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Supplement 5. Beyond Minimum Clinical 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.

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

Michael Souter, MB, ChB, FRCA[1], Anne W. Alexandrov PhD, CCRN, ANVP-BC, NVRN-BC[2], Mehmet Akif Topcuoglu MD[3], David Greer, MD, MA[4], Ariane Lewis, MD[5], Gene Sung, MD, MPH[6], Sylvia Torrance, BSc[7], Sam D. Shemie, MD[8]

1) University of Washington, Seattle, USA; 2) University of Tennessee Health Science Center, College of Nursing, Memphis, USA; 3) Hacettepe University, Department of Neurology, Ankara, Turkey; 4) Boston University School of Medicine, Boston, USA; 5) NYU Langone Medical Center, New York, USA; 6) University of Southern California, Los Angeles, USA; 7) Canadian Blood Services, Ottawa, Canada; 8) McGill University, Montreal Children's Hospital, Montreal, Canada

Key words: brain blood flow, brain death, ancillary testing, confirmatory testing, cerebral angiography, electroencephalogram, radionuclide angiography, radionuclide perfusion scintigraphy, CT angiography, MR angiography, CT perfusion, transcranial Doppler ultrasound, evoked potentials

Abstract

Introduction Studies to assess for brain blood flow or cerebral activity are sometimes performed when making a determination of brain death/death by neurologic criteria (BD/DNC).

Methods We conducted a review of the literature and formulated recommendations with an expert panel on the use of ancillary or confirmatory studies to go beyond the minimum clinical criteria for determination of BD/DNC.

Results/Conclusion Numerous ancillary or confirmatory tests are used when making a determination of BD/DNC, but they are not all validated or widely accepted. The use of digital subtraction angiography, radionuclide perfusion scintigraphy or transcranial Doppler to assess brain blood flow is favored if testing beyond minimum clinical criteria is required.

Introduction

Although the fundamental components of the clinical exam to diagnose brain death/death by neurologic criteria (BD/DNC) are well-established, confounders may obstruct either completion or interpretation of components of the exam or additional testing beyond the clinical evaluation may be desired or required. The Harvard Committee in the United States transiently adopted technological verification of clinical findings with an initial recommendation to include electroencephalography (EEG) in the diagnostic process [1], but that recommendation did not persist beyond 1969.[2] However, some other countries determined that it was essential for an investigation of neurological viability to accompany the clinical examination. This has been documented previously [3], but the situation is dynamically evolving as technologies arise. Consequently, ancillary testing may be performed due to mandates by national/regulatory bodies or the need to resolve uncertainty of diagnosis. The nomenclature for testing under these varying circumstances is listed in Table 2 and 3. In 2002, a review by Wijdicks found that

diagnostic tests were optional in 52 countries and mandatory in 28.[3] In a more recent review from 2015, 22 of 70 countries reporting national protocols for the diagnosis of BD/DNC mandate the use of an ancillary test.[4] These tests evaluate either brain blood flow or cerebral activity. This section is intended to examine the evidence supporting the varying modalities of testing.

Review of Literature

We reviewed Cochrane, Embase and Medline using the following search terms: ‘brain death’ with any of the terms 'ancillary study', 'EEG', ' electroencephalography', 'cerebral angiography', 'transcranial Doppler', 'CT perfusion', 'scintigraphy', 'SPECT', 'nuclear medicine' ,'evoked potentials', 'CT Angiography', 'MR Angiography', ranging over the last 25 years to July 2017, and excluding animal studies. This generated a list of 299 papers, which were screened for relevance. We discarded review papers providing superficial descriptions of testing methods but retained technical and qualitative reviews to capture experience of both procedural facilitation and barriers to implementation. 199 papers were analyzed in detail, with the addition of an additional 22 after further examination of available literature. Review papers were included if they summarized important findings from earlier studies or drew independent conclusions from older primary papers. 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.

Characteristics of an Ideal Ancillary Test

Young concisely described the attributes of an ideal ancillary test (Table 1).[5] Given that the majority of the world's countries use a diagnosis founded on loss of function of the whole brain [3], there is no one test that satisfies all criteria. Each requires appraisal of individual strengths and weaknesses in their relationship to functional assessment of the brain. That appraisal will be further modified by consideration of the array of confounding conditions, presenting in individual circumstances.

Table 1. Characteristics of the ideal ancillary study in the diagnosis of BD/DNC

1.	There should be no "false positives", i.e., when the test confirms BD/DNC there should be none who recover or who have the potential to recover.
2.	The test should be sufficient on its own to establish that BD/DNC is or is not present.
3.	The test should not be susceptible to "confounders" such as drug effects or metabolic disturbances.
4.	The test should be standardized in technology, technique and classification of results.
5.	The test should be available, safe and readily applied. Testing capability should not be restricted to only a few tertiary academic centers; ideally it could be applied within any intensive care unit (ICU) and the technique should be reliable and mastered without difficulty.

Table 2 and 3 summarize various ancillary testing that can be used in the determination of death by neurologic criteria.

Table 2. Tests of brain blood flow

Test	Diagnostic Criteria	Advantages	Disadvantages	Sensitivity/ Specificity	Comments
Digital Subtraction Angiography (DSA) / Conventional 4 vessel angiography	Absence of contrast within the intracranial arterial vessels	• Gold standard for ancillary tests	• Requires transport to imaging suite • Invasive (requires technical skills) • Renal susceptibility to contrast • Stasis filling – false negative	100% / 100%* [6,7]	Persistence of flow does not contradict comprehensive competent clinical diagnosis; Equipment and operator dependence limits practical use – still used as calibration standard;
Radionuclide Angiography	Absence of radiologic activity upon imaging of the intracranial vault	• Can be performed at bedside • No renal susceptibility to contrast	• Limited evaluation of brainstem • Limited availability	98.5% / 56%[8]	Persistence of flow does not contradict comprehensive competent clinical diagnosis
Radionuclide Perfusion Scintigraphy	Absence of radiologic activity indicating metabolic uptake upon imaging of the intracranial vault	• Can be performed at bedside (planar imaging)	• Limited availability • Planar imaging may limit brain-stem evaluation • SPECT requires patient transport to scanner	Planar: 77.8% / 100% SPECT: 88.4% / 100%* [9]	Uptake of isotope indicates metabolic activity.
Transcranial Doppler Ultrasound (TCD)	Biphasic (oscillating) flow or small systolic spikes on initial assessment of intracranial arterial supply, confirmed or proceeding to absent flow velocity signal on second assessment	• Easily performed at bedside • No contrast required • Can assess carotid and basilar circulations	• Operator expertise required • 10% of patients have no acoustic windows	90% / 98%[10]	Persistence of flow does not contradict comprehensive competent clinical diagnosis

Test	Diagnostic Criteria	Advantages	Disadvantages	Sensitivity/ Specificity	Comments
Computed Tomography Angiography (CTA)	No opacification of intracranial arterial circulation, or deep veins	• Widely available • Relatively quick to perform	• Requires transport to imaging suite • Renal susceptibility to contrast • Stasis filling – false negative	52%-97% / 100%* [11-30]	Persistence of flow does not contradict comprehensive competent clinical diagnosis; Limited consensus on required diagnostic criteria; Small number of studies with lack of reference standard.
Magnetic Resonance Angiography (MRA)	No visualization of intracranial arterial circulation	• Not affected by stasis filling • Visualization improved by gadolinium	• Requires transport to imaging suite • Specialized critical care equipment required in scanner • Time of flight imaging affected by hematoma	93-100%[31-34] / 100%* [31,32]	Persistence of flow does not contradict comprehensive competent clinical diagnosis; Small number of studies with lack of reference standard; Uncertainty about risks of nephrogenic systemic fibrosis [35]

*Specificity is *assumed* on basis of experimental data but should be interpreted with caution[36] given the limitation of studies that reported only on clinically confirmed BD/DNC

