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Hypoxic-Ischemic Encephalopathy Evaluated by Brain Autopsy and Neuroprognostication After Cardiac Arrest

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IMPORTANCE Neuroprognostication studies are potentially susceptible to a self-fulfilling prophecy as investigated prognostic parameters may affect withdrawal of life-sustaining therapy.

OBJECTIVE To compare the results of prognostic parameters after cardiac arrest (CA) with the histopathologically determined severity of hypoxic-ischemic encephalopathy (HIE) obtained from autopsy results.

DESIGN, SETTING, AND PARTICIPANTS In a retrospective, 3-center cohort study of all patients who died following cardiac arrest during their intensive care unit stay and underwent autopsy between 2003 and 2015, postmortem brain histopathologic findings were compared with post-CA brain computed tomographic imaging, electroencephalographic (EEG) findings, somatosensory-evoked potentials, and serum neuron-specific enolase levels obtained during the intensive care unit stay. Data analysis was conducted from 2015 to 2020.

MAIN OUTCOMES AND MEASURES The severity of HIE was evaluated according to the selective eosinophilic neuronal death (SEND) classification and patients were dichotomized into categories of histopathologically severe and no/mild HIE.

RESULTS Of 187 included patients, 117 were men (63%) and median age was 65 (interquartile range, 58-74) years. Severe HIE was found in 114 patients (61%) and no/mild HIE was identified in 73 patients (39%). Severe HIE was found in all 21 patients with bilaterally absent somatosensory-evoked potentials, all 15 patients with gray-white matter ratio less than 1.10 on brain computed tomographic imaging, all 9 patients with suppressed EEG, 15 of 16 patients with burst-suppression EEG, and all 29 patients with neuron-specific enolase levels greater than 67 µg/L more than 48 hours after CA without confounders. Three of 7 patients with generalized periodic discharges on suppressed background and 1 patient with burst-suppression EEG had a SEND 1 score (<30% dead neurons) in the cerebral cortex, but higher SEND scores (>30% dead neurons) in other oxygen-sensitive brain regions.

CONCLUSIONS AND RELEVANCE In this study, histopathologic findings suggested severe HIE after cardiac arrest in patients with bilaterally absent cortical somatosensory-evoked potentials, gray-white matter ratio less than 1.10, highly malignant EEG, and serum neuron-specific enolase concentration greater than 67 µg/L.

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Supplemental content

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Following cardiac arrest (CA), the severity of hypoxic-ischemic encephalopathy (HIE) determines neurologic outcome. In patients with suspected HIE, current guidelines recommend multimodal neuroprognostication.[1,2] Bilaterally absent cortical somatosensory-evoked potentials (SSEPs),[3-7] serum neuron-specific enolase (NSE) concentration above a critical threshold,[8,9] highly malignant electroencephalographic (EEG) patterns,[10-13] and a gray-white matter ratio (GWR) based on brain computed tomographic (CT) imaging findings below a critical threshold[14-18] reliably estimate poor outcome. Neuroprognostication studies are susceptible to a self-fulfilling prophecy, as the findings shown with investigated prognostic parameters frequently influence decisions regarding withdrawal of life-sustaining therapy.[19] Neuroprognostication studies with prolonged intensive care and limited withdrawal of life-sustaining therapy could eliminate this bias but may raise ethical concerns owing to the likely high proportion of survivors with severe disability.[20] If a self-fulfilling prophecy exists following current prognostication guidelines, brain autopsies could identify patients with prognostic parameters suggesting poor outcome but without severe HIE. Hence, we studied brain autopsies of patients who experienced CA and investigated the association between the severity of histopathologic findings showing brain damage and premortem neuroprognostication.

Key Points

Question Is there evidence of a self-fulfilling prophecy following current international guidelines of neuroprognostication after cardiac arrest?

Findings In this 3-center cohort study of 187 patients who underwent brain autopsy after cardiac arrest, histopathologically determined severe hypoxic-ischemic encephalopathy was noted in patients with bilaterally absent cortical somatosensory-evoked potentials, gray-white matter ratio of brain computed tomographic imaging less than 1.10, highly malignant electroencephalographic patterns, and serum neuron-specific enolase concentration greater than 67 µg/L.

Meaning The findings of this study appear to support the practice of neuroprognostication according to current international guidelines.

Methods

We retrospectively identified patients who died following CA and had undergone premortem prognostic investigations and brain autopsy in 3 academic hospitals (Charité Universitätsmedizin Berlin, Berlin, Germany, 2005-2015; Skåne University Hospital, Lund, Sweden, 2003-2012; and Aarhus University Hospital, Aarhus, Denmark, 2005-2015). Data analysis was conducted from 2015 to 2020. Patients were treated according to local postresuscitation care algorithms including targeted temperature management for 24 hours in 113 patients (60%). The ethical committees at the 3 sites approved this study and waived the need for patient consent. Data were analyzed in protected clinical data systems and deidentified for final storage. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies.

Neuroprognostication was performed according to international algorithms,[2] and withdrawal of life-sustaining therapy decisions relied on repeated neurological examinations and multimodal neuroprognostication. Prognostic parameters included neurologic examination, serum NSE concentrations, SSEP, brain CT, and EEG. Details on investigations and reanalyses for this study are provided in the eMethods in the Supplement.

Blinded from results of prognostic investigations, 2 of us (C.E. and E.E.) reevaluated hematoxylin-eosin staining of formaldehyde-fixed brain sections using the selective eosinophilic neuronal death (SEND) classification[21] of the following regions: neocortex (frontal and frequently parietal, occipital, temporal, insular, and cingulate), hippocampus (subiculum; subfields cornu ammonis CA1, CA2, CA3, CA4, and dentate gyrus), basal ganglia (caudate, putamen, and globus pallidus), mesencephalon (including substantia nigra pars compacta and reticulata), pons, medulla oblongata, and cerebellum. The SEND classification relies on the finding that, after successful resuscitation with cerebral reperfusion, red, pycnotic neurons with indiscernible cytoplasmic-nuclear borders indicate neuronal death in a surrounding, spared neuropil without reactive gliosis.[21-23] Using a ×10 objective lens, we analyzed all high-power fields of each slice to quantify SEND scores indicating the level of neuronal death (SEND 0: 0% neuronal death, SEND 1: <30%, SEND 2: 30%-60%, SEND 3: 60%-90%, and SEND 4: >90%).[21] We used the maximal SEND score of all high-power fields of a particular brain region to quantify the HIE level of the entire region. SEND 4 was also assigned in the case of frank ischemic laminar necrosis without viable neurons. We dichotomized patients into no/mild HIE if the maximal SEND score in any cortical or brain stem region was 0 or 1, and severe HIE if there was neuronal death greater than 30% (SEND 2-4) in the cortex and/or brainstem. The no/mild HIE group thus comprised not only patients without any neuronal death (SEND 0), but also patients with HIE in the cerebral cortex (SEND 1, up to 30% neuronal death) in whom neurologic recovery might have been possible if they had survived.

