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REVIEW ARTICLE

Pitfalls in the Diagnosis of Brain Death

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Abstract Since the establishment of the concept of declaring death by brain criteria, a large extent of variability in the determination of brain death has been reported. There are no standardized practical guidelines, and major differences exist in the requirements for the declaration of brain death throughout the USA and internationally. The American Academy of Neurology published evidence-based practice parameters for the determination of brain death in adults in 1995, requiring the irreversible absence of clinical brain function with the cardinal features of coma, absent brainstem reflexes, and apnea, as well as the exclusion of reversible confounders. Ancillary tests are recommended in cases of uncertainty of the clinical diagnosis. Every step in the determination of brain death bears potential pitfalls which can lead to errors in the diagnosis of brain death. These pitfalls are presented here, and possible solutions identified. Suggestions are made for improvement in the standardization of the declaration of brain death.

Keywords Brain death · Coma · Diagnosis

Background

The concept of brain death was introduced in 1959 by Mollaret and Goulon [1], as the deepest form of coma, due to primary brain damage, and without chance for recovery. In 1968, the Ad Hoc Committee of Harvard Medical School described characteristics of irreversible coma and argued for recognition of this state as a new definition for death, i.e., brain death. The criteria included unresponsiveness, absence of movement or breathing, and absence of brainstem reflexes. In addition, an isoelectric EEG was recommended, and hypothermia and drug intoxication were to be excluded [2].

In 1981, the President's Commission for the Study of Ethical Problems in Medicine and Behavioral Research issued a landmark report on "Defining Death" and redefined the criteria for the diagnosis of brain death in adults, encompassing the complete cessation of all functions of the entire brain (i.e., "whole brain concept"), and its irreversibility [3]. The Uniform Determination of Death Act, which was subsequently adopted as federal legislation by most states in the USA, is based on these recommendations. While the criteria formulated in this act set the legal ground for determining death by brain criteria, practical guidelines were not specified. In fact, "the Act is silent on acceptable diagnostic tests and medical procedures. It sets the general legal standard for determining death, but not the medical criteria for doing so. The medical profession remains free to formulate acceptable medical practices and to utilize new biomedical knowledge, diagnostic tests, and equipment" [4].

Brain Death Criteria

Brain death can scientifically be classified as brainstem death or whole brain death. In the USA, whole brain criteria (i.e., irreversible cessation of all brain functions) are used. From a legal perspective, each country, and in the USA each State, has its own legal regulations for death by brain criteria. On the basis of these legal regulations, each individual hospital establishes criteria for the determination of brain death. Since the establishment of brain death

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criteria, a large extent of variability in the determination of brain death in adults internationally, between states and within individual hospitals, has been reported [5]. While the three cardinal features of deep unresponsive coma, absent brainstem function, and apnea are widely accepted as key features, the practical aspects of declaring brain death are not standardized. Major differences exist in the requirements of the specialty and training of the assessing physician, the number of observers, timing of the first clinical brain death examination, specifics of the clinical examination, requirement of a repeat examination, duration of observation, and whether and which confirmatory tests are accepted, mandatory, or facilitative [6–9].

In 1995, the American Academy of Neurology (AAN) published evidence-based practice parameters for determining brain death in adults [10]. These guidelines require irreversible absence of clinical brain function, with demonstrable clinical or neuroimaging evidence of a catastrophic irreversible brain injury sufficient to cause brain death, and exclusion of reversible confounders (metabolic disturbances, temperature $<32°C$, intoxication). If these prerequisite criteria are met, the cardinal features expected in brain death are coma, absent brainstem reflexes, and apnea. Confirmatory tests are not mandatory but recommended in the presence of conditions that might interfere with the clinical diagnosis, or in cases of an equivocal diagnosis.

In the USA, brain death declarations are made according to criteria determined by individual hospitals. Despite clear guidelines, the clinical application holds a certain potential of pitfalls that the assessing clinician may encounter.

Clinical Examination

In various clinical scenarios, the nervous system is acutely and dramatically affected, and the impression of a devastating neurological condition can arise. High cervical cord injury, fulminant Guillain–Barré-syndrome, organophosphate intoxication, baclofen toxicity, lidocaine toxicity, and delayed vecuronium clearance have all been reported as clinical mimics of brain death [7, 11–18]. Neurological findings such as absent brainstem reflexes [15], even including unresponsive pupils [17], may occur temporarily and may present as a false positive sign for the examining physician, particularly if they are not accustomed to performing full neurological and brain death examinations. However, in none of these case publications was a complete brain death examination performed according to the current AAN Practice Parameters. In most of the cases, at least one actual discrepancy from a full brain death examination can be found, such as constricted pupils (instead of pupils which are fixed in mid-size) in a case of organophosphate poisoning [14]. Furthermore, one of the cardinal rules of brain death determination is to know the underlying cause of the neurological state, and to know that the cause is irreversible. Strict adherence to the required examination protocol and the criteria for the diagnosis of brain death can avoid such misinterpretation.

