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Acurácia da angiografia por tomografia computadorizada no diagnóstico de morte encefálica

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Programa de Neurologia

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ABBREVIATIONS LIST

4PS	4-point score
AEP	Auditory evoked potentials
BD	Brain death
BIS	Bispectral index
CFM	Conselho Federal de Medicina
CNS	Central nervous system
CRDTAS	Cochrane Register of Diagnostic Test Accuracy Studies
CTA	Computed tomography angiography
CTP	Computed tomography perfusion
DSA	Digital subtraction angiography
EA	Encephalic anoxia
EEG	Electroencephalogram
GBS	Guillain Barré syndrome
HGS	Head gun shot
ICP	Intracranial pressure
ICVs	Internal cerebral veins
IN	Intracranial neoplasia
IST	Intracranial sinus thrombosis
NMPT	Nuclear medicine perfusion test
QUADAS	Quality assessment of diagnostic accuracy studies
SAH	Subarachnoid hemorrhage
SPVs	Superior petrosal veins
TBI	Traumatic brain injury
TCD	Transcranial doppler
VS	Venous score

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ABSTRACT

Lima SPB. *Computed tomography angiography accuracy in brain death diagnosis* [thesis]. São Paulo: "Faculdade de Medicina, Universidade de São Paulo"; 2020.

Object: The present study was designed to answer several concerns disclosed by systematic reviews, indicating no evidence to support the use of computed tomography angiography (CTA) in the diagnosis of brain death (BD). Therefore, the aim of this study was to assess CTA for the diagnosis of BD and to define the optimal tomographic criteria of intracranial circulatory arrest. **Methods:** A unicenter prospective, observational case-control study was undertaken. Comatose patients (Glasgow Coma Scale ≤ 5), even subjects presenting with the first signs of BD, were included. CTA scanning of arterial and venous vasculature and transcranial Doppler (TCD) were performed. A neurological determination of BD and consequently determination of case (BD group) or control (no-BD group) was conducted. All personnel involved with assessing patients were blinded to further tests results. CTA accuracy was calculated based on the criteria of bilateral no visualization of the internal cerebral veins and the distal middle cerebral arteries, the 4-point score (4PS), and an exclusive criterion of absence of deep brain venous drainage exclusively, the venous score (VS), considering only the internal cerebral veins bilaterally. **Results:** A total of 106 patients were enrolled in this study; 52 patients did not have BD, and none of these patients had circulatory arrest observed by CTA or TCD (100% specificity). Of the 54 patients with a clinical diagnosis of BD, 33 met the 4PS (61.1% sensitivity), whereas 47 met the VS (87% sensitivity). The accuracy of CTA was time-related, with greater accuracy when scanning was performed less than 12 hours prior to the neurological assessment, reaching 95.5% sensitivity with the VS. **Conclusions:** CTA can reliably support a diagnosis of BD. The criterion of absence of deep venous opacification can confirm the occurrence of cerebral circulatory arrest.

Descriptors: Tomography, X ray-computed; Computed tomography angiography; Transcranial Doppler; Neurologic examination; Brain death; Tissue donors; Tissue and organ procurement; donors; Cerebral angiography; Transplants.

RESUMO

Lima SPB. *Acurácia da angiografia por tomografia computadorizada no diagnóstico de morte encefálica* [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2020.

Introdução: Testes auxiliares para o diagnóstico de morte encefálica (ME) são frequentemente necessários. A angiotomografia computadorizada (ATC) é um método para avaliar a presença de parada circulatória intracraniana em pacientes com ME. No entanto, o uso de ATC permanece controverso nesse cenário, devido à falta de estudos controlados. Objetivos: Avaliar a acurácia da ATC para o diagnóstico da ME e definir os critérios tomográficos compatíveis com parada circulatória intracraniana. Métodos: Estudo prospectivo, incluindo pacientes comatosos (Escala de Coma de Glasgow [ECG] ≤ 5), e submetidos à angiotomografia computadorizada (ATC) e Doppler transcraniano (DTC). Posteriormente à obtenção dos exames, procedeu-se à determinação neurológica da morte encefálica, interrompendo a avaliação neurológica ao primeiro sinal negativo para ME. Os examinadores de cada etapa (ATC, DTC e avaliação clínica) estavam cegados entre si para os resultados das demais avaliações. Resultados: Em um total de 106 pacientes incluídos, 52 não apresentavam critérios clínicos de ME, e nenhum desses pacientes teve parada circulatória encefálica observada por ATC ou DTC. Dos 54 pacientes com ME positiva, 33 preencheram os critérios de ausência bilateral das artérias cerebrais médias distais e veias cerebrais internas (escore de 4 pontos, sensibilidade 61,1%), enquanto 47 preencheram os critérios de ausência exclusiva de drenagem venosa profunda do cérebro (escore venoso, sensibilidade 87%). O DTC apresentou dois falso-negativos (sensibilidade de 96%). O tempo de intervalo entre as avaliações mostrou ser fator influenciador dos resultados, havendo maior acurácia quando os exames complementares foram realizados a menos de 12 horas antes da avaliação clínica. Conclusões: O uso de ATC é confiável para o diagnóstico de ME. O critério de ausência de opacificação da drenagem venosa profunda confirma parada circulatória cerebral.

Descritores: Tomografia computadorizada por raios X; Angiografia por tomografia computadorizada; Ultrassonografia Doppler transcraniana; Exame neurológico; Morte encefálica; Doadores de tecidos; Obtenção de tecidos e órgãos; Angiografia cerebral; Transplantes.

1 INTRODUCTION

1 INTRODUCTION

1.1 Brain death definition

Neurological determination of death, or brain death (BD) is defined as the irreversible cessation of all encephalic functions, that is, the verification of an irreversible unreactive coma of known structural etiology, the absence of brain stem reflexes, including the ability to breathe[1].

The concept of BD has been globally accepted, both by the scientific community and by society; however, there is still controversy in its clinical application[2-5]. There is not a uniform criterion in many of its aspects, which include, ethical, legal, religious and cultural differences amongst regions and countries[6-13].

Diagnosing BD is sorely important especially because of the families' right to be informed of their loved ones' situation, and to decide about withdrawal of futile therapies. Despite of these, in some locations, diagnosing BD is mandatory only in cases of potential organ donors[14].

1.2 BD epidemiology

BD is a high prevalent situation worldwide, although its real numbers have not been synthetized. In the United States of America, BD is reported to be diagnosed each year between 1% to 2% of all deaths and in 5% of patients with acute brain injury[15]; whereas a systematic review disclosed over 10% of BD because of hypoxic injury after cardio-pulmonary resuscitation, this number represents 12% of hospital deaths approximately[16].

Up to 2010, 50% of brain deaths were not identified in Brazil, considering an underestimated rate of 70 brain death cases per 1 million inhabitants per year. Since then, approximately 70% of cases have been identified. Similar

results have been reported in Europe and Canada, where 35% of the patients who died by devastating brain injury were not reported as such, and thus the possibility of organ donation could not even be considered[17,18].

The development of techniques such as multimodal monitoring, hypothermia and decompressive craniectomy for traumatic and nontraumatic encephalic injury has shown to be effective to manage and control intracranial hypertension (ICH) and improve brain perfusion[19]. Despite these improvements, targeted therapies on physiological and metabolic improvements are still lacking to the date, and many of critical patients develops high disability, minimally conscious states, vegetative states or BD[20,21].

In Spain, the country in the world with current highest transplantation *per capita* index (43,4 transplants per million inhabitants), a 10-year audit revealed 2.3% of brain deaths between all hospital deaths[22], whereas in neurocritical specialized units this number raised to 33%[23].

A survey performed in the year of 2016 comprising 8345 patients admitted in the intensive care units at *Hospital das Clínicas* of São Paulo University concluded that amongst 438 deaths, just 89 (20%) BD diagnoses were concluded demonstrating the lack of proper diagnosis.

1.3 Pathophysiology of BD

Mainly, BD is produced because of a severe primary brain injury, such as in traumatic brain injury (TBI) or intracranial hemorrhages[24]. Other central nervous system (CNS) etiologies include intracranial neoplasm, hydrocephalus and infections, while systemic BD causes are encephalic anoxia in consequence of cardiac arrest, acute liver failure, sepsis, pro-thrombotic diseases, and metabolic disturbances, such as severe hypoglycemia. The common pathway for BD of any etiology is the development of massive CNS ischemia (figure 1).

It is mandatory, in BD diagnosis, to exclude any condition that might confound the subsequent examination of cortical or brain stem function, such as hypothermia, drug intoxication, brainstem encephalitis, Guillain-Barré syndrome, locked-in syndrome or severe metabolic encephalopathy.

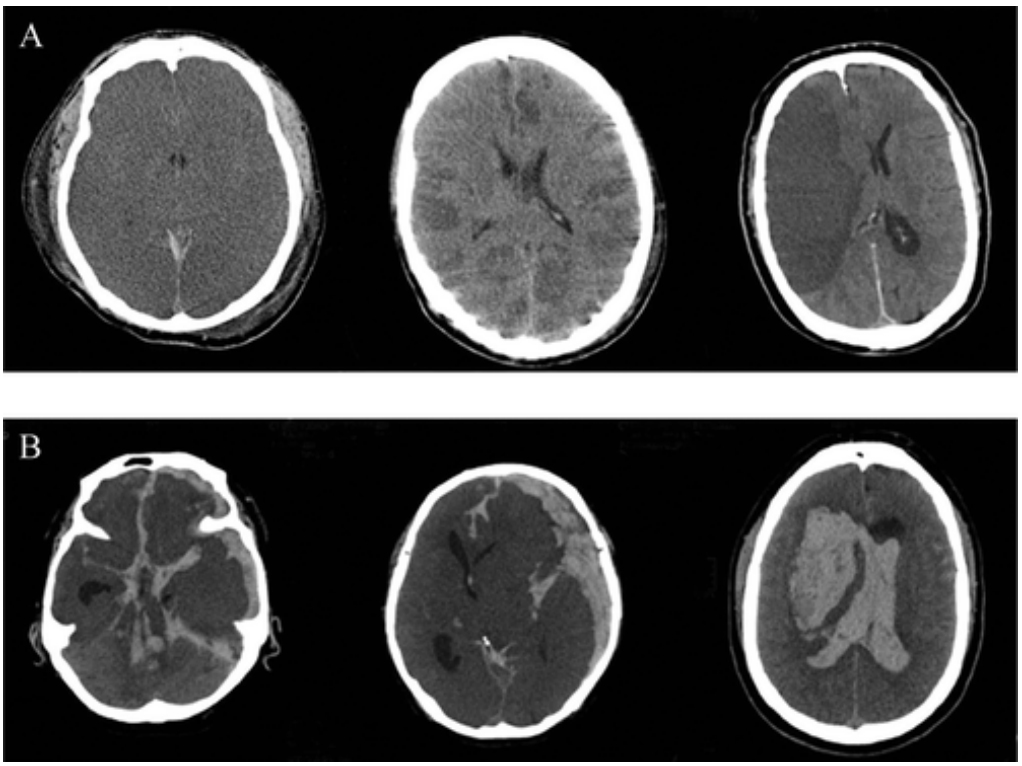


Figure 1 - Ischemic (panel A) and hemorrhagic (panel B) brain injury resulting in brain death. Panel A - from left to right: diffuse cerebral edema; generalized swelling and extensive ischemia; and large right middle cerebral artery distribution ischemic stroke. Panel B - from left to right: acute subarachnoid and intraventricular hemorrhage with subdural hematoma; large left subdural hematoma with midline shift and diffuse brain edema; large right basal ganglia bleed with intraventricular extension, midline shift, and diffuse brain edema (with courtesy of Dr. Spinello).

Relentless brain injury conduces to glial and neuronal membrane dysfunction, unstable ion pump and free water entrance to the cells, promoting edema and mass effect, raising intracranial pressure (ICP) and diminishing cerebral perfusion pressure (CPP). Intracranial hypertension (ICH) produces cerebral vascular auto-regulation impairment and vice-versa[25], which worsens with brain blood barrier disruption[26]; these situations can make intracranial volume raise continuously, conducting to more ischemia and cells death.

Cerebral circulatory interruption commences in vessels with the slowest blood flow, as the capillaries and small veins, which are more susceptible to intracranial hypertension[27]. When ICP reaches systolic arterial pressure values, there is the arrest of brain circulation and perfusion. Even after decompressive craniectomy (DC), BD is preceded by a severely reduced CPP, supporting loss of cerebral perfusion as a critical step in BD pathophysiology[28]. A cerebral blood flow (CBF) below 35 mL/100 g/minute correlates with cessation of neuronal protein synthesis; below 20 mL/100 g/minute with modification of synaptic transmission and absence of EEG activity, and <10 mL/100 g/minute with irreversible damage and neuronal death[29].

1.3.1 Cerebral death

The brain weighs approximately 2% of the body weight, consumes approximately 20% of the circulating oxygen to maintain its metabolic activity, which represents 15% of the cardiac debit[30]. It is comprised 86 billion neurons, 19% of which located in the cerebral cortex. In addition, there are 85 billion non-neuronal cells that provide integral support activities for the neuron[31]. This high demanded energy and complex prosencephalon network performs cognitive and integrative functions, in addition to hormonal balance[32]. Cognition is based in arousal and awareness to allow for reactions to sensory input and interactions with the environment. Hence, the destruction of human cortex leads to impairment of arousal and all the functions which depend on it (memories, emotions, attitudes, judgment and perception). Moreover, the loss of integrative function prevents a person from maintaining the physiological responses that allow maintenance of homeostasis, protective reflexes, and life.

Hormonal balance is controlled through a feedback loop between the brain and endocrine system. Neurons in the brain have receptors for hormones, which act to influence neuronal function and gene regulation that control the hypothalamic-pituitary axis. After brain injury, dysfunction of the hypothalamic- pituitary axis leads to neurogenic diabetes insipidus with a fall

in vasopressin, aggravating the decline of hemodynamic stability and increasing hypovolemia. In severe brain injury there is a high decrease of adrenocorticotrophic, thyroid hormone levels (particularly T_3), insulin and cortisol levels, which are associated with widespread cellular and mitochondrial dysfunction, promotion of inflammatory and immunologic activation[33].

1.3.2 Brainstem death

The brainstem integrates the most basic reflexive protective responses. The loss of brainstem function leads to hemodynamic, metabolic, and immunologic compromises[34]. Initially, a massive sympathetic stimulus “the catecholamine storm” leads to increased cardiac output and oxygen consumption followed by increasing of arterial blood pressure, heart rate, and multi-organ vascular resistance. This autonomic storm is followed by a second paralytic autonomic collapse with profound reduction in sympathetic neural output, loss of vascular tone and, consequently peripheral resistance, which leads to hypotension and diminished cardiac output compromising the perfusion of organs[35].

1.3.3 Systemic and metabolic changes

Brain-dead bodies have impaired capacity for homeostasis. Temperature, for instance, can be maintained at levels compatible with life, but only with the help of blankets, and still at a few degrees below normal. Blood pressure can also be maintained, with some sort of pharmacological assistance, as the “sympathetic storm” invariably develops[36]. Steroids are also needed to help maintain blood volume. Proportionate growth has been seen in the bodies of brain-dead children, but to a severely impaired degree[37].

In catastrophic brain damage with progressive and extensive ischemia, the concomitantly anaerobic metabolism conducts to tissue acidosis, with decreased cellular ATP and lactate accumulation conducting to free radical formation, free fatty acids oxidation and increased cytosolic calcium, leading to membrane changes, affecting the mitochondria and finally, apoptosis. Moreover, systemic phenomena as decreased insulin secretion and depletion of liver glycogen with hyperglycemia, are commonly seen in BD[38]. If arterial hypotension is present, the mentioned metabolic and inflammatory events following BD are aggravated, spreading to organs like kidneys and liver.

The activation of complement (further explained below) may explain myocardial damage also, as observed with elevation of troponin I serum levels. Immune factors infiltration in graft cells activates p53 and nuclear factor kappa B cell enhancer (NFkB) complex pathways, leading to the expression of a proinflammatory gene profile. Recent research on treatment of BD donor with complement antagonists reduced ischemia-reperfusion injury and acute graft rejection[39,40].

BD as consequence of TBI presents a more remarkable participation of the coagulation system. After cerebral injury, tissue-related thromboplastin is released, which initiates fibrin clotting via the extrinsic pathway, whereas activated platelets release procoagulant factors and inflammatory proteins. In rat kidneys, von Willebrand factor and fibrinogen are upregulated and accompanied by fibrinogen deposition in peritubular and glomerular capillaries. P- and E-selectin expression and tubular injury are becoming evident as early as 30 minutes after BD[41].

1.3.4 Immunologic and inflammatory reactions

A significant proportion of the current literature relies on animal models, interpretation of which is limited by both interspecies immune system

differences and experimental environment, moreover, interindividual variation in immune response has been demonstrated in human studies[42].

However, the main immunologic effects associated with neuronal and glial secondary injury mechanisms include a wide variety of processes such as depolarizations and disturbances of ionic homeostasis, release of excitatory neurotransmitters (e.g. glutamate), mitochondrial dysfunction, neuronal apoptosis, lipid degradation, and initiation of inflammatory and immune responses, among others. These neurochemical events generate a host of toxic and pro-inflammatory molecules such as prostaglandins, oxidative metabolites, chemokines and pro-inflammatory cytokines, as interleukin (IL) 1β, IL-6, IL-18, tumor necrosis factor α and IFNγ, lymphokines, increased expression of cell adhesion molecules P/E-selectin, widespread microvascular endothelial changes, macrophage-associated activation factors and other serum components as the coagulation system and complement cascade (particularly C3), both acting in the vascular endothelium, leading to lipid peroxidation, blood–brain barrier (BBB) disruption and the development of cerebral edema. The associated increase in intracranial pressure can contribute to local hypoxia and ischemia, secondary hemorrhage and herniation and additional neuronal cell death via necrotic and apoptotic mechanisms. Although each secondary injury mechanism is often considered to be a distinct event, many are highly interactive and may occur in parallel[43].

1.4 Death and brain death: history enhances diagnostics importance

Since over 800 years ago, when Maimonides codified the diagnosis of death as absence of the heart beat and respiration with cooling of the body[44], the understanding of death remained unchanged until the second half of last century. Thus, mistaking the diagnosis of consciousness disorders[45,46] or even death has been indicated by ancient reports of changed positions of corpses at exhumation and observation of scratches in coffins interiors (figure 2)[47,48];

even nowadays, there are reports of death, misdiagnosis, especially in cases of exogenous intoxication[49,50], and other death mimic situations[51].

Figure 2 - A vault built in 1890 for prevention of alive burials (Windsor, 1921)

Presentation of an unconscious, paralyzed patient, with fixed dilated pupils, no response to deep painful stimuli, and absent deep tendon reflexes leads most physicians to consider BD; however other causes that mimic death, such as poisoning should be considered. One example would be the substance tetrodotoxin (TTX), which is one of the most powerful neurotoxins known. It is about 1200 times more toxic to humans than cyanide and has no known antidote. This toxin binds to the sodium channels of the excitable tissues in the human body (muscles and nerves) and the inhibition of sodium ions through the channels effectively immobilizes these tissues. The severity of the symptoms induced by the TTX is dose dependent, being present for example in pufferfish of the family *Tetraodontidae* (figure 3), in the Californian newt *Taricha torosa*, starfish and other sea fruits, and produced by marine bacteria also. TTX is both water-soluble and heat stable so cooking does not negate its toxicity; rather it increases its toxic effect. Despite these, pufferfish remains a delicacy served in Asian restaurants, with frequent reports of intoxications.

Additionally to fish toxins envenomation, death or even brain death has been clinically misdiagnosed in cases of snakebites[52,53]. Progressive descending paralysis is characteristic of systemic envenoming by elapid snakes (cobra and kraits). Neuroparesis is due to pre-and post-synaptic blockade in krait and post-synaptic blockade in cobra bite. Muscles innervated by cranial nerves are involved earlier. The pupils and diaphragm are the most resistant to toxins. Ophthalmic manifestations of neurotoxin envenomation usually have the following sequence: ptosis, loss of facial muscle expression, partial ophthalmoplegia (usually VI cranial nerve with loss of eye movement toward midline and diplopia on lateral gaze), complete ophthalmoplegia with a fixed forward gaze and lastly fixed dilated pupils.