Table 3. Tests of electrophysiological function

Test	Diagnostic Criteria	Advantages	Disadvantages	Sensitivity/ Specificity	Comments
Electro-encephalography (EEG)	• No detectable electrical activity (equal to or greater than 2 microvolts) over a 30-minute period	• Non-invasive • Can be performed at bedside	• Predominantly cortical assessment • Electromagnetic environmental noise can erroneously suggest cerebral electrical activity • Confounded by sedation, hypothermia, toxic states, metabolic disorders	53-80.4% / 97% [6,37]	Concerns on confounding and interobserver variability limit use - may be more specific used in conjunction with multimodality evoked potential testing
Somatosensory Evoked Potentials (SSEP)	• Bilateral absence of N20 waveform over sensory cortex (with preserved waveforms prior to P14) more than 6 hours after onset of coma	• Non-invasive • Can be performed at bedside • Less susceptible to sedation than EEG	• Confounded by cervical spinal cord injury, isolated brainstem lesions, sedation, hypothermia.	100% / 78%[38]	Limited specificity as isolated test – may be helpful as component of multimodality evoked potential testing, used in conjunction with EEG
Auditory Evoked Potentials (AEP)	• Bilateral absence of waveforms over auditory cortex beyond wave 1	• Non-invasive • Can be performed at bedside • Less susceptible to sedation than EEG	• Confounded by sedation, profound hypothermia, isolated 8th nerve or brainstem lesions • Limited to auditory cortex	NA / NA	Not useful as isolated test – may be helpful as component of multimodality testing
Visual Evoked Potentials (VEP)	• Bilateral absence of waveforms over visual cortex with preserved electroretinogram	• Non-invasive • Can be performed at bedside • Less susceptible to sedation or hypothermia than EEG	• Confounded by sedation, retinal or optic nerve lesions • Limited to visual cortex	NA / NA	Not useful as isolated test – may be helpful as component of multimodality evoked potential testing

Tests of Brain Blood Flow

Studies for brain blood flow testing are complicated by the varying definition of the reference standard for comparison. The absence of brain function by clinical determination is fundamentally based on the absence of brainstem function, and some ancillary tests may have limited ability to evaluate infratentorial pathology. A 2019 retrospective study found that either electroencephalography (EEG) or computed tomography angiography (CTA) confirmed the clinical determination 98% of the time when the pathology was supratentorial, but this decreased to 34% of the time in patients with primary infratentorial pathology[39].

Studies that use an ancillary test as the comparator may be limited by the detection of flow in patients that have no discernable clinical brain function. This is particularly relevant given the paucity of metabolic reserve within the brain, whereupon a cessation of function follows interruption of flow. If brain blood flow is arrested, and does not resume, the brain remains in an irreversible state of non-function. However, demonstrable flow does not mean that there is residual function – it simply inhibits the use of that particular test to support a diagnosis of death.

Blood flow may be reduced in the setting of decreased metabolic demand due to injury, sedation or hypothermia, but cannot be reduced below critical values (around 6 mls/100g/min) without inducing neuronal death.[40] Consequently, assessment of blood flow has a decisive role in confounding circumstances such as induced or accidental hypothermia, endocrine/metabolic disturbances or the presence of anesthetic agents.

Currently, digital subtraction angiography (DSA), radionuclide techniques and transcranial Doppler (TCD) are commonly incorporated into guidelines. However, other techniques that have not undergone substantial scrutiny have been incorporated into clinical practice including computed tomography angiography (CTA), and magnetic resonance angiography (MRA). Other methods, like CT perfusion (CTP), xenon CT (XeCT), MR spectroscopy (MRS), diffusion-weighted MRI (DWI), and functional magnetic resonance imaging (fMRI), are being studied as potentially useful in the diagnosis of BD/DNC.

Digital Subtraction Angiography (DSA)

Illustrated in Figure 1, DSA has historically been considered the reference standard of ancillary testing with 100% specificity and 100% sensitivity [6,7,41], although that latter assertion may be challenged in view of preserved brain blood flow in a number of patients fulfilling clinical criteria.[42-45] DSA eased the technical difficulties associated with conventional selective angiography, and established reliability early on. [41,46] However, the requirements for procedural skills, time and transport to the angiography suite as well as the risks of thrombosis, vasospasm and contrast nephropathy led to recognition of the need for quicker, less invasive and/or portable methods.[6,47] Despite this, angiography continues to be used as a reference standard.[19,48,49] It has also provided crucial insights into the phenomenon of circulatory arrest of the brain [41], furnishing initial evidence that observation of proximal arterial filling did not progress to venous opacification. That arrest of contrast passage has subsequently been corroborated as a lack of effective perfusion [7,45], and illustrates the importance of appropriate timing in the selection of image capture. Mitigation of intracranial tamponade does occur as a consequence of decompressive craniectomy, significant skull fracture, placement of external ventricular drains or expandability of the infant skull, and this may limit subsequent ability to diagnose BD/DNC. [44]

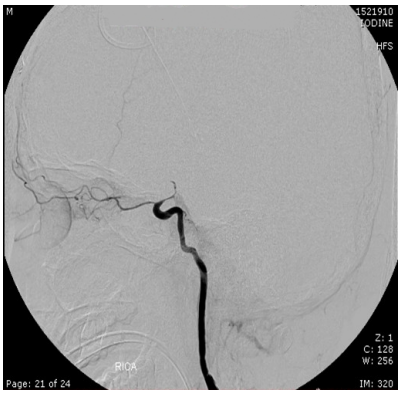


Figure 1. Selective internal carotid angiogram of arrested intracranial blood flow using digital subtraction angiography[50]

Radionuclide Techniques

Nuclear medicine provided the opportunity to administer tracer compounds that would signal their passage by radiological emission through the circulation of interest. Technetium 99 compounds can be divided into lipophobic and lipophilic formulations.[51] Lipophobic agents such as Tc-99m pertechnetate, Tc-99m pentetic acid (DTPA) and Tc-99m gluceptate have been used for radionuclide angiography – their movement through the brain's circulation being detected by collimated detectors. They remain in the vasculature and do not penetrate into brain tissue, and as such may suggest flow in the absence of metabolic activity.[52] The sagittal sinus may also be weakly perfused by leptomeningeal circulation, confusing assessment of intracranial flow.[51] An additional major limitation is the inadequate identification of blood flow through the posterior fossa, which fails to meet the whole brain standard [53-55] and has resulted in false positive results.[8,56]

These issues can be resolved with the use of lipophilic compounds. Tc-99m hexamethyl-propyleneamineoxime (HMPAO; exametazime) and Tc-99m N, N'-1,2-ethylenediylbis-L-cysteine diethyl ester (ECD) are the two common radiopharmaceuticals used for this purpose, listed in order of popularity of use. Both agents diffuse across the lipophilic blood-brain barrier and are then metabolized to lipophobic compounds and retained within the brain.[54,55] This active process is, as such, an indicator of metabolic uptake within the brain, illustrated by appropriately timed (delayed) images, and the technique is often termed radionuclide perfusion scintigraphy in contrast to RCA.

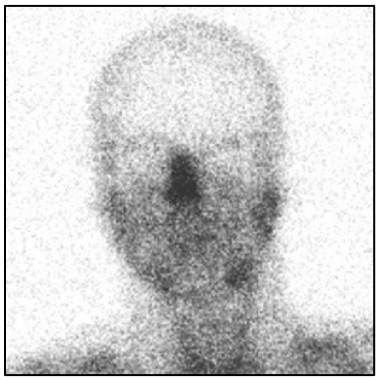


Figure 2. Radioisotope scan showing 'empty skull' and 'hot nose' signs[57]

It has consequently proven a more popular alternative to DSA, with comparable accuracy [58,59], but allowing faster diagnosis than DSA [60], and an improved, though still limited, resolution of the brainstem in comparison to lipophobic agents.[8,61-63] The retention could potentially serve as a confounder if repeated examinations are required, and 24-48 hour intervals between evaluations are suggested.[64] An absence of flow is apparent as an 'empty skull' or 'light-bulb' sign.[64,65] Diversion of blood flow from the intracranial circulation results in increased density of isotope around the pharyngeal venous plexus – the so called 'hot-nose' sign (see Figure 2) – but this is actually not specific to BD/DNC.[66]