Absence of facial movements in response to noxious stimuli and cerebrally mediated motor responses to pain in all extremities are required features for the clinical diagnosis of brain death. A common source of ambiguity is spontaneous and reflex movements in brain-dead bodies, which are widely encountered and display ample variety.

Clinical observations in the cranial nerve distribution include facial myokymias, transient eyelid opening, ocular microtremor, and cyclic pupillary constriction and dilatation in light fixed pupils [18–20]. The exact origin of such movements is often unclear. In the case of a patient without evidence of brainstem function, but slow eye opening in response to painful stimulation of the ipsilateral nipple, the authors hypothesized that the eyelid opening may have been a mere function of Muller's muscle rather than a reflection of brainstem function. The eyelid opening persisted for 9 days before the patient succumbed to asystole [18]. In the case of a patient with absent brainstem reflexes apart from unequal light-fixed pupils which displayed independent periodic constriction and dilatation, it was felt that this was likely not a central process due to the asynchrony. An etiology in the peripheral nervous system, such as arising either in the ciliary ganglion or pupillary sphincter due to hypersensitivity to neurotransmitters in the setting of denervation, may be a plausible explanation, but is yet unproven [20]. Similarly, facial myokymias may be muscle contractions as a result of denervation, or deafferentation of the facial nucleus due to a supranuclear lesion [21]. However, falsely interpreting facial movements as peripheral phenomena or reflex movements could lead to a false positive diagnosis of brain death.

With regard to limb or torso movements, there are a set of spontaneous or stimulus-induced movements that are spinally mediated and not cerebral in origin. The AAN Practice Parameters describe certain movements which do not preclude the clinical diagnosis of brain death, including spontaneous movements of limbs (other than pathological flexion or extension response), respiration-like movements, deep tendon reflexes, superficial abdominal reflexes, triple flexion response, and the Babinski sign. These spinal movements can be categorized in monosegmental muscle stretch reflexes, oligosegmental cutaneo-muscular reflexes, and polysegmental spinal automatism patterns [22]. There is significant inter- and intra-individual variability, as well as temporal variability. Most movements seem to occur within the first 24 h, and rarely after 72 h. A recent review

Table 1 Reflexive movements in brain death

Reflexive movements in brain death
Deep tendon reflexes
Triple flexion
Babinski sign
Head turning
Spontaneous arm or leg movements
Respiration-like movements

lists all reports and studies on brain death-associated movements [23]. Information on the incidence of brain death-related movements mostly stems from small case series, and ranges from 13.4 to 79% of patients, with a wide array of phenomenology [21, 24–26]. The movements can be very subtle, such as fine finger tremors, or dramatic, such as the Lazarus sign [27, 28]. Table 1 presents some of the clinical signs that may be observed in brain death.

The pathophysiological basis for complex spinal reflexes remains unclear. Theories include that they are phylogenetically old motor patterns which are set free when uncoupled from brainstem and neocortical control [22], or that they represent hypoxia- and hypercapnia-induced activity of cervical cord neurons, which are isolated from rostral regions [28]. A mechanical stretch of the spinal roots and compression of the spinal cord may play a role [21], as some spinal movements can be triggered by noxious stimuli or neck flexion [29]. A combination of mechanical stimuli and hypoxia may be a trigger, as low arterial pressure is common in brain death [21]. Spinal shock and severe arterial hypotension have been linked to differences in timing of the occurrence of these movements [25, 30].

Some features are characteristic of movements in brain death that can help to distinguish them from voluntary or brainstem-generated motor activity. Reflex movements occur in response to a trigger of limited variation, show a constant pattern of latency and duration, and often habituate with frequent triggering [31, 32]. Both polysegmental spinal reflex patterns and automatisms do not typically occur prior to brain death [22]. The movements are stereotypical and never purposeful. But even if spinal reflex patterns and automatisms can be diagnosed clearly by their stereotypical pattern, they often mock vitality and resemble certain clinical scenarios, such as periodic limb movements during sleep, cough reflex, or decerebrate posturing [33, 34]. This may cause alarm and doubt of the diagnosis of brain death in physicians and nurses, and even more so in families. The variable elicitation mechanisms and the different patterns make systemic classification difficult. Only broad experience with the different forms of possible movements makes a clinical decision about the spinal origin of the observed phenomenon possible [22]. For the experienced examiner, polysegmental spinal reflex patterns and automatism patterns can even be seen as a confirmatory sign for brain death, as spinal reflexes emerge only after a phase of spinal shock after brain death [35].

