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Supplement 2. The Science of Brain Death/Death by Neurologic Criteria

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The Science of Brain Death/Death by Neurologic Criteria

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Abstract

Brain death/death by neurologic criteria (BD/DNC) can occur via a variety of mechanisms, and the underlying pathology and pathophysiology dictate the mechanisms and likelihood of progression to brain death. We provide a review of these mechanisms, as well as specific areas for caution based on the underlying etiology as well as the age of the patient.

Introduction

Brain death/death by neurologic criteria (BD/DNC) can occur via a variety of mechanisms, resulting in a cascade of pathophysiologic events, some of which are still not fully understood. In order to explore these mechanisms and their consequences, a literature search of the Embase and MEDLINE databases was conducted for the time period between January 1992 and July 2017. Subsequent searches were performed to capture relevant articles between July 2017 and April 2020.

The setting for BD/DNC is almost exclusively in-hospital and mainly limited to intensive care units (ICUs). There are no reliable epidemiologic data about the incidence of BD/DNC. Pathophysiologic events can basically be classified according to whether the mechanism that leads to BD/DNC is mediated by an intracranial circulatory arrest or not. In the vast majority of cases, a massive increase in intracranial pressure that surpasses the mean arterial blood pressure causes a drop in cerebral perfusion pressure and finally an absence of brain blood flow. In some specific scenarios, the chain of events leading to BD/DNC is still not fully characterized. This is the case of patients with craniectomy, children with non-ossified fontanelles and in primary posterior fossa lesions. Selective vulnerability of neurons is the key pathophysiologic feature of BD/DNC with preservation of brain blood flow, which may occur after cardiac arrest, CO poisoning and severe hypoglycemia. From the clinical perspective the consequences of both pathophysiologic mechanisms of BD/DNC are the irreversible loss of capacity for consciousness, brainstem reflexes and the capacity to breathe. The anatomical structures that serve these functions are very resilient to injury and the reflexes that are integrated in these structures are lost only if profound damage has occurred. Spinal structures are spared from these processes and spinal reflexes may be preserved in BD/DNC.

Settings, Epidemiology and Causes

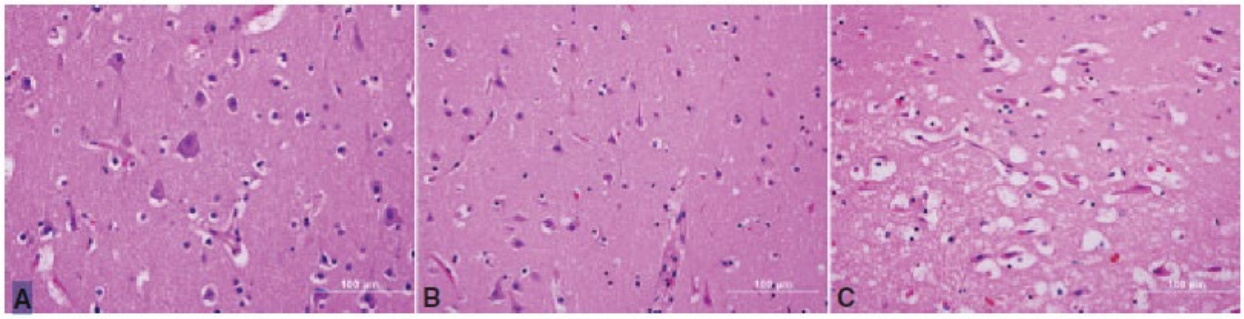
Brain death or death by neurological criteria (BD/DNC) is a condition that can develop in persons with devastating brain injuries who have complete and irreversible loss of all neurological function and therefore require effective respiratory support to maintain oxygenation and circulation with complete independence of brain function. The setting for this condition is almost exclusively in-hospital and mainly limited to intensive care units (ICUs). There are no reliable epidemiologic data about the incidence of BD/DNC worldwide. However, a review of 875 deaths in Swedish ICUs during the last three months of 2007 found that 65 (7.4%) were diagnosed as brain dead, giving an estimated annual incidence of 28.4 per 100,000 inhabitants.[1] Additionally, a review of end-of-life practices in Europe found that BD/DNC accounted for 3.2% of deaths in Northern Europe, 7.6% of deaths in Central Europe and 12.4% of deaths in Southern Europe.[2]