Statistical Analysis

Baseline parameters are given as medians with interquartile range or percentages as appropriate. We illustrated the association between severity of HIE and results of prognostic parameters with scatter and box plots. Using heatmaps, we depicted the distribution of SEND scores in different regions according to findings of neuroprognostication. Data analysis was performed using MATLAB, release 2019b (MathWorks Inc).

Results

Study Population

The **Table** presents demographic details of 187 included patients (117 men [63%]; median [IQR] age, 65 [58-74] years).

Table. Patient Characteristics

Characteristic	No. (%)[a]						
		TTM		HIE			
	All	No	Yes	No/mild	Severe	RoC$^+$	RoC$^-$
Patients	187 (100)	74 (40)	113 (60)	73 (39)	114 (61)	37 (20)	150 (80)
Age, median (IQR), y	65 (58-74)	68 (59-77)	65 (58-73)	65 (58-74)	66 (58-73)	67 (60-76)	65 (58-73)
Male, No. (%)	117 (63)	44 (59)	73 (65)	46 (63)	71 (62)	23 (62)	94 (63)
OHCA, %	42	14	59	25	53	15	48
Cardiac cause, %	49	37	56	44	52	34	52
Shockable rhythm, %	38	37	39	41	36	40	38
tROSC, median (IQR), min	20 (10-30)	10 (5-20)	25 (15-40)	15 (7-25)	25 (15-40)	5 (5-10)	25 (15-40)
Second CA with resuscitation, %	23	27	21	26	21	30	21
RoC, No. (%)	37 (20)	27 (36)	10 (9)	29 (40)	8 (7)	37 (100)	0 (0)
WLST, %	49	40	61	46	57	43	55
Length of ICU stay, median (IQR), d	6 (3-14)	9 (3-25)	5 (3-9)	9 (3-26)	5 (3-9)	25 (14-40)	4 (2-8)

Abbreviations: CA, cardiac arrest; HIE, hypoxic-ischemic encephalopathy; ICU, intensive care unit; IQR, interquartile range; OHCA, out-of-hospital cardiac arrest; RoC, recovery of consciousness; RoC$^+$, temporary recovery of consciousness; RoC$^-$, no recovery of consciousness; tROSC, time from cardiac arrest to spontaneous circulation; TTM, targeted temperature management; WLST, withdrawal of life-sustaining therapy.

[a] Data were missing for OHCA (n = 4), cardiac cause (n = 30), shockable rhythm (n = 47), second CA with resuscitation (n = 1), and WLST (n = 3).

Death occurred within 1 day after CA in 19 patients, 1 to 2 days after CA in 22 patients, and 2 to 3 days after CA in 25 patients. Median duration of intensive care unit (ICU) stay was 6 days. Thirty-seven patients (20%) temporarily regained consciousness during the ICU stay. Age, sex, time to return of spontaneous circulation, cause of CA, proportion of shockable rhythms, and duration of ICU stay did not relevantly differ from those of a recent Berlin study population of patients who died of CA and did not undergo brain autopsy.[24]

Figure 1 provides examples of SEND classification and illustrates the distribution of SEND scores for various brain regions in patients with and without temporary recovery of consciousness. The distribution of SEND scores in subgroups stratified by results of neuroprognostication is presented in **Figure 2** and eResults in the Supplement. Across all patients, HIE severity was greatest in the hippocampus and cerebellum, followed by the cerebral cortex; the severity was least pronounced in the brainstem. Of all patients, 26% had near-complete neuronal death (SEND 4) in the cerebral cortex, 42% and 45% had near-complete neuronal death in the hippocampus and cerebellum, respectively, and 12% exhibited no neuronal death in the cerebral cortex. Severe HIE was found in 114 patients (61%) and no/mild HIE was noted in 73 patients (39%). Of 25 patients with cortical samples and temporary recovery of consciousness without a second successful resuscitation after initial neuroprognostication, 8 had a cortical SEND score of 0 and 14 had a score of 1. The remaining 3 patients had an ICU stay of up to 1 month with pneumonia, sepsis, and intermittent severe hypotension, suggesting the possibility of secondary brain injury after initial neuroprognostication.

