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Supplement 4. Minimum Clinical Criteria for Determination of Brain Death/Death by Neurologic Criteria

This supplementary material has been provided by the authors to give readers additional information about their work.

Minimum Clinical Criteria for Determination of Brain Death/Death by Neurologic Criteria

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Abstract

Introduction Determination of brain death/death by neurologic criteria (BD/DNC) is a complex process. The steps for making a determination of BD/DNC vary around the world.ria

Methods We conducted a review of the literature and formulated recommendations with an expert panel on the minimum clinical criteria for determination of BD/DNC in an effort to avoid false positive declaration of death.

Results and Conclusions Despite variations in some of the details associated with determination of BD/DNC, the core tenets of BD/DNC determination (devastating brain injury resulting in irreversible coma, brainstem areflexia, and the inability to breathe spontaneously) are agreed upon. We provide recommendations and suggestions for the minimum clinical criteria for determination of BD/DNC. Countries/medical societies/institutions should feel free to pursue additional requirements beyond these minimum clinical criteria in accordance with local/regional laws and practices.

Introduction

Determination of brain death/death by neurologic criteria (BD/DNC) is a complex process that requires familiarity and expertise and must be followed diligently in order to avoid mistakes that may lead to false positive declaration of death. However, it is worth emphasizing that the false positive rate in BD/DNC determination is unknown. In this section, we aim to establish the minimum clinical criteria needed to make the diagnosis of BD/DNC in adults based on review of the literature and expert consensus, erring on the conservative side to ensure that there are no diagnostic errors. Establishing minimum clinical criteria for determination of BD/DNC and ensuring the proper documentation through use of a checklist (refer to Documentation of Brain Death chapter), will enhance the rigor of the process. Countries/medical societies/institutions should, of course, feel free to pursue additional requirements beyond these minimum clinical criteria in accordance with local/regional laws and practices.

Methods

Authors conducted the initial literature searches of the Cochrane, Embase and MEDLINE databases for the time period between January 1, 1992 and July 2017. Subsequent searches were performed to

capture relevant articles between July 2017 and April 2020. Because of the significant lack of data from randomized controlled trials or large studies, GRADE evaluation of the evidence was not performed. However, evidence was reviewed by a multidisciplinary group of clinicians (see Introduction chapter) and recommendations were generated according to the following criteria. Strong recommendations ("It is recommended that") were based on expert consensus that clinicians should follow the recommendation unless a clear and compelling rationale for an alternative approach was present, and where actions could be adopted as policy. Even though most evidence in this area is limited and of low-quality, strong recommendations were made as a precautionary, conservative approach, to prevent premature or erroneous determinations of death (false positives). Conditional or weak recommendations ("It is suggested that") were generated when there were potentially different options and the best action may differ depending on circumstances, patients, resources or societal values, or where there is a need for further evidence or discussion among clinicians and stakeholders. In cases where there was insufficient evidence and the balance of benefits versus harms was neutral, no recommendations were made.

I. Prerequisites and Confounders

Determination of BD/DNC around the world begins by establishing that 1) the clinical history, etiology and neuroimaging demonstrate that the person has suffered irreversible devastating brain injury leading to loss of all brain functions, and thus is compatible with BD/DNC; and 2) there are no confounders that could make the person appear to have irreversible brain injury, when, in fact, this is not the case.[1,2] There have been several reports of reversible mimics of BD/DNC in the setting of diffuse leptomeningeal carcinomatosis,[3] fulminant Guillain-Barré syndrome and its Miller-Fisher or axonal sensorimotor neuropathy variants[4-12] (also including a recent case with overlap with Bickerstaff brainstem encephalitis),[13] rabies encephalitis,[14] snake bites,[15,16,17] botulism,[18] severe hypokalemia[19] and high cervical cord injuries.[20] Reports of drug, metabolic and hemodynamic derangements falsely suggesting BD/DNC have also been described.[21-25] See Table 1 for a list of confounders to the determination of BD/DNC.

Table 1. Examples of confounders of the clinical diagnosis of brain death

Disease Process	Possible exam components confounded
Hypothermia	Complete exam
Muscular paralysis	Complete exam
Sedation/analgesia	Complete exam
Hypoxia	Complete exam
Hypotension	Complete exam
Hypoglycemia	Complete exam
Endocrine or metabolic abnormality	Complete exam
Basal skull fracture with hemotympanum	Oculovestibular reflex
Facial trauma	Pupillary response, oculovestibular and oculocephalic reflexes, cranial pain response
Pulmonary injury/ disease	Apnea test
Cervical spine injury	Corporal pain response, apnea test
Anophthalmia	Pupillary, corneal, oculovestibular and oculocephalic reflexes

