SUPPLEMENTAL MATERIAL

Supplemental Methods

Patients: Study personnel were notified via pager about arrival of patients with suspected acute stroke. The first 75 patients were enrolled consecutively. For the final 25 patients, enrollment focused on patients with a higher suspicion of stroke to ensure that at least half of enrollees had a stroke.

Entry criteria were suspected stroke admitted to the ED of UC Irvine Medical Center, symptom onset ≤24 hours prior, age ≥18 years, and English- or Spanish-speaking. Exclusion criteria were presence of major neurological/psychiatric diagnosis, and contraindication to EEG.

EEG was initiated in the ED except for four patients in whom consent was obtained in the ED and then EEG was immediately collected in the ICU due to clinical mandate. Final diagnosis was based on the judgment of the clinical stroke service in the discharge summary.

Data acquisition: The current study employed a small, portable, wireless, battery powered, dry-electrode system (the Quick-20 EEG system) previously found to have excellent sensitivity compared to gel-lead systems and demonstrated utility in clinical and research studies[3, 6]. The Quick-20 EEG system employed in the current study was specially customized for use in the ER, using 17 leads of the 10-20 system, an approach selected in part because our prior EEG study of patients examined early (mean of 6.6 hours) after stroke onset found 20 leads had equivalent diagnostic sensitivity as 256 leads[3, 6]. Compared to the standard system, two electrodes (O1 and O2) were removed and replaced with foam pads to enable data collection from a supine patient. The resulting 17-channel array consisted of Fp1, Fp2, F7, F3, Fz, F8, F4, C3, Cz, C4, P7, P3, Pz, P8, P4, T4, and T3. The reference and ground electrodes were placed adjacent to Fp1 and Fp2, respectively, since these forehead locations maximize the probability of good electrode contact and enable reliable re-montaging for analysis.

Each patient's head was measured to identify the site of Cz (intersection of nasion-inion line with L/R preauricular line), then the Quick-20 was placed with the Cz lead overlying this site. During recording, subjects were instructed to direct their gaze, if capable, towards the center of a fixation-cross displayed at the end of their gurney. To decrease artifacts in the EEG signal, patients were instructed to minimize all movements during EEG recording, as possible. ED physicians approved each enrollment as well as indicated the time when EEG could be acquired in order to avoid interruptions in acute care delivery.

EEG data were collected at the standard rate of 500 samples/sec corresponding to an EEG bandwidth of DC-131 Hz and transmitted wirelessly to a computer running Cognionics Data Acquisition software. The EEG amplifier was configured at the standard gain of 3 for a total input range of +/- 833 mV for immunity against electrode offsets and rapid recovery to movement and contact-loss artifacts. The Quick-20 includes a real-time impedance check to assist with electrode preparation. For this study, the threshold for contact was set to ≤200 kΩ. We noted the time to initiate EEG recording once the decision to record EEG was made, which included headset preparation and placement, initialization and setting up recording software, as well as lead adjustments if necessary. EEG acquisition took a median of 13 minutes in total but improved with increasing familiarity and averaging less than 10 minutes for the second half of subjects, requiring as little as 36 seconds (Figure I).

Of the 105 subjects enrolled, 95 of the EEG recordings obtained by a single examiner (FE). The remainder were obtained by undergraduate students who were on call to the ED. Limited expertise was needed to acquire EEG data: all individuals who recorded EEG participated in a single 2-hour training session prior to the start of the study. Additionally,

the Cognionics data acquisition software provides an intuitive user interface, and the real-time and continuous readout of electrode impedance helped with subject set-up and enabled quality control.

EEG pre-processing: EEG data were exported to MATLAB 2015a 7.8.0 (MathWorks, Inc., Natick, MA) for offline analysis, including filtering and removal of noise. Initial processing steps included applying a second-order 50 Hz low-pass Butterworth filter and 0.2 Hz high-pass Butterworth filter. Continuous EEG data were then binned into 180 sequential, non-overlapping, one-second epochs. Visual inspection was used to identify and remove channels as well as epochs with artifact such as noise from overt movement or speaking, and high-voltage low-frequency signals due to eye movement or blinking.