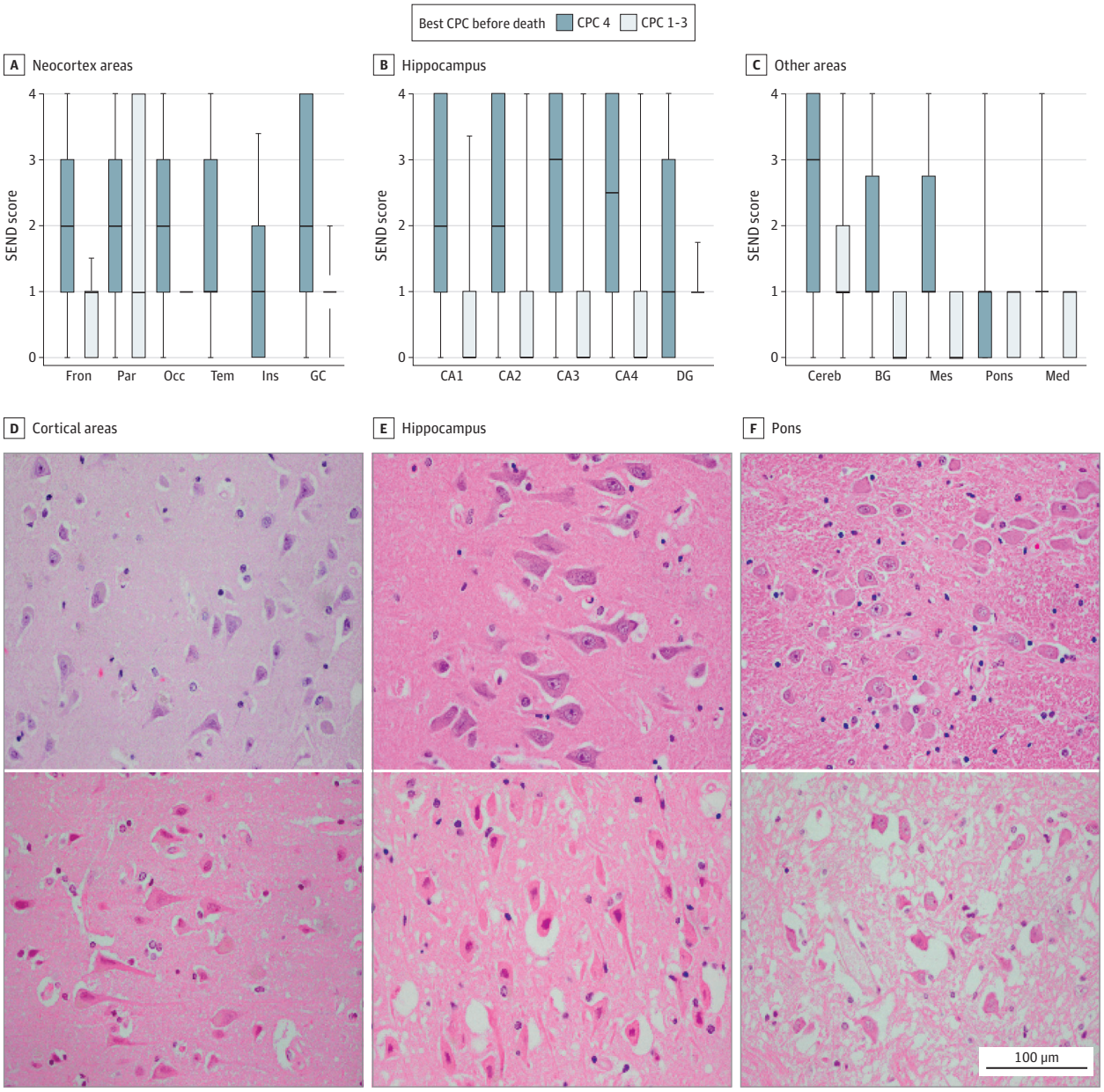
The severity of HIE increased with serum NSE concentration (Figure 2B). **Figure 3** shows that the highest serum NSE concentration obtained more than 48 hours after CA in patients with no/mild HIE was 67.0 µg/L, except for 2 patients with no/mild HIE who had plausible confounders for NSE elevation (eResults in the Supplement provides details). All other 29 patients with higher NSE concentrations more than 48 hours showed severe HIE. Of 114 patients with severe HIE, only 8 patients (7%) had NSE concentrations less than 17 µg/L 48 to 96 hours after CA. Of these 8 patients, 4 initially regained consciousness and 3 developed a second CA with resuscitation indicating possible secondary brain damage after initial NSE measurement.

Median time to SSEP was 84 hours (interquartile range [IQR], 49-123). The severity of HIE increased with decreasing SSEP amplitudes (Figure 2C). Median amplitudes were 0 µV (ie, absent: IQR, 0-0.4) in patients with severe HIE (n = 34), and 2.5 µV (IQR, 1.7-4.0) in patients with no/mild HIE (n = 24). In patients with no/mild HIE, the lowest cortical SSEP amplitude was 0.5 µV; all 26 patients with lower amplitudes showed severe HIE (**Figure 4**A). Accordingly, all 21 patients with bilaterally absent SSEPs had severe HIE. The highest amplitudes noted in 2 patients with severe HIE were 2.7 and 3.5 µV. The latter patient regained consciousness 6 days after CA and was discharged from the ICU with cerebral performance category 2, indicating regained consciousness with moderate cerebral disabilities, on day 11, but died due to a second CA in the cardiology ward on day 14.

Brain CT imaging findings were available for 122 patients. Of these, 73 had severe HIE and 49 had no/mild HIE. The severity of HIE increased with decreasing GWR (Figure 2D). The lowest GWR in a patient with no/mild HIE was 1.13 (Figure 4B). All 15 patients with a GWR less than 1.10 had histopathologically determined severe HIE. A total of 73% to 79% of patients with GWR less than 1.10 had near-complete neuronal death in the cerebral cortex and hippocampus. The majority of patients with GWR greater than 1.3 (n = 15) had no/mild HIE.

Electroencephalographic tracings were available for 81 patients, with 56 showing severe and 25 showing no/mild HIE. For standard EEGs, median time from CA to EEG was 4 days (IQR, 2-7 days). The severity of HIE varied with EEG pattern (Figure 2E and F, and Figure 4C). HIE was most severe in

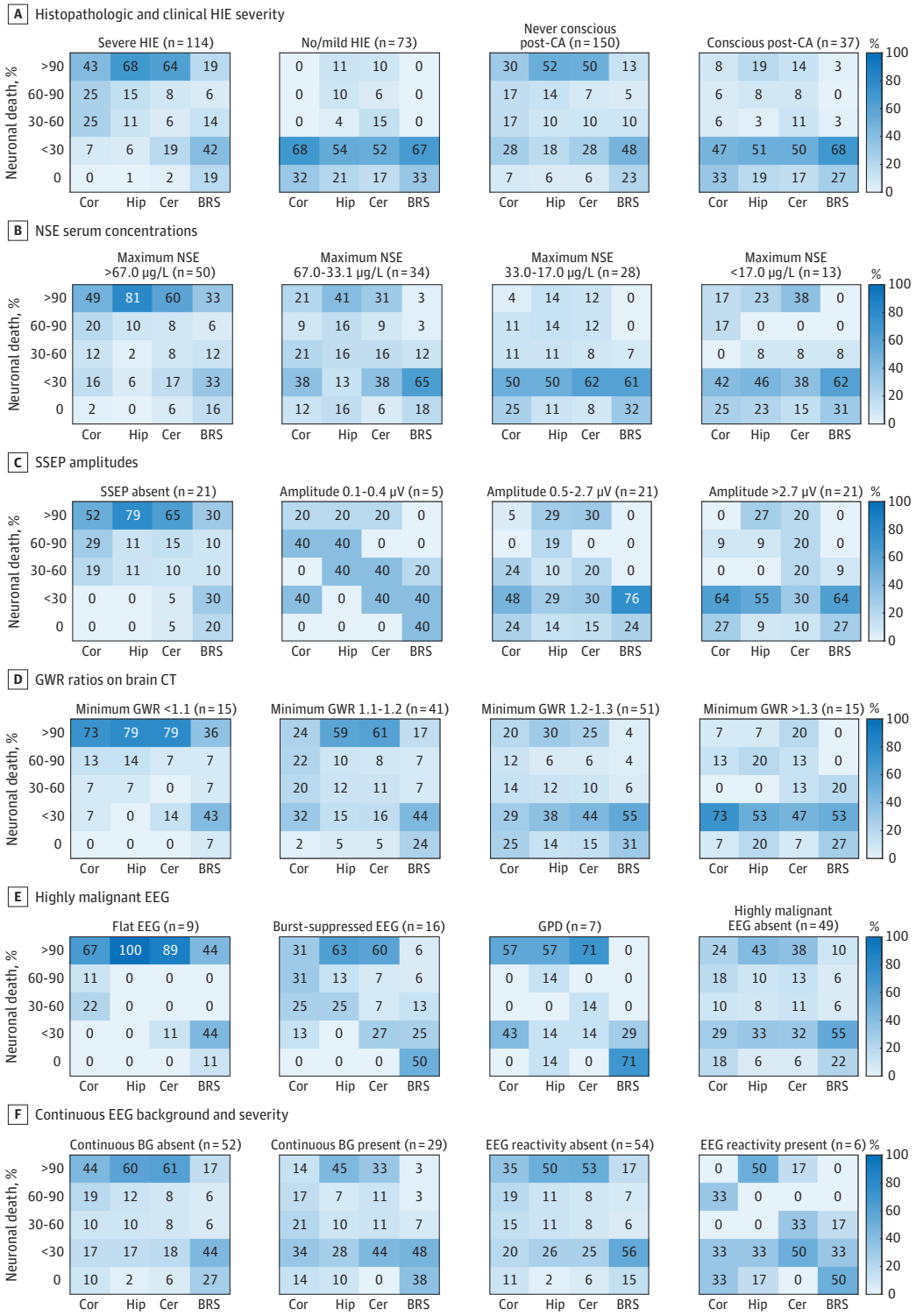
Figure 1. Association Between Microscopic Brain Damage and Best Cerebral Performance Category (CPC) After Cardiac Arrest