There have been numerous reports of drugs, alcohol or prescription medications interfering with a determination of BD/DNC as they can cause both reversible coma and transient brainstem areflexia.[21-23,26-34] Non-sedative prescription medications that have been implicated as BD/DNC evaluation confounders include valproic acid, tricyclic antidepressants, buproprion, baclofen, and several antibiotics (aminoglycosides, tetracyclines, minocycline, vancomycin, isoniazide or ethambutol).[26-32] Organophosphate insecticides can also lead to deep coma with absent brainstem reflexes, but recovery is possible after 5-15 days.[33] It is worth noting that fixed and dilated pupils are not consistent with the presence of opioids, and that Narcan administered to decedents can elicit spinally-mediated reflexes. Because the effects of these substances are reversible, it is widely advised to delay a determination of BD/DNC if these substances are present in a person's system and continue to support the person until these substances are cleared.[21,22] However, clearance may be a slow process and can be delayed for days in the setting of poor renal or hepatic function. For some substances, such as benzodiazepines, levels can be serially monitored with immunoassays and high-performance liquid chromatography or gas chromatography-mass spectrometry.[35-37] Some hospitals dictate specific levels for sedatives prior to a BD/DNC determination, such as a barbiturate level <10 mg/L, midazolam level <10μg/L or thiopentone level <5 mg/L.[38,39] If levels for a particular substance cannot be measured, protocols advise either determination of clearance based on the substance's half-life or utilization of ancillary testing.[39-41] Half-lives of drugs whose clearance 1) frequently needs to be considered when making a determination of BD/DNC and 2) often cannot be assessed with quantitative or qualitative toxicology tests are listed in Table 2.

Table 2. Half-lives of drugs that may need to be considered when making a determination of BD/DNC

	Drug	Half-life[42]	
Opioids	Fentanyl	3.3-4.1 hours	↑CPBS, Aged, Prem; ↔Child
	Oxycodone	2.1-3.1 hours	
Sedatives	Dexmedetomidine	2 hours	
	Diazepam	30-56 hours	↑Aged, LD; ↔HTh
	Lorazepam	9-19 hours	↑LD, Neo, RD; ↔Aged, CPBS, AVH; ↓Burn
	Midazolam	1.3-2.5 hours	↑Aged, Obese, LD; ↔Smoking
	Pentobarbital	15-50 hours	
	Phenobarbital	81-117 hours	↑LD, Aged; ↓Child; ↔Epilepsy, Neo
	Thiopental	8-10 hours	
	Propofol	2.3-4.7 hours	A much longer terminal $t_{1/2}$ was reported following prolonged IV infusion.
	Zolpidem	1.7-2.1 hours	↑Aged, LD; ↔RD; ↓Child
Other	Baclofen	2.8-4.7 hours	
	Bupropion	10-11 hours (7.9-18.4)	↑Aged, LD; ↔Alcohol

AVH Acute viral hepatitis; CPBS cardiopulmonary bypass surgery; HTh Hyperthyroid; LD Chronic liver disease; Neo neonate; Prem Premature infants; RD renal disease.

However, it is worth noting that the pharmacodynamic and pharmacokinetic properties of drugs are altered when persons are critically ill, have impaired hepatorenal function or were treated with targeted temperature management, so projections of clearance based on half-life data derived from less ill persons or normal volunteers may be inaccurate.[43] A recent position statement from the American College of Medical Toxicology (ACMT) emphasized that 5 half-lives is a minimum, and one should wait longer in the setting of overdose, delayed absorption, delayed elimination or interaction with another agent.[44]

One other class of drugs that can falsely suggest BD/DNC is neuromuscular blocking agents. Non-depolarizing neuromuscular blocking agents do not readily cross the intact blood–brain barrier, but they cause skeletal muscle paralysis.[24] Because of this, some protocols recommend that presence of paralytics be excluded by using a train-of-four stimulator to demonstrate that 4 twitches are present prior to conducting a determination of BD/DNC.[39] In addition, it is reasonable to take into account the pharmacokinetic properties of the neuromuscular blocking agent used, any confounders to their metabolism, and the presence or absence of deep tendon reflexes.[45]

While many protocols indicate that severe metabolic derangements could compromise the clinical evaluation and should be excluded, the definition of "severe" is vague. There are no universally agreed upon established levels for electrolytes, creatinine, glucose or hepatic enzymes. Nonetheless, protocols frequently recommend correcting severe acid-base disorders, acute hepatic failure, acute renal failure with severe uremia, hyper- or hypokalemia, hyponatremia, hyper- or hypophosphatemia, hypermagnesemia, hypercalcemia, hypo- or hyperglycemia, and severe hormonal deficits (like severe myxedema or adrenal failure) based on subjective judgement before a determination of BD/DNC is performed.[38-41,46]

Lastly, the need for normal temperature and blood pressure prior to a BD/DNC evaluation is widely described, but the definition of "normal" varies.[38,40,41,47,48] A temperature of 36ºC or above and a systolic blood pressure of at least 100 mmHg were embraced by the 2010 American Academy of Neurology (AAN) Guidelines.[40] In the United Kingdom, a temperature ≥ 34ºC and a mean arterial pressure (MAP) > 60 mmHg are required.[39] In Australia and New Zealand, the temperature must be > 35ºC and the systolic blood pressure must be > 90 mmHg or the MAP > 60 mmHg.[41] The AAN Guidelines are currently the most conservative, but nonetheless, their recommended values are easily achievable in most patients.[40] Much could be gained by standardizing temperature and blood pressure parameters worldwide.