Taken together, pitfalls in the diagnosis of brain death in the clinical examination can mostly be avoided by completeness, experience, and—if further doubt exists—by delaying/repeating the clinical declaration of brain death or using ancillary testing.

Metabolic and Toxic Factors

The determination of brain death is often complicated by the presence of drugs that may mask central nervous system activity. The AAN Practice Parameters request exclusion of medical conditions that may confound the clinical assessment, and stipulate that there be no drug intoxication or poisoning. Toxic levels of drugs are listed as a possible pitfall in the diagnosis of brain death.

Several aspects are to be considered: There is wide variation in pharmacological behavior in critically ill patients, and up to fourfold differences in cerebral effects for a given blood concentration have been reported [36]. Mechanical ventilation may decrease hepatic and renal blood flow, which may in turn decrease the clearance of drugs [37]. Prolonged neuromuscular blockade or sedation may occur even after the drug is withdrawn and the measured concentration has dropped below the therapeutic level [12, 38, 39], one possible factor being the accumulation of metabolites [37]. An additional influence on drug effect and clearance with head injury, anoxia, cardiovascular instability, and presence of other drugs is not well known [40, 41]. For these specific situations, as well as generally in the interpretation of measured drug levels in presumed brain death or the presence of organ failure and drug clearance, the literature provides little guidance. Of the few explicit data, one study argued for waiting at least four half-lives of elimination of the relevant drug in the absence of factors known to delay excretion, whereas another study suggested waiting for 3–4 days [42, 43].

From a practical viewpoint, toxicological samples have to be collected prior to administering any drugs in order to achieve correct results. This is most often impossible, and complicates the interpretation of screening assays. Urine samples provide only retrospective data on renally-excreted substances and are not quantitative. Plasma samples are quantifiable, but do not measure tissue concentrations in the central nervous system. As long as there is a lack of data or a different method for estimating drug effects in the setting of a catastrophic brain injury, the clinical brain death examination should be delayed until a persisting drug effect is essentially ruled out, or supported by ancillary testing.

Table 2 Pitfalls in the clinical examination

Clinical problem	Potential solutions
Proximate cause unknown	Further radiological/laboratory testing
Drug intoxication	Further time for drug clearance; ancillary testing
Metabolic disturbances	Correction of underlying problem, ancillary testing
Severe facial trauma	Ancillary testing
Dilated or constricted pupils	Rule out drug effect
Movements indeterminate as spinal or cerebral	Ancillary testing

Table 2 provides an overview of the pitfalls in performance and interpretation of the clinical examination, and possible strategies to avoid these.

Apnea Testing

The apnea test is required for the diagnosis of brain death. Limitations for a successful apnea test are uncertainties and mistakes in performance and interpretation, and complications during testing.

The AAN Practice Parameters provide a stepwise protocol for the proper performance of the apnea test. Prerequisites prior to test initiation include euthermia, systolic blood pressure greater than 90 mmHg, euvolemia, and normal PCO_2 and PO_2 [10]. Deviations from these prerequisites, such as hypotension or inadequate preoxygenation, or other unfavorable pretest conditions such as acid-base abnormalities, electrolyte abnormalities or arrhythmias, are common in the critically ill and brain-dead bodies [44]. In the setting of such unfavorable conditions, complications during apnea testing occur significantly more often (39 vs. 15% in the largest published series), compared with when none of these conditions are present [45, 46]. Sedatives, especially benzodiazepines, can suppress the respiratory center and affect both pretest conditions (such as blood pressure and CO_2) and the outcome of the apnea test [43]. Therefore, attention to pretest conditions and optimizing these factors can minimize adverse effects during apnea testing.

The first steps of the actual apnea test are to connect a pulse oximeter and disconnect the ventilator, deliver 100% oxygen through a catheter or a cannula at the level of the carina once the ventilator is disconnected, and then monitor for respiratory movements [10]. Complete disconnection from the ventilator is essential, as the presence of minor fluctuations of airway pressure can be falsely interpreted as true ventilatory efforts in patients who otherwise meet criteria for brain death [47].

The complication rate of apnea testing has been reported to be as high as 21% [48]. Common complications are hypoxemia, bradycardia, and arterial hypotension [6, 49, 50]. The oxygenation technique of delivering 100% oxygen through a catheter at the carina has been associated with severe pulmonary complications such as subcutaneous emphysema, pulmonary barotrauma, and tension pneumothorax leading to cardiac arrest [51–53]. In addition, compromising respiratory conditions such as neurogenic pulmonary edema or aspiration pneumonia are common in brain-dead patients, so that increased FiO_2 and PEEP are often required to maintain a safe oxygenation level [49]. The use of a T-piece or continuous positive airway pressure has been evaluated as alternative technique in the setting of hypoxemia, and was found to be less prone to complications than the oxygen catheter technique [54].

