

Outcome-Blind EEG Microstate Architecture Identifies a Prognostically Relevant State After Cardiac Arrest

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Research Article

Keywords: EEG microstates, cardiac arrest, coma, hypoxic-ischemic brain injury, global explained variance, neurological prognostication

Posted Date: September 30th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10683416/v1>

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Additional Declarations: No competing interests reported.

Abstract

Objective

Electroencephalography (EEG) is central to neurological assessment after cardiac arrest, yet conventional approaches predominantly characterize amplitude, frequency content, continuity, suppression, reactivity, or predefined pathological patterns. EEG microstates provide a complementary framework by describing quasi-stable configurations of the global scalp electric field. We investigated whether an outcome-blind microstate decomposition of EEG from comatose survivors of cardiac arrest identifies reproducible spatial states associated with neurological outcome.

Methods

We performed a secondary analysis of the I-CARE/PhysioNet database. After prespecified EEG eligibility screening, 567 subjects were included. Microstate reconstruction was performed outcome-blind using 19-channel EEG, a fixed 300-s analysis window, GFP-peak sampling, and polarity-invariant clustering across $K=2-10$. After model-order evaluation, the $K=4$ solution was frozen and backfitted at the patient level. Associations between microstate metrics and neurological outcome were subsequently evaluated using unadjusted, multivariable, dose-response, and resampling analyses.

Results

The primary outcome analysis included 567 patients: 209 with favorable and 358 with unfavorable neurological outcomes. M2 global explained variance (GEV) was higher in patients with favorable outcome (median 0.0852 vs. 0.0564; rank-biserial correlation=0.185; $p=0.000243$; false-discovery-rate [FDR]-adjusted $p=0.000568$ within the four-state GEV family). M2 GEV was inversely associated with CPC severity (Spearman $\rho = -0.144$; $p = 0.000583$; FDR-adjusted $p = 0.001419$). After adjustment for age, sex, and shockable rhythm in the extended cohort without return-of-spontaneous-circulation (ROSC) time ($N=535$), each 1-SD increase in M2 GEV was associated with greater odds of favorable outcome (OR 1.30, 95% CI 1.07-1.58; $p=0.0092$). In the complete-case model additionally adjusted for ROSC time ($N=292$), the association remained significant (OR 1.47, 95% CI 1.10-1.97; $p=0.0087$). A graded association was observed across M2 GEV quartiles without evidence of nonlinearity. Results remained directionally stable under winsorization, exclusion of distributional extremes, heteroscedasticity-consistent standard errors, bootstrap resampling, leave-one-out analysis, and repeated leave-five-out perturbations. By contrast, M2 coverage, mean duration, and occurrence were not significantly associated with outcome after multiplicity correction.

Conclusions

An outcome-blind decomposition of EEG after cardiac arrest identified a frozen microstate, M2, whose GEV was reproducibly associated with neurological outcome. The prognostic information associated with M2 was related primarily to the degree to which this spatial configuration explained the global EEG field rather than to its conventional temporal parameters. EEG microstate architecture may provide a complementary description of residual large-scale electrophysiological organization after hypoxic-ischemic brain injury. External validation is required before clinical application.

INTRODUCTION

Neurological prognostication remains one of the most consequential challenges in the care of patients who remain comatose after cardiac arrest. Hypoxic-ischemic brain injury produces heterogeneous disturbances of cerebral electrical activity, ranging from profound suppression and discontinuity to relatively organized background activity, epileptiform abnormalities, and evolving patterns over time. Continuous EEG therefore occupies an important position within multimodal prognostication, although no isolated EEG feature should be interpreted independently of clinical context and complementary prognostic modalities (Nolan et al. 2021; Sandroni et al. 2021; Westhall et al. 2016; Ruijter et al. 2019).

Standardized EEG background classification has improved the reproducibility of post-cardiac-arrest EEG interpretation, and prospective studies have shown that early and serial EEG can contribute substantially to outcome assessment (Hofmeijer et al. 2015; Westhall et al. 2016; Tjepkema-Cloostermans et al. 2019; Hirsch et al. 2021). Nevertheless, conventional clinical EEG categories and most quantitative approaches emphasize amplitude, continuity, frequency composition, epileptiform activity, or combinations of features rather than the rapid spatial organization of the global electric field.

Large multicenter datasets have created new opportunities to characterize post-cardiac-arrest EEG quantitatively. The International Cardiac Arrest REsearch consortium (I-CARE) assembled continuous EEG recordings and clinical information from comatose patients treated at seven academic hospitals in the United States and Europe, together with neurological outcome assessed using the Cerebral Performance Category scale (Amorim et al. 2023a, 2023b). PhysioNet provides the infrastructure for reproducible access to these physiological data resources (Goldberger et al. 2000).

EEG microstate analysis provides a framework for studying spatiotemporal organization at the scale of the whole scalp electric field. Microstates are recurring configurations that remain quasi-stable for brief periods before transitioning to another configuration (Lehmann et al. 1987; Pascual-Marqui et al. 1995; Michel and Koenig 2018). Their segmentation treats the instantaneous topography as the fundamental observation, complementing frequency-domain and channel-wise approaches.

Microstate research has demonstrated reproducible developmental patterns, relationships with resting-state networks, changes across sleep and anesthesia, and abnormalities in neurological and psychiatric disease (Koenig et al. 2002; Britz et al. 2010; Musso et al. 2010; Van De Ville et al. 2010; Brodbeck et al. 2012; Khanna et al. 2015; Rieger et al. 2016; Custo et al. 2017; Artoni et al. 2022a). Recent work has also highlighted the potential relevance of microstate dynamics to altered states and disorders of consciousness (Fingelkurts et al. 2012; Bréchet et al. 2022; Artoni et al. 2022b; Topluş et al. 2024).

A major methodological concern arises when electrophysiological states are discovered and evaluated using the same clinical outcome information. Selecting model order, choosing maps, or relabeling topographies according to outcome may introduce circularity and inflate apparent clinical associations. For prognostic applications, electrophysiological architecture should ideally be identified independently of the outcome subsequently used to evaluate its clinical relevance. Topographic analysis also requires explicit attention to polarity invariance, spatial normalization, and appropriate comparison of maps (Murray et al. 2008; Michel and Koenig 2018).

This distinction is particularly important in catastrophic brain injury and disorders of consciousness. Detectable or organized electrophysiological activity should not automatically be equated with consciousness, neurological recovery, or preservation of a particular cognitive network. Clinical guidelines similarly emphasize multimodal assessment and caution in interpreting neurophysiological findings in disorders of consciousness (Kondziella et al. 2020). The interpretation of residual brain activity in severe neurological injury requires explicit separation between physiological observations and conclusions concerning consciousness or prognosis (Machado et al. 2018; Machado 2026).

In the present study, we applied an outcome-blind, patient-balanced microstate pipeline to EEG recordings from the I-CARE cohort. Model order was evaluated across $K = 2-10$ without access to neurological outcome, and topographic stability was assessed using patient-level bootstrap resampling. After freezing the final $K = 4$ solution, the resulting maps were backfitted to individual patients. Only then were outcome data unlocked. Our primary hypothesis was that the expression of one or more independently derived microstates, quantified by GEV, would be associated with neurological outcome. We further examined clinical adjustment, dose-response, state specificity, temporal specificity, influential observations, and resampling robustness.