Establish that Loss of Brain Function is Total and Constant Over Time

Once it has been established that 1) the clinical history, etiology and neuroimaging demonstrate that the person has suffered irreversible devastating brain injury leading to loss of capacity for consciousness (i.e. coma) due to an etiology capable of causing BD/DNC and 2) there are no confounders that could make the person appear to have irreversible brain injury, it must be established that the loss of brain function is total and constant over time. To accomplish this, some countries' guidelines require an observation period before a BD/DNC exam is even considered in order to assure irreversibility.[1,49] The common lengths of observation range from 2-24 hours.[1] The observation period is particularly important after cardiac arrest because there can be slow recovery of brainstem function after successful resuscitation. This is also the case after treatment with targeted temperature management.[25,50] (Refer to the chapter on Determination of Brain Death after Treatment with Targeted Temperature Management.) As with all criteria for BD/DNC determination, the waiting period is arbitrary; clinicians must always come

back to the cardinal rule – if irreversibility is uncertain, determination of death should not proceed; whatever time is necessary to ensure irreversibility should be taken.

Recommendations and Suggestions

1. It is recommended that pathological conditions, confounders and/or reversible conditions that may mimic BD/DNC be excluded prior to commencing a determination of BD/DNC.
2. It is recommended that, prior to commencing a determination of BD/DNC, it must be demonstrated that the person has an established neurological diagnosis, the nature and severity of which is capable of resulting in the irreversible loss of the capacity for consciousness, the irreversible loss of all brainstem reflexes, and the irreversible loss of the ability to spontaneously breathe in the face of a carbon dioxide and acidosis challenge.
3. It is suggested that prior to making a determination of BD/DNC, there be
    a. neuroimaging evidence of intracranial hypertension (severe cerebral edema and herniation), or
    b. intracranial pressure measurements that equal or exceed the mean arterial pressure.
4. It is suggested that in the absence of herniation on neuroimaging, caution be taken when considering an evaluation for BD/DNC.
5. It is suggested that the following prerequisites be met before an evaluation for determination of BD/DNC is performed:
    a. The person should have a minimum core temperature of 36ºC, as defined by esophageal, bladder, rectal or central venous or arterial catheter temperature measurements, with use of a warming blanket, automated temperature regulation device, thermal mattress, warmed fluids and/or warmed oxygen as needed,
    b. Adults should have a systolic blood pressure of ≥ 100 mmHg, or a mean arterial pressure ≥ 60 mmHg and there be age appropriate targets in pediatrics, with use of vascular volume, vasopressors and/or inotropes as needed.
6. It is recommended that the following confounders be eliminated before an evaluation for determination of BD/DNC is performed:
    a. Pharmacologic paralysis must be excluded through use of a train-of-four stimulator if available, or assessment of the presence of deep tendon reflexes if a train-of-four-stimulator is not available.
    b. The influence of central nervous system (CNS) depressing medications including toxins, taking into consideration the elimination half-life that may be prolonged by organ dysfunction and/or hypothermia, be excluded by:
        i. Use of a toxicology screen if there is concern for a toxic exposure, and
        ii. Serially measuring drug levels to ensure they do not exceed the therapeutic range, and, even if within the therapeutic range, are not felt to confound the clinical examination, or

iii. Allowing 5 elimination half-lives to pass before an evaluation for BD/DNC be made (assuming normal hepatic and renal function), or

iv. Performing ancillary testing in addition to the complete clinical examination and apnea test if there is concern about prolonged or unknown drug elimination.

c. If alcohol intoxication is suspected or confirmed, the alcohol blood level must be ≤ 80 mg/dL.

d. Severe metabolic, acid-base and endocrine derangements that could impact the examination must be corrected. If these derangements cannot be corrected and are judged to be potentially contributing to the loss of brain function while the complete clinical examination and apnea test are consistent with BD/DNC, ancillary testing should be performed to confirm this determination.

7. It is recognized that interventions to decrease intracranial pressure, such as hyperosmolar therapy, ventricular drainage and decompressive craniectomy, should be applied when clinically indicated during therapeutic phases of care. It is recommended that if these types of interventions are not indicated for the treatment of devastating brain injury, they should not be performed simply for the purpose of demonstrating irreversibility of the clinical state.

8. It is recommended that an adequate observation period take place prior to clinical testing for BD/DNC.

a. A minimum of 24 hours is recommended specifically for anoxic brain injury after resuscitated cardiac arrest. (Refer to the chapter on Determination of Brain Death after Treatment with Targeted Temperature Management for guidance on determination of BD/DNC in the setting of hypothermia.)

b. The time period for other brain injuries has not been established and should be determined on a case-by-case basis. As a general rule, clinicians should err toward caution, and if there is uncertainty about the potential reversibility of the clinical state, for any reason, the observation time should be the time felt necessary to exclude reversibility without any doubt.