After about 8 min of disconnection from the ventilator, PO_2, PCO_2, and pH should be measured. If respiratory movements are absent, and the PCO_2 is greater than 60 mmHg (from a baseline of 40 mmHg or less), or has risen more than 20 mmHg above the baseline, the apnea test is positive [10]. The time span of 8 min is a theoretical approximation and has been deducted from calculation of the duration of a CO_2 rise of 20 mmHg in the absence of respiratory effort, but with normally functioning lungs. In actual practice, the rate of CO_2 rise displays high inter- and intra-personal variability and often is difficult to predict [42]. On the other hand, this may result in the need to repeat the apnea test, thus posing additional hazards and stress to the patient. Alternatively, the time span may be too long, and hypoxemia and respiratory acidosis may occur and lead to hemodynamic instability.

Another area of uncertainty is the application of the apnea test in CO_2-retainers. For one, eucapnia as one of the prerequisites for the apnea test may not be easily reached [10]. Second, in patients with chronically elevated PCO_2 concentrations, either primary from a chronic respiratory disease or secondary to metabolic derangements, a PCO_2 of 60 mmHg may not represent a sufficient respiratory stimulus, as the baseline PCO_2 is only few points lower [55]. Thus, applying the same guidelines in these situations may provoke the pitfall of a false positive interpretation of the apnea test.

Several variations of the apnea test have been suggested in order to reduce complications. Administration of exogenous CO_2, the use of end-tidal capnometry to monitor the CO_2 rise, or capnography to detect CO_2 release offer the advantages of better estimation of the changes in the blood gas composition during the apnea period [55]. Transcutaneous CO_2 monitoring has also been described to predict the target $PaCO_2$, to shorten the apnea test and reduce adverse events [56]. In order to avoid repeated arterial blood gas

Table 3 Pitfalls in the apnea test and corrective measures

Clinical problem	Potential solutions
Hypoxia, hemodynamic instability	Ancillary testing
CO_2 retention	Attempt to achieve patient's baseline CO_2; ancillary testing
Ventilator registers breath during testing	Repeat test with ventilator disconnected
Hypoxia during testing	Abort test, pursue ancillary testing
Hemodynamic instability during testing	Abort test, pursue ancillary testing
Inconclusive test (PCO_2 does not reach threshold level)	*Clinically stable*—repeat testing for longer period of time after repeat pre-oxygenation
	Clinically unstable—ancillary testing

testing, continuous intra-arterial and transcutaneous blood gas measuring were compared with in vitro arterial blood gas analysis during apnea testing. There were several technical difficulties and no clear advantages, so that neither procedure could replace the standard method of arterial blood gas testing [57].

Despite the requirement of an apnea test as part of a complete brain death examination, apnea testing is not always performed (one series showed that 11.6% of 129 clinicians skipped apnea testing [48]). Only the minority of clinicians, 12.4% in this series, performed the apnea test according to published guidelines. Clinicians in full-time academic practice more often adhered to guidelines for apnea testing.

Table 3 provides an overview of the pitfalls in performance and interpretation of the apnea test, and possible strategies to avoid these.

Ancillary Testing

The ancillary tests used in the confirmation of brain death represent two principles: confirmation of loss of bioelectrical activity of the brain (EEG, EP), or demonstration of cerebral circulatory arrest. Currently, no validated blood tests are available in the diagnosis of brain death.

Ancillary tests are necessary if certain parts of the neurological examination or the apnea test cannot be reliably performed, or their validity is drawn into question. Officially recommended tests are conventional angiography (showing no intracranial filling), EEG (showing absence of electrical activity for 30 min), transcranial Doppler (TCD) (showing small systolic peaks or reverberating flow), Tc99 HMPAO SPECT scan (showing an absence of brain uptake of tracer), and SSEP (with bilateral absence of the N20-P22 response to median nerve stimulation) [10].

With regard to flow studies, an increase of intracranial pressure resulting from diffuse brain edema is thought to be the primary cause for cessation of cerebral perfusion and blood flow [58]. Digital subtraction angiography is widely regarded as the gold standard. It is typically performed with the catheter tip in the aortic arch and contrast injection into each of the four arteries supplying the brain. At least two injections, 20 min apart, must show an absence of filling of all four arteries as their course becomes intracranial [6]. Angiography bears the disadvantages of being an involved, time-consuming, expensive procedure that cannot be performed at the bedside. Furthermore, contrast administration may cause harm by triggering allergic reactions or renal damage and possibly an increased rejection rate in organ recipients following such studies [59]. A false negative angiogram despite a lack of brain function may occur if there is more cellular toxicity than major edematous swelling, and thus no cessation of cerebral flow, as reported in a case of a patient who was resuscitated after acute benzodiazepine intoxication, and had persistent cerebral blood flow [60]. Reaching a certain diagnosis can be achieved by adding a confirmatory test of different modality (in the reported case, an EEG).