Figure 3 - Pufferfish (fugu), contains a toxin that is 1200 times more lethal than cyanide (https://innovationcompounding.com/naturally-dangerous-11-foods-to-fear/)

In 1947, when Claude Beck performed the first successful defibrillation of a human heart[54], death was made “reversible.” In 1950, Bower and Bennett developed positive pressure ventilation[55]. The first large scale produced ventilator, the Bird Mark 7, was introduced in 1955[56]. The development of mechanical ventilation and advanced life support therapies allowed the emergence of an unprecedented clinical condition: patients with irreparably compromised encephalon still preserved their main hemodynamic and ventilatory functions, exclusively due to the intervention of medical technology. Some would recover; others would survive with mild or severe disabilities or,

worse, remain in a vegetative state or in coma. Accurate prognostication was difficult but imperative.

In 1954, Robert Schwab, a neurologist at Massachusetts General Hospital, was one of the first to recognize this when evaluating a comatose patient with a massive brain hemorrhage on a respirator. “The question was, ‘Is this patient alive or dead?’ Without reflexes, without breathing, and with a total absence of evidence of an electroencephalogram, we considered the patient was dead in spite of the presence of an active heart maintaining circulation. The respirator was therefore turned off and the patient pronounced dead”.

Mollaret and Goulon, in 1959, when evaluating 23 patients in coma without response to the pain stimulus, without reflexes of the brainstem and with isoelectric electroencephalogram, named this condition as “*coma dépassé”*, the state just prior to the individual's total death[55], despite the fact that the same situation had been considered by Schwab five years before as death of the central nervous system, or brain death, being the first to use such an expression. Withholding life-saving technology was considered unethical and potentially illegal[57]; the “hopelessly unconscious” were considered essentially dead for some, whereas still alive for others, and the absence of definite guidelines on this issue promoted almost 20 years of constant debate.

In 1963, Guy Alexandre, a Belgium surgeon proposed 5 conditions for BD diagnosis: (1) complete bilateral mydriasis; (2) complete absence of reflexes, both natural and irresponsive to profound pain; (3) complete absence of spontaneous respiration for 5 minutes after cessation of mechanical respiration; (4) falling blood pressure, necessitating increased amounts of vasopressive drugs; and (5) a flat electroencephalogram. He emphasized that these conditions must be met, before the kidney removal can be considered.” Hence, Alexandre proposed one precondition (severe craniocerebral injury) and 5 criteria for BD diagnosis[58].

In that decade, more precisely 1966, Plum and Posner introduced the term “locked-in syndrome” to refer to a neurological condition associated with injury of the ventral pons, commonly an embolic occlusion of the basilar artery.

The syndrome was composed by preservation of consciousness and eye movements and blinking, with inability to perform any other action. Such diagnostic could be of challenge and missed if voluntary vertical eye movement is not assessed in patients who seem unresponsive[59].

However, only after the first heart transplantation performed in December 1967, the decision for a comprehensive and reliable definition of the irreversible coma state arose. The Harvard Medical School in 1968 published the first formal criteria for diagnosis of brain death. The Royal College of Medicine in Great Britain introduced the apnea test in 1976, the need for knowledge of irreparable damage to brain tissue, as well as the exclusion of reversible causes for coma, the demonstration of absence of brain stem reflexes, and the option to use other complementary tests besides the electroencephalogram. In this consensus, spinal reflexes were also excluded as impediments to the diagnosis of BD[60].

Later, in 1995, the American Academy of Neurology (AAN) published a review of the medical literature defining the criteria used today, and in 2010, the same committee published a new review, including studies beginning in 1996, to find some recovery of neurological functions after the diagnosis of BD, and none was found [34].

In Brazil, the first BD resolution in 1991 excluded children under two years of age, even though the concept had been approved in the literature for children over 7 days of age since 1987. Decree-Law No. 9,434, 1997, provides for the removal of organs, tissues and parts of the human body for the purpose of transplantation and delegates, in its article, the Federal Medical Council (CFM) the normalization of the diagnosis of BD. This standardization was established in the CFM's publication guideline 1.480/97[61], and updated in 2017[62]. Since the elaboration of BD criteria according to Harvard school, these have been modified in order to raise the rigor to perform this diagnosis.

1.5 BD mimics

It is essential that professionals from emergency rooms and intensive care units be able to recognize and assess correctly comatose patients. Each potential factor involved must be brought to light, to avoid mistaken diagnosis. As mentioned above, locked-in syndrome, rattlesnakes and pufferfish envenomation have been reported mimicking BD, but those examples are not the only ones. Guillain-Barré syndrome, hypothermia, exogenous intoxication and hypokalemia[63] have also been involved. Spinal reflexes may be complex and lead to misinterpretations on brain death diagnosis[64-66].

1.5.1 Guillain-Barré Syndrome

The Guillain-Barré syndrome (GBS), is commonly an ascending acute inflammatory polyneuropathy, despite this entity may present a wide range of varieties. GBS has been reported mimicking BD [67,68]. The variant Miller-Fisher syndrome per example, in addition to the limbs palsy, these patients develop ophthalmoplegia, and Bickerstaff encephalitis points to disturbance of consciousness and even brain stem reflexes suppression[69]. The occurrence of a complete “locked-in” syndrome harbored in GBS is not an infrequent situation, been reported previously. The key for avoiding misdiagnosing this condition, and consequently a disastrous outcome, is always to proceed with caution when obtaining clinical history of comatose patients[70,71].

1.5.2 Hypothermia

Hypothermia has been described as the condition most capable of BD simulation, in out of hospital cases or even after post-cardiac arrest hypothermic rescue therapy. Moreover, drug metabolism in this situation is slow, and CNS depressors may last active for longer periods[72]. Hypothermia is defined as body temperature less than 35°C and it is divided into 3 stages: mild hypothermia, when the body temperature is between 35°C and 32°C, moderate hypothermia when the body temperature is between 32°C and 30°C,

and deep hypothermia when the body temperature is less than 30°C, but even with 32ºC is possible the pupils response to light to be absent, with abolition of brain stem reflexes in sequence. However, the encephalic impact on extreme hypothermia may be reversible.

Therapeutic hypothermia post-cardiac arrest has become a standard procedure due to its neuroprotective effect. Diagnosing BD in these patients is more challenging, and an ancillary test disclosing cerebral circulatory arrest should be performed instead of the EEG[73]. The neuroprotective effect of hypothermia is affiliate to reduction of the cerebral metabolic rate, release of excitatory transmitters, ions influx, lactic acidosis and vascular permeability, mechanisms particularly important in the ischemic penumbra.

The AAN guidelines indicate to exclude assessing patients with hypothermia; the neurological assessment should be performed exclusively after normalization of core temperature[34].

1.5.3 Drug Intoxication

Despite absence of brain stem reflexes is possible, but pupillary response to light should remain in drugs overdoses, formal determinations of brain death have been reported in cases of intoxication with bretylium[74], tricyclic antidepressants[75], valproic acid[76], baclofen[50,77,78] and barbiturates[79].

The AAN guidelines also adopted in Brazil, recommend excluding the presence of a CNS-depressant drug effect by history, drug screen, and calculation of clearance using 5 times the drug's half-life, provided that the elimination of the drug is not interfered with by other drugs, organ dysfunction, or hypothermia, if available, drug plasma levels below the therapeutic range[80].

If the particular drug, or even the cause of coma are not known, but high suspicion persists, the patient should be observed for 48 hours to determine whether a change in brain stem reflexes occurs; if no change is observed, a test of cerebral blood supply should be performed[81,82].

1.6 Diagnosis of brain death: current picture

Aristotle defined soul as being a body (matter) having life in its interior, being three levels of the soul, in degree of relevance, the inferior, or nutritive, refers to the capacity for nourishment, reproduction and growth, then the soul sensory, related to the perception and the movements that only a living being can present, and the superior, or rational, the intellect, that differs the human beings from other living beings[83].

Metaphysician Thomas Aquinas defined the human being as a composite of soul and body and, the determination of death requires incontrovertible evidence that the body has ceased all the operations that correspond to the soul's proper capacities. The evidence of this is the body's loss of its integrative organic unity and the criterion for determining when this loss occurs is the irreversible cessation of whole-brain functioning[84].

The absence of respiratory effort or heartbeat is clearly understood by most people as synonymous with death. However, the main difference between humans and other living beings is the continuous activity of a highly specialized and interconnected network of trillions of neurons located in the brain. damage to the brain less than total brain death can result in loss of capacity for rational operations, whereas total brain death results in the loss of the radical capacity for conscious sensation[85].

BD has been an accepted but not consensual fact in the world[10,86]. Five years ago, more precisely, December 2013, a particular case extrapolated frontiers in constant debate[5]. Jahi McMath, a teenager from California, USA, received the diagnosis of BD after suffering cerebral anoxia as a complication of an abundant bleeding after tonsillectomy for treatment of obstructive sleep disorder. This diagnosis was made according to accepted standards, by two physicians, one being a neurologist, in addition to an electroencephalogram (EEG) that demonstrated the absence of cortical electrical activity. The stalking began because the family disagreed with the diagnosis, and a legal battle was started to prevent the disconnection of Jahi's breathing apparatus that

culminated to the patient's transferring to another hospital in another state (New Jersey), whose local laws allow the family will to prevail over the medical decision[11].

After viewing over four dozen independent videos of McMath, Dr. Alan Shewmon, a pediatric neurologist, declared her technically alive, in minimally conscious state in June 29, 2017[87], stating that the girl follows movement commands and exhibits "other proof of life". McMath died on June 22, 2018. She was having internal bleeding due to kidney and liver failure, so her doctors removed her from life support, allowing her to die.

Again referring to Aristotle's philosophies, albeit millennial, they bring to light the possibility of distinguishing between the true persistent vegetative state in which the individual preserves only part of his nourishing soul, and the case of Jahi in which the suffering of her brain was apparently sufficient to produce an intermediate state between the sensitive and rational soul, not being able to breathe naturally, but keeping intact its hypothalamic-pituitary-ovary-uterus axis, not demonstrating electrical activity to the EEG, but presenting slight movements of upper and lower extremities only in the presence of his mother.

The diagnosis of brain death is primarily based on clinical examination, including the evaluation of brainstem reflexes and an apnea test. The implementation of ancillary testing to clinical examination for the BD diagnosis has been adopted in many countries around the world and it has become obligatory in some of them[88-91]. Indeed, the absence of electrical activity on electroencephalography, or the lack of brain circulation on echographic and/or imaging exams are often used to confirm the diagnosis of BD or when the clinical assessment cannot be adequately performed, e.g. severe facial trauma, severe damage to the brain stem exclusively, when therapies like artificial coma and hypothermia have been applied, or the impossibility to perform the apnea test because of severe hypoxemia[92].

In Brazil, BD is legally recognized as equivalent to death[17], and the complete evaluation of this condition must be performed in every hospitalized individual that raises the suspicion of interruption of the neurological functions.

Just as the diagnosis of a particular disease is important for every individual in distress, the same importance is found in the definition of encephalic death. This represents a reduction of dysthanasia, psychological stress to which the patients' relatives are subjected, expenses with resources improperly applied to the artificial maintenance of life, as well as an increase in humanitarian donation of organs and tissues to those who hopefully wait in the endless queues.

As equivalent to death, BD diagnosis is mandatory when a strong suspicion is present, for the reasons stated above. On the other hand, some scholars believe the withdrawal of life support brings more suffering to the patients' families, and state that prior to proceeding to BD assessment, an informed consent should be obtained from relatives[3].

To perform a diagnosis of BD in Brazil, it is necessary to meet all of the following criteria; (1) to know the cause of coma, (2) to remove hypothermia or central nervous system (CNS) depressants, (3) to perform the evaluation of apperceptive coma and brain stem reflexes, absence of supraspinal motor activity by two different doctors and with an interval of 1 hour for those over 2 years of age, 12 hours for children between 1 and 2 years incomplete, 24 hours for babies between 2 months and 1 year of incomplete life and 48 hours for babies between 7 days and 2 months, including one apnea test for all ages, and finally, (4) perform an auxiliary graphical examination that demonstrates the absence of electrical activity, metabolism, or cerebral blood flow, and, for children under 1 year of age, only the EEG is accepted.

As stated above, contrary to the British concept that for BD the absence of brain stem activity is sufficient(60), the American concept considers the absence of activity in the entire brain, and the coma finding is not influenced by external factors as evidence of absence of cortical function[93]. In Brazil, currently, the concept of interruption of the function of the whole brain is the rule. However, unlike what is practiced in some states of the United States of America, the absence of cortical viability must necessarily be demonstrated by a complementary examination in order to justify possible controversies due to

different degrees of education of the population, veracity of information or religious beliefs.

1.7 Organ transplants: history and ethics

Successful organ transplantation and BD, which came first? Although not directly linked, history demonstrated that the ultimate definition of BD was finally achieved because of the interest in organ disposal and transplantation.

Eduard Zirm, an Austrian ophthalmologist, performed in 1905 the first corneal transplant, restoring the vision of a man who had been blinded in an accident. On December 23, 1954, the first successful live Inter kidney transplant was led by Dr. Joseph Murray and John Merril at Brigham Hospital in Boston[94]. One kidney was transplanted from Ronald Herrick into his identical twin, Richard, who lived for another eight years after the procedure. Dr. Murray, concerned and not wishing to be the promoter of unethical behavior, said; "As physicians motivated and trained to restore people's health, it becomes a diversion to our targets to risk the health of a healthy individual, no matter how pure our purposes". These same doctors performed the first successful kidney transplant from a deceased donor in 1962[95].

In the following year, 1963, the first successful lung transplant was led by Dr. James Hardy at the University of Mississippi, removing the lung from a cadaveric donor by cardiorespiratory criteria to the consequence of acute myocardial infarction. The first successful liver transplant was led by Dr. Thomas Starzl at the University of Colorado in 1967. Extraction of the liver was performed from a cadaveric donor immediately after cardiorespiratory arrest. In that decade, the debate around ethical questions concerning transplants intensified, for the withdrawal of a vital organ would invariably lead the provider to death, and "who would have the power to decide on the life of a human being?". The very unsatisfactory results obtained by the first transplants, possibly due to the difficulty of preserving an organ withdrawn from a cadaveric donor, increased the attention of the medical community with respct to

individuals suffering from irreparable severe neurological disease under ventilatory support that maintained the viability of their other organs, an entity devoid of definition, considered by some "irreversible coma" and by others "brain death".

It can be said that the climax of the discussion was due to the first successful heart transplant, led by Dr. Christian Barnard at Groote Schuur Hospital in Cape Town, South Africa. Dr. Barnard, due to the urgency of the transplant he was about to perform, administered potassium to a patient whose brain's activity had stopped due to severe head trauma, causing cardiac paralysis, and then removing her heart[96].

Subsequently, Harvard Medical School published in 1968, an attempt to define irreversible coma as a new criterion for death, because "obsolete criteria for defining death could lead to controversy and obstacles to obtaining organs for transplants". Finally, in 1981, the American Congress approved the new definition of death, which included cardiopulmonary or neurological criteria, opening the way for the removal of organs from patients whose vital functions were maintained exclusively by artificial means[97].

1.8 Organ transplants: current aspects

After a period of experience, organ transplantation, evolved successfully to a stage that guidelines are very consistent and currently the main issue worldwide is organ availability[98]. Figure 4 shows current Brazilian organ transplantation waiting list. To date, organ sale is illegal everywhere, except in Iran[94,99]. Consequently, organ donation is crucial for transplants, and the degree of confidence in the diagnosis of BD is directly related to acceptance for organ donation. Around the world, campaigns have been done to educate and raise rates of organ donation[100] (figure 5).

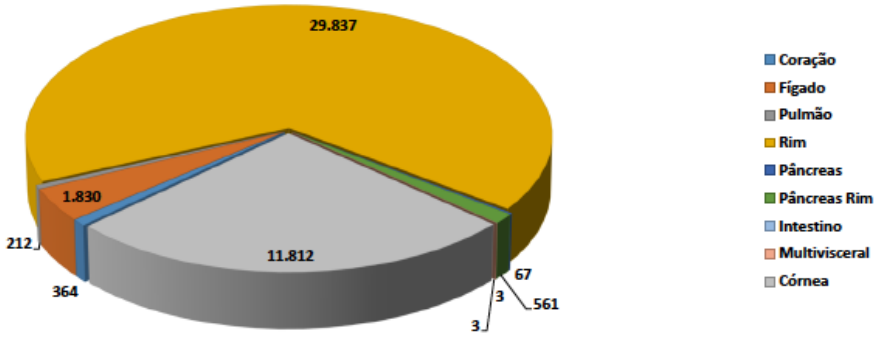


Figure 4 - Real numbers and percentages of patients waiting for transplantation in the year of 2019 in Brazil. Yellow: kidney, light gray: cornea, orange: liver, green: kidney plus pancreas, blue: heart, dark blue: pancreas, light blue: intestine, dark gray: lung, dark yellow: multi-organ. Source: Organ Procurement Central, Brazil http://saude.gov.br/saude-de-a-z/doacao-de-orgaos#estatisticas.

Although the US transplant waitlist consists of roughly 115000 people, this represents only a fraction of the true need for organ replacement. Only approximately 50000 people are added to the US transplant waitlist each year, yet over 700000 US deaths per year are attributable to end-stage organ disease[101]. Some estimates suggest that as many as 30% of deaths in the United States could be prevented by organ replacement with supply and technology constraints removed. The unmet need is far greater worldwide; globally, deaths from organ impairment or from other causes theoretically addressable by organ transplantation are above 15 million per year[102].

Figure 5 - Organ donation campaigns in Brazil (left) and Italy (right). Available at www.transplantes.pe.gov.br and http://gallery.wacom.com/gallery/49400611/AIDO-organ-donation-campaign.

Altruism is an integral part of organ donation and transplantation; hence, it remains a key principle for it. In no other surgical field does solidarity play

such a crucial role, transforming the sacrifice and grieving into therapy and life. Altruism, solidarity, and collaboration among different subjects are particularly embedded in the complex multidisciplinary structure of transplantation, which is an emerging field that brings profound ethical issues.

The complexity of this structure starts with the initial care of the patient at risk of evolving to BD. Many patients exhibit a state known as "imminent brain death" from which they might pass to the status of possible organ donors[103,104]. This condition should be clearly defined and recognized in critically ill patient care services, and it should be emphasized that these patients are not brain dead and thus must receive the required intensive care until irreversibility is confirmed[14,17]. Posterior to BD confirmation, the potential organ donor needs to be cared to the most physiological conditions possible, as these individuals usually present an expected pattern of complex multiple organ failure, hence an appropriate support to the donor before and after brain death can increase the number and quality of organ donors[105]. The medical management of organ donation can be broadly divided into cardiovascular, hormonal and metabolic derangement management, temperature and respiration maintenance, hematological parameters, and nutritional support. Early identification of organs for donation helps to optimize the medical strategies[106].

Many advances in transplant procedures enabled the patient with end stage organ disease to have an outcome that conventional therapy alone could not have achieved. In Brazil, heart, liver, lung, pancreas, cornea, and kidney transplants added up to 2,588 procedures in 1995. In 1997, with the establishment of the National Transplant System (SNT), the number went up to 3,777. Since then, the growth of a decentralized network of collaborators operating at a national, regional and intra-hospital level, raised the number of transplants in Brazil to 22,737 in 2012. The permanent challenge is to keep the efforts to continuously improve Brazilian population’s education about organ donation and brain death definitions, as well as training physicians to better recognize and prioritize this condition[107].

Mortality from violent causes has significantly increased in Brazil, as well as the number of deceased-donor organ transplantation (figure 6)[108]. Besides

primary CNS diseases, injuries by external mechanisms are frequent causes of BD, and for being more often in the younger population, mostly, these donors provide healthy organs. A significant number of head-injured trauma patients are likely to present a positive toxicology; however, this condition is not related to diminished organ donation and poor graft outcomes[109].

Nevertheless, the greater availability of organ donors is not the only factor to be considered for the growing number of transplant procedures; there is also the effective job done by the integrated transplant network that has been decisive in the materialization of this outcome.