II. Clinical Testing

It is universally agreed that the clinical evaluation for determination of BD/DNC includes an assessment for coma and an evaluation of brainstem areflexia to demonstrate that: 1) pupils are fixed in a midsize or dilated position, and are nonreactive to light (as determined with the naked eye, magnifying glass or a pupilometer)[1,2,39-41,51] (some have used 4 mm as the cutoff for the smallest pupillary diameter, but this is arbitrary and smaller pupils have been reported to be compatible with BD/DNC, although the clinician should always be alert to the possibility of drug intoxication with smaller pupils[52]); 2) the corneal, oculocephalic and oculovestibular reflexes are absent; 3) there is no facial movement to noxious stimulation; 4) the gag reflex is absent to bilateral posterior pharyngeal stimulation; 5) the cough reflex is absent to tracheal suctioning; and 6) there is no brain-mediated motor response to noxious stimulation of the limbs.[1,2,39-41] Some protocols include performance of an atropine test to assess the lack of unopposed

vagal tone inhibition at the sinoatrial node through blockade of muscarinic receptors (i.e. lack of tachycardic response >3% above the baseline heart rate after 2-3 mg of atropine is administered intravenously), but this test is not frequently used, and can affect pupillary reflexes.[1,2,39-41,53]

Although, by definition, there is no brain- or brainstem-mediated activity in a person who is brain dead, the presence of spinal reflexes in this population is well-described. Spinal reflexes can be minor or dramatic and their duration varies. They have been reported to occur in 13-75% of decedents.[54-66] A systematic review and categorization of these movements in 235 decedents by Spittler et al. showed significant inter-individual and intra-individual diversity.[59] In another study of 278 BD/DNC examinations, 42 (15%) persons had polysegmental spinal reflex or automatism patterns.[59] Sometimes, it is difficult even for experienced practitioners to identify the origin of these movements, especially if they are complex.[54,59] The most common movements described are finger and toe jerks,[55] undulating toe flexion, triple flexion,[56] increased tendon reflexes (in legs more than arms),[57,65] plantar flexion, and myoclonus.[58] The presence of spontaneous abdominal contractions needs to be carefully assessed to distinguish spontaneous breathing from spinal reflexive activity.[61] Head turning during the BD/DNC exam in response to nociceptic stimuli below the foramen magnum is also a spinal reflex.[67-69] The Lazarus sign can occur spontaneously or be elicited by various stimuli (respirator removal, passive neck flexion, oculocephalic reflex testing or hypotension),[54,55,62-64] but it is rarely seen (it only occurred in 3-12% of decedents in some series).[56-58] Repetitive leg movements mimicking periodic leg movements during sleep have also been reported.[65] A list of some of the spinal reflexes that have been described is provided in Table 3.

Table 3. Described spinal reflexes in BD/DNC

Reflex	Description
Decerebrate-type movements[54]	Spontaneous extension of the extremities
Extensor posturing[54]	Back arching to the left or right
Eyelid opening[54]	Opening of the eyelids after nipple stimulation
Fasciculation[56]	Twitching of contiguous groups of muscle fibers
Head turning[54,67-69]	Intermittent head turning from side to side every 10-30 seconds with or without extension of the upper extremities
Hugging[54]	Flexion of the trunk and movement of the arms in a hugging-like manner
Lazarus sign[54-58,62-64]	Bilateral arm flexion, shoulder adduction and hand raising to chest, face or endotracheal tube with dystonic posturing of the fingers
Limb elevation[54]	Raising of limbs off the bed
Myoclonus[56]	Twitching or contraction of a muscle or group of muscles
Plantar response[56]	Plantar flexion
Pronator-extension[56]	Pronation and extension of the upper extremity
Respiratory-like movements[54]	Adduction of both shoulders followed by a slow cough-like movement
Repetitive leg movements[65]	Slight flexion of the leg and foot
Thumbs Up Sign[70]	Isolated thumb extension
Triple flexion[56]	Flexion of the thigh, leg and foot
Undulating toe[54]	Slow flexion then extension of the toes

Recommendations and Suggestions

1. It is recommended that BD/DNC first and foremost be a clinical determination.
2. It is recommended that an assessment for determination of BD/DNC be made in all persons with devastating brain injuries who are believed to potentially meet criteria for BD/DNC, regardless of whether or not they are potential organ donors.
3. It is recommended that all of the following neurological assessments be performed as part of the minimum determination of BD/DNC. If a portion of the clinical exam cannot be done, it is recommended that the remainder be completed to the fullest extent possible. If any aspect of the clinical examination cannot be completed (except as stipulated below), but the exam, to the extent completed, is consistent with BD/DNC, ancillary testing is recommended.

    a. Coma – there is no evidence of arousal or awareness to maximal external stimulation (including noxious visual, auditory and tactile stimulation).