There is some disagreement between studies on the utility of tomographic processing of HMPAO images (Single Photon Emission Computed Tomography - SPECT). Some studies claim better resolution of the posterior fossa [67-69], while others argue a lack of definite proof for that assertion.[44,70,71] SPECT findings correlate well with anatomical damage seen on CT imaging.[72] Critics point out that using SPECT mandates transport of potentially unstable patients to the nuclear medicine department, and portable gamma cameras with bedside injection of Tc-99m may present an attractive option for patients who are unsafe to travel, although positioning mobile cameras for both anterior and lateral planar images can present problems in the ventilated critical care patient.[71,73] Of note, the false positive rate of planar imaging in non-BD/DNC patients is unknown, as studies have been limited to patients who had BD/DNC confirmed by other means.[74]

There is a growing body of opinion favoring the use of SPECT [9,59,64,75], leading to an increasing appearance within national guidelines.[65] The improved image resolution has been used to successfully promote understanding of the diagnosis to families.[76] SPECT has been proposed [59] and used as a reference standard [77], illustrating concerns on the accuracy of EEG.[78] There remains some debate on its accuracy in infants and the newborn.[64,69,79]

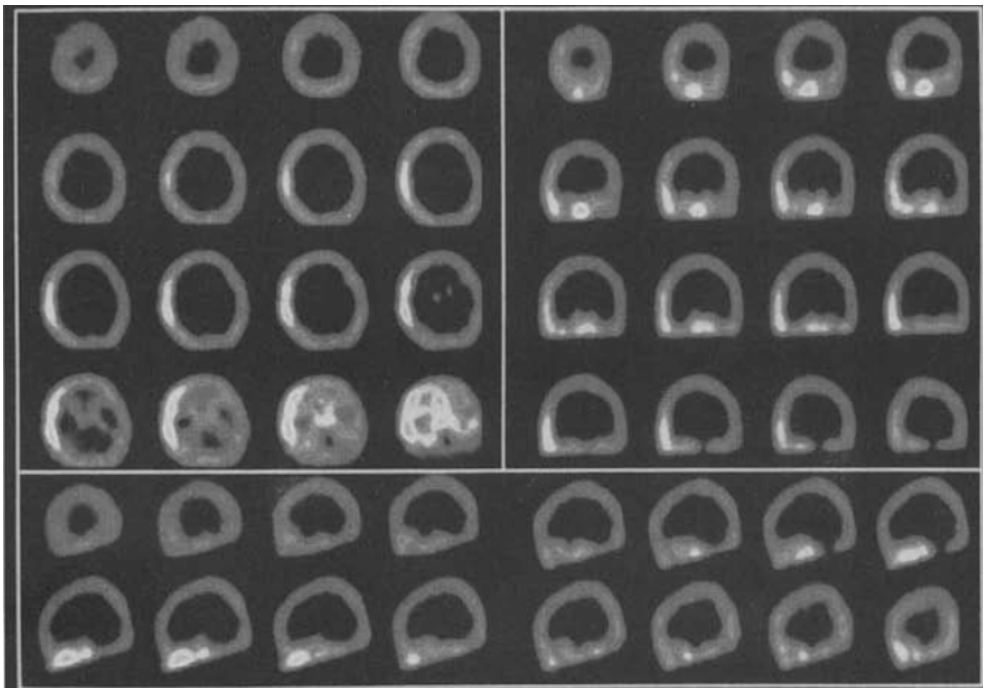


Figure 3. SPECT scan in brain death confirming lack of intracranial uptake of any tracers [34]

Computed Tomography Angiography (CTA)

CTA has generated a great deal of interest by offering the possibility of quickly and easily verifying arrest of the brain's circulation. It is technically fast to perform, but still, at present, requires transport to the radiology suite. Dupas, in 1998, provided one of the first methodologies for interpretation of a CTA image to either support or refute BD/DNC.[15] In a series of 14 persons diagnosed as brain dead on the basis of clinical findings and EEG corroboration, he identified a lack of opacification in the pericallosal and terminal arteries of the cortex as well as internal cerebral veins, the great cerebral vein, and the straight sinus – identified at two time points after injection of contrast. This assessment further required the use of superficial temporal artery opacification as a quality indicator of extracranial perfusion. The authors claimed 100% sensitivity for declaration of death. Several countries (including France) have subsequently adopted CTA as a valid ancillary test, although the United States has not. There have been multiple case reports and small series attesting to an improved ability to resolve situations where diagnosis was confounded.[19,77,80-89] Contrast induced renal toxicity does not appear to be a frequent problem.[19,20,23,77] As with other flow assessment techniques, there may be limitations in BD/DNC confirmation in subjects with large craniectomy.[90,91] A number of studies have also manifested concerns for accuracy [11,16,17], with considerable variation on reported sensitivity (listed in Table 2) and one report of false positive results.[92] That has provoked significant debate over the methodology employed.

One of the main problems is the appearance of 'stasis filling' – contrast is thought to flow to a point where intracranial impedance halts progression, but subsequent permeation occurs into the distal vessel, leading to opacification in the absence of meaningful forward flow or perfusion.[15,93] This phenomenon was observed in DSA originally [45,94], and is characterized by a shorter intracranial length of vascular contrast filling and is increased with time since contrast injection.

The originally proposed 'two phase' protocol established a baseline CT, and then took images 20 seconds after contrast injection to identify the first phase of arterial filling. The subsequent venous phase required image acquisition starting around 55-60 seconds after contrast.[15] Studies have subsequently varied in their adherence to this timing, with some actually advocating for ignoring the venous phase, judging it irrelevant [12,23,24,77], or advancing it to 40 seconds after contrast.[12] At the opposite extreme, Leclerc suggested concentrating exclusively on the venous phase [21], while, more recently, others have advocated a return to dynamic assessment.[83] Alternatively, Suarez-Kelly suggested an interesting concept in defining a threshold of Hounsfield units by which to judge the filling indicative of meaningful arterial perfusion. This improved sensitivity to 97% and specificity to 100% in what remains a single study.[95]

Equally significant problems in heterogeneity have arisen when considering the methodology on actual interpretation of the acquired images – problems confirmed on the systematic reviews conducted to date.[96-98] Driven by the contribution of intracranial arterial segmental length to the possibility of stasis filling, different researchers have attempted to resolve the confounder by selectively disregarding certain vessels associated with greater frequency of opacification in the proximal arterial tree. Opacification of the superior sagittal sinus has long been interpreted as a consequence of circulation through the meningeal vessels or by bridging veins.[11,15] Consequently, there have been varying opinions on which other vessels to use when looking for an absence of opacification that may most closely define cerebral circulatory arrest (see Figure 4).

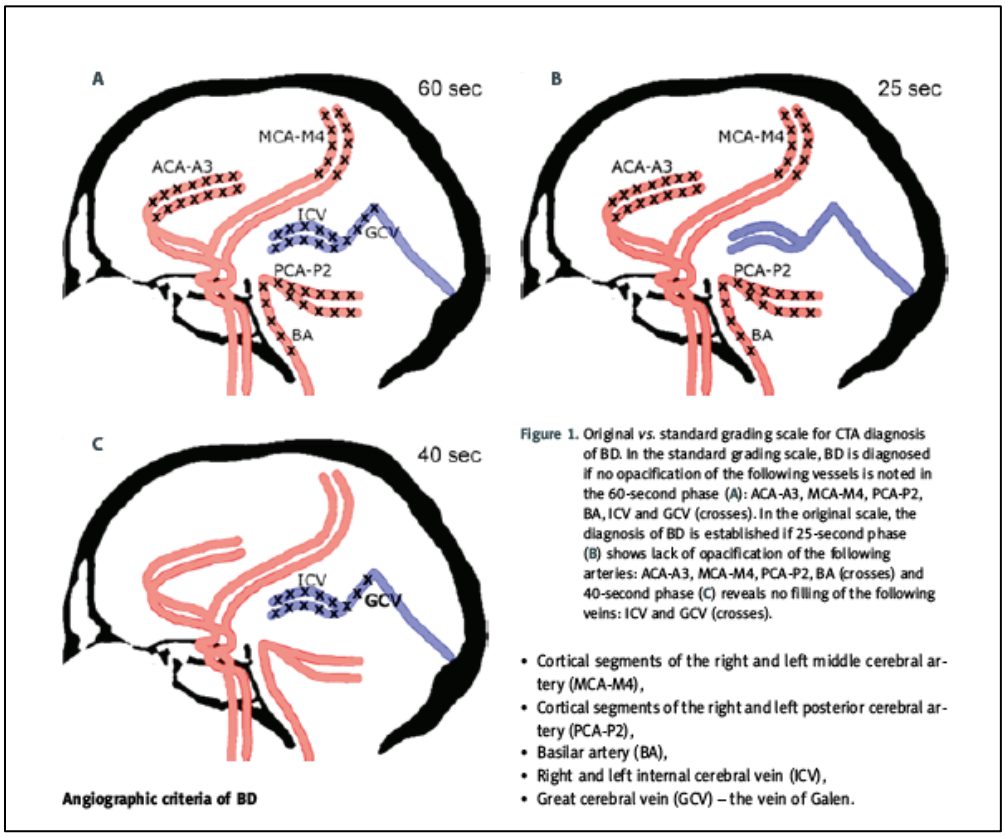


Figure 4. Example of variation in methods of assessing brain blood flow on CTA, depending on choice of anatomical vasculature and time of imaging [13]