Semiquantitative selective eosinophilic neuronal death (SEND) scores (SEND 0: 0% neuronal death, SEND 1: <30%, SEND 2: 30%-60%, SEND 3: 60%-90%, and SEND 4: >90%) of brain areas (A-C) are displayed for different best cerebral performance category (CPC) scores after CA. Patients temporarily regaining consciousness (CPC 1-3) are depicted in white box plots and patients without recovery of consciousness (CPC 1-3) in gray box plots (median, interquartile range, 5th and 95th percentile whisker bars). Typical hematoxylin-eosin–stained sections from cortical areas (D), hippocampus (E), and pons (F) are illustrated, upper row SEND 0, lower row SEND 4. BG indicates basal ganglia; CA, cornu ammonis; Cereb, cerebellum; DG, dentate gyrus; Fron, frontal; GC, gyrus cinguli; Ins, insula; Med, medulla oblongata; Mes, mesencephalon; Occ, occipital; Par, parietal; and Tem, temporal.

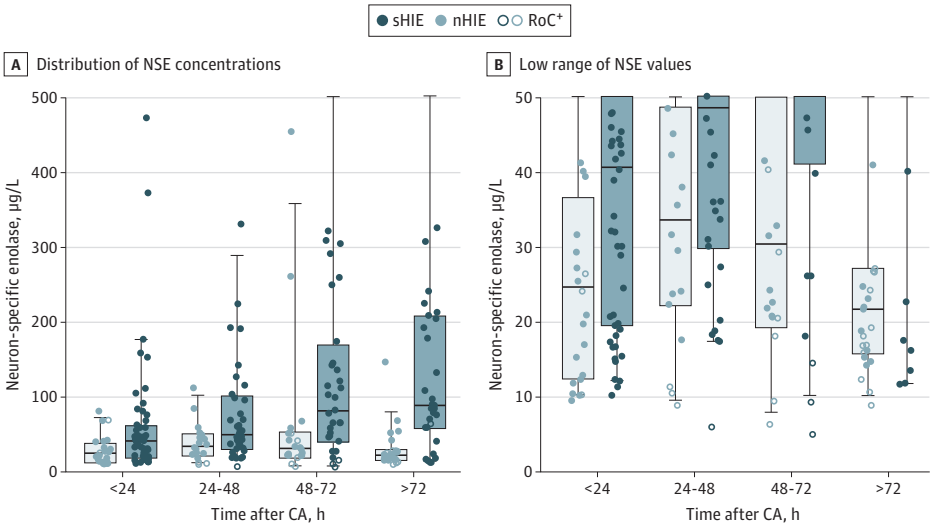
patients with suppressed EEG, followed by patients with burst suppression and generalized periodic discharges superimposed on a suppressed background. All 9 patients with suppressed EEG and 15 of 16 patients with burst suppression showed severe HIE. Among 7 patients with generalized periodic discharges, 3 individuals had SEND 1 in the cortex. However, all 4 patients with burst suppression or generalized periodic discharges and SEND 1 in the cortex had more severe damage in the hippocampus and/or cerebellum. Further details for the single patient with no/mild HIE with burst suppression and 3 patients with no/mild HIE with generalized periodic discharges on suppressed background are provided in the eResults in the Supplement. Among patients with a continuous background, 14% had SEND 4 in the cerebral cortex and 45% had SEND 4 in the hippocampus. This finding indicates that near-complete neuronal death in at least 1 part of

Figure 2. Association Between Histopathologic Severity of Hypoxic-Ischemic Encephalopathy (HIE), Prognostic Parameters, and Regaining of Consciousness



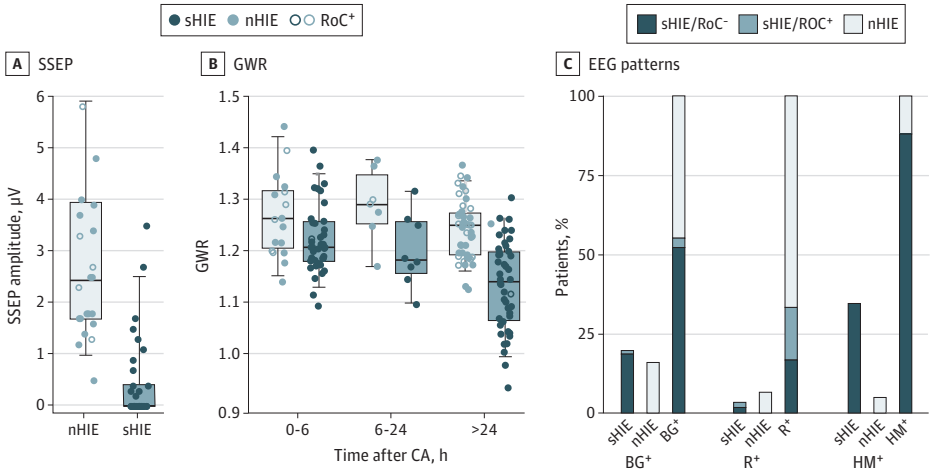
Heatmaps displaying the histopathologic severity of HIE for cortical (cor), hippocampal (hip), cerebellar (cer), and brainstem (BRS) regions using the selective eosinophilic neuronal death (SEND) classification (SEND 0: 0% neuronal death, SEND 1: <30%, SEND 2: 30%-60%, SEND 3: 60%-90%, and SEND 4: >90%). In each heatmap, columns indicate brain region, rows indicate severity of HIE, numbers in the field and field colors indicate the percentage of patients, and the heading indicates the subgroup according to HIE severity dichotomization, clinical course (A) and prognostic findings (B-F). BG indicates background; CA, cardiac arrest; EEG, electroencephalography; GPD, generalized period discharges; GWR, gray-white matter ratio; NSE, neuron-specific enolase concentration; and SSEP, somatosensory-evoked potentials.