    b. Pupillary reflexes

Test	Shine a bright light into each of the person's eyes, looking for pupillary constriction and measuring the diameter of the pupils. Use of a magnifying glass and/or pupillometer is suggested.
Response consistent with BD/DNC	There should be absence of ipsilateral and contralateral pupillary response, with pupils fixed in a midsize or dilated position (~4-6 mm), in both eyes.
Considerations	• Constricted pupils are not consistent with BD/DNC and suggest the possibility of drug intoxication or locked-in syndrome. • Pupils can be any shape (round/oval/irregular). • Corneal trauma or prior ophthalmic surgery may influence pupillary reactivity and preclude adequate evaluation, necessitating ancillary testing. • Ocular instillation of drugs may artificially produce transiently nonreactive pupils. • In the setting of anophthalmia or inability to see the pupils, ancillary testing is recommended.

    c. Oculocephalic (OCR) and oculovestibular (OVR) reflexes

Test	OCR: Rotate the head briskly horizontally to both sides. There should be no movement of the eyes relative to head movement. Testing vertically is optional. OVR: Examine the auditory canal for patency and an intact tympanic membrane. Elevate the head to 30° to place the horizontal semicircular canals in the correct vertical position. Irrigate with ≥ 30 mL of ice water for at least 60 seconds using a syringe or a syringe attached to a catheter placed inside the canal. Test both sides separately and with a 5-minute interval between to allow the endolymph temperature to equilibrate.

Response consistent with BD/DNC	Detection of any extraocular movements is not compatible with BD/DNC.
Considerations	• Confirm the integrity of the cervical spine before proceeding with the OCR test. If the OCR cannot be performed, but the OVR is performed and there are no extraocular movements, ancillary testing is not required. • Ensure the integrity of the tympanic membrane. Presence of a ruptured tympanic membrane does not negate the clinical testing but may risk introducing infections in the ear. • A fracture of the base of the skull or petrous temporal bone may obliterate the response on the side of the fracture, and ancillary testing is recommended in this instance. • Severe orbital or scleral edema or chemosis may affect the free motion of the globes, and ancillary testing is recommended in this instance. • In the setting of anophthalmia, ancillary testing is recommended.

d. Corneal reflex

Test	Touch the cornea of both eyes with a cotton swab on a stick at the external border of the iris, applying light pressure and observing for any eyelid movement.
Response consistent with BD/DNC	No eyelid movement should be seen.
Considerations	• Care should be taken to avoid damaging the cornea. • In the setting of anophthalmia, severe orbital edema, prior corneal transplantation or scleral edema or chemosis, ancillary testing is recommended.

e. Motor responses of the face and limbs

Test	Apply deep pressure to all of the following: i. the condyles at the level of the temporomandibular joints ii. the supraorbital notch bilaterally iii. the sternal notch iv. all four extremities, both proximally and distally. Insert a cotton swab on a stick in each nostril to perform nasal tickle testing.

Response consistent with BD/DNC	Noxious stimuli should not produce grimacing, facial muscle movement or a motor response of the limbs other than spinally-mediated reflexes. Noxious stimuli above the foramen magnum should not produce any movement in the face or body. Noxious stimuli below the foramen magnum should not produce any movement in the face but may elicit spinal cord reflexes.
Considerations	• The clinical differentiation of spinal responses from brain-mediated motor responses requires expertise. Consultation with an experienced practitioner is recommended if the origin of a response is unclear. Alternatively, if interpretation is unclear, ancillary testing is recommended. • Ancillary testing is recommended if a person has a pre-existing severe neuromuscular disorder, such as amyotrophic lateral sclerosis or a pre-existing severe sensory neuropathy. • Ancillary testing is not required if a person does not have all four limbs; absence of a limb does not preclude motor testing to pain on that side of the body. • Severe facial trauma and swelling may preclude evaluation of facial motor response, so ancillary testing is recommended in this setting.

f. Gag and cough reflexes

Test	Stimulate the posterior pharyngeal wall bilaterally with a tongue depressor or suction catheter. Stimulate the tracheo-bronchial wall to the level of the carina with deep endotracheal placement of a suction catheter.
Response consistent with BD/DNC	Absence of cough and gag reflexes.
Considerations	• The efferent limb for the cough reflex includes the phrenic nerve, which may be injured in persons with high cervical cord injuries, so ancillary testing is recommended in this setting.