Table 4 illustrates the array of scoring systems used to differentiate opacification as a result of genuine flow from that of stasis filling, in the definition of circulatory arrest. Dupas proposed the original 'seven point score'.[15] Combes found a lower sensitivity than Dupas (at 69.7%), but considered this a consequence of inadequate examination detail of the brain's circulation, and included the posterior arterial circulation to produce a 'ten point score'.[11] Leclerc initiated avoidance of anterior vessels due to a high frequency of stasis filling, concentrating instead on the middle cerebral arteries and internal cerebral veins.[21] This approach was subsequently confirmed by Frampas and popularized as the 'four point score'.[20] Other variants have arisen and are listed in the table, e.g. the predominant score used in a number of German papers returned to comprehensive assessment of all arteries , but excluded venous assessment [23-27]; researchers in Poland suggested the re-addition of anterior and posterior circulation assessments to the 4 point scale [22]; most recently Marchand in France suggested the replacement of supratentorial venous assessment by infratentorial venous assessment, using the superior petrosal vein.[28]

According to a recent meta-analysis, preservation of supratentorial blood flow with CT angiography evidence of filling of the cortical branches of the middle cerebral artery, the internal cerebral veins, or both, is seen in 15%–16% of all patients diagnosed clinically as BD/DNC, not solely those who have isolated brainstem lesions.[99] The relevance of these results are debatable because there is still no consensus on the technical criteria for CT angiography as anxiliary test and considerable variation on reported sensitivity in the diagnosis of BD/DNC.

The conclusion to be drawn from this somewhat confusing range of disparities is that while CTA obviously possesses considerable potential for use as a rapid ancillary test in BD/DNC, it requires further consensus on the phases and timing of image acquisition, as well as consensus upon and validation of the interpretation criteria subsequently used. It also requires validation in comparison to "gold standard" BD/DNC cerebral perfusion tests (SPECT, conventional angiography). This is in alignment with the conclusions of the available systematic reviews.[96-98]

Table 4. Radiological scores for determination of circulatory arrest using CT angiography

Vessel	10-point scale	7-point scale	4-point scale	Polish scale	German scale	'Revised' 4-point scale
ACA-A3 (bilaterally)	2	2		2	2	
MCA-M4 (bilaterally)	2	2	2	2	2	2
PCA- P2 (bilaterally)	2				2	
BA	1				1	
VA (bilaterally)				2		
ICV (bilaterally)	2	2	2	2		
GCV	1	1				
SPV						2
Possible total	10	7	4	8	7	4
(Range of) Reported Sensitivities	52	52-84	75-96.3	41.7	93-97	95
Studies Using Score	11-14	13-18	13,14,17-21,29	22	23-27	28

R, right; L, left; ACA, anterior cerebral artery; A3, 3rd division of ACA; MCA, middle cerebral artery; M4, 4th division of MCA; PCA, posterior cerebral artery; P2, 2nd division of PCA; BA, basilar artery; VA, vertebral artery, ICV, internal cerebral vein; GCV, great cerebral vein; SPV, superior petrosal vein. One point is given for non-opacification of each vessel.

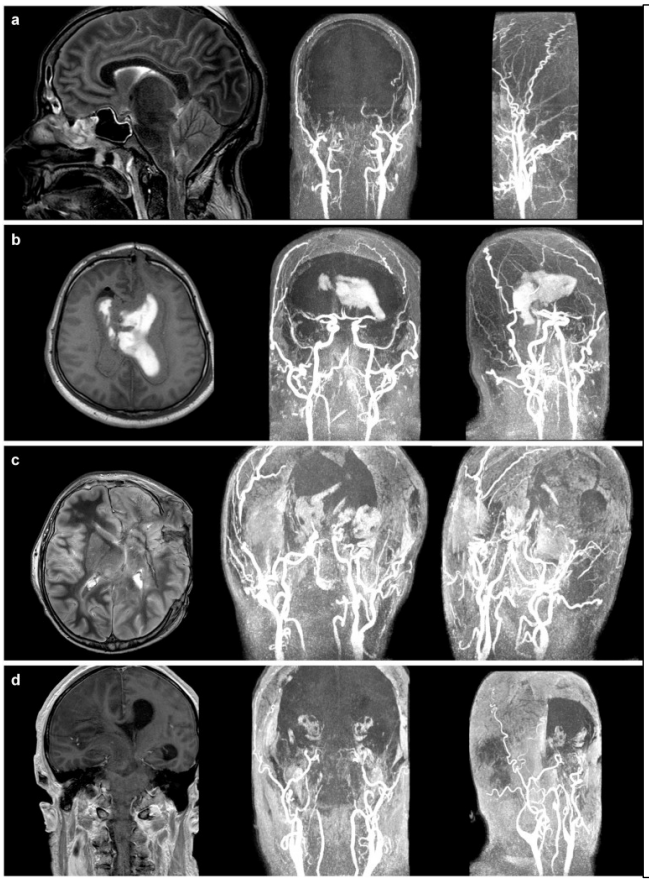
Magnetic Resonance Imaging (MRI) and Angiography (MRA)

The technology of magnetic resonance imaging has excited interest in its potential to assist in the declaration of BD/DNC.[34,100-105] It allows anatomical imaging (MRI) as well as examination of blood flow – the latter ability developed as a consequence of investigation into, and compensation for, flow-based artifact, and corresponding development of angiography sequences. These are time-of-flight and phase contrast imaging. If flow decreases, there will be less difference in the signal intensities between static tissue and moving blood. For that reason, time-of-flight imaging is less reliable in low perfusion states.[33,104,106-108] A contrast agent may also be administered to shorten T1 times and provide delineation of perfused vasculature [106], and appears to be more resistant to time-of-flight errors.[33,100] A 'hot nose' sign may also be apparent.[101,108]

However, similar to DSA and CTA, MRA (whether with contrast or not) requires the identification of flow in the external carotid artery branches in order to clearly demonstrate that the lack of cerebral flow (in the internal carotid and basilar arteries) is pathological as opposed to artifactual.[33,107] As with all other forms of flow-based assessment to date, it appears susceptible to reductions in craniovascular impedance, in registering flow despite clinical and electrophysiological signs of death.[33]

Increased water, as signaled by change of apparent diffusion coefficient (ADC), has been suggested as a marker of BD/DNC.[109] Consequently, susceptibility-weighted imaging (SWI) and diffusion-weighted imaging (DWI) have been investigated for use as diagnostic modalities.[110] Unfortunately, they remain nonspecific markers of neurological pathology and may indicate pathologies other than BD/DNC.[32,107,111] Anatomical changes of herniation and grey/white differentiation may be suggestive [31,32,34,101-104] – whether these constitute irreversibility (and if so, after how long) is uncertain. Absence of intracranial arterial flow with identification of the extracranial carotid vessels is consistent with BD/DNC [31,32], but requires further validation across a wider range of persons.

