was 57 ± 58 mg.

3 pigs were treated with norepinephrine (Arterenol®) because of hypotension. In one pig treatment with norepinephrine was started 13 hours after brain death and 2 pigs were treated with norepinephrine from the 14th hour after brain death onwards. All of them were treated with 4 mg of norepinephrine administered with a perfusion pump.

2 pigs required treatment with desmopressin (Minirin®) due to diabetes insipidus. One pig received desmopressin once 12 hours after brain death, and one pig was treated with desmopressin twice 10 and 14 hours after brain death. One pig received one bolus of 1 µg of desmopressin, the other pig was given 2 boluses of 0,5 µg.

One pig was treated with dopamine because of persistent hypotension. It received 5 mg once 14 hours after brain death had been confirmed.

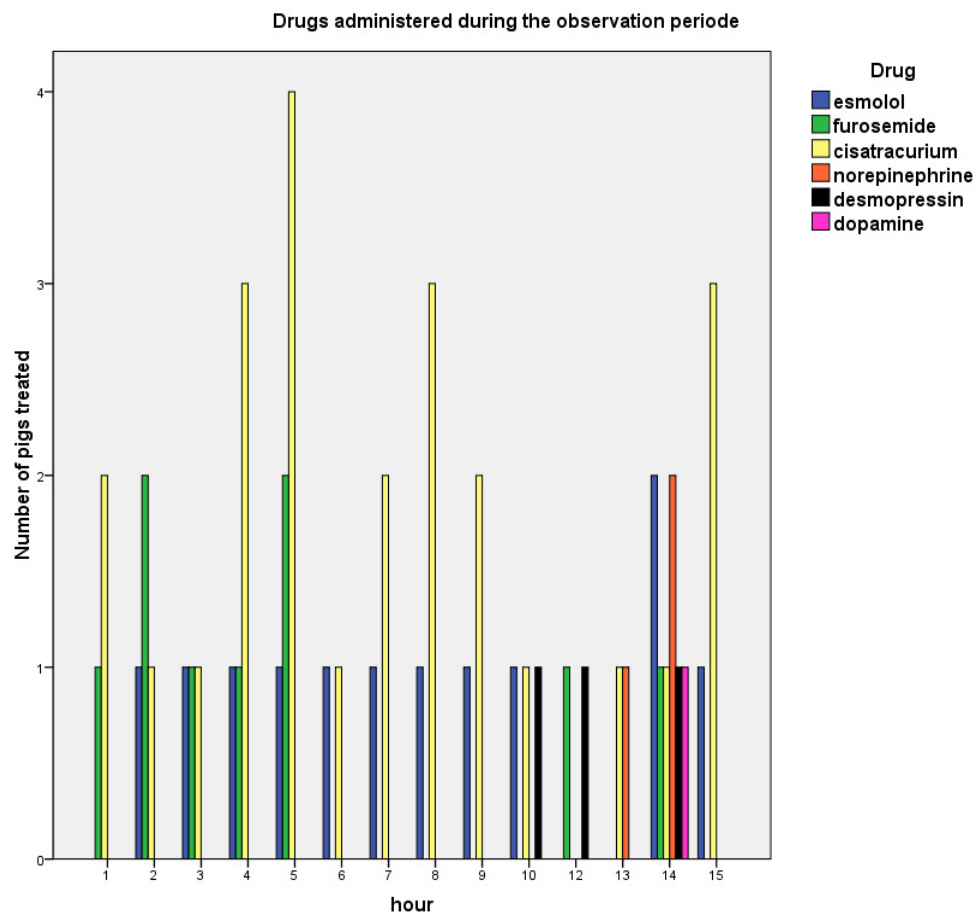


FIG. 16 This figure shows the number of pigs treated with each specific drug at all points in time.

8 DISCUSSION

Today solid organ transplantation is the standard therapy for diseases that lead to fatal end stage organ failure. The greatest source for allografts are cadaveric donors. (1)

Brain death is defined as the irreversible loss of all functions of the brain including the brain stem and the cerebellum and is equal to the death of the individual. (4)

Most frequently it is the result of severe head injury, intracerebral hemorrhage or aneurysmal subarachnoid hemorrhage. (6)

The rise of ICP caused by the mass lesion leads to brain herniation, which takes different forms depending on the location of the lesion. The results are disruptions of cranial nerve functions, palsies and coma. As soon as cerebral blood flow ceases entirely, complete brain failure occurs. (7, 8)

The diagnosis of brain death is found primarily via thorough clinical neurological examination of the brain death. First of all evidence of an acute cerebral damage has to be assessed. No more than three points on the Glasgow Coma Scale and the absence of all brain stem reflexes are mandatory before the apnea test is to be performed which shows the failure of the brain dead patient's respiratory centre. The tests, which are used to confirm brain death, include the assessment of an isoelectrical 30-minute EEG and the confirmation of the stop of cerebral perfusion, using transcranial Doppler ultrasonography and angiographical methods. (4, 5, 10)

Even though this guideline for diagnosing brain death is already well established, not all organs from brain dead patients can be used for transplantation, even if consent to organ donation is available, and not all grafts will resume function in the recipient.