III. Apnea Testing

Apnea testing is part of nearly all protocols for determination of BD/DNC.[1,2,39-41] The goal of the apnea test is to allow the serum carbon dioxide to increase and the cerebrospinal fluid pH to decrease to a level that would normally maximally stimulate the respiratory centers in a functioning medulla.[71] If there is no medullary function, the person will not make any respiratory effort in the setting of profound hypercarbia and acidosis. Although hypoxia depresses neuronal metabolism, it does not stimulate the central chemoreceptors to trigger ventilation in adults.[72]

Prerequisites

The generally accepted prerequisites for apnea testing include 1) absence of spontaneous respirations (i.e. breathing above the set ventilator rate), 2) normotension, 3) normothermia, 4) euvolemia, 5) eucapnia, and 6) absence of hypoxia.[1,2,39-41] Hemodynamic instability, hypoxia and high ventilator requirements may dissuade practitioners from performing apnea testing, as evidenced by the results of a large series of BD/DNC decedents in which an apnea test was not performed in 7% of the patients because of hemodynamic instability or poor oxygenation at baseline. Polytrauma resulting in BD/DNC was significantly more common in persons in whom apnea testing was not attempted.[73]

With regards to performing an assessment for absence of spontaneous respirations, it is worth noting that ventilatory autotriggering can falsely make it appear that a person is breathing spontaneously. This occurs when the ventilator is set on a spontaneous breathing mode (pressure support ventilation) and either extrinsic or intrinsic factors generate sufficient change in airflow or negative pressure to exceed the trigger threshold and lead to a delivered breath.[74-79] Extrinsic causes for autotriggering include excessive condensation in ventilator tubing, endotracheal tube leak, chest tubes, and random artifacts or noise in the ventilator circuit, while intrinsic causes include cardiogenic oscillation, especially in a hyperdynamic cardiovascular state,[77] or abdominal muscle contractions representing spinal reflexes.[61] However, autotriggering can be mitigated by setting the flow or pressure trigger above the threshold value and confirming absence of breathing while observing the person off the ventilatory circuit.[75]

Procedure

The most commonly employed procedure for apnea testing is disconnection from the ventilator while monitoring for spontaneous respirations, with or without the provision of oxygen.[1,2,39-41] Prior to testing, the person is pre-oxygenated for 10-15 minutes and the ventilator settings are adjusted such that the $PaCO_2$ normalizes at 35-40 mmHg (4.7-5.3 kPa) (eucapnia) or mild hypercapnia ($PaCO_2$ 45 mmHg, 6.0 kPa). Once the $PaCO_2$ is appropriate, the endotracheal tube (ETT) is disconnected from the ventilator. When the oxygen insufflation method is used, a small cannula is advanced through the ETT to approximately the level of the carina to deliver oxygen at 4-6 L/min. The chest and abdomen are exposed to allow practitioners to closely observe the person for spontaneous respiratory movements. If any respirations are seen, the test is aborted, as it is clear that the person is not brain dead.

If there are no spontaneous respirations, an arterial blood gas is drawn eight to ten minutes after initiation of the test. If point-of-care testing is available and the person is stable, they can be kept off the ventilator until it is determined that the $PaCO_2$ has risen adequately. If it is not, they are reconnected to the ventilator when the arterial blood gas is sent. The specific $PaCO_2$ goal varies, but the goal is generally $\geq$ 50 or $\geq$ 60 mmHg ($\geq$ 6.7 or 8.0 kPa). Many protocols also indicate that a higher target is needed in the setting of prior CO_2 retention (such as 20 mmHg (2.7 kPa) above a known chronic baseline $PaCO_2$).[1,2,39-41,80] In some cases, end-tidal continuous capnometry or transcutaneous monitoring is used to monitor the carbon dioxide level during apnea testing, but it is important to note that this value may not accurately reflect the arterial $PaCO_2$.[81-83]

In some cases, a pH goal is provided. The specific target varies; some guidelines aim for < 7.4 or $\leq$ 7.28.[39,80] Notably, it is cerebrospinal fluid acidosis not acidemia that triggers respiratory movements,

and while arterial and cerebrospinal fluid hydrogen concentration are similar in the setting of hypercapnia, arterial and cerebrospinal fluid bicarbonate concentration are not.[72,84]

If the $PaCO_2$ does not reach the target level, and point-of-care testing is used, the test is continued for another 5 minutes, then the arterial blood gas is rechecked. If point-of-care testing is not used, the test is repeated for a longer period of time (15 minutes) after repeat pre-oxygenation and re-establishment of normocarbia if the person is stable. If the test cannot be repeated due to instability, an ancillary test or testing using alternative methods is needed.[39,40,85] Alternative methods of apnea testing include: 1) use of continuous positive airway pressure (CPAP) 10 cm H_2O, and 100% O_2 12 L/min provided directly by the ventilator, through the use of a T-piece tube with a CPAP valve at the outflow end, or through the use of a T-piece system with a *collapsible* reservoir bag and adjustable outflow resistance;[40,86,87] 2) use of bulk diffusion (provision of 100% FiO_2 on high flow (40-60 L/min) at the orifice of the endotracheal tube via the ventilator circuitry (with CPAP set at zero or with the ventilator off and disconnected from the expiratory humidity trap);[88] or 3) carbon dioxide augmentation.[82,89] Use of a T-piece circuit entails connection to the endotracheal tube or a flow-inflating anesthesia bag with titration of end expiratory pressure. Tracheal insufflation is less favored, particularly in younger children/infants/newborns due to carbon dioxide washout. The oxygen diffusion method is considered the most common technique, and safe,[90] although a recent head-to-head comparison with CPAP showed a relative preservation of oxygenation with the latter technique.[91] Apnea testing in the prone position has been reported in a hypoxemic BD/DNC patient.[92]