The key barrier to implementation of this modality is the logistical challenges presented by transport into the unforgiving environment of the MR scanner, mandating compatible equipment.[108] Patients in critical care setting usually present with several known and more often unknown contraindications for MR scanners. Capital costs and time overheads also aggravate problems of use.[33] Uncertainty about risks of nephrogenic systemic fibrosis in potential survivors poses another challenge for gadolinium-enhanced MRA.[112]



a) T2-weighted sagittal image showing tonsilar herniation due to global brain edema. Gadolinium-enhanced MR angiography demonstrated no arterial blood flow in the intracranial circulation.

b) T1-weighted axial image showing diffuse gyral swelling, intraventricular hemorrhage and a fracture of the anterior skull resulting from severe traumatic head injury. Gadolinium-enhanced MRA illustrated no intracranial blood above the level of the proximal A2-segment of the ACA and the M2-segment of the MCA.

c) T2-weighted axial image depicts left-sided unilateral decompressive craniectomy showing midline shift after massive hemispheric infarction due to vasospasm resulting from a subarachnoid hemorrhage. Gadolinium-enhanced MRA showed no evidence for brain blood flow while extracranial vessels exhibit normal circulation.

d) The coronal T1-weighted image shows decompression after a hemorrhagic stroke affecting the right hemisphere. Gadolinium-enhanced MRA confirmed the absence intracerebral arterial blood flow.

Figure 5. Contrast enhanced MRI/MRA of two patients diagnosed with clinical brain death
(Adapted from Luchtmann et al, 2014)[33]

Transcranial Doppler (TCD)

TCD allows measurement of brain blood flow velocity, and, as such, has been used as an ancillary investigation in the diagnosis of BD/DNC for over two decades. It is easily portable to the bedside, and relatively inexpensive in terms of equipment outlay. It has established credibility in the assessment of blood flow through basal arteries. Although classically TCD would not, by definition, capture posterior fossa flow, extension to include insonation of the vertebral and basilar arteries will provide insight into brainstem perfusion, and should be performed for assessment of whole BD/DNC.[113] However that practice has not been universal [114] and has led to variable reports on sensitivity and specificity.[6,115-117]

TCD can be used to examine dynamic changes in circulation, and has been used to qualify other investigational tests [23,25-27,118,119], as well as providing confirmation of cerebral circulatory arrest (CCA) in indeterminate circumstances.[90,120] Routine use in a critical care unit can provide early identification of CCA and provoke appropriate diagnostic examination.[121]

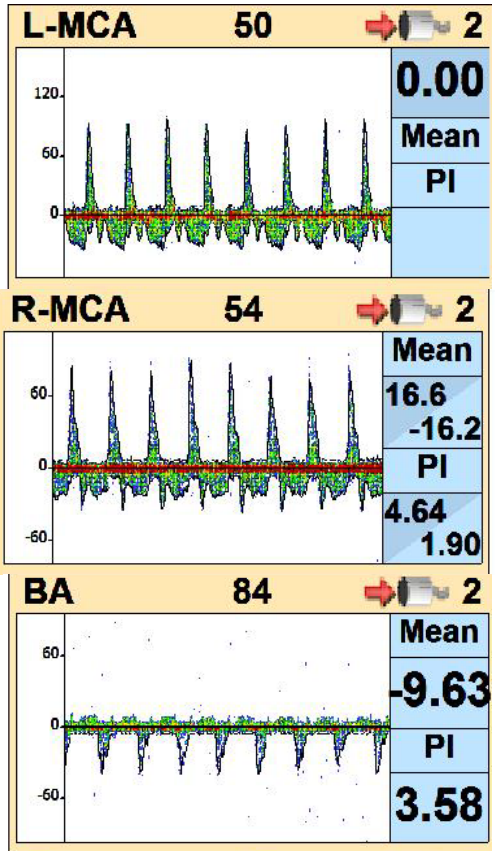


Figure 6a. Biphasic (oscillating) flow velocities on TCD consistent with intracranial circulatory arrest (M. Souter, personal files)

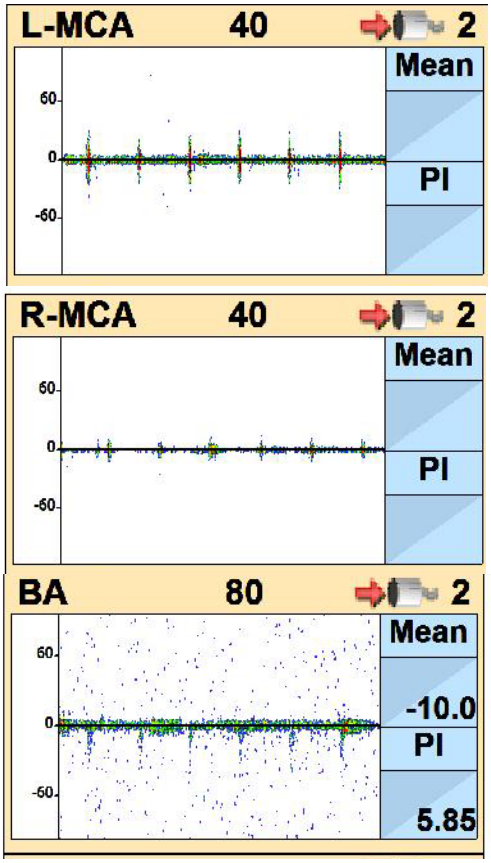


Figure 6b. Isolated systolic spikes consistent with intracranial circulatory arrest (M. Souter, personal files)

The patterns of CCA (see Figure 6) have been described by Ducroq [113], and Hassler.[122] Those studies established some correlation between patterns of Doppler signal spectra and the level of CCA, with oscillating or to-and-fro appearance indicating terminal carotid artery obstruction; systolic spikes indicating infraclinoid carotid obstruction.[113] Similarly, while supratentorial pathology presents with mixed patterns and CCA occurs synchronously with clinical signs, infratentorial pathology presents with systolic spikes, and clinical signs precede CCA in the posterior fossa.[123]

If an initial examination reveals no waveform, it is then impossible to know whether this is BD/DNC or sonic bone impermeability given that up to 10% of the population do not have adequate temporal bone windows [113,124], more so in women than men.[116] Circulatory arrest can thus only be established in the presence of some preceding signal on earlier examination that indicated flow, establishing the presence of an adequate window. Consequently, two exams are normally required to make a diagnosis of CCA. Use of transorbital and transcervical techniques may be useful in the absence of temporal windows [124,125]; however, there has been concern for carotid siphon flow detected via the transorbital route that is inconsistent with CCA patterns seen in the other intracranial arteries.[126] Some authorities have suggested explicitly avoiding insonation of the internal carotid artery unless there are no transtemporal windows.[116] Two studies have used an ultrasonic contrast agent to improve arterial insonation [127,128], and recent Spanish guidelines suggest it as a possible step.[129] One preliminary study has used the same contrast agent for ultrasound perfusion assessment.[130] Similarly, the use of color duplex techniques has been suggested to improve detection of cerebral circulatory arrest, but this still remains speculative at present.[48,119] Measurement of CBF volume by duplex sonography of the cerebral vessels has also suggested some discriminative prediction, in a single study that has not been duplicated.[131]

The main barriers to the use of TCD are thus (i) inadequate acoustic bone windows, along with (ii) any decompressive craniectomy that reduces cerebrovascular impedance to blood flow, which normally accompanies the tissue swelling associated with BD/DNC.[84,113,116,132-140]

One case report noted perfusion to the skull base was enough to indicate forward flow in the vertebral artery, in the absence of cerebral perfusion on angiogram.[141] Unexpected flow in persons satisfying all clinical criteria of BD/DNC, and without a decompressive mechanism, may indicate early stages in the irreversible escalation of craniovascular impedance.[133,142] Repeated serial examination may improve detection of CCA [124,143], illustrating a relationship between BD/DNC, the time course of developing intracranial vascular impedance, and TCD findings.[144] Cervical color doppler has recently demonstrated an 80% sensitivity, and may be a useful test in combination with TCD in the future[145].