The most common etiologies of devastating brain injury are hemorrhagic stroke (subarachnoid hemorrhage and intracerebral hemorrhage), ischemic stroke, traumatic brain injury (TBI) and global anoxic brain injury after resuscitated cardiac arrest. Less frequent etiologies are infectious diseases of the central nervous system (bacterial meningitis, viral encephalitis, and brain abscesses), neoplasms (primary brain tumors or metastases), obstructive hydrocephalus and metabolic causes of brain edema (e.g., hepatic encephalopathy, acute hyponatremia). Rare causes include carbon monoxide poisoning, which accounts for less than 1% of BD/DNC cases, and hypoglycemia.[3,4] The proportion of each etiology and trends over time are strongly dependent on local criteria for referral to ICUs.[5,6] Published data are mainly based on organ donation registries and quality programs that excluded cases not eligible for donation. The primary etiologies of BD/DNC reported in a large donation quality program were cerebrovascular diseases (53.8%), traumatic brain injury (20.1%), post-anoxic encephalopathy (15.5%), neurological neoplasms (2.1%), and other diseases (8.4%).[7] Risk factors for progressing to BD/DNC after out-of-hospital cardiac arrest include age, female gender, neurological cause for arrest, duration of low flow period >16 minutes, and need for vasoactive medications at 24 hours post-arrest.[8] APACHE II and SAPS II scores have also been used to predict progression to BD/DNC in neurocritical care patients.[9]

Neuropathology of BD/DNC

In the mid-1970´s, neuropathologists coined the term “respirator brain” to describe the pathological features of cases with devastating brain injuries kept on prolonged ventilator support, which have since been considered the pathologic hallmarks of BD/DNC.[10-12] On inspection, uncal and tonsillar herniation may be present, and the brain appears grossly swollen, dusky, discolored and congested. In a time-dependent pattern, which reflects selective vulnerability to ischemia, hypoxia, tissue shifts, and compression, there is progressive softening of brain tissue due to autolytic changes, eventually leading to liquefaction and fragmentation. These changes may not be uniform; the earliest affected portions of the brain are the upper brainstem, diencephalon and cerebellum, with a relative preservation of the medulla. The spinal cord is usually spared, but at times softness and duskiness of the cervical cord and medulla can occur.[13] All these changes are time-dependent and become apparent after several days. At this stage, brain tissue will be difficult to fix pathologically, even after long immersion in formalin. However, nowadays decedents are usually kept on the ventilator for shorter periods of time and total brain necrosis is rarely

observed.[14] If the brain is fixed early after circulatory arrest, neuropathologic findings will be quite unremarkable.[14,15]



(A) Normal appearing pyramidal neurons. (B) Scattered neurons with ischemic changes, including contracted, hypereosinophilic cytoplasm and nuclear changes. (C) Diffuse ischemic changes.

Figure 1. Neuronal ischemic changes (Reproduced from Widjicks et al, 2008[14])

Pathophysiology of BD/DNC

Different kinds of devastating brain injuries may end in BD/DNC, but pathophysiologic events can basically be classified according to whether or not the mechanism that leads to BD/DNC is mediated by the development of intracranial hypertension. Experimental data, clinical neuroimaging and intensive neuromonitoring has given a strong support to the basic principles of these mechanisms and the correlation with clinical findings. There are some specific and rather exceptional scenarios where the chain of events is still not fully understood.

BD/DNC with Absence of Brain Blood Flow

The brain accounts for approximately 2% of total body mass but consumes roughly one quarter of resting total body oxygen and demands approximately 14 % of the total cardiac output at rest.[16,17] Basic physical principles are applicable to the understanding of cerebral circulation. Ohm's law predicts that flow (Q) is proportional to the pressure gradient between inflow and outflow (ΔP), divided by the resistance to flow (R). In the brain, inflow pressure is the mean arterial pressure (MAP) and outflow venous pressure is the pressure measured in the thin-walled veins located in the subarachnoid space. These tiny cortical veins reflect the pressure of surrounding cerebrospinal fluid (CSF), equating to intracranial pressure (ICP). If resistance to flow is constant, cerebral perfusion pressure (CPP) can be described as: CPP = MAP – ICP. This links cerebral perfusion to ICP dynamics, which is critically dependent on the anatomical fact that the brain, its vascular network and the CSF compartment are contained within a rigid bony skull and a membranous dura mater with a very limited potential for expansion. The average adult intracranial volume is 1,472.9 ml (SD ± 117.2 ml) for males and 1,321.7 ml (SD ± 108.3 ml) for females;[17] the brain parenchyma accounts for about 85% of that volume, blood for 8-10 % (100–130 ml; 15% arterial, 40% venous and 45% in the microcirculation) and the CSF about 5% (75 ml). ICP reflects the volume of the three compartments.[18]

