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DOCTORS IN TRAINING

Physiological Care of the Organ Donor

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Abstract

Since the advent of the first successful organ transplant, year-on-year recipient numbers have steadily increased in the UK. Unfortunately, however, for each organ transplanted there necessitates an organ to be donated and as such these are extremely precious commodities. This article aims to summarise the management of potential organ donors following brainstem death in the intensive care setting from a UK perspective. A series of predictable pathophysiological changes occur following brainstem death, which if left untreated, can have a catastrophic impact on donor organs and result in them becoming unsuitable for transplantation. The article details the aforementioned pathophysiological changes in order of occurrence, and then discusses their management in line with the recommendations from the NHS Blood and Transplant authority and current literature.

Key words: organ transplantation; tissue and organ procurement; critical care; brain death; UK

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Introduction

Since the first successful organ transplant in 1954, recipient numbers have risen steadily (Barker and Markmann, 2013). Although initially limited to renal transplantation, advances in surgical techniques and an improved understanding of organ rejection have led to the transplanting of more organs of greater variety and thus a rising demand for donor organs (Barker and Markmann, 2013). UK donation rates fell during the coronavirus disease 2019 (COVID-19) pandemic and, despite the introduction of a donor 'opt out' system, have yet to return to fully recover (Fig. 1) (NHS Blood and Transplant authority, 2024). Unfortunately, demand therefore continues to exceed supply (Citerio et al, 2016; Curtis et al, 2021; NHS Blood and Transplant authority, 2024). Given that donor organs are thus an extremely precious commodity, scrupulous care and attention must be paid to optimising their function in the intended donor. This article briefly reviews the pathophysiology of organ death and describes how this is managed in order to optimise potential donor organs.

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Types of Donation

There are, broadly three types of organ donation; (i) after brainstem death (DBD); (ii) after circulatory death (DCD—previously referred to as donation after cardiac death, or non-heart beating organ donation); and (iii) living donation.

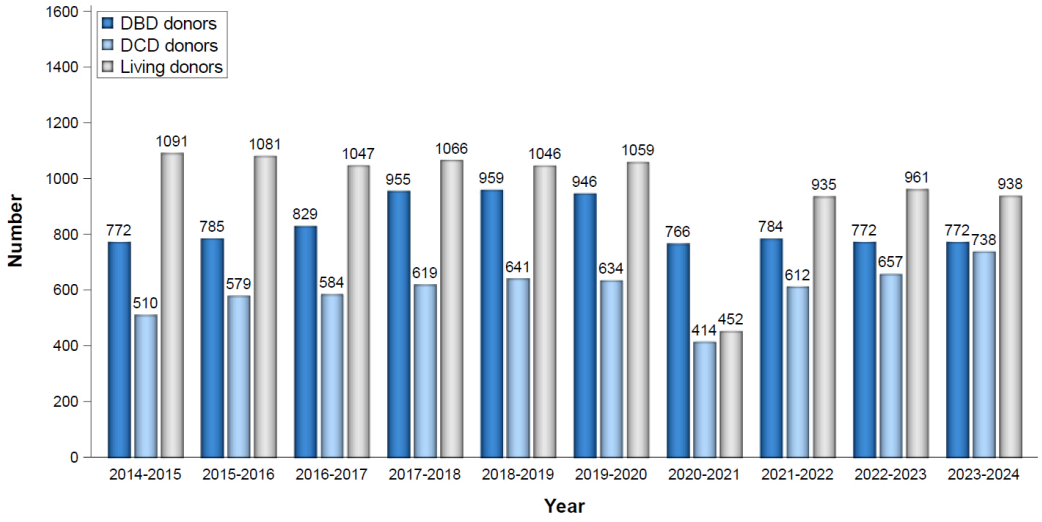

Fig. 1. Number of deceased and living donors in the UK, 1 April 2014–31 March 2024. Reproduced with permission from NHS Blood and Transplant authority (2024), [Organ and Tissue Donation and Transplantation Activity Report 2023/2024]; published by [NHS Blood and Transplant authority]. DBD, donation after brainstem death; DCD, donation after circulatory death.

As DBDs are the primary source of most cadaveric donor organs, and because (due to the nature of their death) these donors are optimised for organ retrieval in the intensive care unit (ICU), this article will predominantly focus on them. For completeness, other donor types are briefly mentioned.