METHODS

Study Design and Data Source

This study was a secondary analysis of the publicly available I-CARE database hosted on PhysioNet (Amorim et al. 2023a, 2023b; Goldberger et al. 2000). I-CARE contains clinical information and continuous EEG recordings from comatose patients following cardiac arrest. The present analysis was designed specifically to separate electrophysiological model discovery from clinical outcome testing. All microstate model-order evaluation, clustering, topographic stability analyses, and final map freezing were performed without using neurological outcome. Outcome information was incorporated only after the final microstate solution had been frozen and backfitted.

The clinical master cohort comprised 601 patients. After applying the prespecified requirement for at least 300 s of analyzable EEG, 567 patients were retained, and 34 were excluded. All 567 eligible patients passed GFP extraction and entered the outcome-blind microstate reconstruction. Following model-order evaluation, the $K = 4$ solution was frozen and backfitted at the patient level for the primary outcome analyses. Adjusted analyses included 535 patients in the base model without ROSC time and 292 patients in the ROSC-extended complete-case model. GFP, global field power; ROSC, return of spontaneous circulation.

Construction

The clinical master dataset contained 601 unique subjects. Application of the prespecified EEG eligibility criteria, including availability of the 19-channel montage and at least 300 s of analyzable EEG, excluding 34 subjects at preflight, leaving 567 subjects for the primary microstate analysis. GFP-peak extraction was successfully completed in all 567 eligible recordings. These 567 subjects constituted the outcome-blind cohort used for microstate model reconstruction and subsequent patient-level backfitting. Clinical outcome was not used during microstate model estimation, model-order evaluation, or freezing of the $K = 4$ solution.

EEG Preprocessing

EEG processing used 19 standard scalp channels in the fixed order Fp1, Fp2, F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1, and O2. The first 300 s of eligible EEG were used to provide a standardized observation interval across patients. Signals were filtered from 2 to 20 Hz and transformed to an average reference. Processing was implemented with MNE-compatible procedures (Gramfort et al. 2013).

Outcome-Blind GFP Sampling and Microstate Clustering

Microstate clustering was based on EEG topographies at GFP peaks, where the global scalp electric field is strongest and topographic signal-to-noise is favorable (Lehmann et al. 1987; Pascual-Marqui et al. 1995; Michel and Koenig 2018). To prevent patients with greater numbers of available GFP peaks from dominating the group solution, sampling was patient balanced and capped at 500 GFP peaks per patient. Neurological outcome played no role in GFP sampling.

Polarity-invariant modified k-means clustering was performed using Pycrostates/MNE-compatible procedures (Artoni et al. 2022a; Gramfort et al. 2013). Candidate model orders from $K = 2$ through $K = 10$ were evaluated. Because EEG microstate polarity is arbitrary, inversion of map polarity was not treated as a distinct physiological state. No synthetic fallback maps were permitted.

Bootstrap Assessment and Freezing of $K = 4$

Model-order stability was evaluated using patient-level bootstrap resampling. Entire patients, rather than individual GFP peaks, were resampled to preserve the hierarchical structure of the data. For each bootstrap solution, estimated centroids were aligned to the reference solution using Hungarian assignment maximizing absolute spatial correlation (Kuhn 1955). This prevented arbitrary cluster ordering from being interpreted as instability.

Candidate microstate solutions were evaluated without neurological outcome information. Model-order assessment preceded freezing of the final K = 4 architecture and all subsequent clinical analyses.

Following outcome-blind model-order and stability assessment, K = 4 was selected and frozen. The four resulting maps were retained under their raw identities M1, M2, M3, and M4. They were not retrospectively renamed according to canonical A/B/C/D classes, and no biological interpretation of raw cluster identity was used to select or relabel a state.

Panels A-D show the four frozen centroids M1-M4. Centroid polarity is arbitrary; positive and negative values reflect spatial orientation rather than physiological activation or deactivation. The lower panels characterize the real frozen M2 centroid at the channel level.

Patient-Level Backfitting and Microstate Metrics

The frozen K = 4 maps were backfitted to individual EEG recordings without refitting the group centroids. Patient-level metrics included GEV, coverage, mean duration, and occurrence. GEV quantifies the proportion of global topographic variance explained by a microstate while weighting observations by GFP and therefore reflects both spatial correspondence and the strength of the global electric field (Pascual-Marqui et al. 1995; Michel and Koenig 2018).

Clinical Outcome and Statistical Analysis

After clustering, model-order selection, stability analysis, freezing, and backfitting were complete, clinical outcome was unlocked. Favorable neurological outcome was defined as CPC 1–2 and unfavorable outcome as CPC 3–5. CPC was additionally evaluated as an ordinal outcome.

Continuous microstate variables were compared between outcome groups using rank-based methods, with effect size expressed as rank-biserial correlation. Associations with ordinal CPC were assessed using Spearman correlation. False discovery rate was controlled using the Benjamini-Hochberg procedure within prespecified analysis families (Benjamini and Hochberg 1995).

Logistic regression evaluated whether the principal microstate association persisted after adjustment for clinical covariates. The extended cohort excluded ROSC time and included age, sex, and shockable rhythm (N = 535). The full complete-case model additionally included ROSC time (N = 292). Continuous M2 GEV was standardized so that odds ratios represent the change in odds of favorable outcome per 1-SD increase.

Dose-response was evaluated across M2 GEV quartiles, and potential departure from linearity was tested by comparing nonlinear and linear specifications. State specificity was assessed by symmetrical M1-M4 analyses, a joint four-state GEV model, and direct coefficient contrasts. Temporal specificity was assessed using M2 coverage, mean duration, and occurrence.

Influence and robustness analyses included 1st/99th percentile winsorization, exclusion outside the 1st/99th percentiles, exclusion outside the 2.5th/97.5th percentiles, HC3 heteroscedasticity-consistent standard errors, patient-level bootstrap resampling, leave-one-out analysis, repeated leave-five-out perturbation, and Cook's distance.

RESULTS

Cohort

Of 601 subjects in the clinical master dataset, 567 satisfied the prespecified EEG eligibility criteria and successfully completed GFP-peak extraction, whereas 34 were excluded at preflight. The 567 eligible recordings constituted the outcome-blind microstate reconstruction cohort and were subsequently backfitted using the frozen K = 4 solution. Neurological outcome was available for all 567 subjects included in the primary outcome analysis.

Table 1
Study cohort and frozen analysis
populations

stage	N
Clinical master	601
Passed 300-s preflight	567
Excluded at preflight	34
GFP extraction PASS	567
Primary backfit/outcome cohort	567
Extended adjusted cohort	535
Full adjusted cohort with ROSC	292

Note. Cohort counts are taken directly from the frozen analysis results. The primary backfit/outcome cohort comprised 567 patients who passed the prespecified 300-s EEG preflight. Adjusted analyses used the corresponding complete-case populations.

Outcome-Blind Microstate Architecture

Patient-balanced clustering and bootstrap stability analysis were performed across K = 2–10 without access to neurological outcome. K = 4 was subsequently frozen as the final microstate solution. The resulting centroids were retained as M1-M4 without retrospective assignment to canonical labels. M2 showed a prominent lateral spatial configuration, with the largest absolute centroid amplitudes involving F7, T8, and T7. Because microstate polarity is arbitrary, the signs of these amplitudes indicate spatial orientation rather than physiological activation or deactivation.

Primary Outcome Associations

Median M2 GEV was 0.0852 in patients with a favorable outcome and 0.0564 in those with an unfavorable outcome (rank-biserial correlation = 0.1846, p = 0.000243, FDR-adjusted p = 0.000568). M4 GEV was also higher in the favorable group (median 0.1047 vs. 0.0764; rank-biserial correlation = 0.1826; raw p = 0.000284; FDR-adjusted p = 0.000568), and M3 GEV showed a weaker but significant unadjusted association (median 0.1266 vs. 0.0714; rank-biserial correlation = 0.1455; raw p = 0.003815; FDR-adjusted p = 0.005087). M1 GEV was not associated with outcome. M2 GEV was inversely correlated with CPC severity (Spearman rho=-0.1440; p = 0.000583; FDR-adjusted p = 0.001419).