Risks and Complications Associated with the Apnea Test

There are reports of persons undergoing apnea testing who have developed hypoxemia, hypotension, arrhythmias, cardiac arrest and barotrauma.[73,85,93-97] As a result, guidelines routinely recommend that: 1) the person be observed for desaturation, and the test be aborted if hypoxia develops; 2) the person be observed for hypotension, which can develop as respiratory acidosis is induced due to direct dilatation of the peripheral arterioles and depression of myocardial contractility by CO_2 elevation, and the test be aborted if the systolic blood pressure decreases below normotension despite use of vasopressors.[1,2,39-41,98] Even if the prerequisites for apnea testing are met, the test needs to be aborted prematurely in 1.6-4.8% of the cases.[85,93,99] One study found that younger age and pre-test acidemia (pH 7.29 vs. 7.39) were associated with test completion failure.[99]

The risk for barotrauma leading to pneumothorax, pneumomediastinum or pneumoperitoneum during apnea testing stems from: 1) the fact that oxygen could be trapped inside the lungs if the ratio between the diameter of the cannula and the endotracheal tube is large or there are dry secretions obstructing the flow out; 2) the cannula is placed too deep; or 3) the cannula perforates the trachea.[100-108] An oxygen insufflation study found that endotracheal tube/cannula diameter ratio < 1.75, cannula tip in the distal third of the ETT and O_2 flow rate > 10 L/min were associated with higher airway pressure in the trachea.[100] Other authors have also noted that the internal cannula diameter to endotracheal tube diameter ratio should not be larger than 0.6-0.7.[101] In addition to the risk of barotrauma, it has also been demonstrated that higher oxygen flow rates may result in CO_2 washout, decreasing the speed of CO_2 elevation, or even decreasing the $PaCO_2$ over time.[94,109]

Lastly, it is theoretically possible, although unproven, that secondary injury can occur to the brain during apnea testing due to elevation of intracranial pressure (ICP) secondary to hypercarbic cerebral vasodilation, and that this injury could in fact cause BD/DNC.[110,111] Because of this, some have suggested that apnea test only be performed after ancillary testing shows absent cerebral blood flow.[111,112] Notably, a study by Ma et al showed that 10% of patients with a clinical examination otherwise consistent with BD/DNC showed respiratory effort during apnea testing.[113] A recent study with intracranial monitoring during apnea testing did not show significant changes in intracranial pressure or cerebral perfusion pressure.[114]

Recommendations and Suggestions

1. Because there is concern that apnea testing may elevate intracranial pressure, it is recommended that:
    a. The apnea test be conducted last, after the rest of the clinical evaluation is completed and found to be consistent with BD/DNC, and
    b. It has been determined that the person is not taking any spontaneous respirations when the ventilator is set on a spontaneous breathing mode in a normocarbic state, and
    c. The test is performed by personnel with experience in resuscitation should the patient decompensate during testing.
2. It is recommended that ventilator requirements and pulmonary status be assessed before apnea testing to determine whether a person is likely to tolerate the evaluation.
3. In the setting of a high cervical cord injury, it is recommended that an apnea test not be performed and ancillary testing is indicated.
4. It is suggested that before commencing the apnea test:
    a. The systolic blood pressure be ≥ 100 mmHg or mean arterial pressure be ≥ 60 mmHg in adults (age appropriate targets in pediatrics) with use of vascular volume, vasopressors and/or inotropes as needed,
    b. Temperature be ≥ 36°C, with use of a warming blanket, automated temperature regulation device, thermal mattress, warmed fluids and/or warmed oxygen as needed,
    c. The person be pre-oxygenated with 100% O_2 for at least 10 minutes.
5. It is suggested the minute ventilation be adjusted to establish normocarbia ($PaCO_2$ of 35-45 mmHg (4.7-6.0 kPa)) prior to apnea testing, confirmed by arterial blood gas testing prior to apnea testing.
6. It is suggested that a functioning arterial line be employed to provide continuous blood pressure monitoring and to quickly draw blood gases during apnea testing.
7. It is suggested that the following techniques may be used for apnea testing:
    a. The application of positive airway pressure with the use of CPAP/PEEP may prevent derecruitment and decrease the risk of cardiopulmonary instability, so 100% oxygen can be

delivered to the lungs (i) via CPAP on the mechanical ventilator or (ii) via resuscitation bag with a functioning PEEP valve,

b. Oxygen can also be delivered via the oxygen insufflation method via placement of a tracheal cannula.