Live Donor Organ Donation

Live donor organ donation is the most common source of organs for transplantation in the UK (Fig. 1). Due to the nature of the donor, the range of suitable organs is limited, and activity is largely limited to renal or partial liver transplants. Donors most commonly donate to a relative or friend (termed 'directed' or 'specified' donation), but organs can also be donated to a recipient with whom the donor has no previous relationship (termed altruistic donation). Whilst immunologically matched transplants remain preferable, advances in immunosuppressive and desensitisation methods mean that tissue type matching is becoming less important, and this has enabled transplant teams to make better use of altruistically donated organs. Donor and recipient surgery is usually done in tandem to minimise ischaemic times, and living donor transplantation is associated with the best outcomes in terms of life expectancy, quality of life and graft survival (Bailey et al, 2016; Krishnan et al, 2020).

Donation After Circulatory Death (DCD)

There are two principal types of DCD—the scenario where organs are retrieved from a patient's whose death is diagnosed and confirmed using cardio-respiratory criteria. Uncontrolled DCD refers to organ retrieval after a cardiac arrest that is unexpected and from which the patient cannot or should not be resuscitated. Once death is confirmed, *in-situ* normothermic regional perfusion is initiated, which allows time for consent for organ donation to be explored. Organ retrieval from un-

controlled DCD has not been practiced in the UK since 2016 (Gardiner et al, 2020). Controlled DCD takes place following the ethical withdrawal of life-sustaining treatments. To minimise organ warm and cold ischaemic times, and to preserve their optimal function, care withdrawal is generally best undertaken in the operating theatre with a transplant team ready to operate once death has been confirmed (Corbett et al, 2021). If death does not occur within a four-hour window, the transplant team are stood down and donation does not proceed. Outcomes in transplanted organs are good, with the graft survival at five years for DCD and DBD being nearly equivalent (86% and 87% respectively) (Gardiner et al, 2020).

Donation After Brainstem Death (DBD)

Also known as brain death, donation occurs when brain injury is thought to have caused irreversible loss of the capacity for consciousness and ventilation, but before terminal apnoea has resulted in hypoxic cardiac arrest and circulatory standstill (Academy of Medical Royal Colleges, 2025). This diagnosis is only possible in mechanically ventilated ICU patients, as the normal parameters for establishing cardio-pulmonary death are not suitable. Death is confirmed using neurological criteria in a verification process known as 'brainstem death testing' (Academy of Medical Royal Colleges, 2025). Given the complexity of the organ retrieval process and the sensitivity required, the process is coordinated by specialist nurses for organ donations (SNODs) who aid in donor suitability assessment, act as a link between the transplant retrieval teams and the hospital caring for the donor, collaborate with colleagues at UK transplant centres to identify suitable recipients, and guide and support the donor's family throughout (Corbett et al, 2021; Gardiner et al, 2020).

The Ethics of Organ Donation

Organ donation is an extremely complex and emotive topic. A number of factors including patient demographics, legislation, healthcare funding and/or religious beliefs may influence a patient's decision to become an organ donor (Cooper, 2023; Gardiner et al, 2021). Whilst it is beyond the scope of this article to cover the matter in great detail, we have briefly summarised two legal and ethical principles that underpin deceased organ donation.

Dead Donor Rule

This rule states that '*donors must be determined to be dead before their organs are removed*'. The statement itself is open to interpretation and different countries have dissimilar accepted definitions and criteria for verification of 'death' (Gardiner et al, 2021). In the UK, it has been accepted that death by either neurological or circulatory criteria satisfies this rule, however, this is not the case in many other countries.

Consenting Donor Rule

For organs to be removed from a deceased patient, someone must have provided consent for the organs to be taken. The decision can be either made in ad-

vance by the patient, or by the patient's family after their death. In the UK, if the donor's family are not in agreement with organ donation, then they have the legal right to overturn the donor's advanced wishes. In recent years, the UK has also changed from an opt-in system of organ donation to an opt out system whereby members of the public must now register that they do not wish to be organ donors. This change has raised numerous ethical considerations, including the notion that if people were not aware of the change in legislation, they cannot be 'informed' and as such it calls into question the validity of their consent (Gardiner et al, 2021).