Patient-level M2 GEV is compared between favorable (CPC 1–2) and unfavorable (CPC 3–5) outcome groups. The M2 centroid remained fixed across patients.

Table 2
State-specific association of frozen K = 4 microstate global explained variance with neurological outcome

A. Unadjusted good-versus-poor outcome and ordinal CPC associations											
state	family	metric	N	GOOD median	POOR median	rank biserial GOOD larger positive	p GOOD POOR raw	spearman rho CPC	p CPC raw	p GOOD POOR FDR within family	p CPC FDR within family
M1	GEV	M1_GEV	567	0.056	0.050	0.017	0.730	-0.033	0.433	0.730	0.433
M2	GEV	M2_GEV	567	0.085	0.056	0.185	0.000243	-0.144	0.000583	0.000568	0.001
M3	GEV	M3_GEV	567	0.127	0.071	0.146	0.004	-0.127	0.002	0.005	0.003
M4	GEV	M4_GEV	567	0.105	0.076	0.183	0.000284	-0.142	0.000709	0.000568	0.001

B. Adjusted state-specific logistic regression analyses

cohort	state	family	metric	N	OR per SD	95% CI low	95% CI high	p raw	AIC	p FDR within cohort family
EXTENDED_NO_ROSC	M1	GEV	M1_GEV	535	0.862	0.704	1.056	0.152	601.892	0.203
EXTENDED_NO_ROSC	M2	GEV	M2_GEV	535	1.300	1.067	1.583	0.009	597.050	0.037
EXTENDED_NO_ROSC	M3	GEV	M3_GEV	535	1.046	0.862	1.271	0.647	603.787	0.647
EXTENDED_NO_ROSC	M4	GEV	M4_GEV	535	1.176	0.969	1.428	0.101	601.318	0.202
FULL_WITH_ROSC	M1	GEV	M1_GEV	292	0.853	0.624	1.166	0.319	304.801	0.553
FULL_WITH_ROSC	M2	GEV	M2_GEV	292	1.473	1.103	1.967	0.009	298.513	0.035
FULL_WITH_ROSC	M3	GEV	M3_GEV	292	0.892	0.669	1.188	0.434	305.208	0.553
FULL_WITH_ROSC	M4	GEV	M4_GEV	292	1.090	0.820	1.448	0.553	305.487	0.553

Note. M1-M4 denote the four outcome-blind frozen K = 4 microstate maps and should not be interpreted as canonical A/B/C/D labels. Positive rank-biserial correlation indicates larger values in the good-outcome group. Adjusted odds ratios are expressed per 1-SD higher GEV. False-discovery-rate correction was performed across M1-M4 within each prespecified analysis family and cohort. CPC = Cerebral Performance Category; GEV = global explained variance; ROSC = return of spontaneous circulation; CI = confidence interval.

Adjusted Association of M2 GEV With Outcome

In the extended cohort without ROSC time (N = 535; 196 favorable, 339 unfavorable), each 1-SD increase in M2 GEV was associated with greater odds of favorable outcome after adjustment for age, sex, and shockable rhythm (OR 1.30, 95% CI 1.07–1.58; p = 0.00918). In the complete-case cohort including ROSC time (N = 292; 83 favorable, 209 unfavorable), M2 GEV remained independently associated with favorable outcome (OR 1.47, 95% CI 1.10–1.97; p = 0.00873). After FDR correction across M1-M4 adjusted GEV models, M2 remained significant in both cohorts (extended FDR-adjusted p = 0.0367; full cohort FDR-adjusted p = 0.0349).

Dose-Response Relationship

A graded relationship was observed across M2 GEV quartiles. In the extended cohort, the odds of favorable outcome increased by approximately 37% per quartile (OR 1.37, 95% CI 1.15–1.65; $p = 0.000532$), without evidence that a nonlinear specification improved fit ($p = 0.200$). In the full ROSC-adjusted cohort, the quartile trend remained significant (OR 1.40 per quartile, 95% CI 1.08–1.81; $p = 0.0112$), again without evidence of nonlinearity ($p = 0.810$). Greater M2 GEV was also associated with lower odds of more severe ordinal CPC in both cohorts.

Proportions are shown across increasing M2 GEV quartiles with 95% Wilson confidence intervals and quartile sample sizes.

State and Temporal Specificity

When M1-M4 GEV were evaluated symmetrically in separate adjusted models, M2 was the only state remaining significant after FDR correction in both clinical cohorts. In a joint model containing all four GEV measures, M2 retained a positive coefficient in the extended cohort (OR 1.27 per SD; $p = 0.0189$) and full cohort (OR 1.43; $p = 0.0156$), although the corresponding four-state FDR-adjusted probabilities were 0.0756 and 0.0624. Direct contrasts supported relative enrichment of the outcome association in M2 but did not establish absolute exclusivity relative to M4.

In contrast to M2 GEV, conventional M2 temporal measures did not differ significantly according to outcome. Median M2 coverage was 0.2133 in the favorable group and 0.2205 in the unfavorable group ($p = 0.469$); mean duration was 23.03 vs. 22.90 ms ($p = 0.507$); and occurrence was 8.25 vs. 8.52 ($p = 0.315$). None survived FDR correction.

Influence and Robustness

The adjusted M2 GEV association was robust to multiple perturbations. In the extended cohort, the original OR was 1.30 (95% CI 1.07–1.58; $p = 0.0092$), compared with 1.29 after winsorization, 1.26 after excluding the 1st/99th percentile extremes, 1.45 after excluding the 2.5th/97.5th percentile extremes, and 1.30 with HC3 robust standard errors. Bootstrap resampling produced a median OR of 1.31 with a 95% interval of approximately 1.09–1.62; 99.7% of bootstrap estimates exceeded 1. All leave-one-out and repeated leave-five-out estimates exceeded 1.

Results were comparable in the full cohort. The original OR of 1.47 remained above 1 after winsorization and exclusion of extremes. The bootstrap median OR was 1.49, with a 95% interval of approximately 1.11-2.00; 99.7% of bootstrap estimates exceeded 1. Every leave-one-out and repeated leave-five-out estimate was greater than 1.