Almost 200 years ago, the 'closed box' analogy of intracranial pressure/volume dynamics was recognized and later conceptualized by Harvey Cushing as the Monro-Kellie hypothesis, stating that a change in blood, brain or CSF volume results in reciprocal changes in one or both of the other two.[18,19]

Although some volumetric changes may occur in the acute setting of increased ICP[20], it remains a basic fact that an adult (and most children >2 years of age whereupon cranial bone sutures have fused) consolidated skull cannot expand significantly. Brain edema and rapidly growing intracranial pathology (like hemorrhages) are the most frequent causes of an acute disruption of intracranial homeostasis.

Adaptation to space occupying volume is initially accomplished by shifting CSF from the intracranial to spinal subarachnoid compartment. Approximately 40% of cerebral blood volume is contained in the cerebral veins and dural sinuses, and this venous capacitance can be reduced to accommodate increased intracranial volume even further.[18] But these mechanisms are quickly exceeded, resulting in a decreased compliance and a significant increase in ICP. If the rise in ICP cannot be resolved quickly enough, CPP will drop beyond critical levels, leading to cerebral circulatory arrest. Once cerebral circulation ceases, the brain becomes irreversibly damaged within minutes and starts to undergo aseptic cellular necrosis, leading to autolysis. Invasive monitoring of ICP and CPP has shown that BD/DNC with absence of brain blood flow is by far the most common pathophysiological mechanism of BD/DNC, regardless of the causative disease.[21,22] Standardized experimental BD/DNC models have been developed, allowing sequential measurements of all clinically relevant parameters of intracranial dynamics.[23]

Brain circulatory arrest may occur even before CPP reaches a level of zero due to increased vascular resistance, as shown experimentally by the model of critical closing pressure (CrCP) of cerebral vessels.[24] Critical closing pressure is the sum of ICP and arterial wall tension, and may be estimated by a nonlinear model of cerebrovascular impedance.[25] The arterial wall tension correlates with CPP: at a CPP of 0, the CrCP simply equals ICP; if CPP is positive, a rising ICP will lead to an increase of CrCP.[21,25,26] In a standardized pig BD model, the critical threshold was reached when CPP decreased below 30 mmHg,[27] and in human case series these patterns have been observed with CPP below 20 mmHg.[26] A more recent study in humans demonstrated ICP values > 95 mmHg an CPP < 10 mmHg, and all but one patient had CPP values ≤ 0 mmHg (this patient had a maximum ICP of 145 mmHg).[28] Invasive brain oxygenation ($PbtO_2$) monitoring has shown that brain tissue oxygen tension also decreases as CPP decreases beyond critical levels. The irreversible drop of $PbtO_2$ is characterized by an absence of response to oxygen challenge (100% oxygen for 2 minutes). $PbtO_2$ reaches the zero level almost at the same time as clinical examination becomes completely consistent with BD/DNC.[29] Venous oxygen saturation measured in the jugular bulb also correlates with this end-stage global metabolic failure of the brain in BD/DNC with absence of brain blood flow.[30]

Recently, a different pattern has been described that is characterized by a fall in $PbtO_2$ to zero despite an apparently adequate perfusion pressure as calculated based on ICP and MAP measurements, but with absent brain flow confirmed with nuclear medicine cerebral flow studies. These were persons with TBI and cardiac arrest, and may represent end organ failure and the inability to deliver or use oxygen at the tissue level of the brain.[22]

Decompressive Craniectomy

The rationale for performing a decompressive craniectomy is to stop the otherwise intractable rise of intracranial hypertension due to either focal (as in large hemispheric infarcts) or global (as in global anoxic brain injury) disorders. Once the cranium and the dura are opened, the intracranial pressure/volume dynamics are profoundly modified. Edematous brain tissue eventually bulges through

the craniectomy, and any further increase in ICP will be compensated by a now compliant and expandable intracranial compartment. Even so, some persons continue to deteriorate with rising ICP and may develop BD/DNC. Recent small case series have shown that almost half these persons have a CPP of zero at the time of the first BD/DNC examination. Those who preserve positive CPP measurements have CPP values below the critical threshold of perfusion pressure, and ancillary tests have demonstrated loss of cerebral perfusion.[31]