Pathophysiology of Brainstem Death

Brainstem death signifies the irreversible cessation of brainstem activity. It proceeds in a cephalic to caudal nature and is frequently followed by a predictable pattern of pathophysiological consequences, which, if left unchecked, can lead to multiple organ failure (Fig. 2). Understanding these alterations is crucial for managing brain-dead patients, particularly in the context of maintaining organ viability for transplantation.

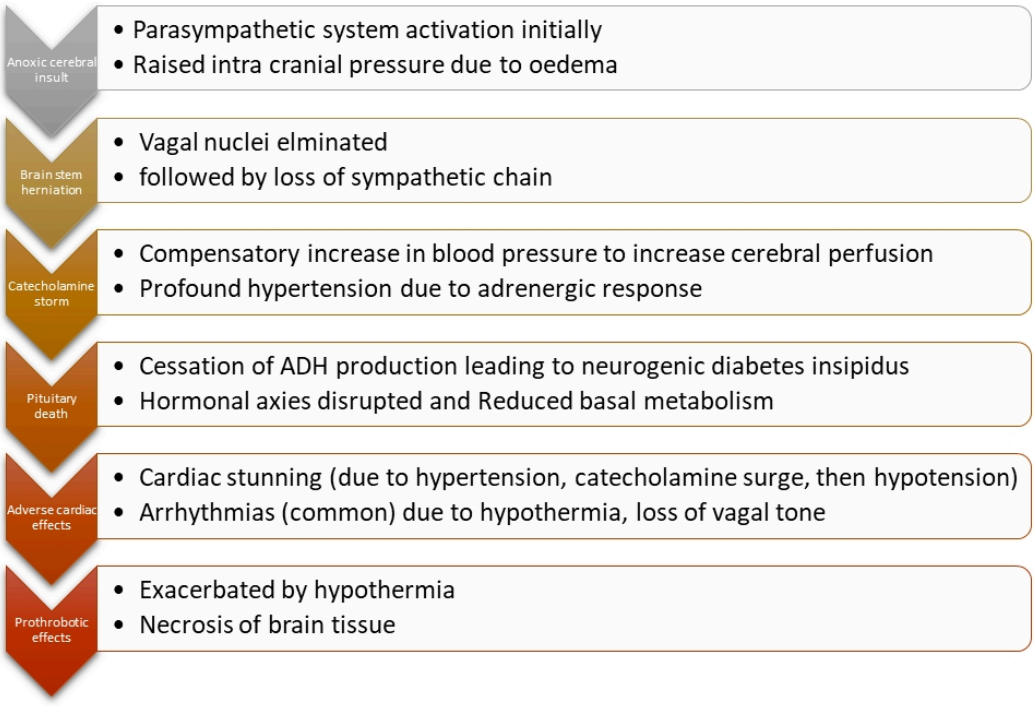

Fig. 2. Pathophysiological progression following brainstem death. Predictable pattern of pathophysiological consequences of brainstem death. The figure was created with Krita v5.2.3 (Krita Foundation, Deventer, The Netherlands). ADH, anti-diuretic hormone.

The Cerebral Insult

An intracranial event (e.g., ischaemic stroke) or extracranial event (e.g., cardiac arrest) occurs, which results in an initial brain injury. Brain anoxia ensues and causes oedema within a fixed-volume cranial vault, which in turn increases intracra-

nial pressure (ICP) (Anwar and Lee, 2019). Raised ICP decreases cerebral blood flow and leads to ischaemia, which in the midbrain region, leads to parasympathetic activation with consequent hypotension and bradycardia. As ICP increases further, pontine ischaemia results in sympathetic activation (and an increase in mean arterial pressure (MAP)), and the brainstem progressively herniates through the foramen magnum (Yoshikawa et al, 2021). This destroys the vagal nuclei, resulting in unopposed sympathetic activity (severe hypertension and tachycardia) described as a 'catecholamine storm' (Meyfroidt et al, 2019). The resultant vasoconstriction increases cardiac afterload, and thus left atrial and pulmonary capillary bed pressures. The rapid increase in pulmonary capillary hydrostatic pressure causes fluid extravasation into the alveolar spaces, which leads to neurogenic pulmonary oedema consequently impairing gas exchange, which will reduce the suitability of the lungs for transplant (Anwar and Lee, 2019; Clarke, 2021).

