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Editorial

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Defining the boundary between life and death: New insights from neuropathology

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"The boundaries which divide Life from Death are at best shadowy and vague. Who shall say where the one ends, and where the other begins?"

Edgar Allan Poe, *The Premature Burial*

In this issue of the *Journal of Neuropathology and Experimental Neurology*, Dr. Folkerth and colleagues provide new insights into the neuropathology of brain death, shedding light on decades-old questions about the boundary between life and death (1). Through the lens of classic light microscopic analysis, they comprehensively characterize histopathological changes affecting the neurons, glia, vasculature, and dura of Ms. Jahi McMath, a 13-year-old girl who was pronounced brain dead after a hypoxic-ischemic brain injury caused by postoperative hemorrhage. Ms. McMath's body was sustained via mechanical ventilation and medical support for 4-and-a-half years after she was pronounced brain dead, raising profound ethical questions that have been the subject of numerous debates (2–5).

Dr. Folkerth and colleagues now help ground these debates about brain death in objective, histopathological evidence, providing a rare glimpse into the structural composition of the human brain, years after brain death declaration. Most knowledge about the science of human brain death, and its sine qua non, the complete cessation of cerebral perfusion, is extrapolated from experimental animal and cell culture models (6), and therefore may be limited by species- and paradigm-specific differences in pathological responses to hypoxic-ischemic injury (7, 8). Direct human observation is of paramount importance to link experimental and mechanistic insights to human neuropathology. To date, however, the few neuropathological investigations of human brain death have raised as many questions as answers.

For example, why do fewer than 100% of neurons show histopathological signs of irreversible cell death in individuals who undergo autopsy within hours or days of brain death (9)? Does this observation reflect the limitations of our conventional staining protocols, or do molecular and transcriptional changes associated with neuronal death precede the morphological changes detected by light microscopy? How can we reconcile the medico-legal concept of brain death with its heterogeneous neuropathology (9–11)? In the chronic setting (i.e., when the body is sustained for years after brain death declaration), do we expect to see complete degradation of the brain, or can its structural integrity persist without function? Most importantly, can cellular and tissue viability be confirmed by the standard light microscope, and if so, does "normal appearing" morphological viability indicate preserved function?

Each of these neuropathological inquiries emerges from a more fundamental question with self-evident clinical, ethical, and societal implications: does our definition of the boundary between life and death matter in clinical medicine today, and why? In art and science fiction, the boundary may be blurred and manipulated. Yet in medicine and law, this boundary must be sharp and definitive (12). For patients with severe brain injuries and their families, there is no diagnosis more profound, or more final, than that of brain death (13). Defined clinically in 1968 (14) and legally accepted by dozens of countries worldwide (15, 16), brain death is pronounced by clinicians when a patient with severe brain injury has experienced "irreversible coma" and the complete loss of brainstem reflexes, including spontaneous respiration (17). Though differences exist between hospital policies for the pronouncement of brain death, such as in the number of neurological examinations required (16, 18), there is international consensus that a human being is medically and legally dead at the moment that brain death is pronounced (19). This definition of brain death is critical for guiding clinical management, such as determining when a brain injury is still reversible and therapeutic interven-

tions can still be effective (20). It is also essential for identifying the ethically appropriate time point to harvest systemic organs for life-saving transplantation.

Moreover, there is a neuroscientific imperative to understand the histopathological hallmarks of brain death. In this endeavor, neuropathology is the bedrock of basic medical science, providing an opportunity to elucidate mechanisms of brain injury and generate hypotheses for testing in cellular and animal models. Yet, for decades, the pathological hallmarks of brain death have remained a matter of debate. This is in part because of their heterogeneity, both temporally and spatially, and in part because of the inability to differentiate primary morphological changes related to stroke, systemic shock, traumatic brain injury, hemorrhage, and tumor from secondary ischemia, edema, inflammation, transtentorial herniation, necrosis, and apoptosis.

Progress in identifying the pathological hallmarks of brain death has also been hindered by a lack of relevant brain specimens available for autopsy (9, 17). Given that mechanical ventilation and cardiovascular support are typically removed within hours of a declaration of brain death (9), there are even fewer neuropathological descriptions of the human brain in individuals whose bodies were supported for months-to-years after brain death (21–23).

The findings reported by Dr. Folkerth and colleagues indicate that the histopathology of brain death is indeed heterogeneous, even within the brain of a single individual, and that complete tissue degradation is not the inevitable outcome. The autopsy of Ms. McMath's brain revealed 2 distinct types of pathological changes, depending on whether the tissue was reperfused. In sections taken from gyral crests and cortical sulci, the extracranial arterioles appeared to reperfuse the tissue, as evidenced by macrophage infiltration, mineralization, and hemorrhages of variable chronicity. The reperfusion of superficial cerebral and cerebellar cortices by the external carotid and vertebral arteries, via meningeal and posterior pharyngeal branches, is a key morphological observation. The angiogenic factors that promote this reperfusion are unknown, as is its human time course.