While individual case reports have described diagnostic failure, a 2006 meta-analysis of 10 qualifying studies demonstrated an overall sensitivity of 89% and specificity of 99%. A number of those studies reviewed did not assess infratentorial circulation. This improved to 95% and 99% when limiting consideration to those studies deemed as high quality.[146] CTA or radionuclide angiography may be faster examinations, given the requirement for two TCD exams separated by at least 30 minutes, but TCD as a bedside evaluation is usually more time efficient than DSA or SPECT given the need to transport persons. The time required to establish a diagnosis of CCA may actually be less than other modalities.[29,137,147,148] There remains however, the question of operator dependence and thus a need for appropriately trained personnel to perform examinations, which may limit accessibility at certain times.[148]

Other Flow Studies

Near infrared spectroscopy (NIRS) has garnered some interest recently for potential as an ancillary test[149], but this is unlikely to be of use as a sole method given its inability to assess the deeper brain structures, including the brainstem. Some of the newer methods have included the use of optical contrast agents. Some have urged caution for NIRS for false negative results (suggesting flow when other ancillary studies have shown no flow)[150]. Cerebral oximetry values have been shown to be lower in BD/DNC patients, but this test should not be used as an ancillary test[151].

Tests of Electrophysiological Function

The generation and/or conduction of electrophysiological potentials within the brain parenchyma are energy dependent processes, and consequently indicate active metabolism. Electrophysiological testing evaluates brain regions or tracts but is currently limited by the inability to evaluate the entire cerebrum and brainstem. However, the requisite amplification and refinement of the small voltages involved result in the problems of excessive noise and external signal contamination.

Electrophysiological testing was the first modality beyond clinical examination that was used to investigate the brain, well in advance of current flow-based and imaging technologies. Given that the generation of biological electrical potentials is a metabolically dependent process, there is some support for the concept of electrophysiological methods demonstrating presence or absence of function of the brain. Similar to blood flow testing with limitations in posterior fossa visualization, the challenge lies in whether the developed modalities are sufficiently sensitive and specific across the entire brain, to the degree required to support (or even substitute for parts of) the clinical diagnosis of BD/DNC.

Electroencephalography (EEG)

EEG (see Figure 7) has an established record in providing ancillary data to support or refute a diagnosis of BD/DNC. It is an inherent and mandatory part of many existing practice guidelines, and, as such, has been used extensively in the prior definition of BD/DNC investigations. [15,23,25-27,64,67,79,84,111,148,152-154] It has good sensitivity, as the presence of electrical activity can be considered an indicator of metabolism and blood flow, but this is restricted to the cerebral cortex and does not encompass the deep cerebrum or brainstem.[78,132,152,155-157] There are also significant problems with contamination errors from electrocardiogram activity, as well as that of electrically noisy environments.[156,158] Unfortunately, there are significant limitations on specificity, as many conditions can give rise to electrical silence of the cortex independent of BD/DNC such as sedation, hypothermia, encephalitis, and metabolic disorders.[155-157] There are also technical specifications, such as the requirement of repeated recording of calibration signals and electrode impedences during on ongoing EEG recording, which are not met by all EEG systems[159]. Additionally, inter-rater reliability is limited.[160] Some have argued that an iso-electric EEG remains sufficiently specific to refute persistent flow [161], but this is difficult to support.

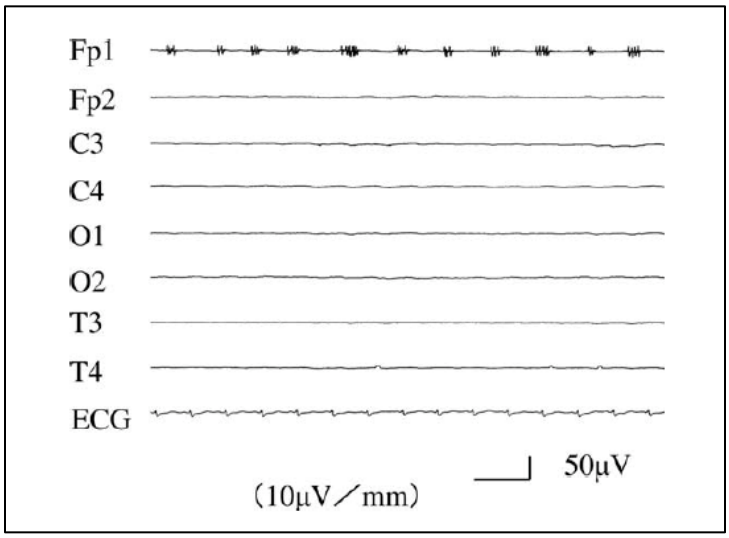


Figure 7. Scalp EEGs and ECG in a brain dead patient (From Okii et al, 2009)[162]

Conversely to its limitations in identifying brainstem pathology, it has proven invaluable in circumstances of spinal cord/brainstem lesions where cerebral activity may still be extant or the pathology is reversible.[163-168] Minimum technical guidelines for the performance of an EEG in BD/DNC diagnosis have been outlined by a number of authors, including Drazkowski [169] and Bleck [170].

Evoked Potential (EP) Monitoring

Data furnished by EEG can be refined by the use of corroborating evoked potential monitoring [38,117,128,155,171] which have been considered an extension of the clinical exam, in that they assess functional conduction of electrical stimuli through brainstem and basal ganglia pathways to cortical processing.[152,172,173] As such, these give insight into the integrity of those areas. They are more resistant to suppression by temperature, sedative agents and metabolic disturbances [152,155], but are compromised by peripheral sensory dysfunction e.g. blindness, deafness, spinal cord injury or isolated brainstem injury.[156] They require demonstration of adequate peripheral sensory transmission, e.g. activity on electroretinogram for visual evoked potentials (VEP), presence of Wave I for auditory evoked potentials (AEP) (see Figure 8.a) and waves prior to P14 on somatosensory evoked potentials (SSEP) [156,172,174] (see Figure 8.b). The combination of EEG and EP can be helpful in the circumstance of persistent flow on TCD or DSA where reduced craniovascular impedance consequent to skull decompression may be playing a confounding role.[64,84,117,133,136,137,140,154]

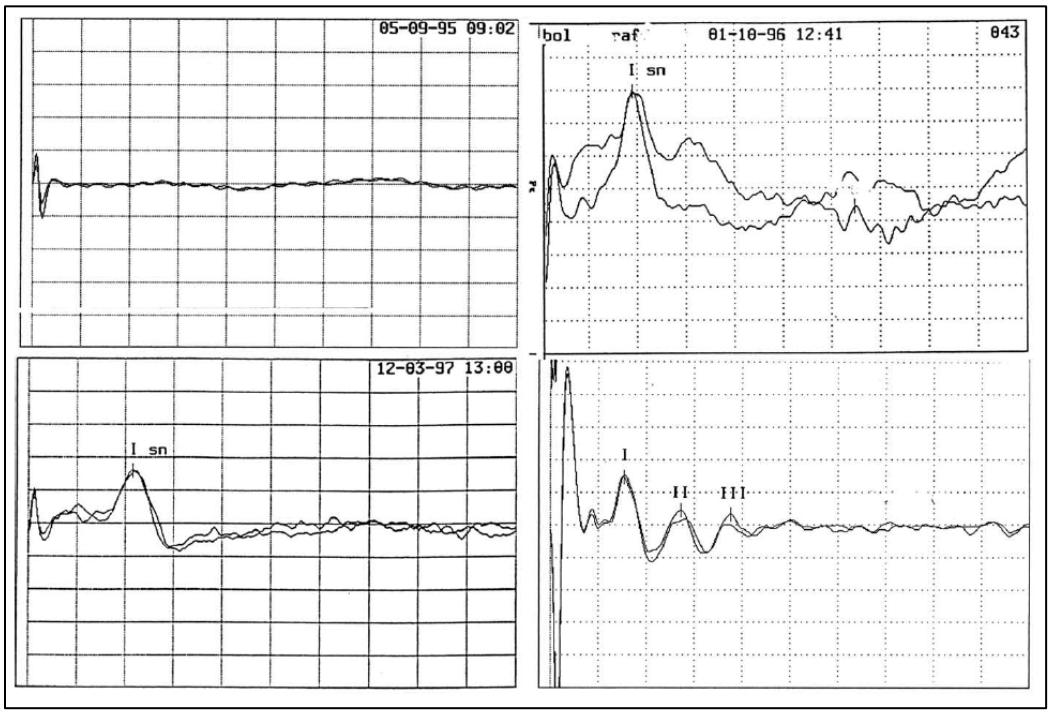


Figure 8a. AEPs in brain dead patients (From Facco et al, 2002)[152]