Posterior Pituitary Death

Endocrine disturbances are a hallmark of brainstem death, driven by hypothalamic-pituitary axis failure. Due to its anatomical position, the posterior pituitary often infarcts as the ICP rises. This classically causes a cessation in the production of antidiuretic hormone at the posterior pituitary giving rise to cranial diabetes insipidus (CDI) (Anwar and Lee, 2019; Meyfroidt et al, 2019). CDI can occur in as many as 80% of patients, with the pronounced polyuria having a potentially catastrophic effect on serum electrolytes and fluid status, which further exacerbates haemodynamic instability (Clarke, 2021; Siah et al, 2019). The anterior pituitary function is often preserved due to its circulation differing from that of the posterior pituitary (Anwar and Lee, 2019), however adrenal insufficiency due to impaired secretion of adrenocorticotropic hormone (ACTH) may be seen and this results in reduced cortisol production, further contributing to hypotension and stress hormone deficiency (Anwar and Lee, 2019).

Adverse Cardiac Effects

The rise and fall in blood pressure due to the catecholamine storm followed by brainstem herniation, impacts myocardial perfusion. This can lead to potentially reversible acute cardiac injury or 'cardiac stunning' (often manifesting as left ventricular dysfunction), the severity of which will depend on the speed of onset of brain death (Anwar and Lee, 2019; McKeown et al, 2012). Arrhythmias are common—initially tachyarrhythmias due to the catecholamine surge, then bradyarrhythmia due to the loss of sympathetic and vagal stimuli (Anwar and Lee, 2019; Meyfroidt et al, 2019). Hypotension is particularly problematic, as it reduces perfusion to vital organs, increasing the risk of ischaemic injury and impairing organ viability (Anwar and Lee, 2019; Zirpe and Gurav, 2019).

Metabolic, Inflammatory and Prothrombotic Responses

The metabolic and inflammatory responses to brainstem death are profound. Brainstem death induces a hypermetabolic state characterised by increased oxygen consumption and glucose metabolism. This state is compounded by a systemic in-

flammatory response syndrome (SIRS), driven by the release of pro-inflammatory cytokines such as interleukins and tumour necrosis factor-alpha (Anwar and Lee, 2019). This inflammatory cascade contributes to endothelial dysfunction, capillary leakage, and a pro-coagulant state, predisposing patients to disseminated intravascular coagulation (DIC). DIC and microvascular thrombosis can further impair organ perfusion, which can be catastrophic for potentially viable organs (Lisman et al, 2011).

Thermoregulatory Failure

The hypothalamus, a key centre for thermoregulation, ceases to function after brainstem death, resulting in poikilothermia, where the body temperature becomes dependent on environmental conditions. Hypothermia is common, arising from the loss of thermoregulatory control, systemic vasodilation, and prolonged exposure during intensive care (Citerio et al, 2016). Hypothermia exacerbates coagulopathies, reduces metabolic rates, and can impair cardiac function, all of which will diminish the quality of potential organs suitability for donation.

Gastrointestinal and Renal Dysfunction

The gastrointestinal system also undergoes significant changes, with gastroparesis and stress ulcers being common. Gastroparesis, caused by autonomic dysfunction, can lead to gastric distension, increasing the risk of aspiration. Stress ulcers, driven by increased gastric acid production and reduced mucosal protection, may result in gastrointestinal bleeding (Meyfroidt et al, 2019). Finally, due to systemic hypotension caused by much of the aforementioned, the kidneys may experience reduced perfusion thereby increasing the risk of acute kidney injury.

Principles of DBD Donor Management

The fundamental physiological aim of managing the DBD donor on the intensive care unit (ICU) is to minimise the degree of organ dysfunction incurred as a result of the pathophysiological processes outlined above.

General Care of the Potential DBD Donor

All patients are closely monitored. Invasive monitoring of blood pressure with an arterial catheter allows tight blood pressure control and facilitates regular assessments of arterial blood gases (Corbett et al, 2021; Zirpe and Gurav, 2019). A central venous catheter is normally placed to allow the administration of potent vasoactive agents and it can also be used to measure central venous pressure. In some cases, particularly those concerning potential cardiac donors, echocardiography or a pulmonary artery catheter may be used to monitor cardiac function (Corbett et al, 2021; Yoshikawa et al, 2021).