This is partly because of the detrimental effects brain death has on the quality of organs and their function.

During brain death a transient massive increase of circulating catecholamines - known as the autonomic storm - occurs, causing severe hemodynamic changes. Although heart rate and blood pressure rise during the autonomic storm due to sympathetic stimulation, still organ perfusion decreases due to a simultaneous rise in SVR. The extent of the impact the autonomic storm has on the organism depends on the speed of the increase of ICP. (11, 12, 13, 14)

After the autonomic storm has passed, blood pressure drops and hemodynamic instability occurs in about 80% of all brain dead patients. Another detrimental effect of the changes in blood pressure and SVR that occurs after brain death is the development of acute pulmonary edema due to a rise in left atrial pressure and SVR along with an abnormal permeability of the pulmonary capillaries. (12, 13, 15, 16, 18)

A lot of research has already been made on the changes that occur in the heart during and after brain death. During the massive rise of SVR in the course of the autonomic storm vasospasm occurs in the coronary arteries as well and can lead to ischemic damage to the myocardium. (12, 15)

The impact of catecholamines during the autonomic storm leads to the production of oxygen derived free radicals which can damage the cell membrane and cause loss of cellular integrity. Furthermore brain death causes a switch from aerobic to anaerobic metabolism causing a reduction in the myocardial energy stores. (12, 13, 15)

The morphologic changes found in biopsies of the hearts of brain dead patients also give evidence of the damage the heart takes from brain death. Microinfarctions, intracellular edema and damaged mitochondria are found and are most severe in the subendocardium of the left ventricle. (11, 12, 13)

One more critical point is the time of reperfusion after engraftment. The massive release of oxygen free radicals causes injury of the myocytes and leads to the production of chemotactic factors that attract leukocytes that consecutively produce proinflammatory cytokines. (13, 18)

The lung also suffers from the effects of brain death and the consecutive period of intensive care. As mentioned earlier, pulmonary edema is often observed in brain dead patients as well as hemorrhage in the alveolar spaces and the interstitium. (11, 13, 15)

In addition the prolonged period of mechanical ventilation increases the risk of the development of pneumonia, which happens in 50% of all intubated patients within one week. (18)

The kidneys are especially sensitive to damage caused by prolonged periods of hemodynamic instability after brain death, and development of ATN is a common finding. The morphologic correlate to this is a marked reduction of the glomerular capillary spaces and necrosis and atrophy of the tubular cells. (11, 13, 16, 18)

Hypernatremia, which is present in more than 80% of all brain dead patients, also appears to have a detrimental effect on long term results in the recipients. (1, 15, 16, 20)

Urine output after brain death is also strongly influenced by the development of diabetes insipidus. The failure of the posterior pituitary gland and the consecutive lack of ADH - leading to massive polyuria of diluted urine - is observed in 30-90% of all brain dead patients. (1, 11, 12, 15, 20)

Although the liver is quite resistant to the changes in blood pressure that occur after brain death, a study by Van Der Hoeven et al. published in 2003 showed that the rate of apoptosis is increased in the liver of brain dead rats. (11, 18, 21) Since the affected cells were primarily hepatocytes, this could be an explanation for the occurrence of intrahepatic cholestasis that is present in some brain dead patients. (13, 21)

Glucose metabolism is also found altered after brain death. Although the beta cells of the Langerhans islets show a normal capacity for insulin, hyperglycemia is often observed in brain dead patients. The most commonly accepted explanation for this is that peripheral insulin resistance occurs after brain death due to alterations in the intracellular postreceptor metabolism. (22)

The small intestine is very sensitive to ischemia, and inflammation as well as an increased rate of apoptosis is present in the jejunum and ileum of many brain dead patients. (18, 23)

The endocrine system experiences alterations after brain death as well. The most common of these alterations is the development of a euthyroid sick syndrome (ESS), which is characterized by a reduction of T_3 and T_4 in combination with unchanged TSH levels. The lack of T_3 is also made responsible for the switch from aerobic to anaerobic metabolism. (11, 13, 15, 18)