Figure 1 shows an angiogram which is performed incorrectly for the diagnosis of brain death, as both carotid arteries are injected simultaneously.

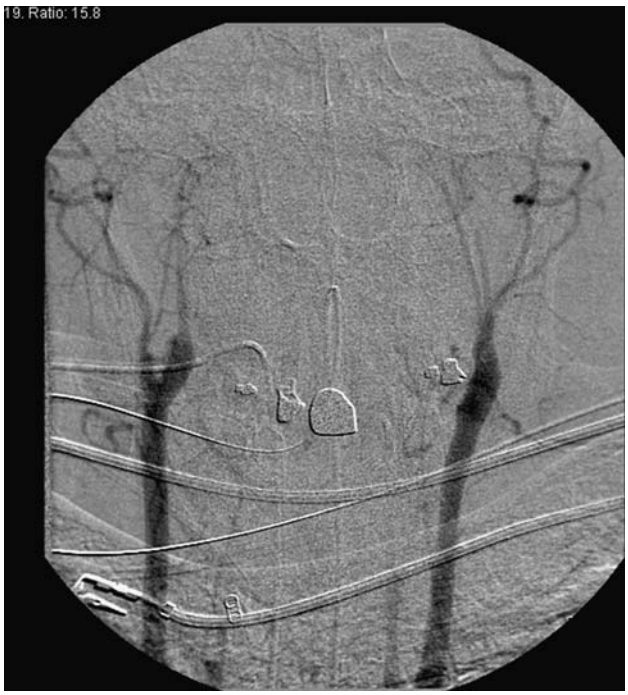

Fig. 1 Conventional angiogram in brain death. Despite the findings of arrest of flow intracranially seen in all four major vessels on this study, it was performed incorrectly. Each of the four major vessels must be separately injected with pressure to ensure that there is no intracranial flow

Other Imaging Modalities

CT with CT perfusion and CT angiography are being evaluated for use as ancillary tests in brain death. Certain features on head CT imaging, such as a midline shift greater than 10 mm, unilateral absence of the ambien cistern, transtentorial herniation, and intraventricular perforation, are predictive of brain death [61, 62]. However, they have not been evaluated in large enough studies and are not felt to be sufficiently specific. CT/CTA could possibly replace conventional angiography [63]; however, sensitivity was only 52.4% in one series [64], and in comparison with angiography, the divergence rate between CTA and angiography was 30% [65].

In MRI and MR angiography, loss of flow voids in the intracranial ICAs, central and tonsillar herniation, diffuse brain swelling, and the absence of any cerebral vessels above the supraclinoid ICA are suggestive of brain death [66]. Non-filling of the ICA on DSA correlates with disappearance of flow voids in the cavernous ICA [67]. As of yet, MRI and MRA, as well as CT and CT/A, need to be validated for use in the determination of brain death in larger studies, and in comparison with a gold standard. At the present time, they are not considered valid ancillary tests, and are not listed in guidelines.

Neurophysiological Methods: EEG and Evoked Potentials

EEG is required to show absence of electrical activity at a sensitivity of 2 μV for 30 min in order to confirm brain death. While EEG is a very reliable test if it displays an isoelectric recording, there are several disadvantages. EEG may be insensitive to detect brain activity in certain areas of the brain: it does not register the electrical activity of the lower brainstem, and may be flat in patients with preserved subcortical function. EEG is very susceptible to false positives: it is sensitive to drug effects [9], and to a greater or lesser degree unreliable in the setting of sedation, hypothermia, toxic or metabolic factors, and artifacts [68]. EEG also is prone to interference, such as with electromagnetic fields in the intensive care unit [9], or as interference between EEG and EMG, especially in brain-dead patients in whom EMG activity may be enhanced. Often, administration of muscle relaxants is necessary to achieve isoelectric EEG readings [69, 70]. Furthermore, a fifth of patients fulfilling clinical criteria for brain death had EEG activity up to 168 h in one series [71]. On interpretation, diagnostic uncertainty arises in up to 20% due to intra- and inter-rater variability [72].