In sections taken from deeper cortical layers and subcortical white matter regions that were not reperfused, the tissue appeared autolytic, with a complete loss of hematoxylin staining of neurons and astroglia. The "faint eosinophilia" that outlined recognizable neurons, glial cells, and vessels in these regions has been described in prior human studies, leading to the term "mummification," as seen in autopsied stillbirths (24, 25). This mummified tissue had preserved structural integrity but contained devitalized cells, a novel insight into chronic postmortem tissue reactions in human brain death. As Dr. Folkerth and colleagues point out, without perfusion there can be no phagocytosis of devitalized cells. Indeed, neuropathological studies performed within days of brain death have reported tissue without neutrophils, macrophages, or reperfusion hemorrhages (26, 27). Dr. Folkerth and colleagues' vivid description of the layering of perfused and nonperfused (mummified) tissue reactions thus suggests an "outside-in" process of revascularization. Even if the reperfusion process is not rapid or robust enough to revitalize the brain, the mere presence of such reperfusion raises profound questions about the dynamic interplay between the infarcted human brain and its extracranial circulation.

Importantly, Dr. Folkerth and colleagues acknowledge that the extent of staining in Ms. McMath's brain was limited (e.g. blocks were not available from the diencephalon or brainstem, which contribute to consciousness [28, 29]). There were also technical challenges experienced during the autopsy, as indicated by the absence of NeuN staining for neurons in well-fixed tissues, possibly due to prolonged fixation or embedding artifacts. As such, the authors humbly and appropriately state that their ability to infer correlations with neuronal function during the 4-and-a-half years after brain death pronouncement is constrained. Nonetheless, these observations provide new insights into the heterogeneous pathological changes of "chronic brain death" and contribute to current neuroscientific debates about the loss of neuronal function at the time that cerebral perfusion ceases.

Animal models of complete cerebral ischemia have established that brain function ceases within seconds of cerebrocirculatory arrest, with high-energy metabolites depleted within minutes (30). Yet recent studies using extracorporeal membrane perfusion, combined with "cocktails" of cell-free perfusates (31, 32), suggest that cells and tissues, including in the brain, can survive hours of ischemia (32). These animal studies have far-reaching clinical implications, as they suggest that the time window for the treatment of cerebral hypoperfusion, and preservation of hypoperfused organs for transplantation, may be longer than previously believed. Tissue death may not follow a single time-defined trajectory, set in motion at 1 time point, or a single molecular cascade mediated by a "death transcriptome" (33). Rather, tissue death more likely reflects a complex interplay of primary and secondary tissue reactions, set in motion by an ischemic event that triggers multiple transcriptome networks underlying heterogeneous morphological changes, as elegantly illustrated with the ever-current and basic tool of the neuropathologist, the light microscope.

Dr. Folkerth and colleagues have done a major service to the discipline of neuropathology by cataloguing the heterogeneous destructive tissue reactions induced by ischemia. These observations build upon the few prior studies of "chronic brain death" and provide a basis for future collaborative research between neuropathologists and basic scientists into the molecular underpinnings of brain death. The heterogeneity and complexity of the neuropathology described by Folkerth and colleagues lend new evidence to the idea that lethal molecular time points precede morphological changes detectable with the light microscope. They may also help explain isolated "islands" of function within the hypothalamic-pituitary axis, as suggested by the onset of menarche in Ms. McMath (34), potentially due to regional differences in vulnerability to ischemia (11).

In summary, Dr. Folkerth and colleagues advance our understanding of the neuropathology of brain death by meticulously reporting a broad spectrum of heterogeneous tissue changes in the brain of a young woman whose body was sustained for 4-and-a-half years after brain death declaration. In doing so, they reinforce the critical role of the neuropatholo-

gist in clinical neurology. Further, Dr. Folkerth and colleagues remind us of the transformational importance of single case reports—a tradition that has provided fundamental observations over hundreds of years of clinical neuroscience (35). Nuanced qualitative observations in a single case may reveal hypothesis generating, mechanistic insights obscured by quantitative data. For brain death—a diagnosis about which the public and press may be understandably confused (36)—the objective evidence and clarity that Dr. Folkerth and colleagues provide are not just welcomed, but essential. In an age of big data and -omics research, the careful microscopic analysis of human tissue still has the potential to provide fundamental answers to age-old questions about the boundary between life and death. The McMath family is to be lauded for consenting to an autopsy study during a painful and difficult time, establishing Ms. McMath's contribution to medical science. We also applaud Dr. Folkerth and colleagues for cementing this contribution with great scholarship, commitment, skill, and compassion.

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CONFLICT OF INTEREST

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