Within their adult donor optimization care bundle, the NHS Blood and Transplant (NHSBT) service recommends regular assessment and documentation of set physiological parameters (Table 1) (NHS Blood and Transplant authority, 2023).

Table 1. The NHS Blood and Transplant authority care bundle: recommended physiological targets for the management of DBD organ donors.

Parameter	Target value
Mean arterial pressure	60–80 mmHg
Cardiac index*	>2.1 L/min/m^2
PaO_2	≥10 kPa**
$PaCO_2$	5–6.5 kPa***
Urine output	0.5–2 mL/kg/hr
Body temperature	36–37.5 °C
Blood glucose	4–10 mmol/L

Target values for numerous physiological parameters as advised by the NHS Blood and Transplant (NHSBT) authority. *Where requested, **Aim FiO_2 <0.4 as able, ***Higher values tolerated providing pH remains >7.25. $PaCO_2$, partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen; DBD, donation after brainstem death.

Cardiovascular Considerations

Hypertension

Intravenous beta-blockers are used to treat catecholamine surge-associated hypertension and tachycardia by blocking adrenergic receptors. As blood pressure can rapidly fall as the brainstem herniates and dies, beta-blockade must be rapidly reversible: intravenous infusions of short acting beta-blockers (e.g., esmolol and labetalol) are sometimes supplemented by hydralazine or glyceryl trinitrate (Anwar and Lee, 2019; Meyfroidt et al, 2019).

Hypotension

DBD patients may be hypovolaemic (e.g., due to shifts in fluid distribution such as that with pulmonary oedema, or due to CDI), and fluid status should be assessed and optimised (using balanced crystalloid, or blood where appropriate prior to commencing vasopressors) (McKeown et al, 2012; Zirpe and Gurav, 2019). Volume replacement with synthetic colloids and starches is associated with delayed graft function, and therefore is not recommended (Anwar and Lee, 2019; McKeown et al, 2012; Zirpe and Gurav, 2019). Vasoactive agents such as dopamine, vasopressin or noradrenaline may be used for the treatment of hypotension due to the loss of sympathetic innervation. Although there is no good quality evidence to suggest superiority of one agent over another, NHSBT recommends first line use of vasopressin—in part because it is catecholamine-sparing properties and for its role in the treatment of CDI (Anwar and Lee, 2019; Siah et al, 2019; Zirpe and Gurav, 2019; Clarke, 2021; NHS Blood and Transplant authority, 2023) .

Cardiac Arrhythmias

Simple measures such as replacement of electrolytes and controlling physiological parameters (e.g., temperature and blood pressure, etc.) are used in the first

instance to correct arrhythmias (Anwar and Lee, 2019). Resistant tachyarrhythmias are treated in a similar manner to critically unwell patients using Advance Life Support (ALS) algorithms (Anwar and Lee, 2019). Bradycardias are treated with adrenaline or isoprenaline infusions: atropine is not used as the loss of cardiac vagal innervation renders it ineffective (Anwar and Lee, 2019; Meyfroidt et al, 2019). All DBD will ultimately develop therapy-resistant terminal arrhythmias. In these cases, cardiopulmonary resuscitation (CPR) should be considered to necessitate successful organ retrieval, particularly if the patient is already in the operating room (Anwar and Lee, 2019). A temporary myocardial injury (or 'stunning') may also occur due to rapid changes in blood pressure and catecholamine surge. This acute drop in left ventricular systolic dysfunction is seen in around 40% of patients, and transthoracic or transoesophageal echocardiography is recommended to assess cardiac function and suitability for donation (Siah et al, 2019). Serial studies are conducted as this phenomenon is commonly transient in nature (Anwar and Lee, 2019).

Respiratory Considerations

Whilst on the ICU, DBD respiratory management revolves around use of lung protective ventilation measures (Siah et al, 2019; The Acute Respiratory Distress Syndrome Network, 2000; Yoshikawa et al, 2021). Minimising inspiratory and peak and plateau pressures reduces barotrauma, and appropriate use of positive end-expiratory pressures promotes alveolar gas exchange. Low ventilatory pressures can also be cardioprotective; a 'stunned' heart may not be able to cope with additional afterload, and as a result raised pressures could precipitate cardiogenic shock (Zirpe and Gurav, 2019). Proning measures may benefit DBDs with hypoxaemia and radiological evidence of atelectasis, with bronchoscopy useful in anatomical assessment of the lungs, assessing for infection, and clearing mucus plugs (Anwar and Lee, 2019; Clarke, 2021; Marklin et al, 2021). Treatment of pulmonary oedema—predominantly through negating the catecholamine storm seen after brain death—is associated with better outcomes after lung retrieval (Anwar and Lee, 2019; Clarke, 2021; McKeown et al, 2012).