Figure 3. Neuron-Specific Enolase (NSE) Serum Concentration and Severity of Microscopic Brain Damage



A, NSE serum concentration in patients with severe hypoxic-ischemic encephalopathy (sHIE) (black dots) and with no/mild HIE (nHIE) (gray dots). Open circles indicate patients without a second cardiac arrest (CA) who regained consciousness before secondary deterioration (RoC+). Results are presented as scatterplots and box plots with interquartile range, median value, and whisker bars representing the 5th and 95th percentiles. B, The y-axis is restricted to the low range of NSE serum concentration.

Figure 4. Association Between Microscopic Brain Damage and Cortical Somatosensory-Evoked Potential (SSEP) Amplitudes, Gray-White Matter Ratio (GWR) of Brain Computed Tomographic (CT) Imaging, and Electroencephalographic (EEG) Patterns



A, Cortical SSEP amplitudes for patients with severe hypoxic-ischemic encephalopathy (HIE) (black dots) and with no/mild HIE (nHIE) (gray dots) are shown. Open circles indicate patients without a second cardiac arrest (CA) who regained consciousness before secondary deterioration (RoC+). Note that all 21 patients with bilaterally absent cortical SSEPs showed severe HIE (sHIE). Furthermore, patients with sHIE with reproducible cortical SSEPs had mainly low amplitudes. B, Association between microscopic HIE severity and GWR in brain. Patients with GWRs lower than 1.13 showed sHIE. Scatterplots and box plots (interquartile range, median value, and 5th to 95th percentiles) illustrate results in A and B. C, Proportion of patients with different EEG patterns: continuous background (BG+), EEG reactivity present (R+), highly malignant EEG pattern according to Westhall et al[12] (HM+) according to histopathology and clinical course. While presence of continuous background activity failed to predict absence of sHIE, most patients with preserved EEG reactivity either had nHIE or regained consciousness. Conversely, the presence of highly malignant EEG patterns indicated sHIE.

the cortex can be observed in a minority of patients who have a continuous EEG pattern. Only 11% of patients with absent EEG reactivity had no cortical neuronal damage (SEND 0).

eResults in the Supplement discuss the histopathologically determined severity of HIE in patients with 2 poor prognostic findings and compare histopathologic severity of HIE in patients with no, 1, 2, or 3 poor prognostic findings. Histopathologic severity increased with the number of poor prognostic findings. Most patients with 2 or more poor prognostic findings had extensive neuronal damage (SEND 3 or 4, >60% neuronal death) in the cerebral cortex and hippocampus.

Discussion

Several pertinent findings were noted in this study. In patients with CA who were treated with modern post-CA care, histopathologic severity of HIE was most severe in the hippo-

campus and cerebellum, second most severe in the cerebral cortex, and least severe in the brainstem. A cortical SEND score of 0 to 1 was compatible with awakening after CA. All patients with bilaterally absent SSEP, a GWR less than 1.10 shown on brain CT, and suppressed EEG, as well as 15 of 16 patients with a burst-suppression EEG, had histopathologically determined severe HIE. On a group level, the histopathologic severity of brain damage was similar in patients with these prognostic findings. Histopathologic severity of HIE increased with the number of poor prognostic findings, supporting a multimodal approach to neuroprognostication. Three of 7 patients with generalized periodic discharges on a suppressed background had a maximal cortical SEND score of 1, indicating less-severe HIE than suppressed or burst-suppression EEG. Except for 2 patients with confounders, all 29 patients with a serum NSE concentration greater than 67 µg/L 48 to 96 hours after CA showed severe HIE. Most patients with high cortical SSEP amplitudes, an NSE within the reference range 48 to 96 hours after CA, or a GWR greater than 1.3 had no/mild HIE. The absolute numbers of patients with individual poor prognostic findings were limited and the functional long-term effect of histopathologic changes cannot be assessed with certainty.

Few studies with small cohorts have examined histopathologic brain damage in patients after CA. Björklund et al[21] established the SEND classification system for histopathologic evaluation of HIE. Our study of 187 patients who underwent autopsy, which, to our knowledge, represents the largest autopsy cohort of such patients with modern post-CA treatment, appears to corroborate and extend the previous findings. Histopathologic brain damage was most pronounced in the hippocampus and cerebellum, followed by the cortex, and least in the brainstem. Overall, 26% of our cohort had near-complete neuronal death (SEND 4) in the cerebral cortex, and 42% to 45% had near-complete neuronal death in the hippocampus and cerebellum. However, 12% of patients had no cortical neuronal death. We related histopathologic damage to clinical examination and results of neuroprognostication. A cortical SEND score of 0 to 1 (0%-30% neuronal death) was compatible with reawakening after CA, indicating that cortical neuronal death per se does not exclude a good neurologic outcome. Only 3 patients with a cortical SEND score greater than 1 awakened after CA, and all 3 had plausible causes for neuronal damage at a later time point. Hence, it is likely that a cortical SEND score of 2 or higher (>30% neuronal death) is incompatible with awakening in most patients. It therefore seems appropriate to categorize patients with cortical SEND 2 or higher as having severe HIE. While patients with no visible neuronal death using the SEND classification have no histopathologic proof of HIE, patients with SEND 1 (up to 30% neuronal death) in the cerebral cortex most likely comprise a heterogeneous group, of which some might have achieved good neurological long-term outcome in case of survival.