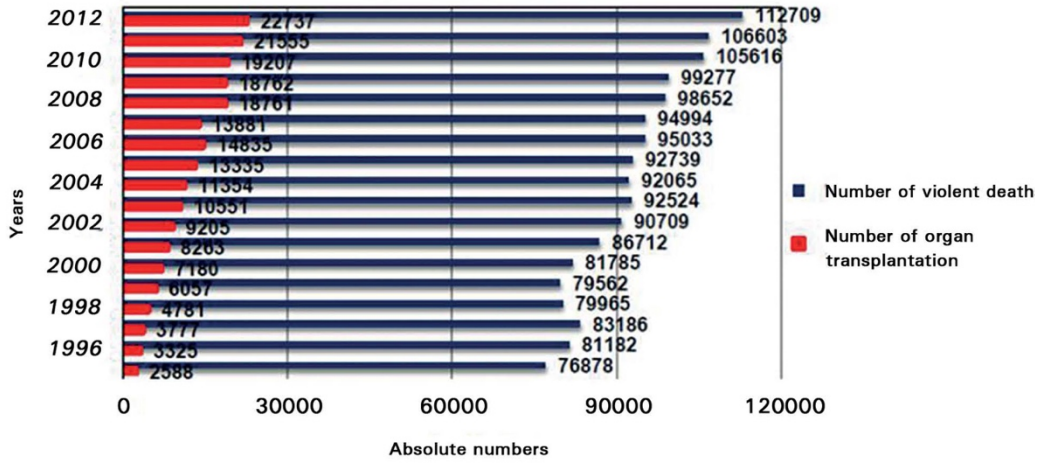


Figure 6 - Violent deaths have raised proportionally as transplants in Brazil, 1995-2012 (Courtesy of Dr. Oliveira)

1.9 Ancillary tests in BD diagnosis

Currently, there are not enough controlled studies on the most commonly used complementary methods for BD diagnosis[93,110-113]. In the United Kingdom, irreversible loss of brainstem reflexes is sufficient for the diagnosis of BD[4,13], and additional examinations are optional[15]. On the other hand, in Brazil, for example, the diagnosis can only be concluded by demonstrating that the whole brain is unfeasible, which makes diagnostic tests mandatory, being TCD, EEG and angiography the most commonly used methods.

Below, we describe some of the most recommended and/or applied BD ancillary techniques worldwide. Besides the techniques listed, jugular venous

oxygen saturation, bispectral index scale (BIS) and ICP register are methods not recommended to the date, despite their ability to alert physicians to the onset of BD[34,114].

1.9.1 Digital subtraction angiography (DSA)

DSA remains the gold standard for BD diagnosis complementation[115,116]. This technique was created in 1927 by portuguese physician Egas Moniz[117]. Catheter angiography is an invasive, time consuming procedure, which needs an experienced neuroradiologist, the availability of an angiography room, patient transportation and the use of high amount of contrast material[118]. As for all techniques that analyses brain blood circulation, it is imperative the blood pressure to be monitored, as such patients can be hemodynamically unstable.

Early recommendations suggested that a certain amount of time, e.g., one minute, should elapse before concluding that the contrast material does not enter the intracranial cavity. A limitation for all techniques in the assessment of brain circulation, is the possibility for brain death to exist without intracranial pressure exceeding mean arterial blood pressure; there can also be a gradual evolution from some intracranial arterial filling, especially in cases of large skull defects and severe damage to the brainstem exclusively[119]. Sawicki et al.[120] investigated the delayed filling phenomenon using CTP and concluded that the mean blood circulation time in this phenomenon is incompatible with neuronal survival.

Radiation exposure isolated to the head is not hazardous in the case of organ donation, and even exposure of the whole body has not been demonstrated to be harmful on transplantable organs to the date. Further limitations of DSA include the need of an expert to perform imaging, the DSA room and device (costly impact), patient transportation.

1.9.2 Transcranial doppler (TCD)

Transcranial Doppler is safe, non-invasive, relatively inexpensive, and can be done without transporting the patient out of the ICU[121-124]. The test requires skill and rigor in its application to insonate the major intracranial arteries. Visualization of systolic peaks or an oscillating or reverberating flow pattern in the middle cerebral arteries and basilar artery is a confirmatory test for cerebral circulatory arrest in brain death diagnosis[125,126] (figure 7). Patients with external ventricular drains, large craniotomies should not be excluded, although in these conditions the exam may be falsely negative, such as with DSA[127,128]. Caution should be exercised with very young children because of skull compliance. In patients lacking good bone windows at TCD, with observed circulatory arrest in the cervical internal carotids and vertebral arteries it could be recommended[121,123,129]. Two meta-analysis found similar results, about 90% sensitivity and 99% specificity for this technique[130,131]. The main limitation of TCD remains in the lack of acoustic temporal windows, its operator dependence, since external arteries may be confounded, decreasing its sensitivity.

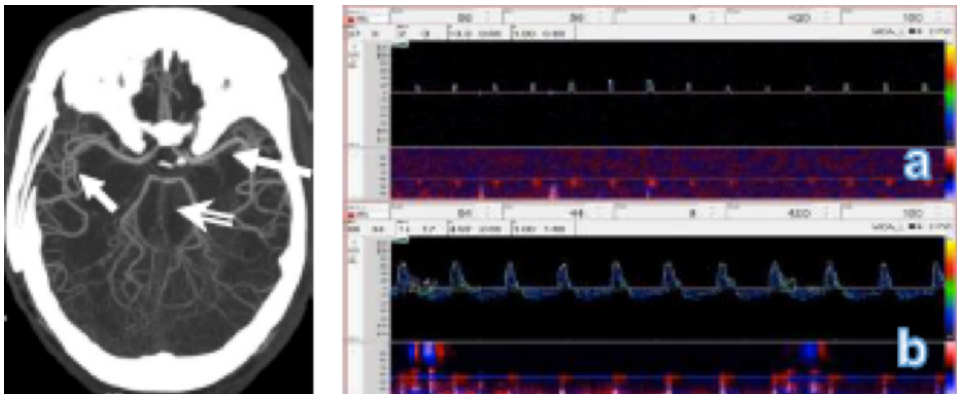


Figure 7 - TCD arterial collapse was verified by the presence of systolic spikes (a) or non-progressive oscillatory flow (b), in the middle cerebral arteries (arrows) and basilar artery (arrowhead)

1.9.3 Magnetic resonance angiography (MRA)

As with CTA, the AAN currently does not support the use of MRA as an ancillary test in the diagnosis of BD[34]. Magnetic resonance imaging (MRI) has the advantage of not requiring potentially nephrotoxic contrast material. Patients with brain death have widespread reductions in the apparent diffusion coefficient (ADC)[132,133]. Magnetic resonance angiography appears to identify brain death with a relatively high sensitivity and specificity, regardless of whether it is performed using time-of-flight algorithms or gadolinium enhancement. As with other forms of angiography, there may be some filling of proximal intracranial vessels[134,135]. One study performed MR venography, with the finding that brain dead patients sometimes had filling of the superior sagittal sinus, which was thought to be due to drainage from meningeal vessels[134]. The large duration of image acquisition, availability, patient transportation, costs and mainly the inability to recognize blood flow if extreme slow, are the main limitations of MRA.

1.9.4 Electroencefalogram (EEG)

EEG was the first ancillary technique applied to assess BD. It measures the summation of synaptic potentials in the cerebral cortex closest to the brain surface. As with physical examination, subcortical structures are not necessarily well assessed. The characteristic EEG finding in brain death is electrocerebral inactivity or electrocerebral silence, which is defined as the absence of EEG activity exceeding 2 μV in amplitude[136], when recording from scalp electrode pairs 10 or more centimeters apart, with interelectrode impedance below 10,000 Ohms.

However, its character liable to artifacts such as the contamination from the power supply and ECG sedative drugs or other factors has made this method less recommended[110,111,137,138]. There are few studies that examine the validity of EEG in brain death[139]. Furthermore, in the hostile electrical

environment of the ICU, some electrical signals are recorded for which the source cannot be identified, even though they probably do not arise from the brain (more false negatives). This probably leads to the considerable variation in inter-rater agreements as to whether electrocerebral silence is present or not.

Because of the limitations of EEG, many jurisdictions no longer consider it to be an appropriate confirmatory test in the diagnosis of brain death, favoring modalities that assess brain blood flow[140].

1.9.5 Nuclear medicine perfusion test (NMPT)

Radionuclide imaging techniques are frequently applied to brain death assessment[141,142]. The agents penetrate brain parenchyma in proportion to regional blood flow with SPECT, being currently 99mTc- labelled hexamethylpropyleneaminoxime (HMPAO) or ethyl cysteinate dimer utilized, because these agents remain per hours in the parenchyma. This technique demonstrates the presence or absence of brain perfusion even of the posterior fossa[143], which is more interesting than displaying encephalic circulation exclusively[113,144].

Despite a promising technique, studies on this subject to the date are few and of small samples[145]. Bertagna et al. have shown persistence of viable spots of brain tissue in patients clinically brain-dead on SPECT[146]. Obviously, the main limitation of this method is the lack of availability and practicality, since it demands a short half-life radioisotope to be performed and being also high costly.

1.9.6 Somatosensory evoked potentials (SEP)

SEP and brainstem auditory evoked response (BAER), like EEG, have limitations in their suitability as ancillary tests. This technique tests only a

discrete region of the brain, with high sensitivity, despite poor specificity[147], such that the absence of EPs does not prove brain death.

Each test activates a discrete sensory pathway. Consequently, evoked potentials (EPs) allow examination of specific areas of interest in the brainstem, but these are individually highly restricted specific pathways[148]. Therefore, EPs do not test the functional integrity of other central nervous system structures. Discretely placed proximal lesions along the pathway may selectively eliminate the evoked responses from that site onwards, while sparing other brain stem structures.

Unlike EEG signals, the early components of SEPs are minimally affected by sedative drugs and anesthetics. However, drugs and metabolic derangements affect middle and late somatosensory and auditory potentials. Wave I of BAERs, generated in the cochlear nuclei outside the brain stem, assures that the auditory signal has been processed up to the brain stem. Its absence could relate to end organ dysfunction, e.g., damage to the inner ear by trauma. Thus, when wave 1 is absent, BAERs are not appropriate for assessment for BD.

Although EPs offer some advantages over EEGs, they do not seem to be sufficient stand-alone tests for brain death. Some have argued that the combination of BAERs and SSEPs offer greater assurance of an accurate diagnosis of brain death. However, since BAERs can only be applied in a minority of patients because of the necessity of requiring wave I to be present bilaterally, it is unlikely that this will be practical. The sensitivity and specificity of the combined use also needs to be determined. Studies of the value of EPs in brain death are very limited in scope[149]. Most have examined a group of clinically brain-dead patients to examine the association with the presence or absence of responses.

1.9.7 Computed tomography perfusion (CTP)

CTP is able to report isolated brainstem death[90]. The technology is capable of discriminating between severe hypoperfusion (2%, 1.2 mL/100

g/minute) from an absence (0%). CTP has been used to evaluate stasis filling phenomenon observed in CTA and DSA, showing nonviability of the brain, indicating that stasis filling does not preclude the diagnosis of BD. Whole-brain CTP is a highly sensitive and specific method for diagnosis of BD. CTP used together with CTA may increase the sensitivity of the test, instead this technique is less available than CTA, also demands patient transportation and contrast administration[150].

1.10 Computed tomography angiography (CTA)

In 1998, Dupas et al. introduced the use of contrast-phase computed tomography on the evaluation of BD[151]. At the beginning, the understanding was that the absence of visualization of the pericalosal arteries, the middle cerebral arteries (MCAs) and the internal cerebral veins (ICVs) bilaterally, as well as the Galen vein were required to issue a BD diagnosis with this technique, this score was termed 7-point score. The visualization of any of these vessels should preclude BD diagnosis by CTA technique. In 2006, Leclerc et al.[152] concluded that the best CTA criteria for confirming BD is to verify the absence of opacification of the cortical portions of the ACMs and the ICVs (figure 8), and the French Society of Neuroradiology in 2007 elaborated the first consensus on the diagnostic criteria of BD by CTA[153]. In addition, the consensus of French experts advises to perform CTA of the chest and abdomen in potential organ donors in order to detect possible inadvertent lesions[154,155] in transplantable organs.

In 2010, Young[156] suggested that cerebral venous drainage should be the focus of investigations in BD because of the presence of cortical contrast uptake in a number of correctly diagnosed patients. Moreover, Marchand et al. in 2016 proposed a modified venous 4PS based on the evaluation exclusively of superior petrosal veins (SPVs) and ICVs, with the effort to assess infra and supratentorial drainage[157], obtaining 85.7% sensitivity with this revised score, and 98.1% of absence of ICVs opacification.

Between 2015 and 2016, Brasil et al., Taylor et al. and Kramer et al. concomitantly published meta-analyses regarding the use of CTA and CTP for the diagnosis of BD. These reviews concluded that the routine use of CTA as a diagnostic method for BD is not recommended until the criteria of BD by means of CTA have been prospectively validated[1,158,159]. The articles included in all reviews were the same, with the exception of one abstract[160], and one retrospective study of CTP in 11 patients[161], identified by Kramer et al.[162].

The quality check of the studies obtained through QUADAS 2[163] tool revealed methodological deficiencies, especially due to the lack of information on (1) how the clinical evaluation was performed, (2) whether the radiologists involved were "blind", and (3) whether the patients were selected consecutively. In addition to the above, (4) the interval between CTA or TCP and the reference test was too long in some cases, and (5) CTA and TCP were exclusively performed in patients diagnosed with clinical brain death. Only one study[164] included controls in ideal conditions for comparison between groups, although we noted the risk of selection bias. We also observed heterogeneity among the studies with reference to the criteria used to define an examination as positive for BD. Moreover, the reference tests used were different within the same study in some of these.

The reviews supported the hypothesis that CTA could be used as an adjunct method of intracranial circulation interruption in those cases when a patient had a complete clinical evaluation compatible with this diagnosis. However, when it comes to the decision to withdraw ventilatory and medical support from an individual, it is imperative that the method used obtain false positives close to zero[165], and with the studies published to the date, CTA specificity was not measured.

Limitations of CTA observed in previous studies rely on impossibility of this technique to adequately evaluate brain microvasculature, but in the vast majority of BD cases gathered by the systematic reviews, it has demonstrated a total absence of visualization of the cerebral arteries or only visualization of proximal portions of the Willis polygon vessels instead[1].

A phenomenon previously described as stasis filling in angiographies was reported in these studies in a similar way. This phenomenon is due to the fact that a small portion of the contrast medium can be led into the skull by collaterals from the external carotid artery[116,166]. Consequently, proximal portions of the Willis polygon vessels can be contrasted even in patients with brain death. In a postmortem study, the blood filling of the large basal arteries of the encephalon was verified for up to 24 hours after cardiac arrest[167]. Sawicki et al. verified the inconsistence of stasis filling with CBF[27]. Hypothetically, deep cerebral venous thrombosis could be an impediment to CTA, although damage to the basal and midbrain veins rarely obstructs deep drainage completely[168].

CTA presented similar characteristics to other methods used to evaluate brain blood flow[169]. It is not able to measure average blood flow time or show blood vessels if blood flow is too slow. However, similar to other methods for assessing cerebral blood flow, including DSA, the cerebral blood flow may not be completely interrupted at the time of the clinical diagnosis of BD. This is particularly true if intracranial pressure has not yet risen to critical levels, leading to cessation of intracranial circulation[115,170]. In cases of cranial defect, such as extensive fractures or after decompressive craniectomy, time to complete BD by means of the 4PS can be extended at least until the soft tissues undergo maximum stretching. This condition may allow the cortical arteries to be filled by contrast for a longer time, although the deep venous drainage, appears to cease precoceously[157].

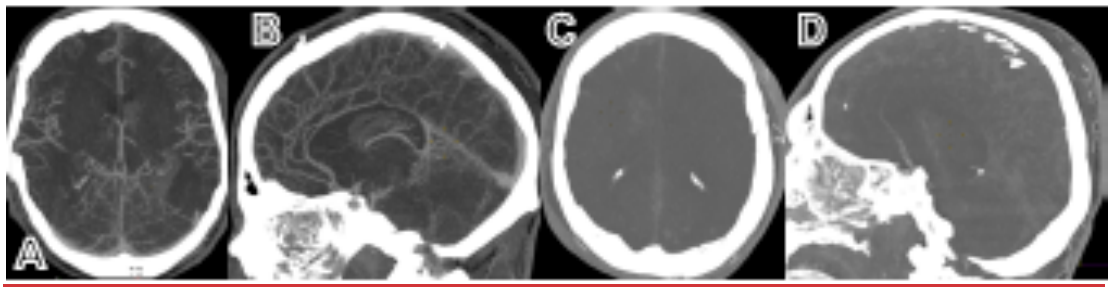


Figure 8 - A and B showing intracranial opacification. C and D do not show intracranial distal arteries or encephalic drainage

In recent years, many studies on the use of computed tomography angiography (CTA) for BD diagnosis have been published[141,151,152,171-179], leading this method to be accepted by some scientific societies[175,180,181]. However, it has been neglected by others because of controversial results[93,165,170,182-184].

The initial score based on the absence of opacification of 7 vessels (7-point score) proposed by Dupas et al. in 1998[151], including the pericalosal arteries, MCAs, ICVs and basilar artery, given 1 point for each vessel visualized and needing 0 points to confirm BD, has been replaced by the 4-point score based exclusively in the absence of opacification of the MCAs and ICVs[185]. Nevertheless, CTA has not been investigated in large controlled studies for the diagnosis of BD[88,186].

2 STUDY HYPOTHESIS

2 STUDY HYPOTHESES

The three systematic reviews published on this subject indicated lack of evidence for CTA in this setting, although it has been suggested as a promising method to confirm intracranial circulatory arrest[88,159,162]. Moreover, Brasil *et al.* in meta-analysis of 321 patients observed the absence of visualization of deep brain drainage through the internal cerebral veins should be the CTA criteria of higher sensitivity. The hypotheses of reliability and definition of best criteria motivated the present study, which aimed to evaluate prospectively the accuracy of CTA in the diagnosis of BD and to define the most reliable CTA criteria for this purpose. This study was elaborated as an effort to satisfy as many reported gaps as possible, by means of an unprecedented methodology, verifying CTA criteria currently applied for this purpose, and the possibility of proposing new criteria.

3 OBJECTIVES

3 OBJECTIVES

3.1 Primary

To assess CTA reliability for BD diagnosis

3.2 Secondary

To define the optimal tomographic criteria of intracranial circulatory arrest.

4 METHODS

4 METHODS

4.1 Study population

This was a prospective, controlled, observational study approved by the local ethics committee, and the legally authorized representative (LAR) consent for each patient was included. The study protocol followed the Standards for Reporting of Diagnostic Accuracy Studies (STARD) statement[187] (Addendum A). The study protocol was registered on ClinicalTrials.gov under number 12500913400000068. Our inclusion criteria were deep comatose patients without signs of BD (i.e., Glasgow coma scale [GCS] 4 or 5 and pupillary reactive GCS 3) and deep comatose patients presenting with the first signs of BD (i.e., GCS 3 with mydriasis) who were admitted in the intensive care unit (ICU) of Hospital das Clínicas of São Paulo University, Brazil, between January 2014 and January 2018. The clinical assessment for BD, as the reference standard, defined each patient to belong to the BD group (case) or no-BD (control) group. Our exclusion criteria were contraindications to contrast injection (i.e., known allergy or acute kidney injury stage ≥ 2 [AKIN ≥ 2][188]), hypothermia (Tax < 35ºC) arterial hypotension (i.e., mean arterial pressure below 65 mmHg) whether prior or during CTA scanning, absence of intracranial sonographic window for TCD assessment, LAR refusal and BD already being diagnosed.

4.2 Study design

Intensive care physicians and the neurosurgeon were involved in the selection of eligible patients, (RAGO, PFT, RAB, LMSM and WP) forwarding them to CTA scan and TCD. TCD was used as our comparative technique due to its practicability, high sensitivity, and specificity[131]. An experienced

transcranial Doppler (TCD) operator (SB) performed all sonographic assessments, and two physicians blinded to the TCD and CTA findings repeated the neurological evaluation for each patient (figure 9). Physicians were aware not to proceed with the complete neurological evaluation for BD until CTA and TCD had been obtained. Two neuroradiologists (GG and DMN) evaluated all CTA images. TCD and CTA analyses were performed blindly with regard to the patient's clinical status. For study purposes, neurological assessment was always aborted when a non indicative sign of BD was present (i.e., GCS 4 or 5, GCS 3 with reactive pupils, etc.). The reasons for including no-BD patients were for CTA specificity calculation and to avoid influence on the image interpretations of the neuroradiologists.

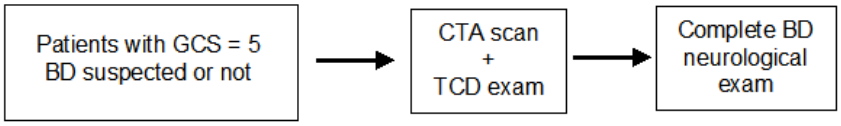


Figure 9 - Flowchart. Obtaining index study imaging (CTA) was fundamental prior to complete BD assessment
BD: brain death, CTA: computed tomography angiography, GCS: Glasgow coma scale, TCD: transcranial doppler.

4.3 Clinical data

We collected demographic data and the Simplified Acute Physiology Score 3[189] on admission (addendum E). Patients with traumatic brain injury (TBI) had the severity of trauma reported by the Marshall scale[190] (addendum F), and subarachnoid severity was reported using the Fisher CT scale[191] (addendum G). We also recorded age, gender, and the cause of brain injury.