Table 3
Robustness and resampling analyses of the association between frozen M2 global explained variance and good neurological outcome

A. Primary and sensitivity logistic regression analyses										
cohort	analysis	N	beta	SE	OR	95% CI low	95% CI high	p	AIC	
EXTENDED_NO_ROSC	ORIGINAL	535	0.262	0.101	1.300	1.067	1.583	0.009	597.050	
EXTENDED_NO_ROSC	WINSOR_1_99	535	0.255	0.099	1.291	1.063	1.568	0.010	597.359	
EXTENDED_NO_ROSC	EXCLUDE_1_99	523	0.229	0.099	1.258	1.035	1.529	0.021	584.770	
EXTENDED_NO_ROSC	EXCLUDE_2p5_97p5	507	0.370	0.104	1.448	1.182	1.774	0.000354	553.867	
EXTENDED_NO_ROSC	ROBUST_SE_HC3	535	0.262	0.097	1.300	1.075	1.571	0.007	597.050	
FULL_WITH_ROSC	ORIGINAL	292	0.387	0.148	1.473	1.103	1.967	0.009	298.513	
FULL_WITH_ROSC	WINSOR_1_99	292	0.365	0.143	1.440	1.088	1.907	0.011	299.273	
FULL_WITH_ROSC	EXCLUDE_1_99	286	0.293	0.146	1.341	1.007	1.786	0.045	291.998	
FULL_WITH_ROSC	EXCLUDE_2p5_97p5	276	0.367	0.144	1.443	1.088	1.915	0.011	280.460	
FULL_WITH_ROSC	ROBUST_SE_HC3	292	0.387	0.127	1.473	1.147	1.891	0.002	298.513	

B. Bootstrap and case-deletion stability analyses

cohort	N	q01 M2 GEV	q99 M2 GEV	robust covariance	bootstrap success	Bootstrap OR median	OR boot mean	Bootstrap OR 2.5%	Bootstrap OR 97.5%	% OR > 1	LOO success	LOO OR min
EXTENDED_NO_ROSC	535	0.000	0.446	HC3	1000	1.305	1.314	1.088	1.620	99.7	535	1.256
FULL_WITH_ROSC	292	0.000	0.466	HC3	1000	1.486	1.502	1.112	2.005	99.7	292	1.401

Note. Odds ratios quantify the association with good neurological outcome per 1-SD higher frozen M2 GEV. Sensitivity analyses include 1st-99th percentile winsorization, exclusion of the 1st-99th percentile tails, exclusion of the 2.5th-97.5th percentile tails, and HC3 heteroskedasticity-robust standard errors. Bootstrap, leave-one-out (LOO), and leave-5 analyses assess the stability of the M2 GEV association to resampling and case deletion. GEV = global explained variance; ROSC = return of spontaneous circulation; HC3 = heteroskedasticity-consistent covariance estimator; CI = confidence interval.

Bootstrap Stability Across Model Orders

Outcome-blind patient-level bootstrap analyses characterized reproducibility across model orders. Complete K = 3–10 stability results are shown in Fig. 6. These analyses were completed independently of clinical outcome and were used to characterize microstate architecture rather than optimize a prognostic

endpoint.

Bootstrap centroids were aligned to reference maps using polarity-invariant spatial matching. Outcome was not used in the stability analysis.

DISCUSSION

In this secondary analysis of a large multicenter cardiac-arrest EEG dataset, an outcome-blind microstate pipeline identified a frozen $K = 4$ architecture in which the GEV of one state, M2, was reproducibly associated with neurological outcome. The principal result was not merely an unadjusted separation between outcome groups: M2 GEV remained associated with outcome after clinical adjustment, showed a graded quartile relationship, correlated with ordinal CPC, and remained directionally stable under extensive perturbation and resampling.

A central strength is that M2 was not discovered by searching for the map that best separated favorable and unfavorable outcomes. Model-order assessment, clustering, bootstrap stability analysis, map freezing, and backfitting were completed before neurological outcome was introduced. This ordering reduces a direct form of circularity that can occur when biomarker construction and clinical testing use the same labels.

The dissociation between M2 GEV and conventional temporal microstate measures is particularly informative. Coverage, duration, and occurrence were not associated with outcome after multiplicity correction, whereas M2 GEV was. GEV incorporates topographic correspondence and GFP weighting; it therefore reflects the degree to which a state explains the global electric field rather than simply the amount of time spent in that state (Pascual-Marqui et al. 1995; Michel and Koenig 2018). The clinically relevant feature may therefore be the strength or fidelity with which the M2 configuration is expressed during high-GFP epochs.

This interpretation is compatible with the broader microstate literature, in which recurring topographies have been linked to transient large-scale network organization and to changes in vigilance and consciousness (Britz et al. 2010; Musso et al. 2010; Van De Ville et al. 2010; Brodbeck et al. 2012; Custo et al. 2017; Bréchet et al. 2022; Artoni et al. 2022b). However, those studies do not justify assigning the present M2 map to a specific canonical network. The frozen M2 centroid should be interpreted first as an empirical sensor-space state.

The state-specificity analyses also require a nuanced interpretation. M2 was the only state that remained significant after FDR correction across separately adjusted M1-M4 models in both clinical cohorts. However, M3 and M4 demonstrated unadjusted associations, and M2 was not significantly different from M4 in every direct contrast. We therefore interpret the result as relative enrichment of the adjusted outcome association in M2 rather than proof that M2 is the only $K = 4$ state carrying clinically relevant information.

The frozen M2 centroid displayed a prominent lateral configuration, with the largest absolute amplitudes at F7, T8, and T7. These are sensor-space descriptors. Microstate polarity is arbitrary, and a negative or positive centroid amplitude cannot be equated with regional deactivation or activation. Likewise, scalp topography alone does not identify a unique intracranial generator (Murray et al. 2008; Michel and Koenig 2018). We therefore deliberately avoid eLORETA or cortical-network claims in the present manuscript.

Current post-cardiac-arrest prognostication emphasizes background suppression, burst suppression, continuity, reactivity, epileptiform patterns, and their evolution over time within a multimodal strategy (Hofmeijer et al. 2015; Westhall et al. 2016; Ruijter et al. 2019; Tjepkema-Cloostermans et al. 2019; Nolan et al. 2021; Sandroni et al. 2021). Microstate analysis addresses a different level of EEG organization: recurring configurations of the whole scalp field. The present findings therefore suggest complementarity rather than replacement of established EEG markers.

The modest incremental discrimination observed after adding M2 GEV should be interpreted cautiously. M2 GEV is not proposed as a stand-alone prognostic classifier. Its present value is primarily as evidence that a spatial property of global EEG organization carries outcome information beyond conventional temporal descriptors of the same microstate. External validation is essential before any clinical threshold or decision rule is considered.

The results also intersect with the broader literature on altered states and disorders of consciousness. Microstate organization changes with sleep, anesthesia, and disorders of consciousness (Brodbeck et al. 2012; Fingelkurts et al. 2012; Bréchet et al. 2022; Artoni et al. 2022b; Toplutaş et al. 2024). Nevertheless, residual electrophysiological organization, consciousness, and prognosis are distinct concepts. This distinction is central to clinical guidance on disorders of consciousness (Kondziella et al. 2020) and to debates concerning interpretation of residual brain function in catastrophic neurological injury (Machado et al. 2018; Machado 2026).

The use of patient-balanced GFP sampling is another methodological strength. Without balancing, patients contributing many GFP peaks could disproportionately determine the group solution. Capping contributions at 500 peaks per patient made the clustering pool more representative at the patient level. Patient-level bootstrap resampling similarly respected the hierarchical structure of the data, while Hungarian matching avoided conflating arbitrary cluster order with topographic instability (Kuhn 1955).

Several limitations should be emphasized. This is a secondary observational analysis, and the M2-outcome association has not been externally validated. ROSC time had substantial missingness, reducing the fully adjusted cohort to 292 patients. Although fixed-sample and larger non-ROSC analyses were reassuring, residual selection bias is possible. The analysis used a standardized 300-s EEG segment, whereas post-anoxic EEG evolves dynamically. Sedation, temperature management, antiseizure treatment, timing relative to arrest, and other intensive-care factors may influence microstate organization. The 19-channel montage provides clinically realistic but limited spatial sampling. No anatomical source-localization claim is made. Finally, observational prognostication studies remain vulnerable to treatment-limitation and self-fulfilling-prophecy effects.