Infant Cranium

In infants, the presence of non-ossified fontanelles modifies the initial compensation of intracranial hypertension, resulting in a distortion of cerebral hemodynamic patterns that are different from children and adults with an inextensible skull. Initially, as the intracranial pressure rises, the fontanelles compensate the volume expansion by becoming less soft and compressible, a classic clinical sign of intracranial hypertension. The compensatory capacity is inversely proportional to the velocity of development of intracranial hypertension, giving rise to complex and less predictable pressure/volume dynamics. With the increase in intracranial pressure, a rhythmic pulse may be observed on the fontanelles, an expression of the heart's flow transmitting the systolic wave to the cerebrospinal fluid. The most frequent observation on transcranial Doppler (TCD) ultrasonography is the presence of a reverberant flow, with higher velocities than in adults that may also persist until final clinical BD/DNC determination. When the large fontanelles are manually compressed, the TCD patterns resemble closely those of adults with BD/DNC.[32,33] After full ossification of fontanelles at the age of two years, the pathophysiology of BD/DNC with absence of brain blood flow is expected to be the same as in adults.

Posterior Fossa Circulatory Arrest Mediated BD/DNC

Persons with primary posterior fossa devastating lesions may retain supratentorial blood flow at a time when they meet clinical BD/DNC criteria. This uncommon scenario is usually transitional. At follow up, these persons consistently lose supratentorial cerebral brain fluid and global intracranial circulatory arrest ensues.[34] The mechanisms for this progression are not well characterized. It may be due to transtentorial herniation, acute hydrocephalus, or primary failure of cardiovascular control centers in the medulla.

BD/DNC with Preservation of Brain Blood Flow

The brain's function and viability depend on a constant supply of blood, oxygen and glucose. The brain is metabolically unique in its use of fuel relative to other organs because it lacks the ability to store useful amounts of fuel and thus requires a constant supply of energy metabolites. Approximately 50% of the energy used is for synaptic activity, 25% is for restoring ionic gradients across the cell membrane, and the remaining energy is spent on biosynthesis.[35] Although glial cells account for almost half of the brain volume, they account for less than 10% of total cerebral energy consumption. The majority of energy is consumed by neurons, which makes these cells especially vulnerable to deprivation of oxygen, glucose or blood flow. Selective vulnerability of neurons is the key pathophysiology feature of BD/DNC with preservation of brain blood flow.

Cardiac Arrest

About two-thirds of persons admitted to the hospital after cardiac arrest die before hospital discharge.[36,37] Most of these deaths are due to hypoxic-ischemic brain injury[38,39] and result mainly from active withdrawal of life-sustaining treatment based on prognostication of survival with a poor neurological outcome.[40,41] According to a recent meta-analysis, among persons with hypoxic-ischemic brain injury who died before hospital discharge, the estimated pooled prevalence of BD/DNC was 12.6% (10.2–15.2%), corresponding to 8.9% (7.0–11.0%) of persons resuscitated from cardiac arrest.[42]

In an animal model, initiation of cardiopulmonary resuscitation (CPR) within 4.5 min after cardiac arrest resulted in reappearance of EEG activity. A delay of 6 minutes or more was consistently associated with a persistent loss of brainstem reflexes and no reappearance of bioelectric brain activity.[43]

Two main types of irreversible brain pathology have been observed in hypoxic-ischemic encephalopathy.[44] The first consists of primary ischemic selective disseminated neuronal death appearing in the form of acidophilic neurons with consequent neuronal loss and reactive astroglial activation. Especially vulnerable neurons in adult human brain include pyramidal cells of the CA1 subfield of the hippocampus, Purkinje cells of the cerebellum, small and medium-sized neurons of the striatum, and layers three, five and six of the neocortex.[45,46] The second type includes microinfarcts in confluent areas of pancellular necrosis associated with perivascular and diffuse tissue sponginess. These lesions show multifocal, perivascular and laminar distribution, with a predilection for the cortical border zones of arterial supply territories, so-called watershed regions, associated with disintegration and loss of GFAP-reactive astrocytes. If brain damage of the first type is dominant and extensive, it may affect even the more resistant brainstem neurons without any significant rise in ICP, and persons may have all clinical signs of BD but preserved intracranial blood flow. In post anoxic BD/DNC with preservation of brain blood flow, extreme precaution must be taken to exclude potentially reversible factors that may contribute to neuronal dysfunction, such as sedatives, paralytics, metabolic disturbances or hypothermia.[47] If the predominant lesions are of the second type, a pattern of multifocal borderzone cerebral infarcts will develop with progressive cytotoxic edema which eventually will lead to elevated ICP, advancing to BD/DNC with absence of brain blood flow.

