Supplementary File S1: Part One:

Understanding Death

Death by cardiopulmonary criteria and brain death criteria

Although the exact mode for determining death has been debated throughout history, for many years the death of a person was signified by the irreversible loss of vital signs of life. [1] Today, the loss of vital signs—namely, the cessation of the heartbeat, respiration, and brain stem function (death by cardiopulmonary criteria)— remains the standard by which physicians declare death for roughly 98% of people.[2] Biologically, cardiac arrest and death by cardiopulmonary criteria are synonymous and reflect the underlying pathophysiological processes that occur in response to ischemia and anoxia after the cessation of the heartbeat. Cessation of the heartbeat and death occur after any severe illness or injury impairs oxygen delivery and/or metabolic activity of the heart, or after any primary cardiac disorder (such as a dilated cardiomyopathy) leads to a fatal cardiac arrhythmia. The underlying biological and pathophysiological process that accompanies the cessation of the heartbeat is medically termed "cardiac arrest" when attempts at resuscitation are actively being made by clinicians and "death by cardiopulmonary criteria" when attempts are not made (e.g., in response to a do-not-resuscitate request), or when resuscitation is deemed to have been futile or unsuccessful. Despite the semantic difference, biologically these terms represent the same underlying pathophysiological processes, albeit at different time points after the cessation of the heartbeat. The immediate absence of blood flow that follows the standstill of the heartbeat leads to the rapid cessation of respiration and brain stem activity, as well as whole-brain function, owing to the cessation of glucose and oxygen delivery to vital organs.[3] A person does not have vital signs of life and appears lifeless; aside from the very rare instances of auto-resuscitation, without interventions this event would usually represent the irreversible end of life and hence death.[4]

The only exception to this method of declaring death is the relatively small group of people who are declared dead by brain death criteria (roughly 2% of all deaths).[2,5] These are individuals who have suffered catastrophic brain injuries, but whose respiratory and circulatory functions are artificially maintained using modern organ-support measures. Consequently, instead of severe brain stem injuries leading to cardiopulmonary collapse and inevitable death by cardiopulmonary criteria, due to modern organ support systems, in severely brain-injured individuals, respirations are artificially maintained using ventilators.[5] This approach prevents respiratory failure and hence the cessation of the heartbeat. Consequently, after the initiation of severe brain injury (e.g. massive hemorrhage and traumatic brain injury), individuals may lose whole brain function (including the brain stem). However, depending on the type and severity of the initial injurious insult, the brain may still maintain the potential for biological recovery. Eventually, the underlying cellular damage progresses to a point whereby the brain is irreversibly damaged and without the potential for biological recovery.[5] At this time, the brain may be considered to have 'died' despite artificial respirations on a ventilator and an active heartbeat. [5] Because the seat of consciousness relates to the brain, since the late 1960s, a small minority of patients (~2% i.e.

those who have suffered devastating brain injuries) have been declared dead using brain-death or neurological criteria, without waiting to meet cardiopulmonary criteria (i.e. the inevitable cessation of respiration and heartbeat), which is delayed while on artificial life support.[5] It is important to point out that the determination of death by cardiopulmonary criteria and brain death criteria typically does not rely on objective markers of irreversible cell death, but rather relies on tests that examine for the absence of organ function.[5] Thus, after the onset of severe brain injuries that lead to loss of whole brain function, time becomes the major factor in determining whether the underlying damaged brain cells have transitioned to a state of biological irreversibility in an individual. Until that point, the potential to restore brain function and hence life remains.

Understanding irreversibility of death: the scientific foundations for exploring death in the 21st century

Today, the concept of an irreversible loss of vital organ function remains the hallmark of definitions of death all over the world. In the United States, the Uniform Determination of Death Act defines death as either the "irreversible cessation of circulatory and respiratory functions" (death by cardiopulmonary criteria) or the "irreversible cessation of all functions of the entire brain, including the brain stem" (death by brain-death criteria).[6] Thus, the key feature in the determination of death, whether from the perspective of the public or the medical profession, remains the issue of an irreversible end of function and hence life (i.e., irreversible loss of vital organ functions).