As prognostic parameters have frequently been used in withdrawal of life-sustaining therapy decisions, a self-fulfilling prophecy may confound neuroprognostication.[19] Our study provides evidence against a self-fulfilling prophecy for several prognostic parameters. All patients with bilaterally absent SSEPs, suppressed EEG, NSE concentration greater than 67 µg/L 48 to 96 hours after CA considering confounders, and GWR less than 1.10 in brain CT images showed severe HIE. The distribution of severity of HIE in patients with these prognostic findings was similar (Figure 2), demonstrating extensive to near-complete cortical neuronal death in most patients.

Except for 1 patient with early high serum NSE concentration and a confounder for that elevation, none of the patients without cortical neuronal death (SEND 0) had any of the poor prognostic findings mentioned above. We found 1 patient with burst-suppression EEG and 3 patients with generalized periodic discharges on suppressed EEG that did not fulfill our strict histopathologic definition of severe HIE. All 4 patients had cortical neuronal death, but less than 30% of neurons were affected; however, there was greater than 30% neuronal death in other oxygen-sensitive regions. We cannot determine with certainty the outcome these patients would have achieved if they had survived.

Our analysis of multimodal neuroprognostication (eResults in the Supplement) indicates that histopathologically determined severity of HIE increases with the number of poor prognostic findings. Most patients with at least 2 strong predictors of poor outcome had greater than 60% neuronal death in the cerebral cortex and hippocampus. Thus, our data suggest that a multimodal approach to neuroprognostication as recommended by current guidelines[1,2] may reduce the risk of falsely assigning a poor prognosis to patients with the potential for recovery.

All 21 patients with bilaterally absent SSEPs showed severe HIE, with 79% of those showing near-complete neuronal death in the hippocampus, supporting findings from clinical studies.[3-7,25] The lowest cortical SSEP amplitude in a patient with no/mild HIE was 0.5 µV, in line with studies on CA survivors.[5,26-28] Amorim et al[29] stated that the false-positive rate of SSEP for predicting poor outcome may be as high as 7%. Our autopsy results do not provide evidence of false-positive, bilaterally absent SSEP, but the absolute number of 21 patients is too small to exclude this possibility.

Previous studies have found different thresholds for predicting poor outcome using NSE concentrations (eg, 50 µg/L 72 hours after CA in a large, prospective multicenter study[8] and 90 µg/L in a large, retrospective multicenter study[9]). The values may differ between laboratories or test kits.[30] In line with previous studies, we found only 2 patients with no/mild HIE but high NSE concentrations more than 48 hours after CA (maximum, 260 and 452 µg/L), both with plausible confounders for NSE level elevation. All other patients with NSE concentrations higher than 67 µg/L more than 48 hours after CA had severe HIE. The 2 exceptions underscore the importance of considering potential confounders for NSE level elevation, such as hemolysis and malignant tumors, and the need for a multimodal approach to neuroprognostication because existing confounders may not be known in an individual patient.

Gray-white matter ratio thresholds to predict poor outcome have varied in previous studies.[15-17] A GWR threshold of 1.10, manually determined with 16 regions of interest, predicted poor outcome with a very low false-positive rate and moderate sensitivity if CT imaging is performed more than 24

hours after CA.[18] Supporting the findings of previous studies, the lowest GWR in patients with no/mild HIE was 1.13 in our autopsy study. A total of 73% to 79% of patients with a GWR less than 1.10 had near-complete cortical and hippocampal neuronal death. A considerable proportion of patients with GWR within the reference range had severe HIE. This finding points to the fact that relevant cortical neuronal death may occur without relevant changes in GWR. However, few patients with a GWR greater than 1.3 had severe HIE, potentially indicating a role of CT imaging in prediction of good outcome. This possibility should be further assessed in future CT imaging studies.

Varying definitions of EEG patterns and moderate interrater agreement have impeded comparability of EEG findings in neuroprognostication studies.[31] Using standardized terminology,[32] studies have established the EEG as an important prognostic test.[12,33] Supporting highly malignant EEG patterns as reliable predictors of poor outcome, we found severe HIE in all 9 patients with suppressed background and in 15 of 16 patients with burst-suppression EEG. Among the highly malignant patterns, the severity of HIE was most pronounced for suppressed EEG with near-complete hippocampal neuronal death in all patients, followed by burst suppression, and least for generalized periodic discharges. Three of 7 patients with generalized periodic discharges on suppressed background showed no/mild HIE (<30% neuronal death in the cortex) according to our definition. However, all 3 patients had higher SEND scores in the hippocampus or cerebellum. It remains unclear whether the degree of HIE would have been compatible with a good outcome in these patients if they had survived.

Based on the selective ischemic susceptibility of pyramidal excitatory synapses to inhibitory interneurons, Tjepkema-Cloostermans et al[34] developed a model in which even a 5% reduction of this cell type caused cortical disinhibition leading to generalized periodic discharges. van Putten et al[35] reported that 20% to 25% of patients with generalized periodic discharges have normal magnetic resonance imaging findings and cortical networks generating generalized periodic discharges can be restored to normal EEG patterns if synaptic damage is limited. Pathophysiologic mechanisms causing burst-suppression EEG are described in the eDiscussion in the Supplement. Our SEND classification does not differentiate between damage of pyramidal cells and interneurons and synaptic damage cannot be analyzed with hematoxylin-eosin staining. However, despite limited patient numbers, our findings suggest that generalized periodic discharges on suppressed background reflects less-severe HIE than the other highly malignant EEG patterns. In line with a recent clinical study,[36] our data on EEG findings underline the need for multimodal prognostication and prolonged intensive treatment should be considered for individual patients with burst-suppression pattern or generalized periodic discharges.

In accordance with previous studies,[12,37] absent EEG reactivity was not highly specific for severe histopathologic HIE in our study, a finding likely explained by sedation effects at the time of EEG. Studies on early, continuous EEG suggest the absence of severe HIE in patients with early recovery of a continuous EEG background.[33] A continuous EEG background was not predictive of absence of severe histopathologic brain damage in our study, most likely owing to the late timing of EEG (median of 4 days after CA).

In 3 studies, cortical SSEP amplitudes greater than 2.5 to 3.0 μV were rarely found in patients with unresponsive wakefulness syndrome and hence indicated absence of severe HIE.[5,26,28] Our autopsy findings are also in line with this observation: only 2 of 21 patients with a cortical SEP amplitude greater than 2.7 μV showed more than 30% cortical neuronal death. Thirteen patients had a serum NSE concentration within the reference range, most of whom had no/mild HIE. These findings support those of a study showing that NSE concentrations less than 17 μg/L on day 3 following CA indicated the absence of severe HIE.[9] Continuous background activity with EEG reactivity is associated with a good outcome depending on EEG timing.[12,33] In our cohort, a continuous background rhythm per se did not permit prediction of the severity of HIE. The number of patients with a continuous reactive EEG tracing was too low to permit conclusions.