8. It is suggested that the apnea test targets during testing be pH < 7.30 and $PaCO_2 \geq 60$ mmHg (8.0 kPa) unless a patient has pre-existing hypercapnia, in which case it should be ≥ 20 mmHg (2.7 kPa) above their baseline $PaCO_2$, if known.
9. It is recommended that apnea testing be aborted if:
    a. Spontaneous respirations are witnessed during apnea testing,
    b. Systolic blood pressure becomes < 100 mmHg or mean arterial pressure becomes < 60 mmHg despite titration of fluids/inotropes/vasopressors,
    c. There is sustained oxygen desaturation below 85%,
    d. An unstable arrhythmia occurs.
10. It is recommended that an arterial blood gas be tested 10 minutes after commencing apnea testing.
    a. If point-of-care testing is available and the person is stable, they can be kept off the ventilator with repeated arterial blood gas sampling every 2-3 minutes until it is determined that the $PaCO_2$ is ≥ 60 mmHg (≥ 20 mmHg above any known chronic baseline $PaCO_2$ in persons with pre-existing hypercapnia).
    b. If point-of-care testing is not available, the person should be reconnected to the ventilator when the arterial blood gas is sent.
11. It is suggested that, while non-invasive capnography may guide the duration of apneic observation, the arterial $PaCO_2$ be used to confirm adequate elevation of CO_2 during apnea testing.
12. If the apnea test is inconclusive (does not reach $PaCO_2$ goals) but the patient was stable during testing from pulmonary and hemodynamic standpoints, it is suggested that the test be repeated after re-establishing pre-oxygenation, normocapnea and a normal pH, and extending the test by several minutes, employing the same technique and parameters as above.
13. It is suggested that prior to aborting the apnea test because of cardiorespiratory instability, an arterial blood gas (ABG) be sent for testing. If the $PaCO_2$ target is met, the apnea test can be considered positive (consistent with BD/DNC).
14. It is suggested that if the apnea test has been aborted because spontaneous respirations are witnessed during testing, apnea testing should be repeated after 24 hours if the clinical exam remains consistent with BD/DNC.
15. If the apnea test is aborted and cannot be repeated safely, it is suggested that either an ancillary test be performed, or apnea testing be attempted after pre-apnea recruitment maneuvers, induction of hypercarbia with CO_2 or carbogen before disconnecting from the ventilator, or utilizing CPAP

to maintain oxygenation.

IV. Number of Examinations

The number of clinical exams required to pronounce BD/DNC varies according to age, hospital, state or country, and generally ranges from 1-3.[1,38,48] Arguments for conducting two exams include mitigation of the potential for diagnostic error and improvement of familial confidence in the diagnosis of BD/DNC. Arguments against waiting for a second exam are that up to 12% of persons may experience cardiac arrest during the waiting period; organ donation consent decreases as the BD/DNC declaration interval increases; and, most importantly, in a review of 1,229 declarations of BD/DNC, none of the persons declared BD/DNC on the initial exam had any evidence of brain activity on the subsequent exam.[115] These concerns might be ameliorated if two examinations take place concomitantly or in relatively quick succession.

Presently, in places in which multiple exams are performed, the number of examiners and timing of the exams varies.[38] In Canada, when organ donation for the purposes of transplant is planned, BD/DNC is determined by at least two physicians. The exams can be done concurrently, but if done at different times, they are full exams including apnea tests at both times.[80] In the United Kingdom, two full consecutive exams (including two apnea tests) by two physicians observing each other on two occasions are required. No inter-observer period is required.[39] In Australia and New Zealand, two full exams (including apnea testing with each) by two different physicians are needed. The tests may be done consecutively, but not simultaneously. There is no requirement for one doctor to be present during the test performed by the other doctor, but such presence is acceptable. No fixed interval between the two clinical tests is required, except where age-related criteria apply (neonates and children).[41] In the United States, only a single examination is required in adults.[40]

Recommendations and Suggestions

1. It is suggested that a single exam, including apnea testing, is the minimum standard for determination of BD/DNC for adults.
2. If 2 evaluations are performed:
    a. It is suggested that an intervening time period is unnecessary because if the prerequisite of irreversibility (which includes an observation period prior to initiating testing) has been satisfied, a second observation period is redundant,
    b. It is suggested that the examinations be performed by two separate examiners,
    c. It is suggested that only 1 positive apnea test be performed in adults.

(See Pediatric and Neonatal Brain Death chapter for recommendations for pediatrics).

Questions to Inform Research Agendas

1. When the presence of drug intoxication is unclear and the pupils are not mid-position to dilated, should naloxone be routinely administered before conducting a BD/DNC determination? What dosing recommendations are reliable?
2. What is the impact of corneal transplant or cataract surgery on ocular examinations?
3. What method of performing apnea testing is associated with the lowest risk of complications?
4. What is the highest $PaCO_2$ and lowest pH at which a person has the potential to breathe?
5. Should there be a different $PaCO_2$ target at high altitudes?
6. Is neuroimaging evidence of severe intracranial hypertension, including the presence and severity of cerebral edema and pontomedullary herniation, predictive of, or correlated with:
    a. Fulfillment of clinical criteria for BD/DNC prior to apnea testing?
    b. Absence of spontaneous breathing during the apnea test?
    c. Absence of brain blood flow on ancillary testing?

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