The only significant change in cytokine distribution after brain death that could be found was an increase of IL-6 which has proinflammatory effects. (24)

Since the slowing down of blood flow after brain death leads to an increased influx of leukocytes into the endothelium and consecutively into the organ tissue, immunogenicity of allografts from brain dead donors is significantly higher than from living unrelated donors. (11)

Because of all those detrimental effects brain death has on organ quality, proper donor care is essential to maximize the number of organs available for transplantation. (1, 18)

Continuous invasive monitoring of blood pressure as well as the assessment of fluid balance and metabolic parameters is mandatory to guide donor care. (1, 25, 26, 27, 28, 29, 30, 31)

Proper fluid resuscitation with restricted use of sodium chloride solutions and the administration of vasoactive drugs like dopamine, vasopressin and norepinephrine are used to control hypotension. (27, 28, 33)

The best way of treating diabetes insipidus is the usage of desmopressin, a synthetic vasopressin analogon, which is highly selective for the V₂ receptor in the epithelia of the renal collecting duct and will not cause vasoconstriction. (1, 28, 29, 30, 33)

A great advance in brain dead donor care has been made by the development of the hormonal replacement therapy. It includes liothyronine, glucocorticoids, vasopressin and insulin and is highly recommended if hemodynamic instability persists despite proper fluid replacement and high doses of vasoactive drugs. (1, 26, 33)

Since mechanical ventilation is required in all brain dead patients, it is necessary to use a ventilatory pattern that preserves the lung for possible transplantation and still maintains acid-base balance. This includes the use of moderate PEEP and low tidal volumes as well as the regular assessment of arterial blood gases. (1, 27, 29, 34)

Regular bronchoscopy for bronchial toilet and the identification of pathogens, which could cause pneumonia in the donor or later the recipient, should be performed as well. (1, 29, 30)

Other measures that should be used to optimize the brain dead donor's condition should include the prevention of hypothermia with external warming devices and the use of warmed intravenous fluids as well as the early therapy of blood loss and coagulopathy. (26, 27, 28, 29, 31, 34)

One current topic of research in donor care is the development of strategies to prevent reperfusion injury and minimize the effects of oxygen free radicals. (28)

Several animal models concerning brain death have already been described in literature. The most commonly used animals were rats, dogs and pigs. (35)

The aims of these studies were often the investigation of the hemodynamic, metabolic and endocrine changes during and after brain death and the effects of hormonal replacement therapy. Several studies concentrated on the impact brain death had on the hearts of the animals.

Brain death was most commonly induced by the same method we used in our study, the inflation of the balloon of an intracranially placed catheter. Donor care consisted of the administration of fluids and vasoactive drugs.

The comparison of animal models of different species has led to the conclusion that not all animals show the same reaction to brain death in regard to blood pressure and heart rate. (12, 14, 15, 36, 37, 38, 39, 40, 41)

In our study we were able to successfully introduce a new brain dead donor model in pigs. It combines the methods of inserting a catheter into the skull to induce brain death, which had been described in other studies with a donor care protocol as it is used on humans. We were able to successfully induce brain death in 10 animals, which was confirmed by a positive apnea test and an isoelectric EEG.

Because of the intensive care treatment the pigs received, however, it seems that in this model changes in blood pressure, heart rate and metabolic parameters were less severe than they had been in prior studies. Still some characteristic changes of these parameters could be observed in this model as well.

Blood pressure differed greatly in baseline values from 131/77 mmHg to 76/50 mmHg and the mean of blood pressure was higher than in the studies of Hing et al. published in 2007 (35) and Mertes et al. published in 1994 (36).

Whereas Mertes and Hing (36, 37) described an increase of blood pressure only during the induction of brain death followed by a continuous decrease, we found that blood pressure also might increase during the observation period, especially 14 and 15 hours after brain death. Mertes (36) found a significant increase of blood pressure during the induction of brain death and a significant decrease 30 minutes and 1 and 6 hours after brain death. In our study, however no significant change throughout the whole observation period occurred. Since the other experiments had an observation time of only 6 hours, it is not easily possible to compare these findings.

In a statistical analysis of the occurrence of hemodynamic instability in human brain dead donors by Naja Zadeh et al. in 2009, it was found that 86% of all donors were hemodynamically unstable after an observation period of $1,7 \pm 1$ days. They also found that the shorter the time between declaration of brain death and organ donation is the fewer patients become hemodynamically unstable. (46)

Heart rate showed an increase until the 2nd hour (93 ± 17 to 147 ± 21 beats/minute), followed by a drop (to 116 ± 12 beats/minute) until the 11th hour after brain death.