However, evidence suggests that irreversibility of organ function in relation to death and, consequently, the understanding of death—whether through cardiopulmonary or brain death criteria—may need to be reconsidered due to recent discoveries.[3,7,8] The major common feature with respect to death by cardiopulmonary and brain death criteria is that after the onset of any severe anoxic or traumatic brain injury (which share a similar pathophysiology) causes loss of whole brain function, there is a period that may last hours and possibly days before the underlying brain cells become irreversibly damaged and "die." Testing for brain death largely examines for the absence of brain function in people with severe brain injuries. However, as the loss of brain function after severe brain injuries may precede the completion of cell death, including necrosis and apoptosis, evidence suggests that in some cases, people correctly declared dead according to brain death criteria may not demonstrate histopathological evidence of brain cell death on autopsy.[9] This suggests that even though untreatable devastating brain injuries had culminated in the loss of whole brain function at the time of the declaration of brain death, the biological/cellular point of irreversibility may not have yet been reached in some individuals. Yet, people were deemed to be irreversibly "brain dead," due to the absence of brain function, together with the lack of treatment options that could inhibit or reverse the devastating injury process. Thus, as future treatments are refined and discovered, many of those who are considered irreversibly dead today – whether by brain death or cardiopulmonary criteria - but have not reached true cellular irreversibility, could have brain function and life restored and thus potentially be revived in the future.

Arguably, the most significant discovery to affect 21st century resuscitation science and the understanding of death has been the realization that human cells do not typically become irreversibly damaged within minutes of anoxia, including in the post-mortem period and that the mechanism of brain damage after anoxia largely relates to the effects of reoxygenation of previously ischemic

tissues.[3,7,8] While irreversible cell death occurs at differing times in different organs, brain cells (if not re-oxygenated) have been shown to be more resilient to the effects of post-mortem anoxia than had been assumed until recently.[7,8,10,11]In a landmark study, Palmer *et al*.[10] demonstrated that cadaveric human brain biopsies obtained 7 h or longer post-mortem can yield viable neuronal stem cells for cell culture. This finding has also been replicated in many animal studies, with some suggesting that it may be possible to culture such cells up to 140 h post-mortem.[11] In 2019, a group of researchers led by Nenad Sestan at Yale University restored multiple aspects of global brain function – including circulation and cellular functions, vascular dilatory activity, active cerebral metabolism and spontaneous synaptic activity - over a period of 6-10 hours in 32 pig brains that had been taken from a slaughterhouse four hours after death (i.e., 10-14 hours post-mortem).[12] This was achieved through controlled perfusion of the brain starting four hours after death. Importantly, they also demonstrated the preservation of cytoarchitecture, as well as attenuation of cell death in the brain. These findings further show that after anoxia and death, cells in the brain do not always become irreversibly damaged and maintain their cytoarchitecture for many hours post-mortem.[12] As the investigators decided to stop the experiment after only 6-10 hours of controlled reperfusion, it is not known whether they could have restored global cortical electrical activity if they had waited longer.

These studies have opened a possible new understanding of irreversibility of death, with implications for neuroscience, critical care, resuscitation science, organ transplantation, philosophy, the social sciences, medical ethics, and society at large. In particular, these findings have led to the recognition that, functionally speaking, the irreversible loss of vital signs of life and hence death transitions through two distinct phases: a) relative (medical/practical) irreversibility and b) absolute (biological/cellular) irreversibility. Relative or medical/practical irreversibility starts after the heart stops and a person is declared dead (in cases of death by cardiopulmonary criteria) and may potentially last many hours to days. This reflects the post-mortem period during which the cytoarchitecture of the cells remains preserved and the underlying cells in the body have not yet become irreversibly damaged in a cadaver and thus could potentially support life again. However, the organs in the body remain non-functional and an individual remains “irreversibly” dead, because the medical means to support life may not be available at a given time or place (e.g., due to disparities in care, different levels of expertise, different perceptions about what may be possible medically, resources, and so on), or may not have yet been discovered scientifically in a given era (e.g. before the discovery of CPR in 1960). In the less common cases of people who have suffered devastating brain injuries leading to brain death, the period of functional irreversibility starts after the injurious insult has led to loss of global brain function or brain stem function (in countries such as the UK), but before the underlying cells in the brain have become irreversibly damaged and “died” (through apoptosis, necrosis or otherwise) and could therefore never support life again, whether now or in the future.

We propose that the in-between period after someone is declared dead, but before the cells in the cadaver have reached a biological point of irreversibility, represents an intriguing gray zone. This is the period in which people may be considered irreversibly dead, but yet maintain the potential to be resuscitated from a scientific and biological perspective. The possibility of a prolonged gray zone before biological irreversibility is established during the post-mortem period provides a possible opportunity for treatments directed at saving lives and brains in the future. These include optimization of oxygen delivery to the heart and brain,

as well as therapeutic opportunities that aim to inhibit cell death pathways after anoxia, ischemia, and traumatic brain injuries, together with their secondary complications, including reperfusion injury and vasospasm.[13–16] Importantly, a gray zone of potential reversibility after death could also provide the opportunity to scientifically explore the human mind and consciousness during the death process.

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