4.4 Definition of BD

The clinical diagnosis of BD was our gold standard, and it was based on: a) the identification of the cause of brain injury; b) the absence of hypothermia

(body temperature < 35ºC); c) the absence of CNS depressors; and d) the absence of brainstem reflexes, such as pupillary reactivity, corneal, oculo-cephalic, oculo-vestibular, cough and gag and a positive apnea test (i.e., acidosis and $PaCO_2$ of at least 55 mmHg with initial values < 40 mmHg). The assessment of brainstem reflexes followed this sequence, with no need to proceed to next reflex evaluation if there was presence of the preceding reflex. In case a patient had been administered sedatives, the evaluation for BD was performed after at least 4 half-lives of drug elimination. The entire clinical assessment for BD was performed twice for each patient by different physicians, observing an interval of at least 6 hours between assessments (addendum H, available at www.portal.cfm.org.br).

4.5 Computed tomography angiography

4.5.1 Imaging protocol

Imaging studies were performed on a 64 or 128 multi-slice CT scanner Brilliance (Philips Healthcare, Best, the Netherlands) or Aquillion CXL128 (Toshiba Medical System Corp., Otawara, Japan). The parameters for CTA acquisition were as follows: 100 kVp, 250 mAs, 200 mm field of view, slice thickness of 0.5 to 0.9 mm, and reconstruction increment of 0.33 to 0.45 mm. The scan was taken parallel to the orbito-meatal line, range extending from C1–C2 to the vertex. A total of 80 mL of non-ionic contrast (iopromide 300 mg/mL) was administered intravenously into the cubital vein, using a power injector at a rate of 4.0 mL/s followed by a 40 mL saline flush at a rate of 4 mL/s.

Three scans were obtained. The first was a non-contrast, followed by an arterial contrast phase, and ending with a venous contrast phase. The arterial scan delay was individually adapted using a bolus-tracking technique. For bolus-tracking, first, a single, nonenhanced low-dose scan at the level of the upper neck was obtained. With the start of contrast administration, repeated

low-dose monitoring scans were obtained every second. When the contrast was first observed in the common carotid artery, the arterial phase scan was automatically triggered without any time delay. Then, after 40 seconds, a venous phase scan was performed.

Multiplanar and three-dimensional maximum intensity projection (MIP) angiographic reconstruction techniques were performed on the images using an iSite Enterprise workstation (Philips Healthcare, Best, the Netherlands). Mean arterial pressure during the scans should be above 70 mmHg. The correct contrast injection was verified by visualizing temporal superficial arteries.

Neuroradiologists scored image analysis difficult in three levels as explained in table 1.

Tabela 1 - Grades of increase in difficult analyzing CTA images

Grade 1:	No difficult in vessels evaluation
Grade 2:	Intracranial artifacts and/or cisternal hemorrhage, high confidence
Grade 3:	Intracranial artifacts and/or cisternal hemorrhage, possibility of misinterpretation

4.5.1 Imaging endpoints

Around 40 1.5 mm slices were obtained in each scan, in cantho-meatal angulation. Imaging reconstruction allowed the evaluation in all three planes (axial, coronal and sagittal). The collected imaging endpoints were opacification of the M4-MCAs and ICVs[175] bilaterally. The 4-point score (4PS) was composed of the observation of both M4 segments and both ICVs (figure 10). As proposed by a meta-analysis, the exclusive observation of both ICVs was defined as the VS, where one point was given for each vessel visualized. A score of 0 corresponded to BD. In both scores, the presence of any of these vessels precluded the diagnosis of BD. These vessels were selected as the tomographic targets, because M4 segments are the most distal arteries

visualized before entering the cerebral parenchyma; whereas, the ICVs are responsible for hemispheric blood drainage (figures 11 and 12).

1 point each for non-opacification of the	7 point score (Dupas et al., 1998)	4 point score (Frampas et al., 2009)
terminal braches (M4) of the right middle cerebral artery	1 point	1 point
terminal braches (M4) of the left middle cerebral artery	1 point	1 point
right pericallosal artery (A3)	1 point	
left pericallosal artery (A3)	1 point	
right internal cerebral vein	1 point	1 point
left internal cerebral vein	1 point	1 point
great cerebral vein	1 point	
sum	7 points	4 points

Figure 10 - CTA scores. The 4-point score is currently recommended by the French Society of Neuroradiology, from Rieke et al.[177]

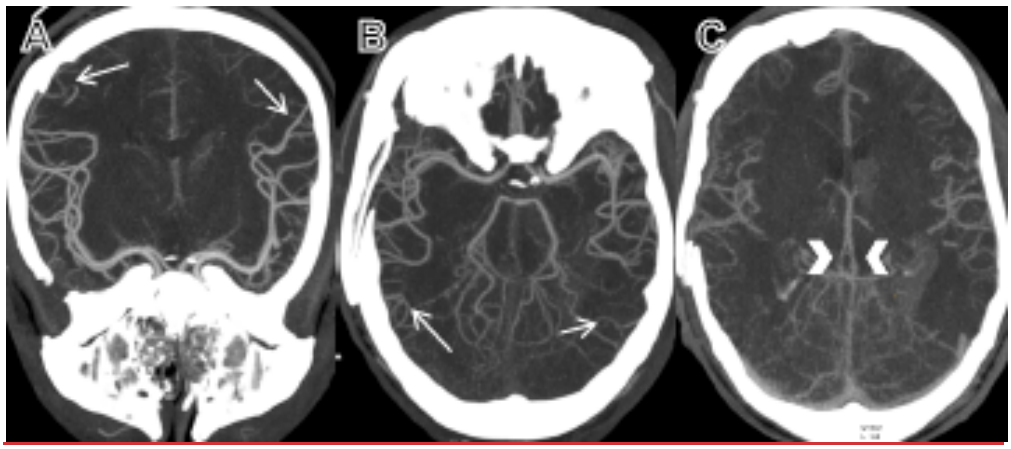


Figure 11 - CTA targets were distal middle cerebral arteries segments (arrows in A and B) and internal cerebral veins (arrowheads in C)

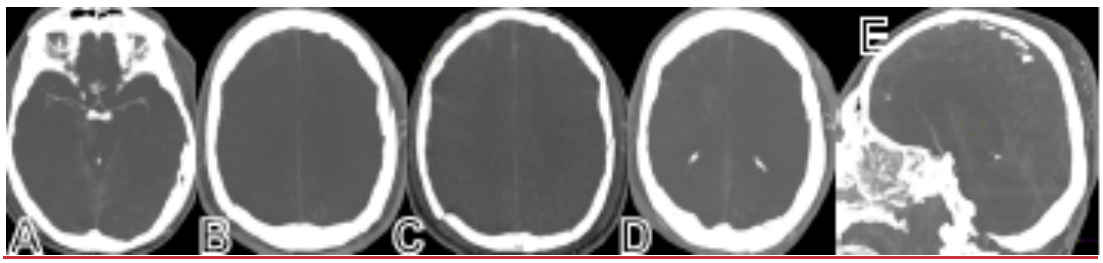


Figure 12 - Brain death. Despite subarachnoid and cisternal hemorrhage, arterial (A, B and C) and venous (D and E) phases revealed absence of middle cerebral arteries and internal cerebral veins

4.6 Transcranial Doppler

BD using blind color TCD (Digi-Lite, Rimed, Ra'anana, Israel or EZ-Dop, DWL, Singen, Germany) was supported if systolic spikes lower than 50 cm/s or non-progressive oscillatory flow (i.e., diastolic velocity = 0 cm/sec or diastolic backflow) were present in the MCAs and artery[192]. As for BD and CTA, the presence of hypothermia or hypotension was avoided before the TCD examination according to inclusion criteria.

4.7 Statistical analysis

The sample size was calculated using the method proposed by Buderer *et al.* [193], after a pilot study. Considering the prevalence of BD in our first 30 cases was 45%, the known CTA sensitivity of previous studies was 85%(171), and an estimated precision of 10%, a sample size of 91 patients was needed to precisely estimate the validity of our index test (i.e., CTA). Considering logistic delays and inappropriate image quality of some CTA exams, we expected a dropout rate of 10%, yielding a final sample size of 100 patients

Continuous variables were expressed as mean and standard deviation (SD) or median and interquartile range (IQR), depending on the data distribution. Discrete variables were expressed as counts and percentages. Differences between groups were compared using Student's t-test, Mann–Whitney U, chi-square or Fisher's exact test, when appropriated. We defined measures of concordance for the CTA exams as agreement's percentage and Cohen's k between the two reading radiologists. Statistical analyses were not performed among clinical assessors because of Brazilian BD protocol mandatorily demands two different physicians to repeat the entire NDD, including the apnea test. In case of disagreement, such patient should be excluded. Likewise, as the reference standard (TCD) was performed in all cases by a single operator, no k score was suitable to calculate.

Sensitivity and specificity were calculated with both the 4-point score (4PS), seeking for opacification of MCA distal right and left plus ICVs right and left, 1 point for each vessel visualized[171], and the venous score (VS), based

exclusively on the visualization of the deep brain drainage, determined by the ICVs. We assumed that circulatory arrest was negative if any of these vessels were observed in CTA. Inter-observer agreement index (*kappa*) was calculated based on both 4PS and VS.

We calculated diagnostic sensitivity, specificity, positive and negative predictive values, likelihood ratios and the area under receiver-operating-characteristic (AUROC) curves of each new diagnostic test, comparing their accuracy with both brain death diagnosis and TCD. In addition, we performed specific subgroup analysis to evaluate the impact of time between tests in their sensitivity and specificity, compared to TCD. All statistical analyses were performed using the Stata software, version 14.1.

5 RESULTS

5 RESULTS

5.1 Study population

From January 2014 to December 2017, 194 eligible patients were enrolled in this study (figure 13). 31 eligible patients were not included because of severe renal failure, 21 because of LAR refusal and 32 because of arterial hypotension at the time of CTA scan. A total of 110 consecutive patients were included. Four patients were lost, in two cases because CTA did not follow scanning protocol properly and two patients presented cardiac arrest prior to accomplishing the neurological assessment. Finally, in the remaining 106 cases, CTA scanning, TCD examination and complete BD clinical assessment were all performed. The time of clinical testing for BD after CTA scanning varied from 0 to 36 hours. However, clinical testing for BD was performed at greater than 12 hours after CTA scanning in only 10 cases.

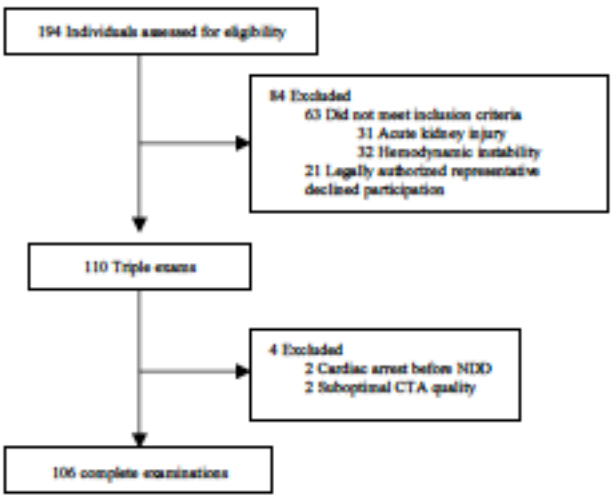


Figure 13 - Patients enrolled and losses description

The characteristics of the study population are reported in Table 2. There were no statistical differences between the BD and no-BD groups with regards to the means of age, gender, admission diagnosis, or severity of health conditions.

Tabela 2 - Baseline characteristics of groups at CTA scan

Subjects	**Brain death**	**No Brain Death**	***p*-value**
N (%)	54 (50.9)	52 (49.0)	
Age, years mean, (SD)	40.07 (18.5)	45.28 (19)	0.15
Male%	66.6	69.2	0.77
Admission diagnosis			
TBI%	57.5	48	0.71
SAH%	26	32	0.14
Stroke%	4	14	0.07
Others%	12.5	6	0.49
Marshall CT score (TBI N)	(31)	(25)	
1-2	2	12	<0,001
3	19	4	<0.001
4	3	1	0.61
5	2	8	0.03
6	4	0	0.11
Hunt and Hess score (SAH N)	(14)	(17)	
1-2	1	5	0.18
3	2	7	0.13
4	5	4	0.69
5	6	1	0.02
SAPS 3 median (IQR)	61.5 (51–74)	57 (45–67)	0.31
GCS%			
3	100	70.6	< 0.001
4	0	21.6	< 0.001
5	0	7.8	0.052

TBI: traumatic brain injury, SAH: subarachnoid hemorrhage, SAPS 3: simplified acute physiology score and GCS: Glasgow coma score

5.2 Clinical results

In all, 52 patients (49%) did not have a clinical diagnosis of BD. The signal against BD in 8 patients was flexion to pain, whereas it was abnormal extension to pain in 7 patients; 37 (71%) of these patients were GCS 3, and of these, 32 presented miotic reactive pupils. Considering the remaining 5 patients who had mydriatic pupils at the time of CTA scanning, 2 presented the absence of the corneal reflex with preservation of the oculo-cephalic reflex, having the BD assessment aborted at this moment; 3 patients had absence of all brainstem reflexes but respiratory movements were observed (figure 14). A total of 19 (36%) control patients died before discharge.

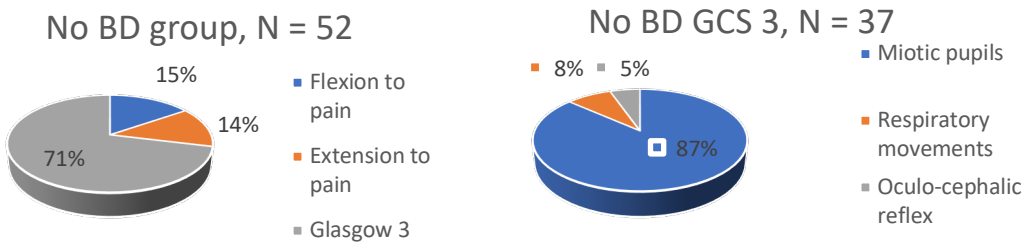


Figure 14 - Characteristics of no BD group

5.3 Ancillary testing results

In the no-BD group, both TCD and CTA showed no false positives (i.e., 100% specificity, figure 15).

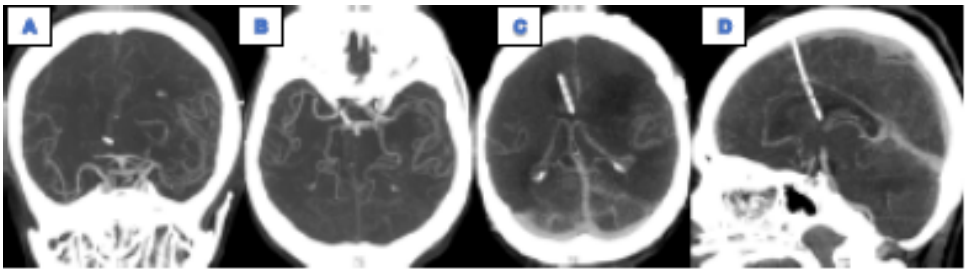


Figure 15 - Patient 49, presenting mydriatic no reactive pupils. NDD and TCD performed immediately posterior to CTA were negative for BD (cough reflex present and inspiratory movements). Accordingly, CTA displayed arterial (A and B) and venous (C and D) flow.

A total of 54 patients fulfilled the clinical criteria for BD. CTA 4PS suggested BD in 33 of them, (i.e., 61% sensitivity), and VS suggested BD in 47 of them (i.e. sensitivity 87%, table 3 and figure 16).

Tabela 3 - Diagnostic test performance

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	AUROC (95% CI)
CTA 4PS	61.1% (46.9-74.1)	100% (93.2-100%)	100% (89.4-100)	71.2% (59.4-100)	Infinity	0.39 (0.28-0.54)	0.81 (0.74-0.87)
CTA ICVs	87% (75.1-94.6)	100% (93.2-100)	100% (92.5-100)	88.1% (77.1-95.1)	Infinity	0.13 (0.06-0.26)	0.94 (0.89-0.98)

PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio, AUROC: area under curve

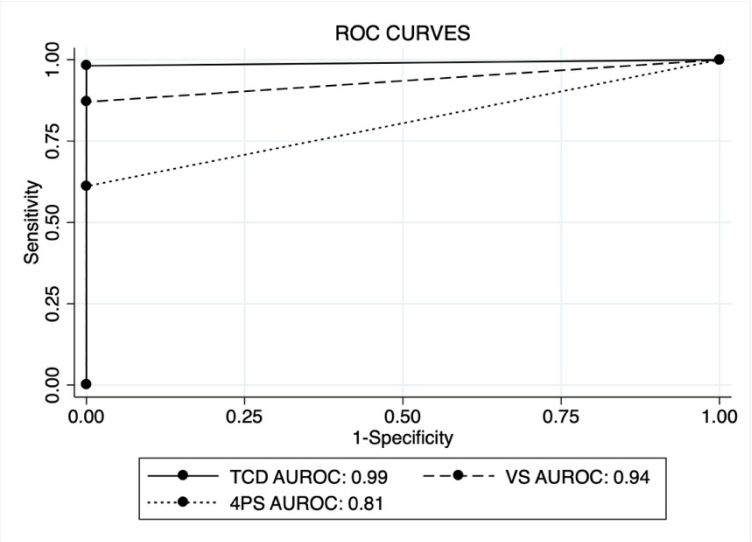


Figure 16 - ROC curve depicting brain computed tomography angiography in predicting the collapse of brain vessels in the included patients
(4PS: 4-point score, AUROC: area under curve, ROC: receiver operator characteristic, TCD: transcranial Doppler, VS: venous score)

TCD was compatible with BD in 52 cases (i.e., 96% sensitivity), displaying systolic spikes or reverberating flow in the MCAs and basilar. None of the patients included in this research had absence of sonographic windows. In the 2 patients that TCD did not show findings of BD, spectral pattern was compatible with residual flow, hence, CTA obtained 12 hours after TCD disclosed intracranial circulatory arrest by both 4PS and VS.

5.4 Ancillary testing in decompressed skulls

Out of twenty patients with decompressive craniectomy (DC) or large skull defects, BD was confirmed in twelve, with circulatory arrest on the TCD and absence of visualization of ICVs in all of them, confirmed a 100% sensitivity for TCD and if applied the VS. Otherwise, sensitivity was lower (41%) in DC cases if applied the 4PS, as seven of these patients opacified distal MCAs bilaterally. Regarding the remaining eight patients with DC and no BD, 6 had pupillary reflexes preserved and the other two, with bilateral mydriasis, presented cough reflex and respiratory movements. These 8 patients had the 4 vessels visualized in CTA images.

5.5 Timing and flow

A decrease in sensitivity was observed if the complimentary test was performed at an interval of more than 12 hours prior to the neurological evaluation. If exclusively considering those patients where CTA scanning was performed close to the neurological assessment for BD, which means a neurological diagnosis of BD an average of 24 hours after the first signs of BD, CTA sensitivity could reach 95.5% and 81.8% by means of VS and 4PS, respectively (figure 17).

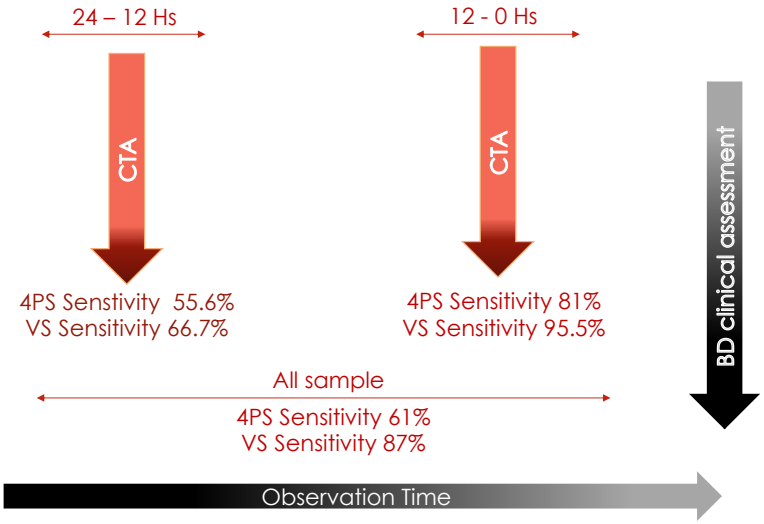


Figure 17 - Sensitivity of both techniques and scores was higher when testing was performed after longer period of observation and closer to clinical assessment

Figure 18 illustrates an example of bias according to our timing protocol. As both TCD and CTA are not influenced by the presence of CNS depressors, exams were performed and delaying to complete NDD was determined by the sedative in use.