SSEP assess the functional integrity of posterior columns, medial lemniscus, thalamus, and the sensorimotor cortex. SSEP are indicative of brain death if bilateral absence of the N20-P22 response to median nerve stimulation is demonstrated [10]. Early absence of the N20/P22 cortical response on SSEP is often predictive of death without awakening. However, in the early phase of brain death, SSEP can be normal. This is attributed to neuronal activity of damaged neurons which are still capable of generating a response, but then disappear over time [73]. P13/14 peaks (reflecting the medial lemniscus) in median nerve SSEPs also can be preserved in brain death early in its course, whereas N18 peaks (reflecting the cuneate nucleus) were completely lost in all brain-dead subjects in a small series [74].

Differences in the P14 potential, which is generated around the foramen magnum and may differ in its caudal and rostral potential, may lead to different results: the greater rostral part of the P14 generator dipole is inactivated in brain death, but the very caudal segments may be intact for some time [75].

In lesions of the upper cervical cord and medulla, SSEP can show arrest of conduction in the spinal cord with present N13 and absent P14 peaks, resembling the pattern observed in brain death. Therefore, additional testing is called for, with a non-cephalic reference, to distinguish between the cervical N13 potential and brain-stem 'N14' negativity [76].

To avoid false positive or false negative results, recording from nasopharyngeal electrodes has been evaluated. This approach was found to be of low invasiveness and permits a near field recording of the lower brainstem and the upper cervical cord [77]. This technique still requires sufficient validation.

Brainstem auditory evoked potentials were found to be 100% sensitive, but only 73.7% specific [78]. BAER flattening may transiently occur after postanoxic coma [79]. In another study, auditory evoked potentials were absent in 70.8% in the setting of a clinical diagnosis of brain death; however, with the combined use of BAER and SSEP it was possible to confirm brain death in 93%, and to exclude the diagnosis of brain death in 5.4%. Evoked potentials have the advantages of being widely available, non-invasive, cheap, able to explore brainstem structures, and feasible in the setting of hypothermia and sedation [80]. Disadvantages are the need for expertise in performance and interpretation, the possibility of false negative results and thus a delay in the diagnosis, difficulty assessing infratentorial lesions, and the possibility of a seeming brain death pattern with lesions in the cervico-medullary junction or transiently after an anoxic insult.

Single Photon Emission Computed Tomography (SPECT)

The absence of radionuclide activity of Tc-99m-HMPAO intracranially on static SPECT images, creating a so-called