Statistical significance of a rise in heart rate was reached 2, 3, 4, 5, 6, 8 and 9 hours after brain death ($p < 0,05$).

In the study of Hing similar findings were noticed with heart rate increasing further until the 6th hour after brain death. (37) Mertes, however, found heart rate only significantly increased during the induction of brain death, followed by a constant decrease to below baseline values. (36)

In our study heart rate showed a second slight increase between the 11th and the 12th hour after brain death (116 ± 12 to 133 ± 34 beats/minute). In humans heart rate also rose from $72,5 \pm 6,3$ to $83,6 \pm 7,4$ beats/minute during an observation period of 3 hours. (15)

In both studies by Mertes and Hing no data about fluid intake and urine output were presented (34, 35). Hing (37) only described that intravenous fluids were applied to treat hypotension and Mertes (36) described an aggressive volume expansion protocol at the end of his experiment, in which he used 20 ml/kg of dextrane infused over 15 minutes.

In our study we found that fluid balance at the time of organ retrieval or the pig's death was positive in all animals with a mean of $+2407 \pm 956$ ml and a range of +560 to +4000 ml and that neither urine output nor fluid requirements changed significantly. It is possible that the aggressive fluid resuscitation therapy in our study reduced the need for vasoactive drugs and that the use of desmopressin kept the effects of diabetes insipidus under control.

Arterial blood gases showed a decrease of pO_2 and an increase of pO_2 between the 6th and the 15th hour after brain death which both reached statistical significance 10 hours after brain death ($p < 0,05$). The mean of pO_2 was higher than the normal pO_2 value in pigs throughout the whole experiment. In our study the highest mean of pO_2 (185 ± 57 mmHg) was found before the induction of brain death. In the study of Hing et al. (37) pO_2 was much higher than in our study with a pO_2 of 336 ± 160 mmHg compared to our 180 ± 48 mmHg, 6 hours after brain death but no significant changes of arterial blood gases could be found in their study.

pCO_2 was lower than the normal value for pigs during the first 6 hours as well as 18 hours after brain death. 15 hours after brain death the highest mean of pCO_2 was found, which was 85 ± 82 mmHg. In the study of Hing pCO_2 was closer to the normal value than in our study, with a pCO_2 of 56 ± 20 mmHg 6 hours after brain death.

These high values of pO_2 and low values of pCO_2 are most likely due to hyperventilation and the high amount of oxygen used.

Since the normal pH value of a pig is similar to humans, the pH values we encountered suggest that all pigs suffered from slight to severe acidosis 3, 10, 15 and 18 hours after

brain death with a mean pH of 7,291 to 7,410. Nevertheless, these changes did not reach statistical significance. Uncharacteristically high pH values were found 6 hours after brain death (7,482). The reason for this did not become clear during statistical analysis.

Mean bicarbonate (HCO_3) remained within the normal range for pigs throughout the whole experiment, except 15 hours after brain death when it rose slightly above the normal value. This increase reached statistical significance ($p < 0,05$).

Since the lowest value of pH occurred at the same time as the highest value of pCO_2 and bicarbonate 15 hours after brain death respiratory acidosis in the pigs at this time is very likely, which might have been due to pulmonary edema.

Neither pH nor bicarbonate have been examined in the studies of Hing and Mertes (36, 37).

Lactate values were inside the normal values for a pig during the whole experiment. They decreased from before the induction of brain death until the 10th hour after. Then a slight increase occurred followed by a further drop. None of these changes were statistically significant. In the study of Mertes (36) as well as in humans (15) arterial lactate values were much higher than in our study. However, in Mertes' study (36) 6 hours after brain death a drop of lactate values (from 5,4 to 4,3 mmol/l) that was not statistically significant was found as well.

Since lactate values remained in a normal range during the whole experiment, no switch from aerobic to anaerobic metabolism could be proved in our study.

Hemoglobin was already low at the beginning of the experiment ($9,17 \pm 2,08$ g/dl) and decreased further during the observation period. Hematocrit was also below normal values but slightly increased towards the end of the experiment. In the study of Mertes hemoglobin was in a normal range at the beginning but also dropped one hour after brain death. The reason for the low levels of hemoglobin and hematocrit might be the repeated blood sampling during the experiment.

Hypernatremia was not observed in the pigs in our experiment. Different to humans, pigs have a physiological range of serum sodium from 135-170 mmol/l so values above 145 mmol/l were not regarded as hypernatremia. However a constant rise of serum sodium was observed which was statistically significant ($p < 0,05$) 6 and 10 hours after brain death.