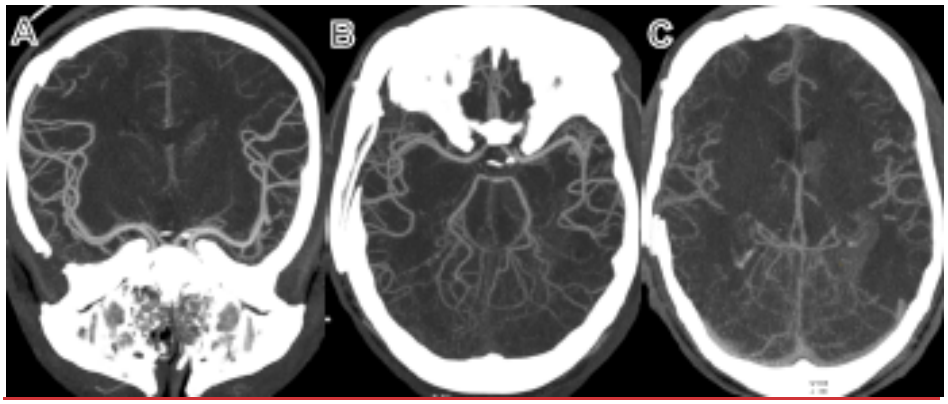


Figure 18 - Patient 24, presenting mydriatic no reactive pupils. CTA performed 30 hours prior to clinical assessment because of thiopental use, with evidence of arterial (A and B) and venous (C) flow. Clinical assessment and TCD performed concomitantly were positive for BD.

Among BD group patients, CTA was performed in 14 cases during CNS depressors administration (N thiopental: 4, propofol: 8 and midazolam 2). Especially in the case of thiopental NDD was performed 48 hours after drug

withdrawal, and CTA was performed within this interval. The others CNS depressors used were propofol, midazolam and fentanyl. If no CNS depressant was in use, NDD was completed immediately posterior to CTA scanning. TCD was always performed from 12 hours before, up to 12 hours after CTA. Table 4 show statistical variances according to the interval between tests.

Tabela 4 - Diagnostic test performance, with lower interval between tests, better results

Time between ancillary tests >5h N = 71							
	Sensitivity	**Specificity**	**PPV**	**NPV**	**LR +**	**LR -**	**AUROC (95% CI)**
CTA 4PS	53.8% (37.2-69.9)	100% (96.6-100)	95.5% (77.2-99.9)	63.3% (48.3-76.6)	17.23 (2.45-121)	0.48 (0.34-0.67)	0.75 (0.67-0.84)
CTA VS	80.8% (60.6-93.4)	100% (96.9-100)	100% (83.9-100)	81.5% (61.9-93.7)	Infinity	0.19 (0.09-0.42)	0.9 (0.83-0.98)
Time between ancillary tests <5h N = 35							
	Sensitivity	**Specificity**	**PPV**	**NPV**	**LR +**	**LR -**	**AUROC (95% CI)**
CTA 4PS	78.6% (49.2-95.3)	100% (83.9-100)	100% (71.5-100)	87.5% (67.6-97.3)	Infinity	0.21 (0.08-0.58)	0.89 (0.78-1)
CTA VS	100% (76.8-100)	100% (83.9-100)	100% (76.8-100)	81.5% (61.9-93.7)	Infinity	0	1 (1–1)

5.6 Tests and interobserver agreements

The results of TCD and the NDD were harmonic with CTA results in 91% of the cases according to the VS, and 86,6% when considered the 4PS, for one neuroradiologist, while 90% and 80% respectively, for the other neuroradiologist. Agreement inter-observer was lower on the evaluation of the 4PS, *kappa* score was 0.77 (strong), while 0.96 (almost perfect) on the VS[194] (table 5).

Tabela 5 - Inter-observer agreement

	Agreement	**Kappa**	**Std. Err.**	**P**
CTA 4PS	89.62 %	0.775	0.095	<0.001
CTA VS	98.11 %	0.962	0.097	<0.001

6 DISCUSSION

6 DISCUSSION

6.1 Literature review

Three systematic reviews have been published on the use of CTA to support the diagnosis of BD[88,159,162]. These systematic reviews agreed that the overall quality of the studies included were low, especially because of the lack of information regarding (1) how a clinical diagnosis of BD was performed, (2) if the radiologists engaged were blinded, and (3) if patients were consecutively selected. Furthermore, (4) the time interval between CTA and the gold standard was excessively delayed in some instances. Additionally, CTA was exclusively applied in patients who were previously considered brain dead. A second limiting factor was the heterogeneity among the included studies, especially in terms of the CTA imaging evaluation criteria used for the diagnosis of BD, whether a 7-point score or 4-point score. Moreover, the ancillary tests used to compare with CTA varied within the same study in some cases. Although low-quality studies were identified, Taylor *et al.* (in their systematic review) explained based on the knowledge obtained until that time, that when performed in patients who had undergone a complete, well-designed clinical assessment for BD, CTA could be assumed as a reliable technique for support of the diagnosis[195].

The role of ancillary testing in BD has been considered exclusively to support diagnostic challenges, such as in severe facial traumatic injuries, in some kinds of poisonous (i.e., rattlesnakes bites and pufferfish ingestion) and exogenous intoxications, Bickerstaf syndrome, and chronic carbon dioxide retainers, and there is no perfect ancillary technique to confirm BD that could replace a neurological examination. Otherwise, as one of the most important diagnoses in medical practice, elevation in diagnostic reliability is profitable for LARs and physicians, indicating the association of ancillary testing with neurological assessments as the best practice currently.

6.2 Study design

This is the first study with three groups of “blinded” evaluators on this subject. Only one study recently included control patients suitable for comparison when evaluating ancillary techniques for diagnosing BD[164]. Patient inclusion was consecutive, obeying the same protocol of neurological evaluation as proposed by the AAN for the entire sample and not varying from the gold standard.

In our study, with respect to patients without BD, CTA was able to adequately exclude intracranial circulatory arrest in all cases, giving no false positives. Another novelty concerns the different methodology to previous studies because our CTA scans were performed prior to the neurological evaluation, preventing neuroradiologists from knowing which patient was effectively brain dead and which one was not. Moreover, as CTA scanning was performed in a “very early phase” of BD evolution, the 4-point score sensitivity dropped in comparison with the study by Garret *et al.*[164], while the sensitivity of the exclusive VS was substantially higher, indicating that the phenomenon of cerebral deep venous compression occurs precociously in BD.

For none of the BD patients, both techniques (i.e., CTA and TCD) were incompatible with BD. Interestingly, we observed higher accuracy of both techniques (CTA and TCD) and scores (4PS and VS) when the exams where performed closer (< 12 hours) to the neurological assessment. The latter may be explained because circulatory arrest depends on the rise of intracranial pressure, while intracranial flow inversely decreases(186) thus, BD may exist with persistent cerebral blood flow (CBF)[196]. Extremely low flow, known as the stasis filling phenomenon, can be falsely interpreted as blood flow[120]. This phenomenon is observed in digital subtraction angiography (DSA)[197], and have been proven not to be real CBF, since the flow time transit is too low to maintain cerebral perfusion[150].

Our prevalence of BD was higher than that of previous reports[198,199], although specific reports on BD statistics among GCS $\leq$ 5 patients exclusively are lacking. Overall mortality in GCS 3 TBI patients with absent pupillary

reflexes is 80%[200], and 90% with reference to the Marshall 3 scale[201]. Some of the no-BD group subjects displayed the first signs of BD, although complete neurological assessment revealed no BD at that moment; these and a large number of no-BD patients died posteriorly, depicting the severity of included patients in this study and the feasibility of comparison between groups.

Our results strongly recommend the absence of ICVs opacification as the most reliable criteria to CTA positivity for BD, just like recent criteria revision concluded[157]. Otherwise, a non-contrast scanning and arterial phase are mandatory in order to observe potential confounders and perform a dynamic vascular assessment. Although *kappa* score was not weak with 4PS as in previous study[185], the evaluation of distal MCA revealed to be more challenging, possibly by stasis filling. To preserve the purity of our results, we did not submit CTA to uncovered review by neuroradiologists, agreeing with the possibility of some divergences in images interpretation between them.

6.3 CTA technique

CTA is a widely used method for evaluating intracranial vascular structures, especially after the advent of the multislice acquisition technique. It is performed with very rapid volumetric multislice acquisitions during and after intravenous infusion of the iodinated contrast medium through injection pump under pressure at high infusion rate, usually 4 to 6 ml/s. Volumetric acquisition with thin sections allows high resolution angiographic reconstructions. Although not a continuously dynamic method with arterial infusion such as digital angiography, the combination of rapid acquisitions with the use of a power injector allows the obtaining of high-resolution angiographic images with high endovascular concentration of the iodinated contrast medium, whereas CTA multiphase acquisitions, prioritizing arterial, venous and late time, are a multiphasic dynamic method that allows adequate evaluation of intracranial arteries and veins[183].

Modern CTA techniques may be timing invariant, superimposing several timeframes, enabling reconstructions independently time of maximal vessel enhancement[150,202]. For this purpose, CT multislice devices monitor the passage of contrast medium through the main cervical arteries, guiding the ideal moment to start arterial acquisition. Thus, even in clinical situations in which the circulation of the arterial contrast bolus to the brain is delayed, such as in intracranial hypertension or heart failure, the equipment will adjust the onset of acquisition to the ideal arterial time, and consequently, to the other times of venous or late acquisition.

In our study, optimum arterial acquisition was initiated based on the monitoring of cervical artery contrast and the venous phase acquisition was scheduled to begin 40 seconds after the arterial phase. In order to rule out any technical problem related to the infusion of the contrast medium, was mandatory to evaluate the adequate opacification of the superficial temporal arteries in all cases.

Most exams were of low degree of evaluation difficult, that is, it was clearly observed if the vessels were contrasted or not. However, some situations in which there was some degree of difficulty, the analysis required more attention to the findings and the technical conditions of the examination, in order to avoid errors of diagnostic interpretation. Acute subarachnoid hemorrhage, diffuse cerebral edema with diffuse reduction of the cortical sulci and artifacts from metallic materials such as firearm projectiles were some of the situations that made the analysis more difficult, especially in the detection of venous contrast. These difficulties however did not compromise the accuracy of the method in this study. We verified in these more difficult cases, the non-contrast phase had their analysis facilitated, especially in the presence of intracranial hemorrhage, so we suggest that the non-contrast phase must always be acquired for brain death.

There is no perfect technique to confirm BD that could replace clinical diagnosis. CTA demands patient transportation, what may be harmful for patients and toilful for ICU personnel[203], faced the large amount of losses by cardiovascular instability in our study. Furthermore, the need of contrast may

worsen renal function. Although a clear behavior of BUN elevation was seen in our study in the first three days after contrast injection, those levels were slowly returning to the initial values, which suggests that the use of contrast may not be a factor of poor outcome for graft transplantation, as previously reported[204].

CTA is not operator dependent, as a technician is able to perform the examination, while a radiologist can evaluate the images remotely. Additional advantages of this technique are the wide availability of devices with 32 channels or more, the readiness in acquiring images, the need of only a single peripheral vein, and its ability for additional scanning of the entire body of a potential organ donor[154]. Otherwise, TCD displays advantages as a repeatable and inoffensive bedside technique.

6.4 Brain drainage

Current research, adopting the 4PS, recommends delaying CTA scan 12 hours after the clinical determination of BD[205]. Otherwise, sensitivity in our study was best with the VS 12 hours prior to neurological assessment. However, the VS would represent cerebral drainage exclusively, and the evaluation of the petrosal veins might be of value to assess the entire encephalic drainage[157], being subject to further research.

CTA has been criticized for not following the same standard of DSA, with lower contrast injection pressure and the hypothesis of insufficient pressure to the contrast to penetrate the skull, in situations of intense intracranial hypertension[183]. Otherwise, the phenomenon of circulatory arrest in the distal capillary and deep brain drainage seems to occur in the early phase of BD. Cortical branches may opacify and falsely transmit the impression of CBF, as selective injection of individual vessels can raise the blood pressure to a degree that it forces contrast material into the intracranial space, especially with high injection flow rates[206]. Additionally, it is not uncommon for DSA to

present proximal filling, even when contrast is injected intravenously rather than intra-arterially[114,207].

In our study, all cases of decompressive craniectomy with positive clinical BD had absence of ICVs contrast filling, what indicates that a brain-dead patient with open skull will often display absence of drainage, despite the fact that DC truly diminished CTA sensitivity if applied the 4-point score, as previous studies pointed[157,208]. Marchand et al. suggested the exclusive assessment of the ICVs associated to the superior petrosal veins (SPV), with the advantage of the absence of visualization of these four veins transmitting notion of entire brain death[157].

6.5 Limitations

The main limitation of this study was the time for completing the clinical assessment for BD. Efforts were made to shorten the time between examinations to reduce the possibility of timing bias. However, this study was designed and executed as closely as possible to the actual practice of tomographic scanning in neurocritical patients, and the highly stressful psychological moment was significant for LAR refusal in participation of the study.

TCD was performed only by one very experienced single operator in all cases. As this technique is highly operator dependent, should be of value to calculate TCD interobserver agreement. Otherwise, the absence of temporal acoustic window was an exclusion criterion, meaning that in the entire sample a sonographic pattern was visualized and recorded, reducing the possibility of diagnostic error.

Analyses of most of the CTA scans were of low degree of difficulty (addendum B-D), that is, it was clearly observed whether the vessels were contrasted or not. However, some situations in which there was some degree of difficulty of the analysis required more detail in the observation of the images, the attention to the findings and the technical conditions of the

examination, in order to avoid errors of diagnostic interpretation. Acute subarachnoid hemorrhage, diffuse cerebral edema with diffuse erasure of the cortical grooves and artifacts from metallic materials such as firearm projectiles were some of the situations that made the analysis more difficult, especially in the detection of venous contrast. These difficulties of analysis, however, did not compromise the accuracy of the method in this study. Inter-observer heterogeneity has been calculated among experienced neuroradiologists only. We believe such images interpretation require specific expertise and training.

Other relevant point was the absence of deep venous thrombosis cases in our sample, to study the possibility of false positives in this condition. Rationally, CTA should be precluded of use in such diagnosis, since this condition would be previously diagnosed by another technique, or even CTA itself, especially if no visualization of the internal cerebral veins was verified. In daily practice, two other conditions are frequently related to delayed circulatory arrest verification by blood flow techniques, namely either BD after exclusive brainstem stroke, or after cardiac arrest[181,209]. However, in our study none of the latter occurred, whereas one case of brainstem stroke was amid our losses, due to suboptimal CTA imaging.

CTA demands patient transportation, which may be harmful to patients and laborious for ICU personnel[203]. Furthermore, the need of contrast may worsen renal function, although the use of contrast may not be a factor for poor outcome for graft transplantation, as previously reported[204]. In our study, hemodynamic instability and impairment of renal function were important contributors to the dropout rate and represented hindrances for utilizing CTA.

7 Impact of Present Research

7 IMPACT OF PRESENT RESEARCH

Around the world in many locations, CTA is the sole technique available to provide support for the diagnosis of BD. Especially in those countries where an ancillary test is mandatory, CTA represents an opportunity to improve ICU strategies and organ donation and transplantation.

8 CONCLUSIONS

8 CONCLUSIONS

CTA is reliable to support BD diagnosis. The criterion of absence of ICVs opacification can confirm the occurrence of cerebral circulatory arrest.

9 ADDENDUM

9 ADDENDUM

9.1 ADDENDUM A - STARD 2015- CTA accuracy on the BD diagnosis. Numbers in reference to the published paper

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	3
	4	Study objectives and hypotheses	4
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	4
Participants	**6**	Eligibility criteria	4
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	4-5
	8	Where and when potentially eligible participants were identified (setting, location and dates)	4-5
	9	Whether participants formed a consecutive, random or convenience series	4-5
Test methods	**10a**	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	7-8
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	6
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	5
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	5

to be continued

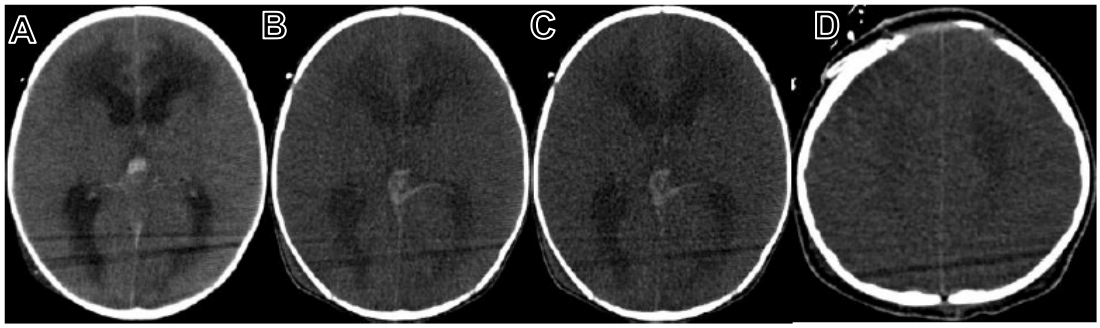
conclusion

Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8-9
	15	How indeterminate index test or reference standard results were handled	7-8
	16	How missing data on the index test and reference standard were handled	7-8
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	NA
	18	Intended sample size and how it was determined	8-9
RESULTS			
Participants	**19**	Flow of participants, using a diagram	10
	20	Baseline demographic and clinical characteristics of participants	10
	21a	Distribution of severity of disease in those with the target condition	10
	21b	Distribution of alternative diagnoses in those without the target condition	NA
	22	Time interval and any clinical interventions between index tes and reference standard	11
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	11
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	10-11
	25	Any adverse events from performing the index test or the reference standard	NA
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistica uncertainty, and generalisability	15
	27	Implications for practice, including the intended use and clinical role of the index test	15
OTHER INFORMATION			
	28	Registration number and name of registry	4
	29	Where the full study protocol can be accessed	4
	30	Sources of funding and other support; role of funders	NA

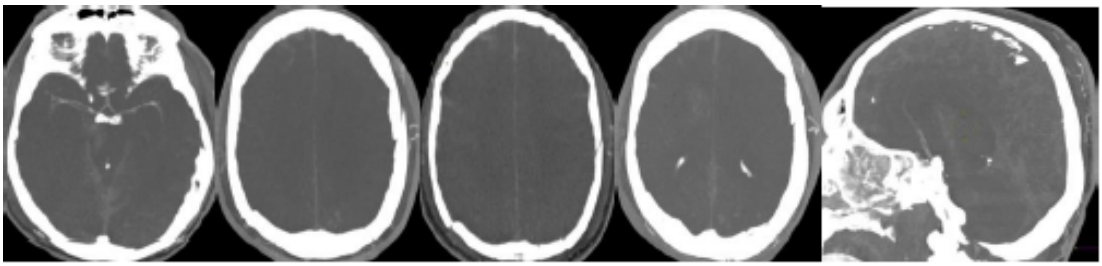
9.2 **ADDENDUM B** - Image analysis difficult SCORE

Grade 1	No difficult in vessels evaluation.
Grade 2	Intracranial artifacts and/or cisternal hemorrhage, high confidence.
Grade 3	Intracranial artifacts and/or cisternal hemorrhage, possibility of misinterpretation.

9.3 ADDENDUM C - Patient 45, cisternal hemorrhage hampering the evaluation of internal cerebral veins, lower level of confidence (grade 3). A and B arterial phase, C and D venous phase



9.4 **ADDENDUM D** - Patient 51, subarachnoid and cisternal hemorrhage. Grade 2



9.5 **ADDENDUM E** - SAPS 3 admission screensheet

Table 2 SAPS 3 admission scoresheet – Part 2

Box II – continued	
ICU admission [12)]	16
Reason(s) for ICU admission	
Cardiovascular: Rhythm disturbances [13)]	–5
Neurologic: Seizures [13)]	–4
Cardiovascular: Hypovolemic hemorrhagic shock, Hypovolemic non hemorrhagic shock. / Digestive: Acute abdomen, Other [3)]	3
Neurologic: Coma, Stupor, Obtuned patient, Vigilance disturbances, Confusion, Agitation, Delirium	4
Cardiovascular: Septic shock. / Cardiovascular: Anaphylactic shock, mixed and undefined shock	5
Hepatic: Liver failure	6
Neurologic: Focal neurologic deficit	7
Digestive: Severe pancreatitis	9
Neurologic: Intracranial mass effect	10
All others	0
Anatomical site of surgery	
Transplantation surgery: Liver, Kidney, Pancreas, Kidney and pancreas, Transplantation other	–11
Trauma – Other, isolated: (includes Thorax, Abdomen, limb); Trauma – Multiple	–8
Cardiac surgery: CABG without valvular repair	–6
Neurosurgery: Cerebrovascular accident	5
All others	0

[12)] Every patient gets an offset of 16 points for being admitted (to avoid negative SAPS 3 Scores).
[13)] If both reasons for admission are present, only the worse value (–4) is scored.