During offline analysis, each of the 17 leads was re-referenced to a bipolar montage consisting of 27 bipolar lead pairs. Each bipolar pair was computed by subtracting the EEG signals recorded from the reference electrode adjacent to FP1.

Clinical variables: Four clinical variables that have established relationships with stroke severity and are easily measured in the prehospital setting were retrieved from the patient's record: age, sex, time from last known well (LKW) to EEG, and Rapid Arterial Occlusion Evaluation (RACE) [3, 6] score.

RACE score was selected to be a clinical variable because, although no single prehospital LVO scale is optimal[7], RACE performs well in identifying prehospital patients with stroke and LVO[4, 8, 9] and can be retrospectively calculated from the NIHSS score using specific guidelines[3, 6]. Other patient data, including NIHSS scores in the ED, were available from the initial neurological consultation; for patients with intracerebral hemorrhage or TIA, a stroke neurologist (SCC) retrospectively estimated values from chart data[3, 6].

Infarct volume: For subjects discharged with a final diagnosis of ischemic stroke or intracerebral hemorrhage, images were retrieved from the electronic medical record (EMR) for analysis. Infarct volume was measured on the first MRI or CT scan (ordered as part of standard of care) that demonstrated the index stroke. Infarcts were outlined using MRIcron (http://www.mccauslandcenter.sc.edu/mricro/mricron) by hand using techniques for which reliability and validity have been described previously[3, 6].

EEG variables: Spectral power was examined within each of the 27 bipolar lead pairs. First, each lead pair's time series was submitted to a discrete fast Fourier transform. Power for each bipolar lead pair was then calculated within a 1-30 Hz frequency band, in 1-Hz bins, and then expressed as relative power. Power was then calculated across five frequency bands: delta (1-3 Hz), theta (4-6 Hz), alpha (7-12 Hz), low beta (13-19 Hz), and high beta (20-30 Hz). Ipsilesional leads were designated as odd numbers (Fp1-T5), while contralesional leads were designated as even numbers.

Statistical analyses: Given the high-dimensionality of the EEG data, we used Lasso[10], a penalized (regularized) regression model, to select a subset of EEG variables in order to develop predictive models based on joint consideration of all variables simultaneously, while reducing the risk of overfitting, which can be associated with models that perform well on one dataset but do not generalize well to new datasets. Additionally, unlike linear regression, Lasso minimizes the influence of outliers. The Lasso procedure requires a tuning parameter (penalty), lambda, which was chosen in a standard way through (five-fold) cross validation. Lasso was implemented using the glmnet package in R, then applied to all 135 lead-band pairs (i.e., each of the 27 bipolar pair leads in each of the five frequency bands), in the same randomly selected 60 subjects.

Of these 135 lead-band pairs, Lasso identified four as important predictor variables for the stroke prediction model (F8-T4 in the alpha band, C3-F3 in the low beta band, Cz-C3 in the high beta band, and C4-F4 in the high beta band) and two as important predictor variables for the stroke with LVO prediction model (C3-F3 in the theta band and T3-F7 in the alpha band). Note that these Lasso-identified EEG variables strongly suggest that the relevant features are EEG, rather than artifact, in origin for two reasons. First, dry electrode systems typically are susceptible to motion, sweat, and electrode pop artifacts, which primarily appear in the delta band and were not identified by Lasso. Second, EEG is

sensitive to EMG artifacts, which are high frequency, but occur primarily in the frontal and temporal locations and were also not identified by Lasso.

Models for identifying acute stroke/TIA were directly compared based on their AUC values. To this end, we use 10-fold cross-validation to estimate SE of AUC for each model, and use paired t-tests to evaluate the statistical significance of differences among AUC's across different models; this was not done for LVO given the number of subjects.