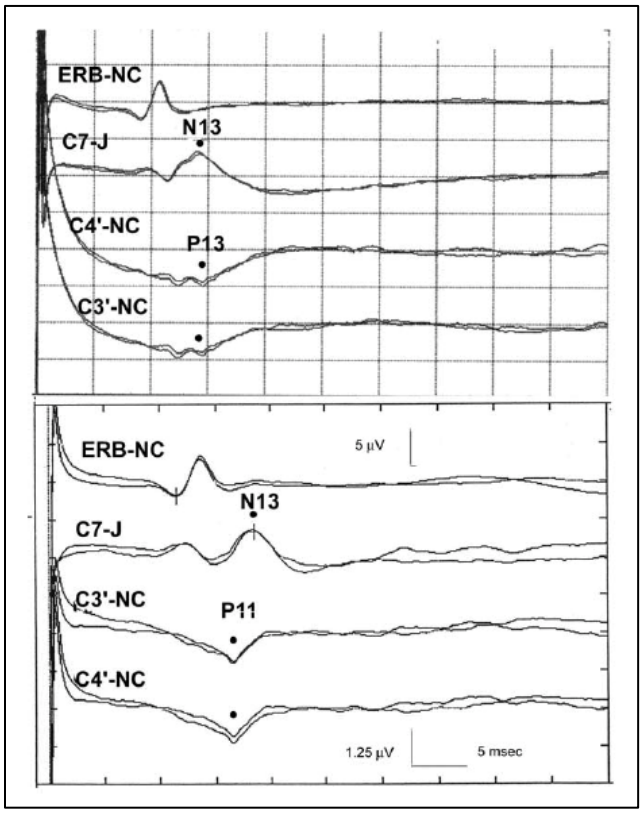


Figure 8b. SSEPs in brain dead patients (From Facco et al, 2002)[152]

EEG and SSEP have proven inadequate to detect brainstem function in one case report [85], and some have speculated that there should be a 'battery' of electrophysiological testing to be truly sensitive to retained function.[174-176] Some authors have proposed that the apnea exam be the *final* part of diagnostic testing, preceded by AEP/SSEP monitoring. This would avoid hypercarbic secondary insults to those still deemed alive by functional neurophysiological conduction.[152,155] Both EEG and EP testing offer bedside testing, and do not require investment in major infrastructure, but do require skilled recording and interpretation.[175,177] The management of cases where EEG and EP offer conflicting results may be difficult to resolve.[178]

Experimental Approaches and Technologies

Given our imperfect understanding of the functions of the brain, and limitations on methods of assessment, we can expect continued reports on the results of new approaches and technologies, aimed at improving confidence in the diagnosis of BD/DNC. These will most probably be based upon elaboration of existing means and methods, but common to all research moving forward is the need to standardize methods of testing and interpretation as early as possible, in order to avoid inefficiencies in knowledge discovery.

- Ultrasound of the eye has been considered a 'ready-made' window into the cerebral circulation, given the intracranial origin of the ophthalmic artery. Consequently, ultrasound assessment of the vessel resistive index (expected to increase with elevated craniovascular compliance) was measured and shown to be different from that of indices derived from transtemporal ultrasound routes. There was risk of bias in that no controls were studied, and concerns for accuracy given that assessment was restricted to the anterior circulation.[179,180]
- Electrical impedance (as distinguished from vascular impedance) of the head has also been measured in a comparison of live and brain-dead persons, but the degree of variability compromised distinction between groups and this test is not currently helpful.[181] A comparison of resting energy expenditure between brain-dead and live persons demonstrated significant differences, but this study has not been replicated and presents logistical challenges in execution outside the research environment.[118]
- CT Perfusion is an interesting accompaniment to CTA, as it offers the ability to distinguish flow opacification from stasis filling.[18,22,84,93,96] CTP is very sensitive in detecting blood flow and can detect decreased perfusion as low as 2-3% in BF and 2% in CBV[182]. CBF < 5 mL/100g/min is consistent with clinical diagnosis of brain death in nuclear scintigraphy studies.[183,184] Similar to the comparison of scintigraphic arteriography and perfusion, it may offer more insights into meaningful capillary level perfusion rather than large vessel opacification in CTA.[18,30,185] Larger coverage on the newer generation of CT scanner allows for evaluation of whole brain using CT perfusion. It has required larger doses of contrast, as well as more processing time [93,96], although different protocols may reduce contrast volume.[18] Considered singly, it may have a lower sensitivity to CCA than CTA [96], although the combination compares effectively.[81,84,93] Like other flow-based techniques, it remains vulnerable to signals of persistent flow where craniovascular impedance is mechanically reduced.[84,93] Perhaps most promise lies in methodology where images acquired for CT perfusion can be reconstructed to provide CTA imaging – thus effectively

reducing time interval and contrast dose.[18] Differential loss of perfusion has been reported on CT perfusion as isolated brainstem death. This remains debatable.[30,186] A large prospective study is currently underway to assess the role of CT perfusion in assessment of BD/DNC (ClinicalTrials.gov: NCT03098511).

- While transcranial magnetic stimulation has been used to produce motor evoked potentials (MEP) in brain dead persons, it does not possess great specificity, given the vulnerability of motor cortex to other conditions.[187] SSEPs are already well developed diagnostic tools and MEPs do not add any advantages for monitoring the brain as a whole.[187]
- The summation of brain blood flow volume is possible by color duplex flowmetry of the extracranial components of internal carotid and vertebral arteries. While there appears to be a reliable threshold of less than 100 mls/minute in distinguishing BD/DNC, the technique is described as technically demanding and this has perhaps led to no further development.[131]
- Bispectral index monitoring (BIS) is essentially a simplified EEG, which is easy to apply and has been used in conjunction with monitoring sedative depth. Given its dependence on EEG frequencies, it is not surprising that monitored values decline in BD/DNC.[188-190] It is, however, prone to electromyographic contamination [188,190], and displays a lack of specificity for BD/DNC, as may be expected.[188,189] If used in an ICU environment, it may serve as an early indicator of a need for further BD/DNC testing.[189,190]
- Brain tissue oximetry ($PbtO_2$) has been monitored with the onset of BD/DNC and drops to zero at that time. It has not been compared to live controls, nor for acute diagnosis in a previously unmonitored person. It offers very focal observations and may not distinguish BD/DNC from local infarction.[191]

Recommendations and Suggestions

There are no recorded instances in the literature of a recovery of neurologic function after a correctly performed neurological examination diagnosing BD/DNC [192] (nothwithstanding the potential for misinterpretation of complex spinal-mediated movements as brain-based motor function [193]). While clinical examination may precede pathophysiological evidence on imaging, flow assessment or electrophysiological testing, conflicts have predominantly arisen from the *timing* of the diagnosis, rather than the diagnosis itself. Facco made the following observation in 2002, which is still pertinent today.

> "As a matter of fact, no investigation is absolutely superior and is to be used exclusively in all cases: the pathophysiology of BD/DNC is complex and skilled physicians are to apply the proper diagnostic tools to achieve the absolute certainty of diagnosis in each case." [152]

Making an appropriate choice of ancillary test depends on the clinician and the clinical circumstances. The most effective method to 'see past' a confounder must be identified using an educated appraisal of the strengths and weaknesses of the array of ancillary tests available.[52,194] The clinical exam may still be considered the most sophisticated way of testing neurologic function in that it deliberately delivers a stimulus to provoke central processing and an efferent response. All ancillary testing infers the integrity of that stimulus-integration-response arc, but does not observe it directly. Having said that, there are

clearly a number of situations which can compromise clinical examination for BD/DNC, and recommendations will be made based on confounding conditions.