Table 1 SAPS 3 admission scoresheet—Part 1

Box I	0	3	5	6	7	8	9	11	13	15	18
Age, years	<40		>=40<60				>=60<70		>=70<75	>=75<80	>=80
Co-Morbidities		Cancer therapy [2)]		Chron. HF (NYHA IV), Haematological cancer [3),4)]		Cirrhosis, AIDS [3)]		Cancer [5)]			
Length of stay before ICU admission, days [1)]	<14			>=14<28	>=28						
Intra-hospital location before ICU admission			Emergency room		Other ICU	Other [6)]					
Use of major therapeutic options before ICU admission		Vasoactive drugs									

Box II	0	3	4	5	6
ICU admission:					
Planned or Unplanned		Unplanned			
Reason(s) for ICU admission	please see Part 2 of the scoresheet				
Surgical status at ICU admission	Scheduled surgery			No surgery [7)]	Emergency surgery
Anatomical site of surgery	please see Part 2 of the scoresheet				
Acute infection at ICU admission			Nosocomial [8)]	Respiratory [9)]	

Table 1 continued

Box III	15	13	11	10	8	7	5	3	2	0	2	4	5	7	8
Estimated Glasgow Coma Scale (lowest), points	3–4			5		6			7–12	>=13					
Total bilirubine (highest), mg/dL										<2		>=2<6	>=6		
Total bilirubine (highest), µmol/L										<34.2		>=34.2 <102.6	>=102.6		
Body temperature (highest), Degrees Celsius						<35				>=35					
Creatinine (highest), mg/dL										<1.2	>=1.2<2			>=2<3.5	>=3.5
Creatinine (highest), µmol/L										<106.1	>=106.1< 176.8			>=176.8 <309.4	>=309.4
Heart rate (highest), beats/minute										<120			>=120 <160	>=160	
Leukocytes (highest), G/L										<15	>=15				
Hydrogen ion concentration (lowest), pH								<=7.25		>7.25					
Plateletes (lowest), G/L		<20			>=20<50		>=50<100			>=100					
Systolic blood pressure (lowest), mm Hg			<40		>=40<70			>=70<120		>=120					
Oxygenation [10),11)]			PaO2/ FiO2 <100 and MV			PaO2/ FiO2> =100 and MV	PaO2<60 and no MV			PaO2> =60 and no MV					

The definition for all variables can be found in detail in Appendix C of the ESM. For names and abbreviations which are differing from those in the ESM, explanations are given below. Generally, it should be noted that no mutually exclusive conditions exist for the following fields: Comorbidities, Reasons for ICU admission, and Acute infection at ICU admission. Thus, if a patient has more than one condition listed for a specific variable, points are assigned for all applicable combinations.
[1] This variable is calculated from the two data fields: ICU Admission date and time—Hospital admission date and time (see Appendix C of the ESM)
[2] Cancer Therapy refers to the data definitions in Appendix C of the ESM: Co-Morbidities: Chemotherapy, Immunosupression other, Radiotherapy, Steroid treatment
[3] If a patient has both conditions he/she gets double points.
[4] Chronic HF (NYHA IV)/Haematological cancer refer both to the data definitions in Appendix C of the ESM: Co-Morbidities: Chronic heart failure class IV NYHA, Haematological cancer.
[5] Cancer refers to the data definitions in Appendix C of the ESM: Co-Morbidities: Metastatic cancer.
[6] Other refers to the data definitions in Appendix C of the ESM: Intra-hospital location before ICU admission: Ward, Other.
[7] No surgery refers to the data definitions in Appendix C of the ESM: Surgical Status at ICU Admission: Patient not submitted to surgery.
[8] Nosocomial refers to the data definitions in Appendix C of the ESM: Acute infection at ICU admission—Acquisition: Hospital-acquired.
[9] Respiratory refers to the data definition in Appendix C of the ESM: Acute infection at ICU admission—Site: Lower respiratory tract: Pneumonia, Lung asbcess, other.
[10] PaO_2, FIO_2 refer to the data definitions in Appendix C of the ESM: Arterial oxygen partial pressure (lowest), Inspiratory oxygen concentration.
[11] MV refers to the data definition in Appendix C of the ESM: Ventilatory support and mechanical ventilation.

Marshall Class		Description
Class I	Diffuse injury I (no visible pathology)	No visible pathology seen on CT scan
Class II	Diffuse injury II	Cisterns are present with midline shift 0-5 mm and/or: lesion densities present no high- or mixed-density lesion > 25 cc: may include bone fragments and foreign bodies
Class III	Diffuse injury III (swelling)	Cisterns compressed or absent with midline shift 0-5 mm, no high- or mixed-density lesion > 25 cc
Class IV	Diffuse injury IV (shift)	Midline shift > 5 mm, no high- or mixed-density lesion > 25 cc
Class V	Evacuated mass lesion	Any lesion surgical evacuated
Class VI	Non-evacuated mass lesion	High- or mixed-density lesion > 25 cc, not surgical evacuated

9.7 **ADDENDUM G** - Fisher score in subarachnoid hemorrhage

Grade 0	No SAH or IVH[1]
Grade 1	Minimal/thin SAH, no IVH in either lateral ventricle
Grade 2	Minimal/thin SAH, with IVH in both lateral ventricles
Grade 3	Dense SAH,* no IVH in either lateral ventricle
Grade 4	Dense SAH,* with IVH in both lateral ventricles

[1]Intraventricular hemorrhage; *Completely filling ≥1 cistern or fissure.
SAH: subarachnoid hemorrhage; IVH: intraventricular hemorrhage.

9.8 **ADDENDUM H** - Brazilian BD protocol changes

Resolution 1.480/97	**Resolution 2.173/17**
Clinical parameters for the onset of diagnosis A non-perceptual coma with absence of supraspinal motor activity and apnea.	**Clinical parameters for the onset of diagnosis** Non-perceptual Coma, absence of supraspinal reactivity, persistent apnea. It should present encephalic lesion of known cause, irreversible and capable of causing brain death, absence of treatable factors that may confuse the diagnosis of brain death. Body temperature higher than 35 º, arterial oxygen saturation above 94% and systolic blood pressure greater than or equal to 100 mmHg for adults.
Observation time for the diagnosis to be initiated Not determined	**Observation time for the diagnosis to be initiated** Minimum of 6 hours When the cause was hypoxic-ischemic encephalopathy, the observation should be 24 hours.
Minimum interval between the two clinical assessments From 7 days to 2 months incomplete – 48 hours From 2 months to 1 year incomplete – 24 hours From 1 year to 2 years incomplete – 12 hours Over 2 years – 6 hours	**Minimum interval between the two clinical assessments** From 7 days to 2 months incomplete – 24 hours 2 months to 24 months incomplete - 12 hours Over 2 years – 1 hour
Confirmation of brain death A) clinical examinations performed by different physicians and complementary examinations performed at varying time intervals; b) Complementary exams should demonstrate absence of cerebral electrical activity, or absence of cerebral metabolic activity or absence of cerebral blood perfusion.	**Confirmation of brain death** A) Two clinical examinations by different physicians, specifically trained to confirm the non-perceptual coma and the absence of brainstem function; b) an apnea test; c) A complementary examination that proves the absence of brain activity. This exam should prove absence of brain blood perfusion, or absence of brain metabolic activity or absence of brain electrical activity.
Training of Medical Examiners A) Decree 2.268/97 (repealed by Decree 9.175/17) established that one of the two physicians confirming brain death should be a neurologist; b) The same decree established that none of these physicians could be part of the transplant team.	**Training of Medical Examiners** A) a physician with a year of experience in the care of patients in coma who has accompanied or performed at least ten determinations of brain death, or who has undergone a training course for Brain death determination; b) One of the physicians specifically trained should be expert in one of the following specialties: Intensive care medicine, pediatric intensive care Medicine, neurology, pediatric neurology, neurosurgery or emergency medicine. c) None of these physicians can be part of the transplant team.

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10 REFERENCES

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11 APENDIX

11 APENDIX

11.1 APENDIX A - Systematic review published by *the Journal Of Neuroradiology, 2016.*

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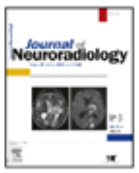

REVIEW

Role of computed tomography angiography and perfusion tomography in diagnosing brain death: A systematic review

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KEYWORDS
Brain death;
Angiography;
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Computed tomography angiography

Summary
Background: Several complications make the diagnosis of brain death (BD) medically challenging and a complimentary method is needed for confirmation. In this context, computed tomography angiography (CTA) and computed tomography perfusion (CTP) could represent valuable alternatives; however, the reliability of CTA and CTP for confirming brain circulatory arrest remains unclear.
Methods: A systematic review was performed to identify relevant studies regarding the use of CTA and CTP as ancillary tests for BD confirmation.
Results: Three hundred twenty-two patients were eligible for the meta-analysis, which exhibited 87.5% sensitivity. CTA image evaluation protocol exhibited variations between medical institutions regarding which intracranial vessels should be considered to determine positive or negative test results.
Conclusions: For patients who were previously diagnosed with BD according to clinical criteria, CTA demonstrated high sensitivity to provide radiologic confirmation. The current evidence that supports the use of CTA in BD diagnosis is comparable to other methods applied worldwide.

Abbreviations: BD, brain death; TCD, transcranial Doppler; EEG, electroencephalogram; ICV, internal cerebral vein; DSA, digital subtraction angiography; CNS, central nervous system.

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Introduction

The increasing demand for organ transplantation, as well as the dependence on cadaveric donors, has increased the need for methods to assist in the earlier diagnosis of brain death (BD). The issue regarding a BD diagnosis is primarily clinical [1], entailing the need to avoid misinterpretation. Therefore, in cases of facial trauma, given that the presence of central nervous system (CNS)-depressing agents, chronic obstructive pulmonary disease, and certain other conditions may compromise verification of brain stem reflexes and positive apnea testing in a comatose patient, a confirmatory test should be applied [2]. However, in many countries, these ancillary tests are legally required in all cases to confirm the diagnosis when BD is considered [3–6].

The absence of brain blood flow or perfusion is often accepted as evidence of BD [4], and many ancillary tests may assist in its detection. Four-vessel digital subtraction angiography (DSA) is considered the gold standard [7–9] because of its high sensitivity and specificity for the identification of brain blood flow. However, this method suffers from serious drawbacks, such as the large quantity of contrast medium needed, its invasiveness, the need for an expert physician and the lack of angiographic equipment in many hospitals.

Computed tomography angiography (CTA) and computed tomography perfusion (CTP) can be used to evaluate intracranial vessels [8–11]. Both methods can acquire images promptly; require only one peripheral vein for contrast administration. The cost is lower than that of DSA, and tomographic devices are more widely available. Despite clear advantages, CTA and CTP are still not approved to assess BD in many countries because of the lack of existing evidence [12,13,30].

If proven, these methods could become routine options for this purpose and might promote organ donation. These conditions justified a systematic review of medical databases to verify the reliability of CTA and CTP for diagnosing BD.

Objective

This review assesses the accuracy of CTA and CTP in confirming BD. Hypothetically, these methods could be added to the BD ancillary test arsenal in areas where they are currently not a routine practice.

Materials and methods

Search strategy

An extensive search was performed of online databases, including MEDLINE, the Cochrane Library, Embase, and LILACS. The Boolean operator ''and'' was used to combine the ancillary test CTA and BD in the search. The Boolean operator ''or'' was used to discriminate similar tests, such as angiography, angiogram, tomography, ancillary tests, neurological death and circulatory arrest. Table 1 describes the results of the search.

The same searches were repeated in all databases, although the number of relevant studies retrieved did not increase.

Table 1 Medical subject headings (MeSH) applied for the search in MEDLINE and their respective results.

MeSH terms	Number of studies identified
CT angiography and brain death	122
Angiography and brain death	890
Angiography and circulatory arrest	493
Angiography and neurological death	349
Angiogram and brain death	918
Tomography and brain death	2944
Ancillary test and brain death	27

Study quality was assessed independently by two authors (SB and MKA). Studies were classified as high- or low-quality or non-valid according to the Dutch Cochrane Center guidelines published to evaluate diagnostic methods. Four criteria were used:

(1) the presence of an independent blind comparison with a reference (gold) standard;
(2) the population studied included an appropriate spectrum of patients to whom the test would be applied in clinical practice;
(3) the inclusion of consecutive patients who fulfilled the inclusion criteria;
(4) a sufficient description of the CTA technique to enable reproduction of the method.

High-quality studies fulfilled all four criteria. Low-quality studies fulfilled criterion (1) (although not necessarily a blind comparison with a reference [gold] standard) and criterion (4). All other studies were categorized as non-valid studies.

Additionally, the studies were submitted to the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS 2, Table 2) tool, an acknowledged instrument recommended for use in systematic reviews to evaluate diagnostic test quality and assess the risk of bias in study design and applicability.

Inclusion criteria and data extraction

The exclusion criteria involved:

- all studies that did not apply CTA or CTP as the confirmatory test for BD;
- studies that were not published in English if it was not possible to contact the authors;
- case reports or very small series (under 10 patients enrolled);
- abstracts, reviews or research protocols;
- animal studies.

Divergence in study selection was resolved by discussion until agreement.

Statistical analysis

For the statistical analyses, only studies that investigated the usefulness of CTA [7,9,14–20] and/or CTP [8,21] in the evaluation of BD in patients were included. Using

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the data of agreement (true positive) and disagreement (false negative) between the tests (CTA or CTP) and the gold standard (varied) in BD patients, the test sensitivity was calculated from a 95% confidence interval using the software CATMAKER calculator (available at www.cebm.net/catmaker-ebm-calculators). It was not possible to calculate specificity due to the lack of false positives (all patients were brain-dead). The meta-analysis graphical expression of the sensitivity of all included studies was performed using the Review Manager (RevMan) computer program software (version 5.3; Copenhagen, Denmark; The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

Although the use of CTA was initiated in 1992, spiral CT has been used since 1989 as a tomographic method to investigate brain circulation [5]; it was therefore included in this review. Small series [22,23] or non-English language studies were included to provide further information regarding BD diagnosis. Two authors [9,19] were contacted to address uncertainties associated with their respective studies.

Many citations from the reference lists of each identified study were assessed, including other reviews regarding the use of ancillary testing of brain blood flow to confirm BD [5,11,24–27].

Results

Number of identified studies

We identified eight observational, prospective, non-randomized series [7–9,15–17,19,21], one case/control study [18], one observational cohort [20] and one transversal retrospective study [14]. All studies were considered to be of low quality according to the applied criteria, and none of the studies was considered to be non-valid, based on agreement among the researchers (SB and MKA).

Characteristics of included trials

The studies were conducted from 1998 to 2014 and included single- and multicenter studies from medical centers in Europe and North America. The retrieved studies included adults of both sexes, and the clinical examinations were consistent with BD resulting from head trauma, ischemic stroke, intracranial hemorrhage, brain tumor, cerebral anoxia, meningitis or cerebral venous sinus thrombosis.

Following the exclusion of patients with hypothermia or who used CNS depressants, patients were selected according to clinical criteria exclusively [7–9], or they had BD confirmed via a clinical evaluation in addition to an ancillary test prior to or after the use of CTA/CTP, such as an electroencephalogram (EEG) [16,19,21], evoked potentials [21], the nuclear medicine perfusion test (NMPT) [15], transcranial Doppler (TCD) [19,21] and DSA [9,21].

Welschehold et al. published four papers from the same unique sample; we selected the study that included the largest number of patients [19]. Another study was designed to submit suspected brain-dead patients to CTA prior to clinical assessment for BD. After clinical confirmation, the patients were subsequently submitted to CTA again [20].

Two series used CTP in addition to CTA [8,21]. A paper describing the use of CTP exclusively for BD diagnosis was not found.

Images were evaluated by radiologists who were blind to the secondary ancillary tests, but the radiologists were not blind to the clinical state of the patients.

Various devices were used in the identified studies, including a Siemens Sensation 64 [8,15], 8-slice GE LightSpeed Ultra [14], 16-slice Somatom Sensation 16 CT [14,16], 32-slice Aquilion20, Multislice Detector Tomographer Aquilion 64 Toshiba [21], and GE High Speed CT Scan [17].

The protocol for contrast administration in all cases was in the temporal superficial arteries or the proximal and middle portions of the arteries from the aortic arch [14]. Images were then acquired at 20, 30 and 60 seconds following contrast injection.

None of the reports described renal dysfunction after contrast administration.

Outcome measures

Table 2 describes the results of the QUADAS 2 signaling questions to assist in judgments regarding the risk of bias.For PAGI: Table 2 should appear in this section

Table 2 QUADAS 2 criteria.

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Dupas et al., 1998	HR	LR	LR	HR	LR	LR	LR
Leclerc et al., 2006	HR	LR	HR	HR	LR	LR	HR
Combes et al., 2007	HR	LR	LR	?	LR	LR	LR
Quesnel et al., 2007	HR	LR	LR	?	LR	LR	HR
Frampas et al., 2009	HR	LR	HR	HR	LR	LR	LR
Escudero et al., 2009	HR	HR	LR	HR	LR	LR	LR
Bohatyrewicz et al., 2010	HR	LR	LR	?	LR	LR	LR
Berenguer et al., 2010	HR	HR	LR	LR	LR	LR	LR
Rieke et al., 2011	HR	LR	HR	HR	LR	LR	HR
Welschehold et al., 2012	HR	LR	LR	HR	LR	LR	LR
Welschehold et al., 2013	HR	LR	LR	HR	LR	LR	LR

LR: low risk; HR: high risk; ?: unclear risk.

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Table 3 CTA criteria for BD from Rieke et al.

1 point each for non-opacification of the	7-point score (Dupas et al., 1998)	4-point score (Frampas et al., 2009)
Terminal branches (M4) of the right MCA	1 point	1 point
Terminal branches (M4) of the left MCA	1 point	1 point
Right pericallosal artery	1 point	
Left pericallosal artery (A3)	1 point	
Right internal cerebral vein	1 point	1 point
Left internal cerebral vein	1 point	1 point
Great cerebral vein	1 point	
Sum	7 points	4 points

A risk of bias concerning patient selection was noted because all studies were conducted on a BD or likely BD population. Furthermore, Berenguer et al. did not submit the entire sample to the apnea test, and no explanation was provided. The risk of bias in reference to the index test is offered as an explanation for the lack of venous phase tomographic assessment in the studies of Escudero et al. and Berenguer et al.

Leclerc et al., Frampas et al. and Rieke et al. compared the results of CTA exclusively with clinical evaluations of the sample, and no reference standard was used. Bohatyrewicz et al. reported a 12- to 48-hour delay between CTA/CTP and DSA. Quesnel et al. did not describe the time between tests, and Combes et al. reported that EEG was available at the time of CTA in just 48% of the sample. All of these studies were categorized as exhibiting unclear flow and timing risk of bias. All other studies, except for Berenguer et al., submitted patients to various reference standards, which could have increased the risk of bias. The applicability concerns were satisfactory for all studies, except those that did not compare CTA or CTA/CTP to another reference standard.

The sensitivity of CTA regarding the confirmation of BD ranged from 52.4 [17] to 100% [8,18]; this range was likely because of the heterogeneity in image interpretation between studies. The criteria (Table 3) were labeled according to a 7-point score (based on the lack of opacification for the pericallosal and cortical segments of the MCAs, internal cerebral veins (ICVs), and 1 great cerebral vein), as proposed by Dupas et al. [18], or according to a 4-point score (based exclusively on the lack of opacification of the MCA cortical branches and ICVs), as proposed by Frampas et al. [7] and accepted by the French Radiology Society [28].

None of the studies described the interval between acquiring and reading the images. Additionally, there was no lack of agreement about CTA scores between the radiologists involved. Inter-observer scores were not evaluated.

As expected, the CTA sensitivity was lower when the 7-point score was used; Combes et al. [17] reported a 30.2% false negative rate. It was not possible to verify CTA specificity because no eligible controls were assessed. Welschehold et al. observed 90% specificity visualizing circulatory arrest at the magnum foramen level in three patients who were considered controls, despite the absence of certain brain stem reflexes in these patients.

A well-known phenomenon called stasis filling, which is primarily associated with DSA, was also observed in CTA. This phenomenon is explained by the pool of contrast medium conducted within the cranial space, especially from the external carotid artery collaterals [7,14,18,29,30]. Hence, proximal Willis polygon vessels may display opacification even in brain-dead patients. Post-mortem imaging studies have observed circulatory stasis of basal vessels even 24 hours after complete death [31].