Four predictor models were evaluated, validated, and compared, looking at presence of acute stroke/TIA (or not) as the dependent measure: **[1] clinical data only**, which examined the 4 clinical predictor measures of interest (age, sex, time from LKW to EEG, and RACE score) using logistic regression modeling; **[2] EEG data only**, which examined the Lasso-selected 4 EEG lead-band pairs (F8-T4 alpha, C3-F3 low beta, Cz-C3 high beta, and C4-F4 high beta band) using logistic regression modeling; **[3] combined clinical and EEG data using logistic regression**, which used 2 clinical variables (the most significant clinical predictor from model 1 plus RACE score given its key significance in the context of prehospital diagnosis[4, 8, 9] plus the 4 Lasso-selected EEG lead-band pairs, using logistic regression modeling; and **[4] combined clinical and EEG data using a deep learning neural network model**. The deep learning neural network model used 3 hidden layers each with 200 neurons and the ReLU (Rectified Linear Unit) activation function. We used 0.5 dropout ratio and L1 penalty to improve generalization. The deep learning model used the same 2 clinical variables and 4 EEG lead-band pairs as in model [3] and was implemented using the h2o package in R.

The same four models ([1] clinical only, [2] EEG only, [3] combined clinical and EEG using logistic regression, and [4] combined clinical and EEG using deep learning) were again examined, this time looking at presence of acute stroke with LVO (or not) as the dependent measure. The same clinical variables were included, as above, and for EEG, the two variables identified by the Lasso procedure (C3-F3 in the theta band and T3-F7 in the alpha band) were included.

Supplemental Results

Subject Characteristics: Of 105 enrollees, five were excluded because <40 epochs were free of EEG artifact, leaving 100 patients with suspected acute stroke among whom 79±36.7 (mean±SD) of the 180 EEG epochs were retained for subsequent analyses.

Of the 50 subjects with acute stroke, 47 had a radiologically confirmed ischemic infarct or hemorrhage. The 3 without radiologically confirmed stroke had each received IV tPA upon ED arrival, and the final discharge diagnosis from the acute stroke service for each was acute ischemic stroke.

For the 37 patients who initially were suspected of acute stroke but had a discharge diagnosis other than acute stroke/TIA, final diagnosis was infection in 8, encephalopathy in 7, migraine in 6, somatoform disorder in 3, Bell's palsy in 2, dizziness in 2, syncope in 2, general weakness in 2, drug intoxication in 1, focal seizure in 1, sickle cell crisis in 1, transient global amnesia in 1, and peripheral neuropathy in 1.

EEG prediction of acute stroke/TIA or not

[1] Clinical variables only: Using the four clinical variables (age, sex, time from LKW to EEG, and RACE score), the regression model had an AUC of 62.3 on the validation group (SE=5). At a specificity of 80%, the sensitivity was 40%. The strongest predictor was the variable "time from LKW to EEG," with longer LKW times associated with a diagnosis of stroke/TIA.

[2] EEG variables only: Of the 27 bipolar lead pairs and five frequency bands, the four lead pairs identified by the Lasso procedure (F8-T4 in alpha band, C3-F3 in low beta band, Cz-C3 in high beta band, and C4-F4 in high beta band) in

the training group (*n*=60) were entered into a regression model to predict acute stroke/TIA. The model had an AUC of 78.2 on the validation group (SE=4). At a specificity of 80%, the sensitivity was 65%. The strongest predictor was power in the high beta band in C4-F4, where lower power was associated with a diagnosis of stroke/TIA.

[3] Combined clinical and EEG using logistic regression: The most significant variable from model [1] (LKW to EEG acquisition), along with RACE score, was advanced into a new model that also included the four EEG variables used in model [2] in order to train a new logistic regression model in the 60-subject training group. In the 40-subject validation group, this combined clinical and EEG model had an AUC of 80.3 on the validation set (SE=6, full model presented in Table I). At a specificity of 80%, the sensitivity was 70%.

[4] Combined clinical and EEG using deep learning: The same six variables used in model [3] were again evaluated but using a deep learning neural network model, which yielded an AUC of 87.8 in the validation group (SE=5). At a specificity of 80%, the sensitivity was 80%.

The three models with EEG had higher prediction value compared to the model with clinical variables only (model [2] vs. model [1], p=.005; model [3] vs. model [1], p=.016; model [4] vs. model [1], p=.004). Differences between the models that included EEG were not significant.

EEG prediction of acute stroke with LVO or not

The same four models were evaluated but with acute stroke with LVO (or not) as the dependent measure. Findings were overall similar, with the model combining clinical and EEG using deep learning again yielding the highest AUC.