Limitations

The study had limitations. Few of the patients who died following CA underwent autopsy. It remains unclear whether our results extend to all patients who have CA. Furthermore, we cannot exclude that patients with falsely pessimistic prognostication were less likely to receive autopsy. Because brain histopathologic samples were obtained postmortem, our cohort may be biased toward severely affected patients. Thus, in subgroup analysis of individual prognostic investigations, the number of patients with low SEND scores was limited. Not all prognostic investigations were obtained in all patients for various reasons, including availability of investigations, death before completion of prognostication, or recovery of consciousness before death. Thus, the comparisons between the different prognostic investigations must be interpreted with caution. Our dichotomization of HIE using the SEND score cannot determine with certainty the functional long-term outcome that patients would have achieved if they had survived. In addition, patients in our severe HIE group might have had a chance for recovery if they had survived. Conversely, it seems likely that individual patients classified as no/mild HIE with up to 30% neuronal death in the cortex may not have regained consciousness if they had survived. Although CA leads to global brain hypoxia-ischemia and thus more homogeneous damage than other brain diseases, we cannot exclude that the samples obtained from the cortex in an individual patient are not representative of the entire cortex. Our dichotomization is supported by findings from patients who temporarily regained consciousness, but a conclusion regarding the functional outcome of the histopathologic changes cannot be drawn. We consider a second CA with return of spontaneous circulation as a confounder in the interpretation of our results. It seems possible, however, that transient episodes of severe hypotension/hypoxia or other unknown factors might have led to secondary neuronal damage after prognostication in some patients, confounding evaluation of HIE severity. To improve postmortem histopathologic examination, future studies could use automated quantification of neuronal death and additional histologic stains to study different cell types, synaptic damage, and

impaired neuronal circuits. We investigated a mixed cohort of patients who received or did not receive targeted temperature management. Prior evidence and pathophysiologic reasoning argue against a large effect of targeted temperature management on the association between results of prognostic investigations evaluated in our study and severity of HIE.[5,8] In addition, we reevaluated all prognostic tests according to definitions provided by current literature. Expertise in performing and interpreting investigations is key in reliable prognostication after CA, and our results are applicable in this context.

Conclusions

In a histopathologic, postmortem analysis of 187 patients who died following CA, we found severe HIE in patients with poor prognostic findings obtained from SSEP, brain CT imaging, EEG, and serum NSE levels, supporting the current practice to predict poor outcome after CA. However, for individual tests, our sample size was limited; thus, our results need corroboration in future studies.

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REFERENCES

1. Callaway CW, Donnino MW, Fink EL, et al. Part 8: post-cardiac arrest care: 2015 American Heart Association guidelines update for cardiopulmonary resuscitation and emergency cardiovascular care. *Circulation*. 2015;132(18)(suppl 2):S465-S482. doi:10.1161/CIR.0000000000000262

2. Sandroni C, Cariou A, Cavallaro F, et al. Prognostication in comatose survivors of cardiac arrest: an advisory statement from the European Resuscitation Council and the European Society of Intensive Care Medicine. *Intensive Care Med*. 2014;40(12):1816-1831. doi:10.1007/s00134-014-3470-x

3. Bouwes A, Binnekade JM, Kuiper MA, et al. Prognosis of coma after therapeutic hypothermia: a prospective cohort study. *Ann Neurol*. 2012;71(2):206-212. doi:10.1002/ana.22632

4. Dragancea I, Horn J, Kuiper M, et al; TTM Trial Investigators. Neurological prognostication after cardiac arrest and targeted temperature management 33°C versus 36°C: results from a randomised controlled clinical trial. *Resuscitation*. 2015;93:164-170. doi:10.1016/j.resuscitation.2015.04.013

5. Endisch C, Storm C, Ploner CJ, Leithner C. Amplitudes of SSEP and outcome in cardiac arrest survivors: a prospective cohort study. *Neurology*. 2015;85(20):1752-1760. doi:10.1212/WNL.0000000000002123

6. Leithner C, Ploner CJ, Hasper D, Storm C. Does hypothermia influence the predictive value of bilateral absent N20 after cardiac arrest? *Neurology*. 2010;74(12):965-969. doi:10.1212/WNL.0b013e3181d5a631

7. Rossetti AO, Oddo M, Logroscino G, Kaplan PW. Prognostication after cardiac arrest and hypothermia: a prospective study. *Ann Neurol*. 2010;67(3):301-307. doi:10.1002/ana.21984

8. Stammet P, Collignon O, Hassager C, et al; TTM-Trial Investigators. Neuron-specific enolase as a predictor of death or poor neurological outcome after out-of-hospital cardiac arrest and targeted temperature management at 33°C and 36°C. *J Am Coll Cardiol*. 2015;65(19):2104-2114. doi:10.1016/j.jacc.2015.03.538

9. Streitberger KJ, Leithner C, Wattenberg M, et al. Neuron-specific enolase predicts poor outcome after cardiac arrest and targeted temperature management: a multicenter study on 1,053 patients. *Crit Care Med*. 2017;45(7):1145-1151. doi:10.1097/CCM.0000000000002335

10. Backman S, Cronberg T, Friberg H, et al. Highly malignant routine EEG predicts poor prognosis after cardiac arrest in the Target Temperature Management trial. *Resuscitation*. 2018;131:24-28. doi:10.1016/j.resuscitation.2018.07.024

11. Rossetti AO, Tovar Quiroga DF, Juan E, et al. Electroencephalography predicts poor and good outcomes after cardiac arrest: a two-center study. *Crit Care Med*. 2017;45(7):e674-e682. doi:10.1097/CCM.0000000000002337

12. Westhall E, Rossetti AO, van Rootselaar A-F, et al; TTM-trial investigators. Standardized EEG interpretation accurately predicts prognosis after cardiac arrest. *Neurology*. 2016;86(16):1482-1490. doi:10.1212/WNL.0000000000002462

13. Duez CHV, Johnsen B, Ebbesen MQ, et al. Post resuscitation prognostication by EEG in 24 vs 48 h of targeted temperature management. *Resuscitation*. 2019;135:145-152. doi:10.1016/j.resuscitation.2018.10.035