Despite these limitations, the combination of outcome-blind state discovery, frozen topographies, symmetric state testing, dose-response analysis, multiplicity correction, and extensive influence analyses provides a rigorous foundation for independent replication. Future studies should test whether M2-like topographies reproduce in external cohorts, examine longitudinal microstate evolution, and determine whether microstate measures add meaningful information to standardized EEG categories and multimodal prognostic models.

CONCLUSIONS

An outcome-blind, patient-balanced analysis of EEG microstates after cardiac arrest identified a reproducible frozen $K = 4$ architecture in which M2 GEV was associated with neurological outcome. The association persisted after clinical adjustment, followed a graded dose-response relationship, was stable to extensive influence and resampling analyses, and was not reproduced by conventional M2 coverage, duration, or occurrence measures. These findings suggest that the degree to which a specific global scalp configuration explains the EEG field may contain information about residual electrophysiological organization after hypoxic-ischemic brain injury. M2 GEV should not currently be regarded as a stand-alone prognostic biomarker or anatomical marker. Independent replication, longitudinal characterization, and evaluation alongside established multimodal prognostic measures are required.

DECLARATIONS

Ethics Approval

This study was a secondary analysis of a publicly available, de-identified research dataset from the International Cardiac Arrest REsearch Consortium (I-CARE) Database hosted on PhysioNet. Ethical oversight, informed-consent procedures, and data-sharing provisions for the original studies were established by the participating institutions and are described in the source dataset and its associated publication. The present study involved no new patient recruitment, intervention, or collection of identifiable information. The secondary analysis was conducted in accordance with the ethical principles of the Declaration of Helsinki and with the data-use requirements of PhysioNet.

Consent to Participate

Not applicable to the present secondary analysis of publicly available, de-identified data. Consent procedures applicable to the original data collection were governed by the participating institutions and are described in the I-CARE source documentation.

Consent for Publication

Not applicable. The present study contains no identifiable individual participant data.

Data Availability

The data analyzed in this study are available through the International Cardiac Arrest REsearch Consortium (I-CARE) Database hosted on PhysioNet (Amorim et al. 2023a), subject to the access, credentialing, licensing, and data-use requirements specified by PhysioNet.

Code Availability

The analyses were performed using a custom Python-based pipeline. The code required to reproduce the principal analyses and figures, together with non-identifying derived results, is available from the corresponding author upon reasonable academic request.

Competing Interests

The author declares no competing interests.

Funding

The author received no funding for this research.

Author Contributions

C.M. conceived and designed the study; developed the methodology; curated and analyzed the data; performed EEG preprocessing, microstate reconstruction and backfitting, statistical analyses, validation, and robustness analyses; generated and interpreted the figures and tables; interpreted the findings; conducted the literature review; and wrote, reviewed, and approved the final manuscript. C.M. is solely responsible for the integrity of the work and the final content of the manuscript.

Acknowledgments

The author gratefully acknowledges the investigators and participating institutions of the International Cardiac Arrest REsearch Consortium (I-CARE) for making the dataset available for secondary research, and PhysioNet for hosting and providing access to this valuable scientific resource.

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Figures

Cohort flow and outcome-blind microstate analysis pipeline

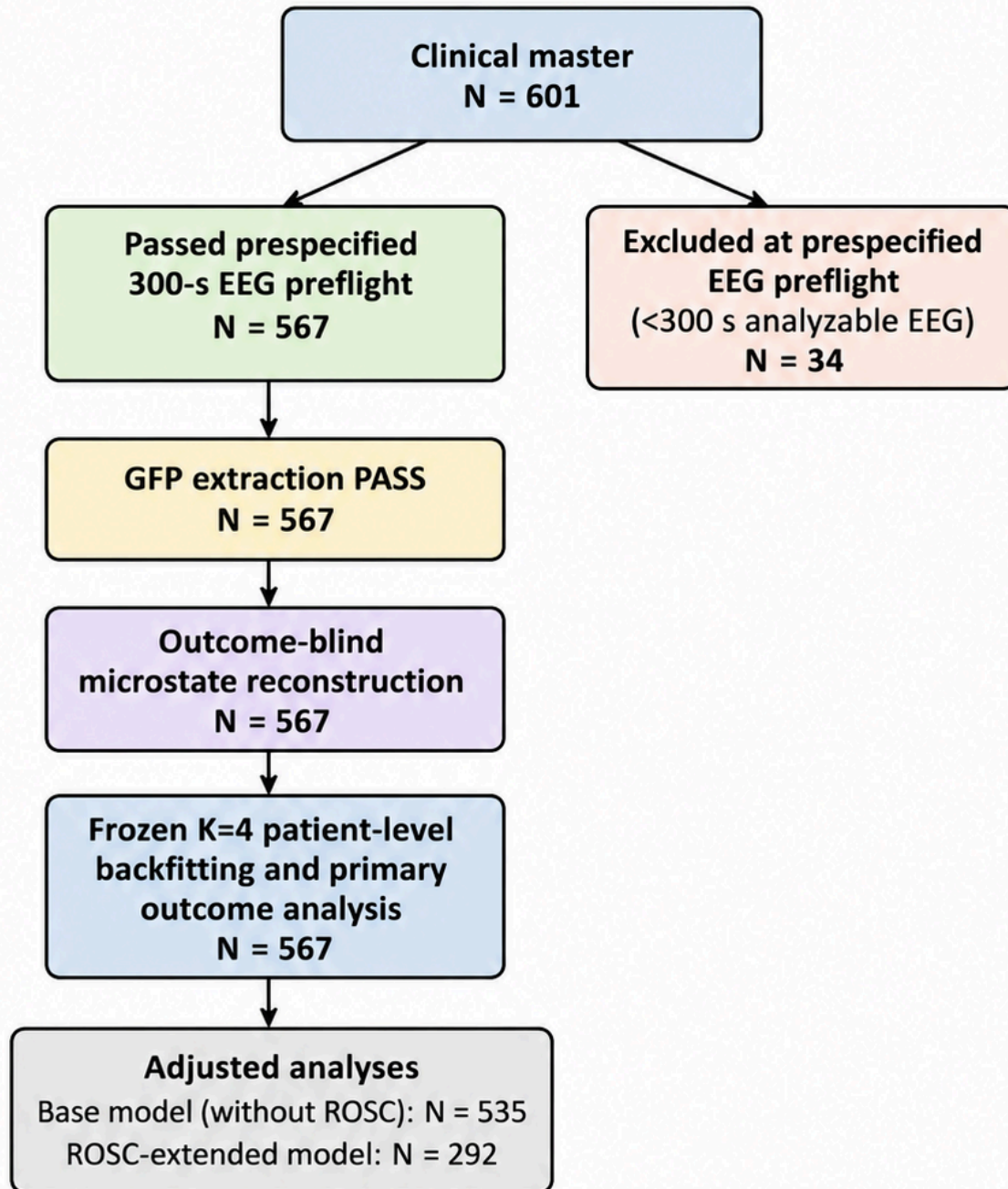


Figure 1

Cohort flow and outcome-blind microstate analysis pipeline.

The clinical master cohort comprised 601 patients. After applying the prespecified requirement for at least 300 s of analyzable EEG, 567 patients were retained, and 34 were excluded. All 567 eligible patients passed GFP extraction and entered the outcome-blind microstate reconstruction. Following model-order evaluation, the K=4 solution was frozen and backfitted at the patient level for the primary outcome analyses. Adjusted analyses included 535 patients in the base model without ROSC time and 292 patients in the ROSC-extended complete-case model. GFP, global field power; ROSC, return of spontaneous circulation.

Cohort flow and construction of the final EEG microstate analysis cohort. Flow diagram showing reconciliation of EEG and clinical records, application of the prespecified EEG-duration requirement, construction of the outcome-blind microstate cohort, and the final population available for outcome analysis.