[1] Clinical variables only: Using the same four clinical variables as in the model [1] used to predict acute stroke/TIA or not, the regression model showed an AUC of 80.4 on the validation group. At a specificity of 80%, the sensitivity was 65%. The strongest predictor was “RACE score”, with higher score associated with a diagnosis of LVO.

[2] EEG variables only: Of the 27 bipolar lead pairs and five frequency bands, the two lead pairs identified by the Lasso procedure (C3-F3 in theta band and T3-F7 in the alpha band) in the training group (*n*=60) were entered into a regression model to predict LVO. The model had an AUC of 68.9 on the validation group. At a specificity of 80%, the sensitivity was 41%. The strongest predictor was power in the alpha band in T3-F7, where lower power was associated with a diagnosis of LVO.

[3] Combined clinical and EEG using logistic regression: The most significant variable from the above clinical model (RACE score), along with time from LKW to EEG, was advanced into a new model that also included the two EEG variables used in the above model [2] in order to train a new logistic regression model in the 60-subject training group. In the 40-subject validation group, this combined clinical and EEG model had an AUC of 77.8 on the validation set (full model presented in Table I). At a specificity of 80%, the sensitivity was 57%.

[4] Combined clinical and EEG using deep learning: The same four variables used in the above model [3] were again evaluated but using a deep learning neural network model, which yielded an AUC of 86.4 on the validation set. At a specificity of 80%, the sensitivity was 76%.

Table I. Combined Clinical and EEG Logistic Regression Models

	Estimate	Std. Error	z value	Pr(>|z|)
Predict Stroke/TIA				
Intercept	1.10	0.92	1.19	0.23
RACE score	0.068	0.12	0.56	0.58
Time from LKW to EEG	0.07	0.04	1.71	0.087
Alpha power F8-T4	-12.47	8.78	-1.42	0.16
Low beta power C3-F3	28.71	10.54	2.72	0.006
High beta power Cz-C3	-21.48	9.41	-2.28	0.02
High beta power C4-F4	-11.70	3.76	-3.11	0.0019
Predict Acute Stroke with LVO				
Intercept	-3.99	2.36	-1.69	.0905
RACE score	0.46	0.16	2.90	0.0037
Time from LKW to EEG	0.046	0.077	0.59	.55
Theta power C3-F3	7.36	9.70	0.76	0.45
Alpha power T3-F7	-19.1	21.6	-0.88	0.38

Table I presents the results of model [3] for prediction of Stroke/TIA and for prediction of Acute Stroke with LVO

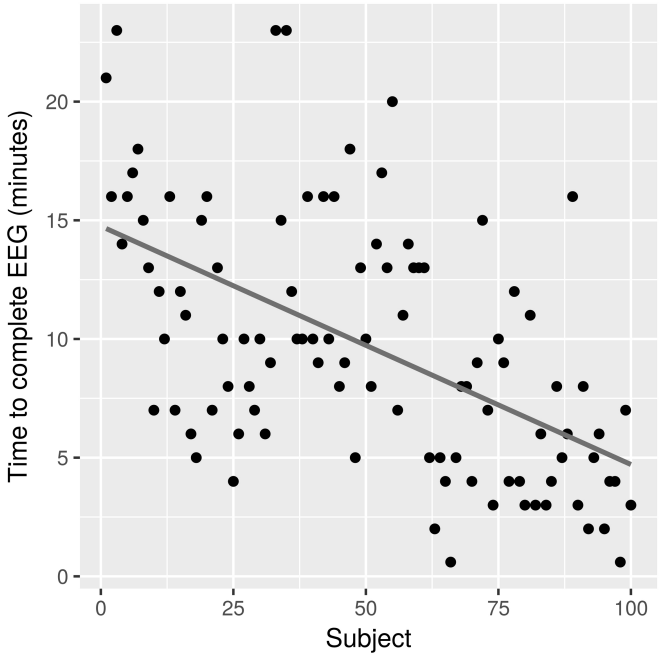

Figure I: The time to initiate EEG acquisition (prepare the EEG system, place EEG leads, make any lead adjustments, and start EEG recording) decreased during the study (r=-0.57, $p<0.0001$).