14. Lee BK, Kim WY, Shin J, et al; Korean Hypothermia Network Investigators. Prognostic value of gray matter to white matter ratio in hypoxic and non-hypoxic cardiac arrest with non-cardiac etiology. *Am J Emerg Med*. 2016;34(8):1583-1588. doi:10.1016/j.ajem.2016.05.063

15. Lee BK, Jeung KW, Song KH, et al; Korean Hypothermia Network Investigators. Prognostic values of gray matter to white matter ratios on early brain computed tomography in adult comatose patients after out-of-hospital cardiac arrest of

cardiac etiology. *Resuscitation*. 2015;96:46-52. doi:10.1016/j.resuscitation.2015.07.027

16. Metter RB, Rittenberger JC, Guyette FX, Callaway CW. Association between a quantitative CT scan measure of brain edema and outcome after cardiac arrest. *Resuscitation*. 2011;82(9):1180-1185. doi:10.1016/j.resuscitation.2011.04.001

17. Scheel M, Storm C, Gentsch A, et al. The prognostic value of gray-white-matter ratio in cardiac arrest patients treated with hypothermia. *Scand J Trauma Resusc Emerg Med*. 2013;21(23):23. doi:10.1186/1757-7241-21-23

18. Streitberger KJ, Endisch C, Ploner CJ, et al. Timing of brain computed tomography and accuracy of outcome prediction after cardiac arrest. *Resuscitation*. 2019;145:8-14. doi:10.1016/j.resuscitation.2019.09.025

19. Greer DM, Rosenthal ES, Wu O. Neuroprognostication of hypoxic-ischaemic coma in the therapeutic hypothermia era. *Nat Rev Neurol*. 2014;10(4):190-203. doi:10.1038/nrneurol.2014.36

20. Kim Y-J, Ahn S, Sohn CH, et al. Long-term neurological outcomes in patients after out-of-hospital cardiac arrest. *Resuscitation*. 2016;101:1-5. doi:10.1016/j.resuscitation.2016.01.004

21. Björklund E, Lindberg E, Rundgren M, Cronberg T, Friberg H, Englund E. Ischaemic brain damage after cardiac arrest and induced hypothermia—a systematic description of selective eosinophilic neuronal death: a neuropathologic study of 23 patients. *Resuscitation*. 2014;85(4):527-532. doi:10.1016/j.resuscitation.2013.11.022

22. Horn M, Schlote W. Delayed neuronal death and delayed neuronal recovery in the human brain following global ischemia. *Acta Neuropathol*. 1992;85(1):79-87. doi:10.1007/BF00304636

23. van Putten MJAM, Jansen C, Tjepkema-Cloostermans MC, et al. Postmortem histopathology of electroencephalography and evoked potentials in postanoxic coma. *Resuscitation*. 2019;134:26-32. doi:10.1016/j.resuscitation.2018.12.007

24. Petzinka VN, Endisch C, Streitberger KJ, et al. Unresponsive wakefulness or coma after cardiac arrest—a long-term follow-up study. *Resuscitation*. 2018;131:121-127. doi:10.1016/j.resuscitation.2018.07.007

25. Oh SH, Park KN, Choi SP, et al; KORHN investigators. Prognostic value of somatosensory evoked potential in cardiac arrest patients without withdrawal of life-sustaining therapy. *Resuscitation*. 2020;150:154-161. doi:10.1016/j.resuscitation.2020.02.029

26. Carrai R, Scarpino M, Lolli F, et al. Early-SEPs' amplitude reduction is reliable for poor-outcome prediction after cardiac arrest? *Acta Neurol Scand*. 2019;139(2):158-165. doi:10.1111/ane.13030

27. Endisch C, Waterstraat G, Storm C, Ploner CJ, Curio G, Leithner C. Cortical somatosensory evoked high-frequency (600Hz) oscillations predict absence of severe hypoxic encephalopathy after resuscitation. *Clin Neurophysiol*. 2016;127(7):2561-2569. doi:10.1016/j.clinph.2016.04.014

28. Oh SH, Park KN, Choi SP, et al. Beyond dichotomy: patterns and amplitudes of SSEPs and neurological outcomes after cardiac arrest. *Crit Care*. 2019;23(1):224. doi:10.1186/s13054-019-2510-x

29. Amorim E, Ghassemi MM, Lee JW, et al. Estimating the false positive rate of absent somatosensory evoked potentials in cardiac arrest prognostication. *Crit Care Med*. 2018;46(12):e1213-e1221. doi:10.1097/CCM.0000000000003436

30. Mlynash M, Buckwalter MS, Okada A, et al. Serum neuron-specific enolase levels from the same patients differ between laboratories: assessment of a prospective post-cardiac arrest cohort. *Neurocrit Care*. 2013;19(2):161-166. doi:10.1007/s12028-013-9867-5

31. Westhall E, Rosén I, Rossetti AO, et al. Interrater variability of EEG interpretation in comatose cardiac arrest patients. *Clin Neurophysiol*. 2015;126(12):2397-2404. doi:10.1016/j.clinph.2015.03.017

32. Hirsch LJ, LaRoche SM, Gaspard N, et al. American Clinical Neurophysiology Society's standardized critical care EEG terminology: 2012 version. *J Clin Neurophysiol*. 2013;30(1):1-27. doi:10.1097/WNP.0b013e3182784729

33. Ruijter BJ, Tjepkema-Cloostermans MC, Tromp SC, et al. Early electroencephalography for outcome prediction of postanoxic coma: a prospective cohort study. *Ann Neurol*. 2019;86(2):203-214. doi:10.1002/ana.25518

34. Tjepkema-Cloostermans MC, Hindriks R, Hofmeijer J, van Putten MJAM. Generalized periodic discharges after acute cerebral ischemia: reflection of selective synaptic failure? *Clin Neurophysiol*. 2014;125(2):255-262. doi:10.1016/j.clinph.2013.08.005

35. van Putten MJAM, Hofmeijer J. Generalized periodic discharges: pathophysiology and clinical considerations. *Epilepsy Behav*. 2015;49:228-233. doi:10.1016/j.yebeh.2015.04.007

36. Forgacs PB, Devinsky O, Schiff ND. Independent functional outcomes after prolonged coma following cardiac arrest: a mechanistic hypothesis. *Ann Neurol*. 2020;87(4):618-632. doi:10.1002/ana.25690

37. Admiraal MM, van Rootselaar AF, Hofmeijer J, et al. Electroencephalographic reactivity as predictor of neurological outcome in postanoxic coma: a multicenter prospective cohort study. *Ann Neurol*. 2019;86(1):17-27. doi:10.1002/ana.25507