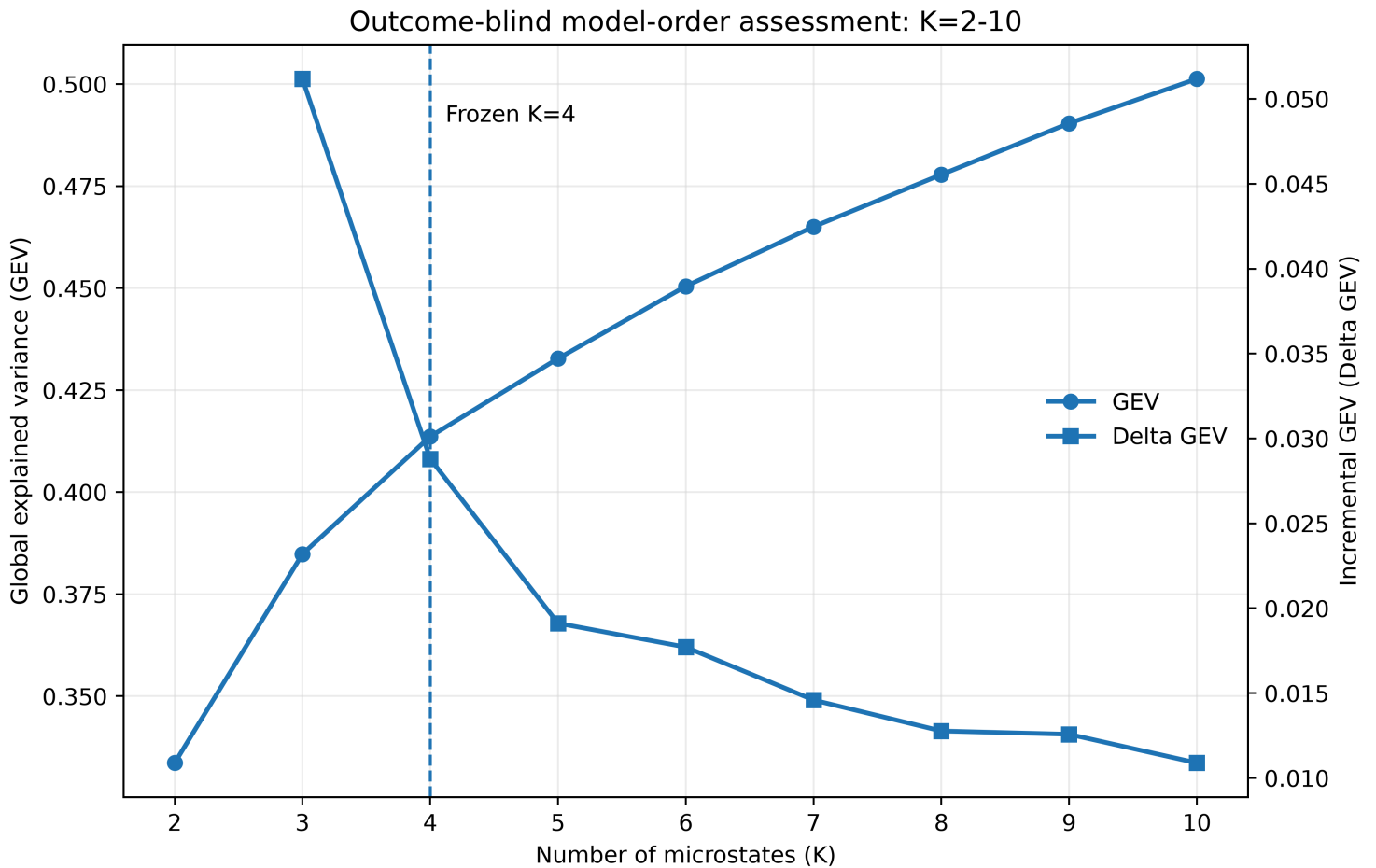


Figure 2

Outcome-blind model-order assessment across K=2-10.

Candidate microstate solutions were evaluated without neurological outcome information. Model-order assessment preceded freezing of the final K=4 architecture and all subsequent clinical analyses.

Following outcome-blind model-order and stability assessment, K=4 was selected and frozen. The four resulting maps were retained under their raw identities M1, M2, M3, and M4. They were not retrospectively renamed according to canonical A/B/C/D classes, and no biological interpretation of raw cluster identity was used to select or relabel a state.

Outcome-blind model-order assessment across K=2-10. Candidate microstate solutions were evaluated without neurological outcome information. Model-order assessment preceded freezing of the final K=4 architecture and all subsequent clinical analyses.

Frozen K=4 microstate solution and spatial characterization of M2

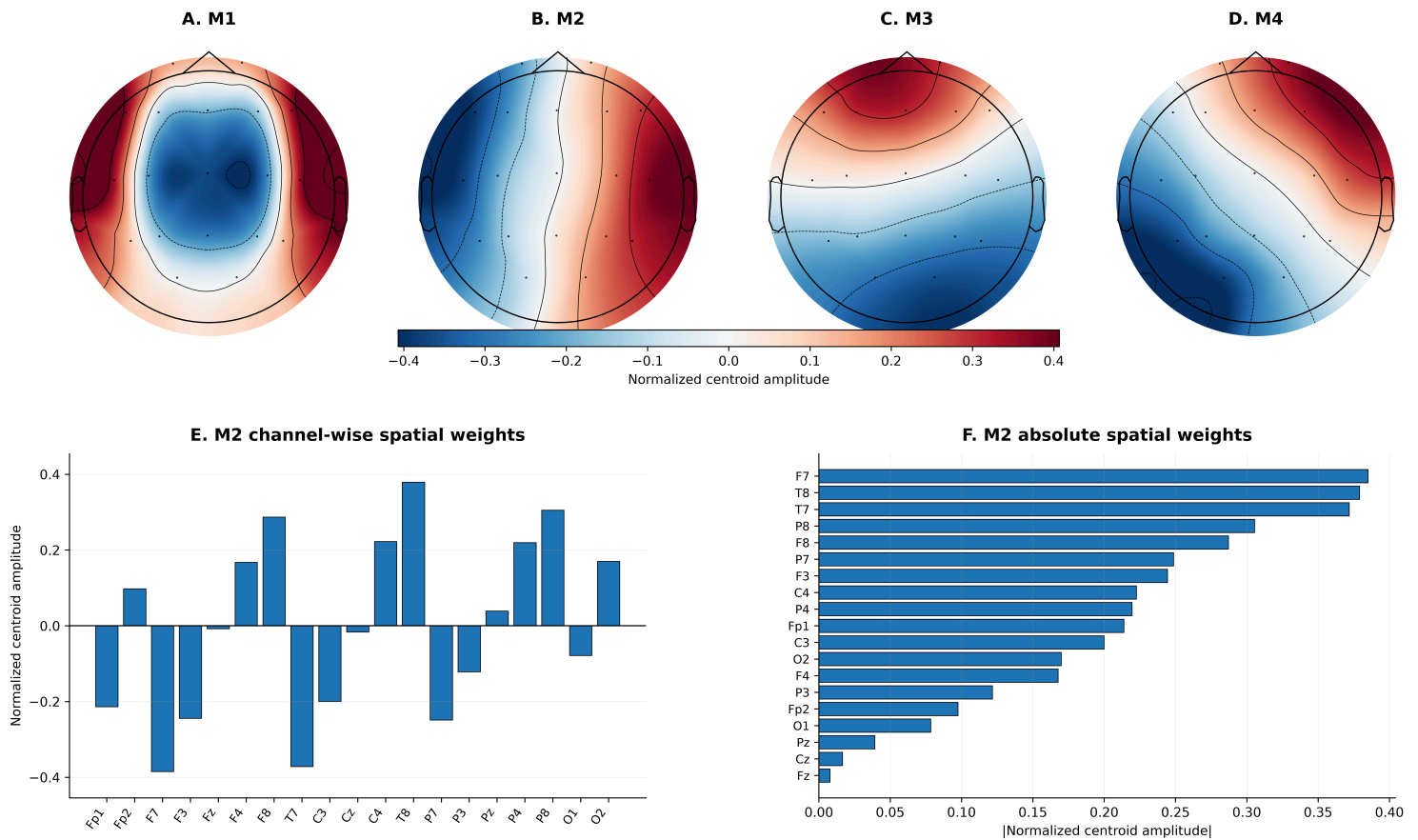


Figure 3

Frozen K=4 microstate topographies and spatial characterization of M2.