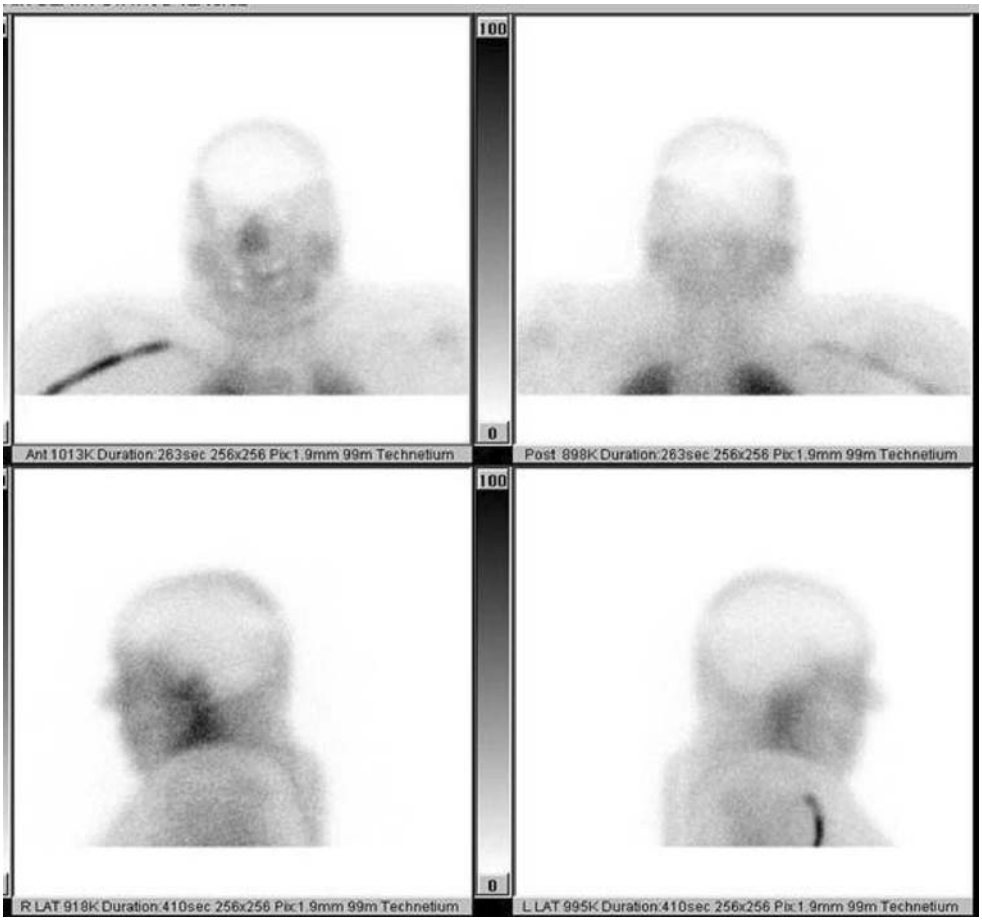

Fig. 2 SPECT in brain death. This study was performed correctly, showing an absence of intracranial tracer uptake. Both anterior-posterior and lateral views were obtained, ensuring adequate visualization of the posterior fossa structures

"hollow skull" appearance, is indicative of brain death [81]. In addition to anterior static images, flow images reveal a lack of cerebral perfusion and uptake, and lateral static images can determine whether tracer is seen in the brain stem and cerebellum. In the setting of brain death, the nuclear flow study shows blood that would have gone to the ICA territory being shunted to the ECA. The nasal area lights up due to multiple vessels in that ECA-perfused territory, and creates the so-called "hot nose sign" [82]. Figure 2 shows a SPECT study confirming brain death ("hollow skull" appearance).

The rates of residual flow vary widely from 3 to 40% depending on the criteria used and selection of patients. The discordant rate between the clinical exam and the initial scan was found to be 17% for cerebral HMPAO fixation, but as high as 38% if both cerebellar and cerebral perfusion need to have ceased completely [83, 84]. In a study using a different tracer, brain death was confirmed in 77%, but patchy cerebral uptake or even a normal scan (5%) was seen in the remainder of patients thought clinically brain dead. There were no false positive results [85, 86].

In a direct comparison between SPECT and angiography, both tests confirmed brain death in 95% on initial examination, and in 100% after 48 h [87]. The most commonly encountered problem with SPECT studies therefore seems that they may be negative early on in the setting of brain death and thus possibly delay the diagnosis.

TCD Sonography

TCD has been studied extensively and is used as an ancillary test in the diagnosis of brain death [88]. With rising intracranial pressure, the pattern of flow changes in the large intracranial arteries. The earliest sign on Doppler examination is an increased pulsatility index, followed by progressive reduction in the diastolic and mean flow velocities. When the anterograde diastolic flow has vanished, an oscillating biphasic flow pattern with retrograde diastolic flow occurs, until diastolic flow has completely disappeared. Small systolic spikes can then be found, but there is no effective forward flow [89]. The sensitivity of TCD in the diagnosis of brain death has been found in the range of 70 to 100% [59, 90–93]. Specificity has been

Table 4 Potential pitfalls in ancillary testing

Ancillary test	Potential pitfalls
Conventional angiography	Requires transport; invasive; contrast nephrotoxicity; must involve injection of all four major vessels
HMPAO SPECT	Posterior fossa may be difficult to visualize; uptake may be affected by hypothermia, barbiturates
EEG	Measures only surface activity; subject to ICU artifacts
TCD	Absence of signal insensitive to loss of flow; requires bilateral and anterior and posterior insonation; cannot be used in setting of craniectomy or EVD
SSEP	Questionable specificity; false negatives early after cardiac arrest
MRI/MRA, CT/CTA	Not validated; slow flow may appear as absence of flow

reported as 97–100% [59, 90–93]. In addition to transtemporal and suboccipital insonation, a transorbital approach has been shown to increase the rate of definite positive results for a diagnosis of brain death [94].

False positive results have rarely been reported. Occasionally, a TCD examination can demonstrate a pattern as seen in cerebral circulatory arrest in patients who are clinically brain dead but still have EEG activity [95]. Another possible pitfall is to equate absence of signal with absence of intracranial circulation, and interpret the study as positive when it actually is non-diagnostic. Lack of signal is found in 5–10% of patients, mostly due to inability to insonate the intracranial arteries with the available technique, and does not allow one to conclude the absence of intracranial circulation [96]. On the other hand, false negative results are quite frequently reported. Persistent flow in the intracranial arteries can be found in as many as 17.4% of patients clinically declared brain dead [90]. In 20% of cases, persistent flow in the ICA may be found despite cerebral circulatory arrest [97]. Signals may also be normal in infratentorial lesions and in patients with anoxic brain injury after cardiac arrest [98]. Furthermore, initial false negative results have been associated with the timing of the TCD exam in relation to the clinical diagnosis of brain death, female gender, and sympathomimetic drug use [90, 92].