Hormonal and Metabolic Considerations

Central Diabetes Insipidus

Central diabetes insipidus related polyuria and subsequent electrolyte derangement can negatively impact donor organs (Siah et al, 2019) and their early management is thus essential. Vasopressin infusions are commonly used due to the dual vasopressor and anti- diuretic effect, however when vasopressin is not indicated, or where it lacks sufficient effect, desmopressin (DDAVP), a potent vasopressin 2 receptor agonist, can be given. One dose of DDAVP is often sufficient to halt CDI, however it may need to be repeated (Siah et al, 2019).

Other Pituitary Hormones

Results of thyroid function tests in DBDs are often consistent with the sick euthyroid hormone picture seen in other ICU patients, and routine supplementation

of thyroid hormones is not recommended unless there is an overriding clinical need (e.g., pre-existing hypothyroidism) (Anwar and Lee, 2019; Siah et al, 2019; Zirpe and Gurav, 2019), and routine.

Hypothermia should be corrected with external warming measures (e.g., blankets or active warming with a goal of 36–37.5 °C) (Zirpe and Gurav, 2019; NHS Blood and Transplant authority, 2023).

Glucocorticoids can be given as a vasopressor sparing agent, particularly where an element of septic shock exists (Clarke, 2021; Meyfroidt et al, 2019). Whilst a study has not demonstrated superiority of one glucocorticoid over another, hydrocortisone is often preferred because it has mineralocorticoid and glucocorticoid activity, which can be beneficial in volume depleted DBDs (Pinsard et al, 2014).

Hyperglycaemia

Hyperglycaemia can result from insulin resistance, unsuppressed gluconeogenesis, or administration of exogenous corticosteroids (McKeown et al, 2012). Uncontrolled hyperglycaemia has been associated with poor renal graft function, and hyperglycaemia may induce an osmotic diuresis and increase the risk of the DBD developing infections (Meyfroidt et al, 2019). It should therefore be managed with variable rate insulin infusion (and potassium administration as needed) (Zirpe and Gurav, 2019). Hypoglycaemia is uncommon, but can be treated with intravenous (IV) glucose.

Haematological Considerations

Given the prothrombotic nature of brain death, prophylactic low molecular weight heparin (LMWH) is recommended in most cases as is mechanical calf compression (Meyfroidt et al, 2019; NHS Blood and Transplant authority, 2023). Should DIC develop, advice from haematologists should be sought and blood products may be required (Anwar and Lee, 2019). A circulating haemoglobin concentration transfusion target of 70 g/L is non-inferior to higher targets and should be used (Meyfroidt et al, 2019; Siah et al, 2019; Yoshikawa et al, 2021).

Renal Considerations

The kidneys are predominantly managed through careful control of the blood pressure. Baseline renal function tests should be taken on admission to the ICU, and these should be monitored at regular intervals throughout. Strict fluid balance monitoring is a necessity, aiming to avoid a positive fluid balance with a urine output of 0.3–0.5 mL/kg/hr. Nephrotoxic drugs and intravenous contrast media should be avoided where possible, and any electrolyte abnormalities should be corrected (Siah et al, 2019).

Hepatic Considerations

Adequate liver glycogen stores have been shown to reduce liver graft loss, and therefore it is recommended that enteral feeding or intravenous glucose supplementation be undertaken to minimise graft rejection. Some literature recommends central venous pressure (CVP) monitoring to assess for hepatic congestion; however, there is no evidence to support this practice (Siah et al, 2019).