1. It is recommended that ancillary testing is required in the following circumstances:
    a. Inability to complete all aspects of the minimum clinical examination, including the apnea test,
    b. Confounding conditions that cannot be resolved,
    c. Uncertainty regarding interpretation of spinal-mediated motor reflexes.
2. It is recommended that the clinical examination be completed to the fullest extent possible prior to conducting an ancillary test.
3. It is suggested that ancillary testing may be used to promote understanding of the clinical determination to families who express resistance or uncertainty.
4. It is recommended that the following brain blood flow-based methods be used for BD/DNC ancillary testing:

    a. *Digital subtraction angiography (conventional 4 vessel cerebral angiography)*. It is recommended that if 4 vessel cerebral angiography is performed, the study illustrates absent filling at the points where the internal carotid and vertebral arteries enter the skull base, with patent external carotid circulation in order to make a declaration of BD/DNC.

    b. *Radionuclide studies.* It is suggested that if scintigraphic techniques are used as an alternative to digital subtraction angiography:
        i. Diffusible radiopharmaceuticals be used preferentially,
        ii. SPECT be chosen over planar imaging,
        iii. Perfusion scintigraphy with anterior and lateral planar imaging be used, with appropriate time intervals to demonstrate static filling of the posterior fossa, if SPECT is not available,
        iv. The study illustrates absence of intracranial isotope in order to make a declaration of BD/DNC.

    c. *Transcranial Doppler.* It is suggested that if transcranial Doppler are used as an alternative to conventional 4 vessel cerebral angiography or scintigraphy:
        i. Two exams be performed ≥ 30 minutes apart,
            - 10% of patients have no acoustic windows. Circulatory arrest can only be established in the presence of some preceding signal on earlier examination that indicated flow, establishing the presence of an adequate window. Consequently, two exams are normally required to make a diagnosis of cerebral circulatory arrest with TCD.
        ii. The exams be performed bilaterally, anteriorly and posteriorly to include both internal carotid arteries as well as the vertebrobasilar circulation,
        iii. The exams illustrate biphasic oscillating flow and systolic spikes with reversal of flow in diastole in order to make a declaration of BD/DNC,
        iv. TCD should not be used for pediatrics in the absence of validation studies.

5. It is recommended that when ancillary testing is performed and demonstrates the presence of brain blood flow, BD/DNC cannot be declared at that time.
    a. It is suggested that repeat examinations be conducted at another point in time if the clinical examination and apnea test continue to be consistent with BD/DNC, or that alternative end-of-life care be considered.
6. It is suggested that electrophysiologic testing with an EEG no longer be utilized routinely as an ancillary test in adults, but that:
    a. It may be required if mandated by regional laws or policy, or craniovascular impedance has been affected by an open skull fracture, decompressive craniectomy, or an open fontanelle/sutures in infants,
    b. If performed as an ancillary test, EEG should be used in conjunction with somatosensory and brainstem auditory evoked potentials given the limitations of EEG for evaluating brainstem function,
    c. It be interpreted in accordance with regional criteria. In the absence of regional criteria, guidance from the following may be considered: American Clinical Neurophysiology Society [195], Bleck [170], The Korean Society of Clinical Neurophysiology [196], Société de Neurophysiologie Clinique de Langue Française [197].
7. It is suggested that CTA and MRA ***not*** be used to support a diagnosis of cerebral circulatory arrest at present, pending further research into the sensitivity and specificity of these modalities.
8. It is recommended that no other modalities be used to support a diagnosis of cerebral circulatory arrest at present, pending further research.
9. It is suggested that conventional 4 vessel cerebral angiography remain the reference standard of ancillary testing, and that it be used for initial validation or research of newer techniques.
10. It is suggested that validation of new ancillary techniques will require assessment in patients fulfilling full and unconfounded clinical criteria for BD/DNC, as well as non-brain dead patients as controls, and should include circumstances of infancy, craniovascular decompression, persistence of CNS depressing medications or intoxication, and hypothermia. Standardized methods of interpretation for each new technique should be developed, founded on principles of monitoring the whole brain, encompassing supratentorial and infratentorial integrity, flow and function.
11. It is suggested that some precedence be given to the further validation of CTA, given its increasing prevalence and usage. Integration with CT perfusion may prove valuable, given recent advances in CT technology.

Questions to Inform Research Agendas

1. What is the validity of CTA, CT perfusion, MR angiography and MR perfusion in determination of BD/DNC?
2. How often and under what conditions does ancillary testing support or contravene a clinical determination of BD/DNC?
3. Does the presence and degree of cerebromedullary herniation on neuroimaging correlate with brain blood flow on ancillary testing?
4. When minimum clinical criteria for BD/DNC are fulfilled, does the absence of herniation on neuroimaging correlate with the presence of brain blood flow on ancillary testing?
5. What are the lower limits of brain blood flow and duration that are associated with cessation of brain function? Can physiologically irrelevant brain blood flow on ancillary testing be defined?
6. Can the lower limits of brain blood flow detection by ancillary testing, including radionuclide, be defined?
7. Can continuous TCD monitoring identify the moment of cessation of brain blood flow and does this correlate with loss of all brain function?
8. Are the findings on the second TCD ever different from the findings on the first TCD if a person meets clinical criteria for BD/DNC?
9. What is the natural history of clinical BD/DNC with preservation of brain blood flow by ancillary testing?
10. What are the potential roles of experimental approaches, including ultrasound of the retina, electrical impedance, transcranial magnetic stimulation, craniovascular flowmetry, and brain tissue oximetry in determination of BD/DNC?
11. What are the minimum requirements for training and expertise in the performance and interpretation of ancillary studies by neuroradiology experts?

Rationale

- All flow based ancillary testing is vulnerable to the logical fallacy of 'flow necessitating function'. Namely, while a sustained absence of flow specifies loss of function, the converse is not necessarily true – the presence of flow does not indicate presence of function.
- Scintigraphic perfusion studies using lipophilic agents have an established history of good sensitivity and specificity. There is evidence that SPECT offers better definition of the brainstem. Barriers to implementation are based on access to appropriate equipment and isotopes. Preparation of the isotopic vehicle does require some time, and it has a limited shelf-life once reconstituted.
- A person’s instability may preclude transport to the nuclear medicine department, but mobile gamma cameras present some challenges in positioning around a ventilated ICU person. Lateral imaging lends specificity to anterior views.

- CTA holds significant promise as a swift and easy testing method, with no evidence that it has significant effect on renal function. Its problem lies in the variability of methods and interpretation employed to date, with real concern for lack of infratentorial assessment in certain scoring systems. More recent scoring systems cater for infratentorial assessment, with good sensitivity, but require validation in larger studies. CTA already has an established role in several national guidelines but requires improved consistency of method and subsequent testing of that method in larger populations, before extension further afield.
- MRA is also interesting but shares the problem of variability in diagnostic method. It requires further development to accurately determine reproducible sensitivity and specificity using gadolinium contrast. Variability of method, as well as concern for the accuracy of time-of-flight-imaging argue against widespread use at present.
- Cerebral angiography has the best sensitivity and specificity reported to date, but is resource intensive, as well as possessing an incidence of associated risks and side-effects. For those reasons alone, it should be reserved for carefully controlled validation studies.
- The frequency of hyperostosis relegates transcranial Doppler to a second line methodology, unless there is a history of demonstrable acoustic windows. In those circumstances, it would then be an appropriate first line choice, without a need for transport. It suffers from the same vulnerability to decreased craniovascular compliance as other flow-based investigations. The use of ultrasound contrast agents is interesting but needs continued methodological development.
- Electrophysiological tests have a long history of use with developed and relatively consistent methodology. EEG is widespread, but confined to detection of cortical and subcortical activity, whereas evoked potentials offer assessment of sensory pathways from the periphery to focal areas of cortex. The accuracy of EEG is affected by any factors reducing or compromising metabolic demand. Evoked potentials are relatively resistant to those factors but may be compromised by individual sensory deficits. The combination of the two has well-recognized sensitivity and specificity.
- CTA is a diffusely prevalent technology with relative ease of access and low operator dependence. It has already been included in some national guidelines. CT perfusion offers some functional insights and can reconstruct CTA images with good definition of brainstem structures. It may be worthy of relative priority in study development.

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