Carbon Monoxide Intoxication

Carbon monoxide's (CO) affinity for hemoglobin is more than 200 times higher than that of oxygen, resulting in the formation of carboxyhemoglobin and shifting the oxyhemoglobin dissociation curve to the left.[48] CO interferes with myoglobin, P450, and other enzyme functions, causing lipid peroxidation through neutrophil activation. It produces oxidative stress manifested by peroxynitrate deposition in endothelium, binds to cytochrome aa3 and disrupts the mitochondrial respiratory chain, can cause neuroexcitotoxicity, and contributes to hippocampal cellular death through apoptosis.[49-51] CO poisoning is common and very often causes neuropsychological sequelae, but few persons develop BD/DNC. It is estimated that less than 1% of all organ donors in Western Europe and the USA are procured from CO-poisoned donors.[3]

Hypoglycemia

Profound, prolonged hypoglycemia can cause BD/DNC. In studies of insulin-induced hypoglycemia in monkeys, 5-6 hours of blood glucose concentrations of less than 1.1 mmol/l (20 mg/dl) were required to produce neuronal damage.[52] Hypoglycemia of that magnitude and duration occurs rarely in people with diabetes. A variety of mechanisms are thought to be involved in the pathogenesis of hypoglycemic neuronal death. These include glutamate release and activation of neuronal glutamate receptors, production of reactive oxygen species, neuronal zinc release, activation of poly (ADP-ribose) polymerase and mitochondrial permeability transition. Animal models have shown that neuronal death may even be triggered by glucose reperfusion and activation of neuronal NADPH oxidase.[53]

Anatomical and Clinical Remarks Relevant to BD/DNC

From the clinical perspective the consequences of both pathophysiologic mechanisms of BD/DNC, i.e. with absent or preserved brain blood flow, are the irreversible loss of capacity for consciousness, brainstem reflexes and the capacity to breath. The anatomical structures that serve these functions are very resilient to injury and the reflexes that are integrated in these structures are lost only if profound damage has occurred.[53] The brainstem has a key role as a through-station for almost all hemispheric input and output and hosts the main centers that generates arousal, as well as respiration. The long-standing concept of an ascending reticular activation system (ARAS) includes the central region of the brainstem extending forward from the pontine reticular formation through the mesencephalic tegmentum and into the caudal diencephalon.[54,55] Several neurochemically specific wake-promoting cell groups within the ARAS have been identified including cholinergic cells in the pedunculopontine and laterodorsal tegmentum, serotoninergic cells in the raphe nuclei, noradrenergic cells in the locus coeruleus, dopaminergic cells in the substantia nigra pars compacta and ventral tegmental area and glutamatergic cells in the midbrain.[56] Projections from these pontine and midbrain wake-promoting cells travel dorsally to activate the thalamocortical systems and ventrally to activate the hypothalamic-cortical and basal-cortical systems. Cell groups in the forebrain also promote wakefulness in addition to these ARAS wake-promoting cell groups such as hypocretin (orexin)-containing cells in the lateral hypothalamus, histaminergic cells in the posterior hypothalamus, cholinergic cells in the basal forebrain, neuropeptide Y–containing cells in the suprachiasmatic nucleus and glutamatergic cells in the ventromedial prefrontal cortex.[56] No single system is responsible on its own for generation and maintenance of wakefulness. The irreversible damage of the structures that host these cells and its projections causes the absence of wakefulness and responsiveness to external and internal stimuli, hindering the collective cortico-subcortical interaction that are essential for conscious awareness.[57,58]

The neuroanatomical basis of brainstem reflexes is well known and offers the possibility of a detailed clinical assessment of the structural integrity of every single portion of the brainstem,[59,60] provided any relevant confounding factor has been appropriately excluded. A bilateral mesencephalic lesion interrupts oculomotor nerve fascicles causing a bilateral oculomotor paralysis including the parasympathetic fibers responsible for pupillary light reflexes. Extensive pontine lesions interrupt both afferent trigeminal fibers and efferent facial nerve fibers responsible for corneal blink reflexes as well as facial motor response or grimacing to noxious stimuli in the trigeminal area. Reflex eye movements to head motion and caloric vestibular stimulation are also very resilient. Absence of these reflexes reflects damage of afferent fibers,