Table 4 shows the total number of BD patients enrolled in each study and the opacified intracranial vessels assessed according to the 4-point score.

Three hundred seventy-one clinically BD patients underwent CTA; 322 patients exhibited the complete absence of intracranial vessel opacification, 40 patients exhibited at least one opacified cortical MCA, and only 9 patients exhibited at least one opacified ICV.

Fig. 1 displays the statistical analyses of the results standardized to the 4-point score.

By standardizing the obtained data to the 4-point score in the 8 studies in which this approach was feasible, we were able to obtain a sensitivity of approximately 87.5%. Forty of the 322 patients exhibited opacification of either a cortical artery or internal cerebral veins. This value is excluding Berenguer et al. and Escudero et al. because they did not evaluate the venous phase and Welschehold et al., 2013 because that study was part of a larger sample. The sensitivity may increase to 97.5% when exclusively considering ICVs.

Discussion

Some conditions are not compatible with the application of clinical criteria for BD, e.g., when a complete examination of brain stem reflexes is impossible, neuromuscular paralysis or heavy sedation is present, or, in some patients, the apnea test is precluded (respiratory instability or high cervical spine injury) or not valid (high carbon dioxide retainers); ancillary tests become essential in these situations. Some countries worldwide legally require complimentary tests in all BD suspected cases [13,32].

Currently, high-quality evidence is missing regarding DSA and the other most accepted techniques [11,12], including single-photon emission computed tomography (SPECT) [33]. In the United Kingdom, the irreversible loss of brain stem functions is compatible with BD [12,34,35], in which the application of an ancillary test is optional [5,36]. Otherwise, in Brazil, for example, in addition to the assessment of the absence of functions in the entire encephalon, ancillary tests remain mandatory; being TCD, EEG and DSA the most commonly used ancillary methods to diagnose BD. TCD and EEG are both operator-dependent exams, and the latter exam is influenced by sedative drugs or other confounding factors [11]. DSA is an invasive method

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Table 4 Results categorized in accordance to the 4 point score.

Series	N	Complete opacification absence	Cortical MCA opacification	ICV opacification
Dupas et al., 1998	14	14	0	0
Leclerc et al., 2006	15	14	1	0
Combes et al., 2007	43	35	7	1
Quesnel et al., 2007	21	13	7	2
Frampas et al., 2009	105	90	15	2
Escudero et al., 2009	27	24	U	NA
Bohatyrewicz et al., 2010	24	24	0	0
Berenguer et al., 2010	22	19	U	NA
Rieke et al., 2011	29	22	6	4
Welschehold et al., 2012	71	67	4	0
Welschehold et al., 2013	(30)	(29)	(1)	0
Total	371	322	40	9

N: sample total; MCA: middle cerebral artery; ICV: internal cerebral vein; U: unclear; NA: not available.

[22] and, similar to TCD and EEG, may be not widely available in hospitals.

Kramer et al. recently published a meta-analysis on the use of CTA to diagnose BD. The authors concluded that the routine use of CTA as a supplementary BD diagnostic test is not recommended until the CTA diagnostic criteria have undergone further refinement and prospective validation [37]. The studies included in both meta-analyses were the same, with the exception of one abstract [38] and one retrospective evaluation of CTP that assessed BD in 11 patients [39] identified by Kramer et al. The overall quality of these studies was low, especially because of the lack of information regarding:

- how a BD clinical diagnosis was performed;
- if the radiologists engaged were blinded;
- if patients were consecutively selected;
- furthermore, the time interval between CTA/CTP and the gold standard was excessively delayed in some instances.

Additionally, CTA and CTP were exclusively applied in patients who were previously considered brain-dead. One study [20] differed from the other studies and may be considered higher quality; however, a risk of bias related to patient selection should be noted. A second limiting factor was the heterogeneity among the included studies, especially in terms of the various CTA imaging evaluation criteria used for BD diagnosis. Moreover, the ancillary tests used to compare CTA/CTP varied within the same study in some cases.

Although low-quality studies were identified in our search, the present meta-analysis of 322 patients indicated 87.5% sensitivity, as shown in Fig. 1. This result could encourage trusting CTA as a reliable technique when performed in patients who first undergo a complete, well-designed clinical assessment for BD, as concluded by Taylor et al. in a systematic review [5]. CTA cannot be used to properly evaluate the encephalic microvasculature; however, in most BD cases, it can be used to identify the complete absence of brain blood flow or the opacification of proximal arterial vessels, as shown in Table 4.

CTA identifies similar behaviors compared with the other methods used to evaluate brain blood circulation [6,10]. This technique is not able to measure the mean blood transit time or visualize intracranial vessels in a case of slowed flow. However, similar to other techniques used to evaluate brain blood flow, including DSA, brain blood circulation may not be completely absent at the time of clinical BD diagnosis. This is especially true if the intracranial pressure has not yet increased to critical levels, which subsequently leads to the complete cessation of intracranial circulation [6,10]. Furthermore, the presence of a skull defect, as in the case of an extensive fracture or decompressive craniectomy, could

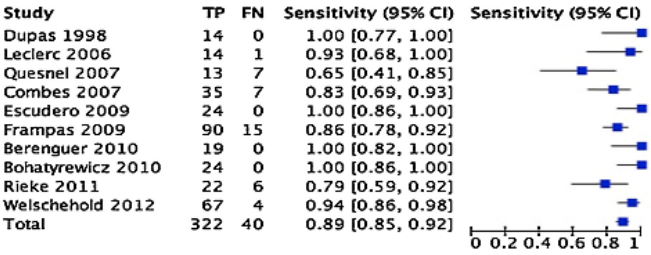

Figure 1 4-point score CTA sensitivity ranged from 65% (one study) to 100% (two studies). Sensitivity was over 80% in 85% of the studies. Mean sensitivity was 86.7% ± 7.8%. TP: true positive; FP: false positive; FN: false negative; TN: true negative.

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delay reaching the 4-point score [32] until the skin stretches to its limit. This condition may make it possible for opacification to be displayed in cortical vessels for a longer time, despite the fact that an absence of deep venous drainage appears to be maintained [40]. The studies evaluated in this review excluded hypothermia or hypotension, and images were obtained at 20, 30 and 60 seconds after contrast ministration, which is sufficient time to observe the contrast transit in the encephalon.

One patient in the entire sample died as a consequence of thrombosis of the superior sagittal sinus. Hypothetically, a deep venous thrombosis (DVT) could preclude obtaining the 4-point score by CTA, although the infarction of the basal ganglia and mesencephalon associated with the opacification of the distal ACM bilaterally may suggest DVT. Additionally, this condition rarely exclusively affects the deep brain drainage system and obstructs it completely [41].

In 2006, Leclerc et al. concluded that the best criterion to confirm BD using CTA was verification of the absence of opacification in cortical MCAs and ICVs, and the first consensus about CTA scores was determined by the French Society of Neuroradiology in the 2007. Furthermore, the French expert consensus has advised extension of CTA scans to the thorax and abdomen of potential organ donors to verify possible lesions that may have previously gone unnoticed [42]. In 2010, Young [43] suggested that brain drainage should be the focus of BD investigations because of the possibility of brain perfusion in a subset of brain-dead patients. Moreover, up to 25% of patients with BD may exhibit residual blood perfusion on SPECT scans in small cerebral areas for various time periods [44]. Sawicki et al. [45] investigated the stasis filling phenomenon using perfusion computerized tomography and calculating the mean blood transit time in BD patients. The authors concluded that this phenomenon is not compatible with neuronal survival. Recent investigations, especially since 2009, have considered exclusive evaluations of distal arterial cortical segments and deep brain drainage as reliable for BD diagnosis [7,9,19,25,28].

Although CTP is able to measure the mean transit time of blood circulation in the brain, this test suffers from drawbacks, such as the need for a greater amount of contrast medium (although the use of contrast medium did not affect graft function or survival [46]), the inability to evaluate all brain tissues, the increased time to obtain and process images, and the decreased sensitivity compared with CTA [11]. Limitations that are common to both methods include the need to transfer these patients to the radiology department and the need for modern CT devices.

CTA evidence level to confirm BD is comparable to other brain blood circulation ancillary tests that are currently applied. Regarding future investigations, we address the need to include a sample of eligible controls to obtain conditions suitable for comparison between groups, to assess inter-observer scores and to calculate the CTA specificity during the evaluation of cortical perfusion and brain drainage.

Conclusions

For patients who fulfill clinical BD criteria, the use of CTA with a 4-point score demonstrated high sensitivity to confirm the diagnosis. However, the use of CTA with a 7-point score is not recommended. Current evidence does not support the use of CTA or CTP as screening exams for BD. CTP does not offer advantages over CTA; however, further investigations regarding BD diagnosis using these methods should be conducted.

Contributorship statement

The idea for the article was of Professor Edson Bor-Seng-Shu. Sergio Brasil performed the literature search, wrote the article, and is the guarantor of the paper. Milena Azevedo performed a parallel search, and Wanderley Bernardo and Luca Bernardo the statistical analysis. Professors Edson and Manoel Jacobsen with the aid of Marcelo Oliveira critically revised and authorized its submission.

Data sharing

There is no unpublished data available from this study. Results have not been presented in any meeting to the date.

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Disclosure of interest

The authors declare that they have no competing interest.

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11.2 APENDIX B - Article published by *the Journal of Neurosurgery, 2019.*

JNS

CLINICAL ARTICLE

Computed tomography angiography accuracy in brain death diagnosis

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OBJECTIVE The present study was designed to answer several concerns disclosed by systematic reviews indicating no evidence to support the use of computed tomography angiography (CTA) in the diagnosis of brain death (BD). Therefore, the aim of this study was to assess the effectiveness of CTA for the diagnosis of BD and to define the optimal tomographic criteria of intracranial circulatory arrest.

METHODS A unicenter, prospective, observational case-control study was undertaken. Comatose patients (Glasgow Coma Scale score ≤ 5), even those presenting with the first signs of BD, were included. CTA scanning of arterial and venous vasculature and transcranial Doppler (TCD) were performed. A neurological determination of BD and consequently determination of case (BD group) or control (no-BD group) was conducted. All personnel involved with assessing patients were blinded to further tests results. Accuracy of BD diagnosis determined by using CTA was calculated based on the criteria of bilateral absence of visualization of the internal cerebral veins and the distal middle cerebral arteries, the 4-point score (4PS), and an exclusive criterion of absence of deep brain venous drainage as indicated by the absence of deep venous opacification on CTA, the venous score (VS), which considers only the internal cerebral veins bilaterally.

RESULTS A total of 106 patients were enrolled in this study; 52 patients did not have BD, and none of these patients had circulatory arrest observed by CTA or TCD (100% specificity). Of the 54 patients with a clinical diagnosis of BD, 33 met the 4PS (61.1% sensitivity), whereas 47 met the VS (87% sensitivity). The accuracy of CTA was time related, with greater accuracy when scanning was performed less than 12 hours prior to the neurological assessment, reaching 95.5% sensitivity with the VS.

CONCLUSIONS CTA can reliably support a diagnosis of BD. The criterion of the absence of deep venous opacification, which can be assessed by use of the VS criteria investigated in this study, can confirm the occurrence of cerebral circulatory arrest.

Clinical trial registration no.: 12500913400000068 (clinicaltrials.gov)

https://thejns.org/doi/abs/10.3171/2019.6.JNS191107

KEYWORDS brain death; computed tomography angiography; transcranial Doppler

Brain death (BD) is defined as an irreversible coma with a catastrophic brain injury and is characterized by unresponsiveness, lack of receptivity, and absence of respiratory movements and brainstem reflexes. After the diagnosis of BD, artificial life support may be terminated.[5,40] Therefore, a rigorous assessment of an individual with a suspicion of BD is mandatory to prevent any source of error in the diagnosis.[14,43]

The diagnosis of BD is primarily based on clinical examination,[12,21] including the evaluation of brainstem re-

ABBREVIATIONS BD = brain death; CBF = cerebral blood flow; CTA = computed tomography angiography; GCS = Glasgow Coma Scale; ICV = internal cerebral vein; LAR = legally authorized representative; MCA = middle cerebral artery; SAH = subarachnoid hemorrhage; TBI = traumatic brain injury; TCD = transcranial Doppler; VS = venous score; 4PS = 4-point score.

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flexes and an apnea test. The implementation of ancillary testing has become mandatory in many countries around the world, whether adopted in cases for which examination for BD is impossible or to help clarify conditions that might mimic compromised cortical or brain stem function.[5,24,44]

Recently, the use of computed tomography angiography (CTA) has been extensively investigated as an ancillary method in the diagnosis of BD[2,3,15,16,23,33,42] and has been adopted by some medical societies, applying the standard proposed by Frampas et al.[16] regarding the lack of opacification of the distal portions (M4) of the middle cerebral arteries (MCAs) and internal cerebral veins (ICVs) as compatible with BD.[10,32,37] However, the use of CTA has been contested due to concerns about its accuracy,[18,45] and in accordance with previous meta-analyses, there is no evidence to support its use in the diagnosis of BD. Although CTA could theoretically be suitable for confirming intracranial circulatory arrest, CTA should be investigated in large controlled studies.[5,6,22,39] One meta-analysis verified the higher sensitivity of CTA if the absence of contrast was observed in the ICVs exclusively.[5] Therefore, by means of what is to our knowledge an unprecedented methodology, in the present study selected neurocritical patients underwent CTA imaging and transcranial Doppler (TCD) followed by neurological assessment, with the purpose of evaluating the accuracy of CTA in the diagnosis of BD and reviewing the CTA criteria currently applied for this purpose.

Methods

Study Population

This was a prospective, controlled, observational study approved by the local ethics committee, and consent from the legally authorized representative (LAR) was obtained for each patient. The study protocol followed the Standards for Reporting of Diagnostic Accuracy Studies (STARD) statement.[29] The study protocol was registered with the ClinicalTrials.gov database (http://clinicaltrials.gov) under the registration number 12500913400000068. The inclusion criteria were deeply comatose patients without signs of BD (i.e., Glasgow Coma Scale [GCS] score 4 or 5 and pupillary reactive GCS score 3) and deeply comatose patients presenting with the first signs of BD (i.e., GCS 3 with mydriasis) who were admitted to the ICU of the Hospital das Clínicas of São Paulo University, Brazil, between January 2014 and January 2018. The clinical assessment for BD, as the reference standard, defined each patient to belong to the BD group (case) or no-BD (control) group. Our exclusion criteria were contraindications to contrast injection (i.e., known allergy or acute kidney injury [AKIN] stage ≥ 2),[26] arterial hypotension (i.e., mean arterial pressure below 65 mm Hg) just prior to CTA scanning, absence of intracranial sonographic window for TCD assessment, LAR refusal, and BD already diagnosed.

Study Design

Intensive care physicians and the neurosurgeon were involved in the selection of eligible patients and sending them to undergo CTA scans and TCD. TCD was used as the comparative technique due to its practicability and high sensitivity and specificity.[11] An experienced TCD operator performed all sonographic assessments, and 2 physicians blinded to the TCD and CTA findings repeated the neurological evaluation for each patient (Fig. 1). Physicians did not proceed with the complete neurological evaluation for BD until CTA and TCD were obtained. Two neuroradiologists evaluated all CTA images. TCD and CTA analyses were performed blindly with regard to the patient's clinical status. For study purposes, neurological assessment was always aborted when a sign not indicating BD was present (i.e., GCS score 4 or 5, GCS score 3 with reactive pupils, etc.). The reasons for including no-BD patients were for CTA specificity calculation and to avoid influence on the image interpretations of the neuroradiologists.

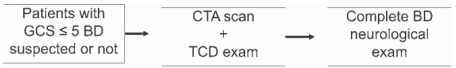

FIG. 1. Flowchart. Obtaining the index study imaging (CTA) was fundamental prior to completion of the BD assessment.

Clinical Data

We collected demographic data and results of the Simplified Acute Physiology Score 3 on admission. Patients with traumatic brain injury (TBI) had the severity of trauma reported by use of the Marshall classification of TBI, and subarachnoid hemorrhage (SAH) severity was reported using the Hunt and Hess grading scale. We also recorded patient age, sex, and the cause of brain injury.

Definition of BD

The clinical diagnosis of BD was our gold standard, and it was based on the following criteria: 1) identification of the cause of brain injury; 2) absence of hypothermia (body temperature < 35°C); 3) absence of CNS depressors; 4) absence of brainstem reflexes, such as pupillary reactivity; corneal, oculo-cephalic, and oculo-vestibular reflexes; and cough and gag reflexes; and 5) a positive apnea test (i.e., acidosis and $PaCO_2$ of at least 55 mm Hg with initial values < 40 mm Hg). The assessment of brainstem reflexes followed this sequence, with no need to proceed to the next reflex evaluation if the preceding reflex was present. If a patient had been administered sedatives, the evaluation for BD was performed after at least 4 half-lives of drug elimination. The entire clinical assessment for BD was performed twice for each patient by different physicians, with an interval of at least 6 hours between assessments.

Computed Tomography Angiography

Imaging Protocol

Imaging studies were performed on a 64 or 128 multislice Brilliance CT scanner (Philips Healthcare) or Aquillion CXL128 (Toshiba Medical System Corp.). The parameters for CTA acquisition were as follows: 100 kVp, 250 mAs, field of view 200 mm, slice thickness 0.5–0.9 mm, and reconstruction increment 0.33–0.45 mm. The

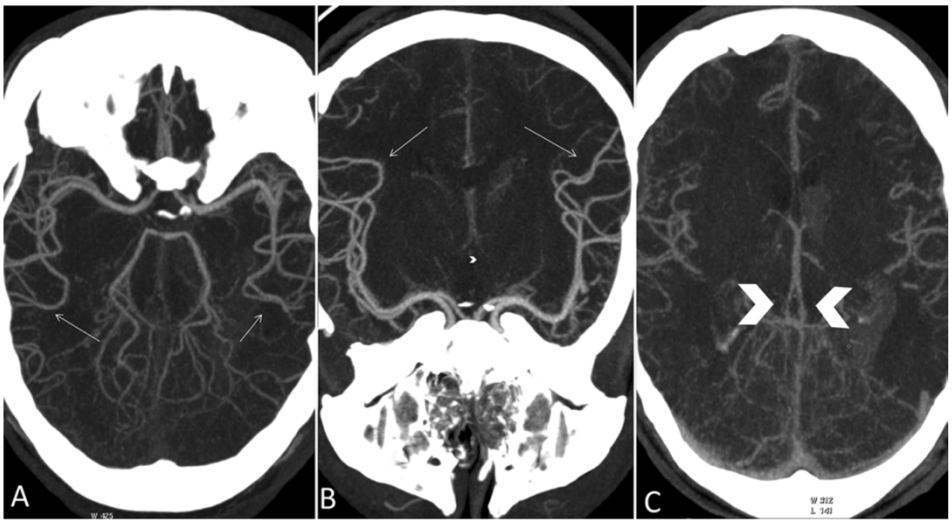

FIG. 2. CTA targets were the distal MCA segments (*arrows* in **A** and **B**) and ICVs (*arrowheads* in **C**).

scan was taken parallel to the orbito-meatal line, with the range extending from C1–C2 to the vertex. A total of 80 mL of nonionic contrast (iopromide 300 mg/mL) was administered intravenously into the cubital vein, using a power injector at a rate of 4.0 mL/sec followed by a 40-mL saline flush at a rate of 4 mL/sec.

Scans were obtained for 3 phases. The first was a noncontrast phase, followed by an arterial contrast phase, and ending with a venous contrast phase. The arterial scan delay was individually adapted using a bolus-tracking technique. For bolus tracking, the first scan, a single, nonenhanced low-dose scan at the level of the upper neck, was obtained. With the start of contrast administration, repeated low-dose monitoring scans were obtained every second. When the contrast was first observed in the common carotid artery, the arterial-phase scan was automatically triggered without any time delay. Then, after 40 seconds, a venous phase scan was performed.

Multiplanar and 3D maximum intensity projection angiographic reconstruction techniques were performed on the images using an iSite Enterprise workstation (Philips Healthcare). Mean arterial pressure during the scans was above 70 mm Hg. The correct contrast injection was verified by visualizing superficial temporal arteries.