Potassium levels, however, were low during the whole experiment except during the 15th hour after brain death. The reason for this hypokalemia might have been renal loss due to diabetes insipidus. For humans, however, the potassium levels we observed were in the normal range.

SO₂ was high during the first 6 hours after brain death. It dropped between the 6th and the 10th hour after brain death with that drop being statistically significant ($p < 0,05$) and then returned to high values. This is in accordance with the high pO₂ levels we observed and is most likely the result of hyperventilation.

Sodium, potassium and arterial oxygen saturation have not been assessed in the other brain death studies on pigs.

The medical treatment the pigs received in our study was very similar to the actual treatment used in human brain dead donors. In the study of Hing the only drug used was norepinephrine and all pigs required this treatment one hour after brain death. (37)

Due to the aggressive fluid resuscitation strategy we used, we were able to delay the point in time when vasoactive support was needed to 13 hours after brain death.

It was necessary, however, to treat tachycardia with esmolol during the whole observation period.

Another interesting finding during this long period of donor care on brain dead pigs was the great amount of muscle relaxant needed to suppress the strong spinal automatisms that occurred. Like in humans these automatisms consisted of movements of the limbs, but also tail and ears of the pig. These movements were observed especially after the 12th hour after brain death induction.

One more interesting change that could be observed was a strong secretion out of the noses and eyes of the pigs. Whether this secretion was due to brain death or caused by the state of being intubated and ventilated remains unclear.

Similar to humans all pigs showed signs of pulmonary edema like rales in auscultation, which could be verified during multiorgan donation.

In humans admitted to ICUs for donor care the baseline values we obtained before the induction of brain death are difficult to assess.

In the future it has to be further investigated if these differences in baseline values do have an effect on the hemodynamic status after brain death.

One measure to further improve this brain death model might be the use of digital recording devices for blood pressure and heart rate. Thereby the development of these parameters in all pigs could be compared irrespective of their baseline values. The change of blood pressure and heart rate during the autonomic storm could also be assessed more precisely by this method.

By using traditional sheets for intensive care, some significant changes of blood pressure and heart rate could have been missed by the observer.

Still our brain dead donor model in pigs has great advantages over previously described models because of the longer observation period and the adoption of therapeutic strategies used on human brain dead donors into an animal model.

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AUSBILDUNG

2003 – 2009 Medizinstudium and der Medizinischen Universität Graz
1. Diplomprüfung 2003
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1995 – 2003 Bischöfliches Gymnasium, Graz
Reifeprüfung mit Auszeichnung bestanden

SPEZIELLE STUDIENMODULE

2008 Chirurgische PatientInnenbetreuung
2008 Case-based Learning in Klinik und Praxis
2007 Stationäre Psychosomatik und Psychotherapie: Krankheitsbilder und Therapieansätze
2007 Klinisch-topografische Anatomie der Eingeweide
2006 Modernste Methoden zur Messung der Bodycomposition
2006 Von der Theorie zur Praxis: Chirurgische Operationslehre
2004 Klinisch-topografische Anatomie der Extremitäten

PRAKTISCHES JAHR

Okt. 2008 – Dez. 2008 Abteilung für Transplantationschirurgie, LKH Graz
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FAMULATUREN

2009	Department of Traumatology, Jagellonian University Hospital in Kraków, Polen
2008	Department of Internal Medicine II and Department of Gastroenterological Surgery, University Hospital Hiroshima, Japan
2007	Unfallchirurgie, UKH Graz
2007	Abteilung für Psychosomatik und Psychiatrie in Bad Aussee, LKH Graz
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2005	Univ. Klinik für Unfallchirurgie, LKH Graz

TÄTIGKEIT

2008	Ordinationshilfe in der Praxis Dr. Theresia A. Knauer, Facharzt für Innere Medizin, Graz
2005 – 2006	Mitarbeit bei der Studie der Steiermärkischen Gebietskrankenkasse „RAUCHFREI... ganz von selbst“ im Rahmen des Raucherentwöhnungsprogramms „Rauchfrei in nur 2 Tagen PDM nach Dr. Grohs“
2005	Tour-Guide für „Die Organisation“ im Rahmen der Eröffnung der renovierten Alten Universität in Graz
2004 – 2006	Promotion- Tätigkeiten rund um die Garage Pfauengarten in Graz für „denkbar&so“
2003	Private Lernaufsicht für 4 Kinder im Alter von 6 bis 14
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