In general, velocities may be affected by marked changes in $PaCO_2$, hematocrit, and cardiac output. The technique requires practice and skill in both performance and interpretation. For example, application of TCD in a patient with an intra-aortic balloon pump (IABP) can lead to false interpretation of results if the IABP is not on stand-by while mean TCD velocities are recorded, or if the net flow velocities are not calculated [99]. TCD can both increase and reduce the delay for a firm diagnosis of brain death [92]; in one series, the reduction of diagnostic delay was by 85%, and in this respect TCD examination was superior to Tc 99m HMPAO brain scintigraphy [100].

Table 4 provides an overview of the pitfalls in performance and interpretation of the ancillary testing.

Outlook and Newer Techniques in the Determination of Brain Death

Monitoring brain tissue oxygenation may allow the clinician to follow the trend toward brain death and to determine the moment of death [101, 102]. Various techniques have been described. For the ratio of jugular to central venous oxygen saturation, sensitivity was found to be 96.6%, specificity 99.3% in one series [103]. With near infrared spectroscopy, extracranial contributions to regional brain oxygenation made a distinction of brain death from viable brain tissue more difficult [104].

Quantitative estimation of diffusion anisotropy of the brain by using diffusion-weighted MRI allows an indirect assessment of changes in axonal function. Anisotropy of fibers decreases after brain death and may be an early indicator if impending brain death [105]. Assessment of heart rate variability in comatose patients showed that the variability collapses as soon as brain death is clinically suggested. While this method seems to be very sensitive, specificity is poor [106].

Bispectral index is an EEG-derived method to estimate brain functionality. Using bispectral index as a supportive measure in the diagnosis of brain death has been suggested, as it was found useful in assessing progression toward brain death [107] and associated with outcome in severe brain injury [108]. While the bispectral index reliably stayed above a certain threshold in patients who did not progress to brain death in one series, it could decrease to zero prior to complete brain death [109]. Apart from that, the bispectral index is EEG-derived and as such shares the pitfalls associated with EEG analysis in the setting of brain death. EMG activity and cardiovascular hyperpulsatility can falsely affect the bispectral index [109].

Laboratory Tests

As of now, brain death cannot be confirmed by any laboratory blood test. Several markers are under investigation, and the most extensively examined is protein S-100b.

Protein S-100b is a calcium-binding protein found predominantly in the cytosol of astroglial and Schwann cells of the CNS, and is highly specific for CNS lesions [110, 111]. Serial measurements of S-100b in patients with severe head injury leading to brain death showed that S-100b concentrations closely correlated with brain damage and survival, with significantly higher levels of S-100b in brain death. S-100b therefore could be an early marker for brain death [112, 113], but will need to be studied in setting other than brain trauma.

Adherence to Guidelines

Accordance with the AAN Practice Parameters as well as with the policies established by individual hospitals is widely variable [8, 114]. In most US States, no specific qualification beyond the medical license is required in order to be able to perform a brain death declaration. The determination of death according to neurological criteria remains one of the most serious roles of a physician, and demands expertise and experience in assessing brain function [115]. Yet surprisingly few hospital policies require specific neuroscientific training for this task [8]. A review of the criteria of 106 hospitals in 42 states revealed that larger hospitals require that the examiner be a neurologist or neurosurgeon more often, and also tend to ask for a greater number of examiners [114]. Whether trained in neurosciences or not, the competence of physicians performing brain death examinations has not been well studied [6]. In a survey assessing the knowledge of the concept of brain death among physicians, one-third seemed to apply the neocortical definition of brain death [116]. In a study of the reliability of the clinical determination of brain death, the accuracy of the clinical examination was found to be 100% when performed by experienced neurosurgery or neurology physicians [117], but this was not compared to the clinical performance of non-experienced staff. There are wide variations in the compliance with guidelines and the clinical criteria actually used to determine brain death compliance with guidelines. Documentation of individual performance has been shown to be poor, and implementation of a check list has been suggested previously to improve performance of brain death examination procedures [8, 118].

Conclusion

Overall, there is a large degree of variability and vagueness in the criteria used to determine brain death and the adherence to these guidelines, as well as a number of possible pitfalls in applying the individual protocols of brain death declaration and the interpretation of the results. This can allow errors to occur at any step of the procedure. Improvements could be made on different levels: (1) greater standardization of criteria for the declaration of brain death; (2) more strict adherence to guidelines by implementation of check lists; (3) neuroscientist participation in performance and education regarding legal and scientific aspects of brain death determination; (4) certification of completion of a course on brain death examination performance; and (5) delaying the diagnosis of brain death, or alternate use of confirmatory tests, in cases in which deviations from guidelines are possible, or doubt exists as to the validity of the exam. Clinicians declaring brain death must be aware of these potential pitfalls so that testing can be performed correctly and without ambiguity.

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