vestibular nuclei, oculomotor nerves and internuclear connections. Extensive lesion of the medulla interrupts fibers of the IX and X cranial nerves causing loss of gag and cough reflexes and also loss of vasomotor and cardio-modulating output. During the process of rostral- caudal neurological deterioration, brainstem compression leads to marked hypertension and bradycardia (i.e., the Cushing phenomenon) reflecting both sympathetic and vagal stimulation. When the entire brainstem becomes ischemic, the vagal cardiomotor nucleus becomes ischemic, with unopposed sympathetic stimulation, leading to tachycardia, hypertension, and high blood levels of catecholamine (i.e., autonomic storm or catecholamine surge). At the onset of brain death, a drop in blood pressure, heart rate and heart rate variability signals the loss of output from cardiac vagal neurons and vasomotor neurons to the cervical and thoracic spinal cord.[60,61]

The ability to breathe depends on a vast array of interconnected neurons located primarily in the medulla oblongata and lower pons.[62] This complex system generates the automatic rhythm for contraction of respiratory muscles at rest, the adjustment of these rhythms to changing metabolic demands, and the highly coordinated output for non-ventilatory behaviors like speaking and swallowing. The exact location of the central pattern generator (CPG) for respiration has been elusive and different models have been proposed. A restricted-site model assigns this role to the pre-Bötzinger complex of the ventral respiratory group, a distributed oscillator model considers more than one CPG that could take over the task in different conditions and an emergent property model considers that no individual region of the medullary ventilator groups is sufficient to generate the rhythms but many of them are necessary for normal breath function.[62] The activity of these neurons depends on their intrinsic pacemaker properties and the influences they receive from chemoreceptors. The major source of feedback for assessing the effectiveness of ventilation are central chemoreceptors primarily sensitive to hypercapnia. The blood-brain barrier has a high permeability to small neutral molecules like CO_2 and arterial hypercapnia rapidly leads to a respiratory acidosis of similar magnitude in the CSF and brain extracellular fluid. Low pH is sensed by these chemoreceptors located near the surface of the ventrolateral medulla, but also in the medullary raphe nuclei, the nucleus of tractus solitarius and pontine locus coeruleus with a high grade of redundancy for this critical system.[62] Peripheral chemoreceptors located in the carotid and aortic bodies are sensitive to hypoxemia and have a role during intense exercise, but they are not essential for maintenance of normal breathing at rest.[62,63] This is the conceptual basis for the apnea testing in which the reactivity of the CPG for respiration is assessed by means of short term marked respiratory acidosis without hypoxemia.[59,61]

BD/DNC does not include the structures of spinal cord caudal to C2, because their location outside the skull spares them from global ischemia of intracranial circulatory arrest. Consequently, spinal reflex and spontaneous movements may be present in brain-dead patients. Finger jerks are the most common movement, but also more complex movements like undulating toe flexion, triple flexion response, pronation-extension reflex and facial myokymia may be seen in up to 39 % of patients.[64] Rarely, seemingly purposeful movements of the upper extremities may occur termed the Lazarus sign, in which the arms flex quickly to the chest from the patient's side, the shoulders adducted, and in some patients, the hands crossed or opposed just below the chin. Spinal movements may be observed in response to noxious stimuli, during transport, in synchrony with mechanical ventilation, during the apnea test or may

be precipitated after several minutes of hypoxia or ischemia when the ventilator is removed terminally.[61,64,65]

Questions to Inform Research Agendas

1. What is the epidemiology (demographics, incidence, point prevalence) of BD/DNC with absent and preserved brain blood flow?
2. What is the precise sequence of events in primary posterior fossa lesions that lead to BD/DNC?
3. Do different primary brainstem pathologies have a different rate of progression to BD/DNC? What is the pathophysiology of this process?
4. In post-anoxic BD/DNC with preservation of brain blood flow, how does one ensure that potentially reversible factors have been excluded? Is an observation period always necessary, and if so, how long should this period be?
5. What is the clinical relevance of critical closing pressure (CrCP) of cerebral vessels?
6. In the setting of decompressive craniectomy, what are the differences in pathophysiology of BD/DNC?
7. Is there any relevant difference in pathophysiology of BD/DNC in children after full ossification of fontanelles and adults?
8. What is the sequence of changes in heart rate variability during the development of BD/DNC? Is heart rate variability analysis predictive of progression to, or confirmation of, BD/DNC?

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