Imaging Endpoints

The collected imaging endpoints were opacification of the ICVs and M4 MCAs[10] bilaterally. The 4-point score (4PS) consisted of the documentation of no observation of both M4 segments and both ICVs. As verified by a meta-analysis, a venous score (VS) was proposed with higher sensitivity[5]; thus, the exclusive absence of observation of both ICVs was defined as the VS, where 1 point was given for each vessel visualized. A score of 0, indicating no opacification of both ICVs, corresponded to BD. In both scores, the visible presence of any of these vessels on CTA precluded the diagnosis of BD. These vessels were selected as the tomographic targets because M4 segments are the most distal arteries visualized before entering the cerebral parenchyma, whereas the ICVs are responsible for hemispheric blood drainage (Figs. 2 and 3).

Transcranial Doppler

BD using blind color TCD (Digi-Lite, Rimed or EZ-Dop, DWL) was supported if systolic spikes lower than 50 cm/sec or nonprogressive oscillatory flow (i.e., diastolic velocity 0 cm/sec or diastolic backflow) were present in the MCAs and basilar artery[1] (Fig. 4). Presence of intracranial Doppler signals were mandatory for inclusion of each patient in this study. As for BD and CTA, hypothermia or hypotension were not present during TCD examination.

Statistical Analysis

The sample size was calculated using the method proposed by Buderer.[8] Considering that the prevalence of BD in our first 30 cases was 45%, the known CTA sensitivity of previous studies was 85%,[16] and there was an estimated precision of 10%, a sample size of 91 patients was needed to precisely estimate the validity of our index test (CTA). Considering logistic delays and the inappropriate image quality of some CTA exams, we expected a dropout rate of 10%, yielding a final sample size of 100 patients.

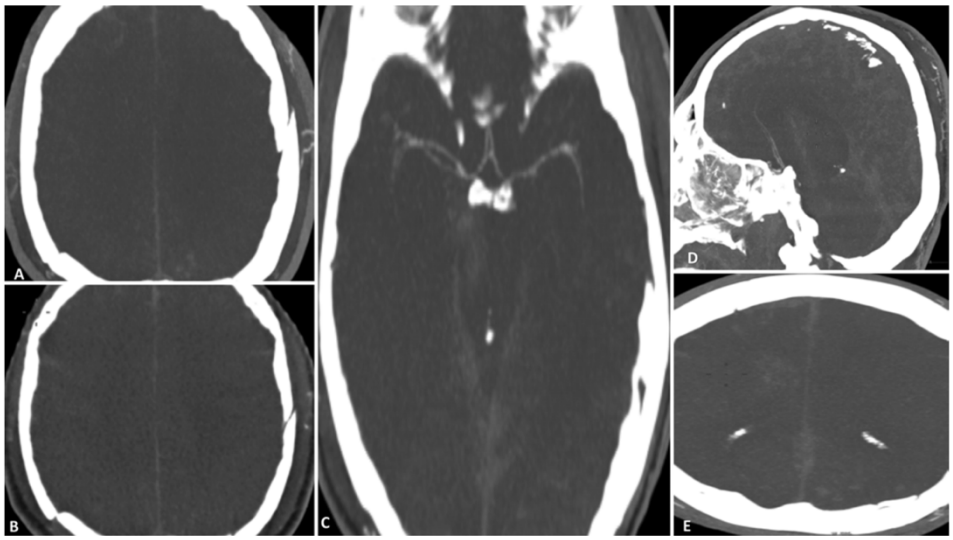

FIG. 3. CTA showing BD. Despite subarachnoid and cisternal hemorrhage, arterial (**A, B, and C**) and venous (**B and E**) phases reveal the absence of MCAs and ICVs.

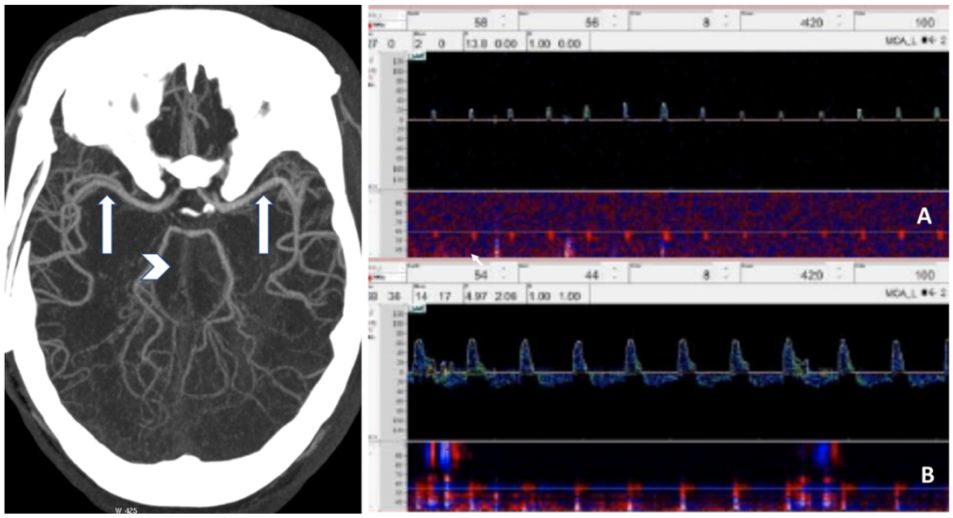

FIG. 4. TCD arterial collapse visible on CTA (*left*) was verified by the presence of systolic spikes (**A**) or nonprogressive oscillatory flow (**B**) in the MCAs (*arrows*) and basilar artery (*arrowhead*). Figure is available in color online only.

Continuous variables were expressed as the mean ± standard deviation (SD) or median and interquartile range (IQR), depending on the data distribution. Discrete variables were expressed as counts and percentages. Differences between groups were compared using the Student t-test, Mann-Whitney U-test, chi-square test, or Fisher's exact test, as appropriate. We defined measures of concordance for the CTA exams as the agreement percentage and Cohen's $\varkappa$ between the readings by 2 radiologists for both 4PS and VS.

We calculated diagnostic sensitivity, specificity, positive and negative predictive values, likelihood ratios, and the area under receiver operating characteristic curves of diagnostic tests, comparing their accuracy with clinical assessment. In addition, we performed a specific subgroup analysis to evaluate CTA statistical variances according to the interval between CTA and clinical assessment. Statistical analyses were performed using Stata software, version 14.1 (Stata Corp). Statistical significance was determined by a $p < 0.05$.

Results

Study Population

From January 2014 to December 2017, 194 eligible patients were enrolled in this study. In all, 31 eligible patients were not included because of severe renal failure, 21 because of LAR refusal, and 32 because of arterial hypotension at the time of CTA scan. A total of 110 consecutive patients were included in this study. Four patients were lost, 2 patients because their CTA scanning protocol was not followed properly and 2 patients who suffered cardiac arrest prior to completing the neurological assessment. Finally, for the remaining 106 patients, CTA scanning, TCD examination, and complete BD clinical assessments were all performed. The time of clinical testing for BD after CTA scanning varied from 0 to 36 hours, although there were only 10 cases for which clinical testing for BD was performed more than 12 hours after CTA scanning.

The characteristics of the study population are reported in Table 1. There were no statistical differences between the BD and no-BD groups with regard to the mean numbers of included patients stratified according to age, sex, admission diagnosis, or severity of health conditions.

TABLE 1. Sample features

Variable	BD	No BD	p Value
No. (%) of patients	54 (50.9)	52 (49.0)	
Age in yrs, mean ± SD	40.07 ± 18.5	45.28 ± 19	0.15
Male	66.6	69.2	0.77
Admission diagnosis			
TBI	57.5	48	0.71
SAH	26	32	0.14
Stroke	4	14	0.07
Other	12.5	6	0.49
Marshall TBI classification			
Total no. of TBI patients	31	25	
Score			
1–2	2	12	<0.001
3	19	4	<0.001
4	4	1	0.61
5	2	8	0.03
6	4	0	0.11
Hunt & Hess SAH scale			
Total no. of SAH patients	14	17	
Score			
1–2	1	5	0.18
3	2	7	0.13
4	5	4	0.69
5	6	1	0.02
SAPS 3 score, median (IQR)	61.5 (51–74)	57 (45–67)	0.31
GCS score			
3	100	70.6	<0.001
4	0	21.6	<0.001
5	0	7.8	0.052

SAPS 3 = Simplified Acute Physiology Score 3.
Values are presented as percentages of patients unless otherwise indicated.

Diagnosis of BD

In all, 52 patients (49%) did not have a clinical diagnosis of BD. The signal against BD in 8 patients was flexion to pain, whereas it was abnormal extension to pain in 7 patients; 37 (71%) of these patients had a GCS score of 3, and of these, 32 presented with miotic reactive pupils. Considering the remaining 5 patients who had mydriatic pupils at the time of CTA scanning, 2 presented with the absence of the corneal reflex with preservation of the oculo-cephalic reflex, and for these 2 patients the BD assessment was aborted at the moment the presence of this reflex was detected; 3 patients had absence of all brainstem reflexes but respiratory movements were observed. A total of 19 (36%) control patients died before discharge. In the no-BD group, both TCD and CTA showed no false-positive results (i.e., 100% specificity).

A total of 54 patients fulfilled the clinical criteria for BD. CTA 4PS suggested BD in 33 of these patients, (i.e., 61% sensitivity), and VS suggested BD in 47 of them (i.e., 87% sensitivity) (Table 2 and Figs. 5 and 6).

TCD showed signs of BD in 52 cases (i.e., 96% sensitivity). None of the patients included in this research had absence of sonographic windows. In the 2 patients in whom TCD did not show findings of BD, CTA showed intracranial circulatory arrest. However, in both cases, CTA was obtained 12 hours after TCD.

Timing and Flow

A decrease in sensitivity was observed if the complementary test was performed at an interval of more than 12 hours prior to the neurological evaluation. If exclusively considering those patients in whom CTA scanning was performed close to the neurological assessment for BD, which means a neurological diagnosis of BD an average of 24 hours after the first signs of BD, CTA sensitivity could reach 95.5% and 81.8% by means of VS and 4PS, respectively (Table 2).

TABLE 2. Statistical results with reference to time interval between tests

	≤12 Hrs Btwn Tests		>12 Hrs Btwn Tests		Full Sample	
Technique	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
TCD	100% (96.1–100%)	100% (93.2–100%)	96% (92–100%)	100% (92–100%)	96% (95.5–99.7%)	100% (95.8–100%)
CTA 4PS	81.8% (67.3–91.8%)	100% (93–100%)	55.6% (21.2–86.3%)	100% (92.5–100%)	61.1% (46.9–74.1%)	100% (93.2–100%)
CTA VS	95.5% (84.5–99.4%)	100% (93–100%)	66.7% (29.9–92.5%)	100% (92.5–100%)	87% (75.1–94.6%)	100% (93.2–100%)

Tests and Interobserver Agreements

Interobserver agreement was higher for the evaluation of the VS ($\varkappa = 0.96$) than for the evaluation of the 4PS ($\varkappa = 0.77$).

Discussion

Literature Review

Three systematic reviews have been published on the use of CTA to support the diagnosis of BD.[5,22,39] A meta-analysis of 322 patients indicated a sensitivity of 87.5% with the 4PS and a sensitivity of 97.5% with the VS.[5] Otherwise, Kramer and Roberts concluded that the routine use of CTA as a supplementary diagnostic test for BD is not recommended until the CTA diagnostic criteria have undergone further refinement and prospective validation.[22] The systematic reviews agreed that the overall quality of the studies included were low, especially because of the lack of information regarding 1) how a clinical diagnosis of BD was performed, 2) if the radiologists engaged were blinded, and 3) if patients were consecutively selected. Furthermore, the time interval between CTA and the gold standard assessment was excessively delayed in some instances. Additionally, CTA was exclusively applied in patients who were previously considered brain dead. A second limiting factor was the heterogeneity among the included studies, especially in terms of the CTA imaging evaluation criteria used for the diagnosis of BD, i.e., whether the evaluation involves a 7-point score or 4-point score. Moreover, the ancillary tests used to compare with CTA varied within the same study in some cases. Although low-quality studies were identified, Taylor et al. (in their systematic review) reported that for the knowledge obtained until that time, the CTA, when performed in patients who first undergo a complete, well-designed clinical assessment for BD, may be considered a reliable technique for support of the diagnosis.[39]

Study Design

To our knowledge, this is the first study of BD diagnosis to use 3 groups of blinded evaluators. Only 1 recent study included control patients suitable for comparison

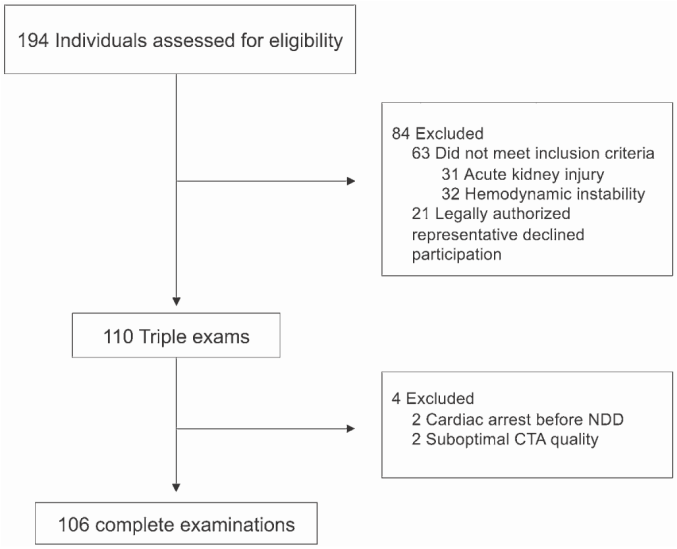

FIG. 5. Flow chart of patients enrolled and reasons for exclusion. NDD = neurological determination of death.

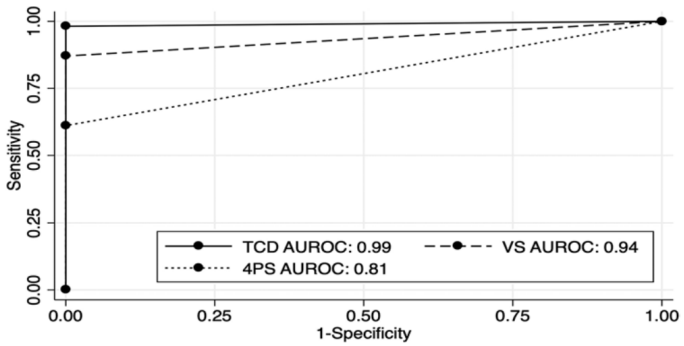

FIG. 6. Receiver operating characteristic (ROC) curve showing sensitivity and specificity of brain CTA in predicting the collapse of brain vessels in the included patients. AUROC = area under the ROC curve.

when evaluating ancillary techniques for diagnosing BD.[17] Patient inclusion was consecutive, obeying the same protocol of neurological evaluation as proposed by the AAN (American Academy of Neurology) for the entire sample and not varying from the gold standard.

In our study, for patients without BD, CTA was able to adequately exclude intracranial circulatory arrest in all cases, giving no false positives. Another novel aspect of the present study concerns the use of a methodology that differs from that used in previous studies, because our CTA scans were performed prior to the neurological evaluation, thus preventing the neuroradiologists from knowing which patient was effectively brain dead and which one was not. Moreover, as CTA scanning was performed in a "very early phase" of BD evolution, the 4PS sensitivity dropped in comparison to what was observed in the study by Garrett et al.,[17] while the sensitivity of the exclusive use of VS was substantially higher, indicating that the phenomenon of cerebral deep venous compression seen in BD leads to early collapse of the ICVs, as shown in our study.

Of the BD patients in this study, there were none in whom both techniques (i.e., CTA and TCD) were incompatible with BD. Interestingly, we observed greater accuracy of both techniques (CTA and TCD) and scores (4PS and VS) when the exams were performed closer (< 12 hours) to the neurological assessment. This finding may be explained by the dependence of circulatory arrest on the rise of intracranial pressure, while intracranial flow inversely decreases[6]; thus, BD may exist with extremely reduced cerebral blood flow (CBF)[30] or the stasis filling phenomenon, which can be falsely interpreted as blood flow.[34] Both phenomena are also observed in DSA[31] and have been suggested not to be real CBF.[35]

In our study the prevalence of BD was higher than that reported for previous studies,[9,28] although specific reports lack BD statistics obtained exclusively among patients with a GCS score ≤ 5. Overall mortality in TBI patients with a GCS score of 3 with absent pupillary reflexes is 80%,[13] and 90% with reference to the Marshall 3 scale.[27] Some of the patients in the no-BD group displayed the first signs of BD, although complete neurological assessment revealed no BD at that moment; these and a large number of no-BD patients died soon after the study, which indicates the severity of illness in all included patients in this study and the feasibility of comparison between groups.

CTA Technique

CTA multiphase acquisition, prioritizing arterial, venous, and late time, is a dynamic method that allows adequate evaluation of intracranial arteries and veins.[18] Modern CTA techniques may be timing invariant, superimposing several timeframes and enabling reconstructions independent of the time required for maximal vessel enhancement.[35,36] Thus, even in clinical situations in which the circulation of the arterial contrast bolus to the brain is delayed, such as in intracranial hypertension or heart failure, the equipment will adjust the onset of acquisition to the ideal arterial time and consequently to the other times of venous or late acquisition.

CTA is not operator dependent, as a technician can perform the examination, while a radiologist can evaluate the images remotely. Additional advantages of this technique are the wide availability of devices with 32 channels or more, the readiness in acquiring images, the need of only a single peripheral vein, and the capacity for additional scanning of the entire body of a potential organ donor.[38] TCD displays advantages as a repeatable and unobtrusive bedside technique.

Brain Venous Drainage

Current research, adopting the 4PS, recommends delaying the CTA scan 12 hours after the clinical determination of BD.[19] Otherwise, the highest sensitivity in our study was observed with the VS 12 hours prior to neurological assessment. However, the VS represents cerebral drainage exclusively, and the evaluation of the petrosal veins might be of value to assess the entire encephalic drainage,[25] an aspect that is under to further research.

CTA has been criticized for not following the same

standard as DSA.[18] Otherwise, it is not uncommon for DSA to present contrast injection as proximal filling, even when contrast is injected intravenously rather than intra-arterially.[7,21] Even with the application of DSA standards, cortical branches may opacify and falsely transmit the impression of CBF, as selective injection of individual vessels can raise the blood pressure to a degree that it forces contrast material into the intracranial space, especially with high injection flow rates,[4] whereas our study indicates that the phenomenon of circulatory arrest in the distal capillary and deep brain drainage appears to occur in the early phase of BD.

Limitations

The main limitation of this study was the time for completing the clinical assessment for BD. Efforts were made to shorten the time between examinations to reduce the possibility of timing bias. However, this study was designed and executed as closely as possible to the actual practice of tomographic scanning in neurocritical patients, and the timing during a highly stressful psychological moment was significant for LAR refusal of participation of the study.

CTA demands patient transportation, which may be harmful to patients and laborious for ICU personnel.[20] Furthermore, the need for contrast material may worsen renal function, although the use of contrast may not be a factor for poor outcome for graft transplantation, as previously reported.[41] In our study, hemodynamic instability and impairment of renal function were important contributors to the dropout rate and represented hindrances for utilizing CTA.

The role of ancillary testing in BD has been considered exclusively to support diagnostic challenges, such as in severe facial traumatic injuries, in some types of poisonous (i.e., rattlesnakes bites and pufferfish ingestion) and exogenous intoxications, Bickerstaff syndrome, and chronic carbon dioxide retainers, and there is no perfect ancillary technique to confirm BD that could replace a neurological examination. BD is one of the most important diagnoses in medical practice, and methods to increase diagnostic confidence are valuable for LARs as well as physicians. Meanwhile, the association of ancillary testing with neurological assessments is currently the best practice.

Impact of Present Research

In many locations around the world, CTA is the sole technique available to provide support for the diagnosis of BD. Particularly in those countries where an ancillary test is mandatory, CTA represents an opportunity to improve ICU strategies and organ donation and transplantation.

Conclusions

CTA can reliably support a diagnosis of BD. The criterion of absence of deep venous opacification can confirm the occurrence of cerebral circulatory arrest.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Brasil, Bor-Seng-Shu. Acquisition of data: Brasil, Gattás, Nunes, de Oliveira, Tierno, Becker, Bassi, Malbouisson, Paiva. Analysis and interpretation of data: Brasil, de Oliveira. Drafting the article: Brasil. Critically revising the article: Bor-Seng-Shu, de-Lima-Oliveira, Taccone, de Oliveira, Nogueira. Reviewed submitted version of manuscript: Nogueira. Statistical analysis: Martins Tomazini. Administrative/technical/material support: Teixeira. Study supervision: Bor-Seng-Shu, de-Lima-Oliveira.

Supplemental Information

Previous Presentations

The manuscript was included as part of the PhD thesis of Sergio Brasil, submitted to the Neurology Department of Hospital das Clinicas, School of Medicine, São Paulo University, Brazil.

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