Panels A-D show the four frozen centroids M1-M4. Centroid polarity is arbitrary; positive and negative values reflect spatial orientation rather than physiological activation or deactivation. The lower panels characterize the real frozen M2 centroid at the channel level.

Patient-Level Backfitting and Microstate Metrics

The frozen K=4 maps were backfitted to individual EEG recordings without refitting the group centroids. Patient-level metrics included GEV, coverage, mean duration, and occurrence. GEV quantifies the proportion of global topographic variance explained by a microstate while weighting observations by GFP and therefore reflects both spatial correspondence and the strength of the global electric field (Pascual-Marqui et al. 1995; Michel and Koenig 2018).

Frozen K=4 microstate topographies and spatial characterization of M2. Panels A-D show the four frozen centroids M1-M4. Centroid polarity is arbitrary; positive and negative values reflect spatial orientation rather than physiological activation or deactivation. The lower panels characterize the real frozen M2 centroid at the channel level.

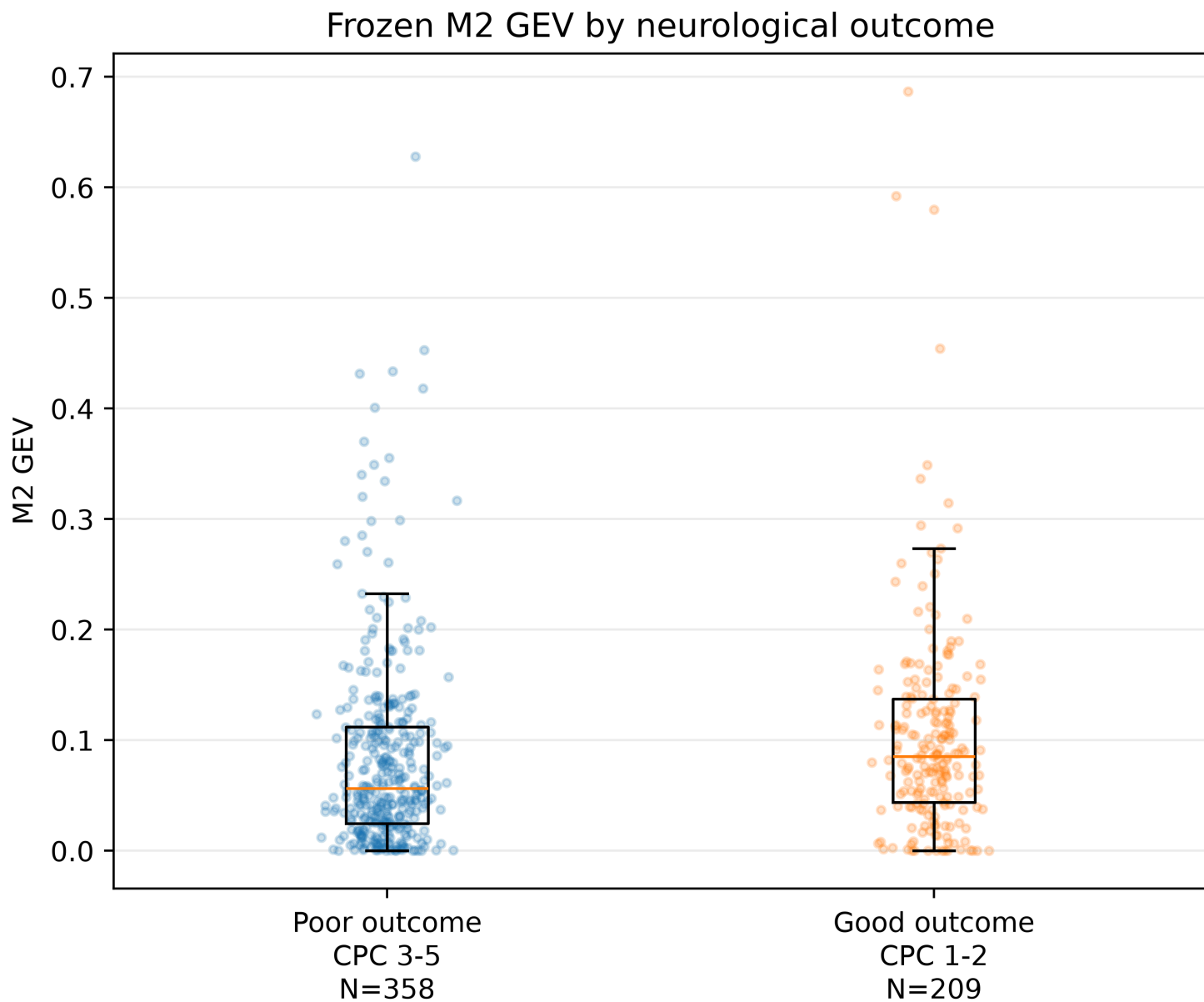


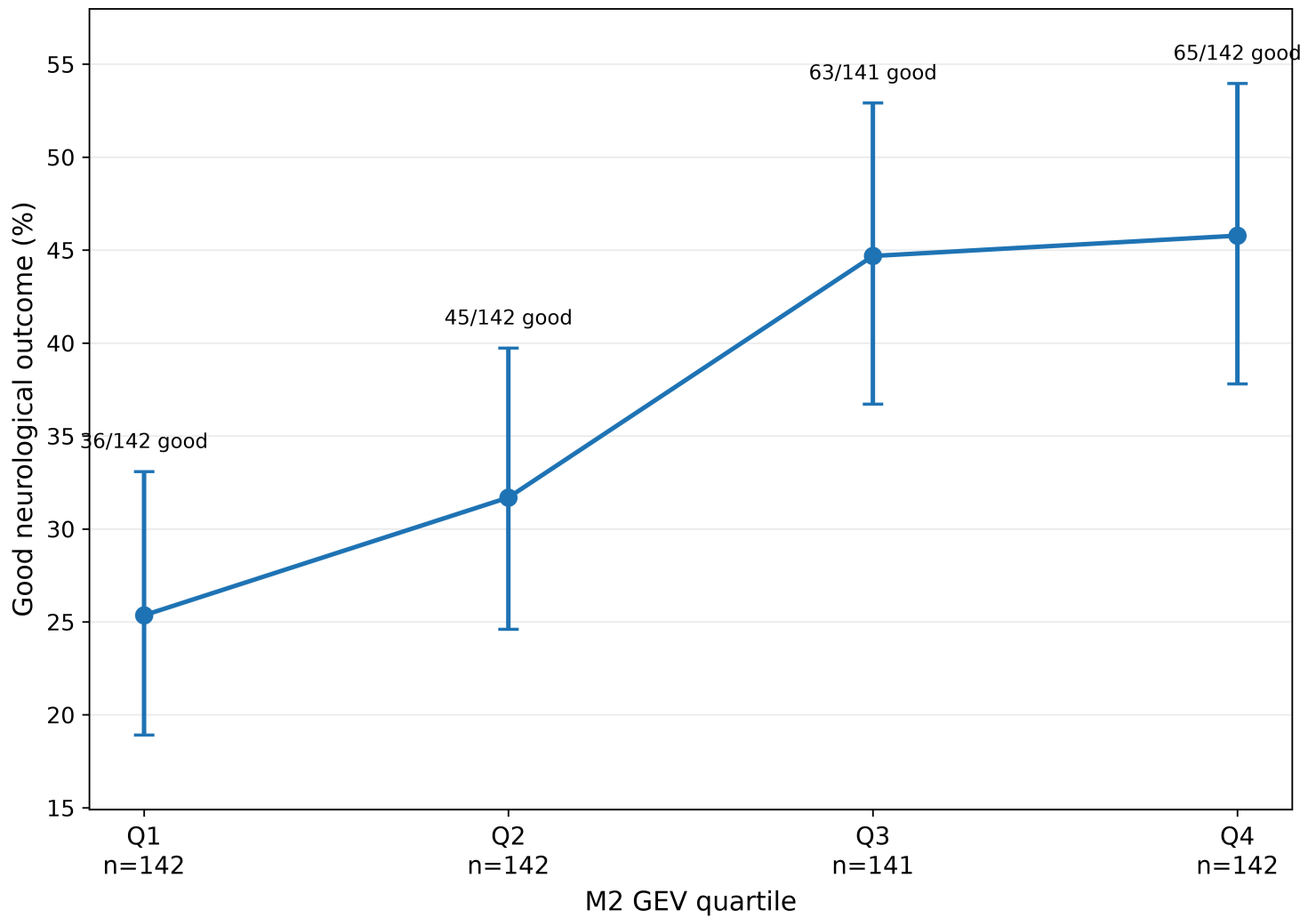
Figure 4

Frozen M2 global explained variance according to neurological outcome.

Patient-level M2 GEV is compared between favorable (CPC 1-2) and unfavorable (CPC 3-5) outcome groups. The M2 centroid remained fixed across patients.