Other Considerations

Infection

Diagnosis of infection in DBDs can be challenging—elevated leukocytes and tachycardia are non-specific (and likely present due to other ongoing processes), and loss of hypothalamic temperature control may stop fevers developing (Anwar and Lee, 2019). Vigilance for infection is thus essential, with management following protocols for other ICU patients except that nephrotoxic antibiotics should be avoided. Bacteraemia is not a contraindication to organ donation, providing pathogen-specific antibiotics have been administered for at least 48 hours prior to retrieval (Meyfroidt et al, 2019; Zirpe and Gurav, 2019). Actual transmission rate of infection between donor and recipient is low and occurs in 1–5% of cases where infection is present It is worth noting that the presence of blood borne viruses is not an absolute contraindication to organ donation so long as there is informed consent from the recipient (e.g., human immunodeficiency virus (HIV) positive donor and to HIV positive recipient) (Clarke, 2021; Corbett et al, 2021; Meyfroidt et al, 2019).

Nutrition

The intense inflammatory response induced by necrosis of the brain and the ensuing sympathetic storm initially induce a period of metabolic stress, and this is followed by a reduction in energy requirements due to a reduction in the basal metabolic rate. Enteral nutrition has been shown to support immune function and prevent loss of muscle mass, and therefore although there are no good quality studies of nutrition in DBD, standard protocols should continue unless advised otherwise by the transplant teams (Anwar and Lee, 2019; Meyfroidt et al, 2019; Yoshikawa et al, 2021).

Dignity, Comfort and Compassion

Optimising the potential organ donor should not be at the expense of providing both the patient and their loved ones with dignity, comfort and compassion. Due to its time-sensitive nature, organ donation can feel hectic and this may be distressing for a donor's loved ones. They should be prepared for the final stages of the process, including withdrawal of life sustaining treatment, in advance, with appropriate support offered. The SNOD team are trained in such care and thus act as a key line of communication between the medical teams and the deceased's relatives.

Practical Considerations

Whilst this article has predominantly focussed on the physiological management of DBD organ donors, we are conscious that there are a wide variety of challenges posed by the logistical aspects of organ retrieval, transport (and associated ischaemic times), and transplantation surgeries. This topic is beyond the scope of this article; however, we would direct readers wishing to know more about such challenges to some excellent articles written by Corbett et al (2021) and Yoshikawa et al (2021).

Conclusion

The pathological process of brainstem death provides unique challenges, which if left untreated, can render the potential donor organs unsuitable for retrieval and transplant. Care of such patients is best undertaken in an intensive care environment where the high ratio of staff to patients, alongside invasive monitoring and organ support, enables careful management of their complex physiology. The NHSBT provides guidance and recommendations for target physiological parameters for management of DBDs, and this can be flexed in response to specific instructions from retrieval teams. Finally, it is essential to remember that organ donation is a complex and highly emotive subject. It is vital that those close to the donor are treated with care and respect, and that attention be paid to their needs alongside the patients. SNOD teams play a vital role in this, and in the communication and co-ordination between all professionals and loved ones.

Key Points

- Donor organs are an extremely precious commodity.
- Following brain death, a predictable series of pathophysiological changes occur, which, if left unchecked, can lead to multiple organ failure.
- Care of the potential DBD donor is undertaken on the intensive care unit where scrupulous attention must be paid to all organs.
- Optimising the potential organ donor should not be at the expense of providing both the patient and their loved ones with dignity, comfort and compassion.
- Specialist nurses for organ donations (SNODs) play an essential role in the complex organ retrieval process and should be utilised.

Curriculum Checklist

This article addresses the following aspects of the Core Medical Training curriculum:

- Organ donation

This article addresses the following aspects of the Core Anaesthetics Training curriculum under the Intensive Care domain:

- Manages the medical/surgical needs and organ support of patients during their critical illness, including the holistic care of patients and relatives
- Manages end of life care within the intensive care environment
- Liaises with transplant services when appropriate, and provides the physiological support of the donor

This article addresses the following aspects of the Core Surgical Training curriculum:

- Management of the dying patient (organ donation aspects)

Availability of Data and Materials

All the data of this study are included in this article.

Author Contributions

DL: primary author of manuscript, responsible for initial composition and literature search, revising drafts with feedback from NGK and HM, final approval of manuscript. NGK: provided feedback for drafts, authored pathophysiology of brain death section of the final manuscript and agreed to final manuscript draft. HM: provided feedback for manuscript drafts and assisted with summarizing and fact-checking, and agreed to final draft. All authors made substantial contributions to conception and design. All authors contributed to revising the manuscript critically for important intellectual content. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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