Frozen M2 global explained variance according to neurological outcome. Patient-level M2 GEV is compared between favorable (CPC 1-2) and unfavorable (CPC 3-5) outcome groups. The M2 centroid remained fixed across patients.

Dose-response across frozen M2 GEV quartiles



Points represent observed proportions; error bars are Wilson 95% confidence intervals.

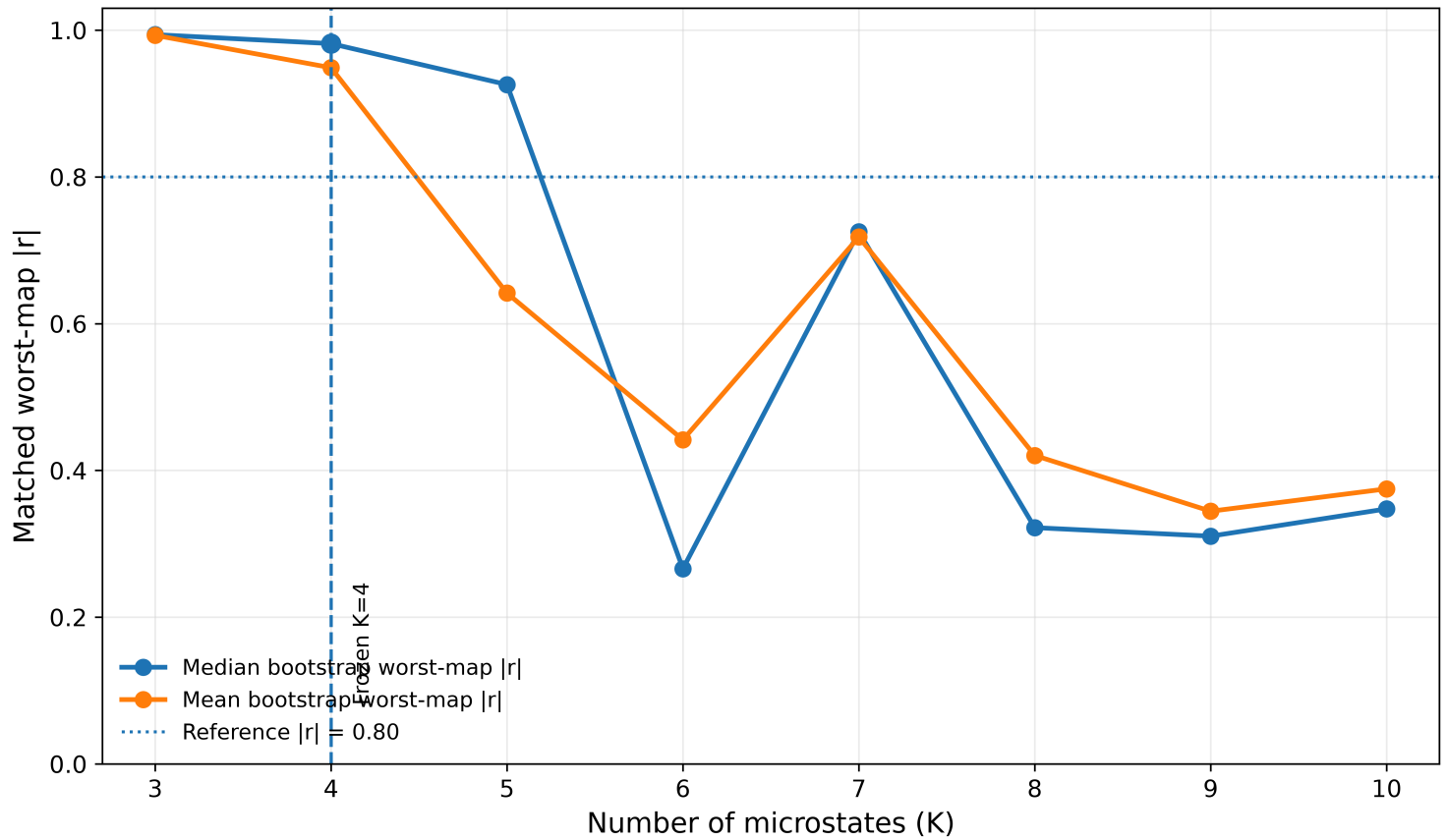
Figure 5

Dose-response relationship between M2 global explained variance and favorable neurological outcome.

Proportions are shown across increasing M2 GEV quartiles with 95% Wilson confidence intervals and quartile sample sizes.

Dose-response relationship between M2 global explained variance and favorable neurological outcome. Proportions are shown across increasing M2 GEV quartiles with 95% Wilson confidence intervals and quartile sample sizes.

Outcome-blind bootstrap topographic stability across model orders



Stability was assessed without outcome information. The horizontal line is a reference threshold, not a post hoc model-selection rule.

Figure 6

Patient-level bootstrap topographic stability across model orders K=3-10.

Bootstrap centroids were aligned to reference maps using polarity-invariant spatial matching. Outcome was not used in the stability analysis.

Patient-level bootstrap topographic stability across model orders K=3-10. Bootstrap centroids were aligned to reference maps using polarity-invariant spatial matching. Outcome was not used in the stability analysis.