Stroke

CLINICAL AND POPULATION SCIENCES

Early Neurological Change After Ischemic Stroke Is Associated With 90-Day Outcome

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BACKGROUND AND PURPOSE: Large-scale observational studies of acute ischemic stroke (AIS) promise to reveal mechanisms underlying cerebral ischemia. However, meaningful quantitative phenotypes attainable in large patient populations are needed. We characterize a dynamic metric of AIS instability, defined by change in National Institutes of Health Stroke Scale score (NIHSS) from baseline to 24 hours baseline to 24 hours ($NIHSS_{baseline} - NIHSS_{24hours} = \Delta NIHSS_{6\text{-}24h}$), to examine its relevance to AIS mechanisms and long-term outcomes.

METHODS: Patients with NIHSS prospectively recorded within 6 hours after onset and then 24 hours later were enrolled in the GENISIS study (Genetics of Early Neurological Instability After Ischemic Stroke). Stepwise linear regression determined variables that independently influenced $\Delta NIHSS_{6\text{-}24h}$. In a subcohort of tPA (alteplase)-treated patients with large vessel occlusion, the influence of early sustained recanalization and hemorrhagic transformation on $\Delta NIHSS_{6\text{-}24h}$ was examined. Finally, the association of $\Delta NIHSS_{6\text{-}24h}$ with 90-day favorable outcomes (modified Rankin Scale score 0–2) was assessed. Independent analysis was performed using data from the 2 NINDS-tPA stroke trials (National Institute of Neurological Disorders and Stroke rt-PA).

RESULTS: For 2555 patients with AIS, median baseline NIHSS was 9 (interquartile range, 4–16), and median $\Delta NIHSS_{6\text{-}24h}$ was 2 (interquartile range, 0–5). In a multivariable model, baseline NIHSS, tPA-treatment, age, glucose, site, and systolic blood pressure independently predicted $\Delta NIHSS_{6\text{-}24h}$ (R^2=0.15). In the large vessel occlusion subcohort, early sustained recanalization and hemorrhagic transformation increased the explained variance (R^2=0.27), but much of the variance remained unexplained. $\Delta NIHSS_{6\text{-}24h}$ had a significant and independent association with 90-day favorable outcome. For the subjects in the 2 NINDS-tPA trials, $\Delta NIHSS_{3\text{-}24h}$ was similarly associated with 90-day outcomes.

CONCLUSIONS: The dynamic phenotype, $\Delta NIHSS_{6\text{-}24h}$, captures both explained and unexplained mechanisms involved in AIS and is significantly and independently associated with long-term outcomes. Thus, $\Delta NIHSS_{6\text{-}24h}$ promises to be an easily obtainable and meaningful quantitative phenotype for large-scale genomic studies of AIS.

Key Words: genome-wide association study ■ phenotype ■ population ■ stroke, ischemic

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Nonstandard Abbreviations and Acronyms

ΔNIHSS$_{6–24h}$	change in score from NIHSS collected within 6 hours of onset to follow-up NIHSS score collected at 24 hours (+/− 4 hours) from baseline examination
AIS	acute ischemic stroke
ESR	early sustained recanalization
GENISIS	Genetics of Early Neurological Instability After Ischemic Stroke
GWAS	genome-wide association studies
HT	hemorrhagic transformation
LVO	large vessel occlusion
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
OR	odds ratio
PH2	parenchymal hematoma type 2
TOAST	Trial of ORG 10172 in Acute Stroke Treatment (stroke etiology designations)
tPA	alteplase

The National Institutes of Health Stroke Scale (NIHSS) measured 24 hours after stroke onset is a strong predictor of functional outcome at 90 days.[1,2] However, between the time of onset and 24 hours, there can be a substantial change in NIHSS scores. Clinically meaningful fluctuations, frequently defined as changes of ≥4 points, within the first 24 hours occur in more than half of patients with acute ischemic stroke (AIS).[3–5] This critical period is a window for preventing neurological deterioration or enhancing improvement.

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Studying early NIHSS change in large AIS populations may be informative, revealing mechanisms underlying neurological instability following cerebral ischemia. Possible mechanisms may include but are not limited to blood pressure variations, collateral circulation, reperfusion (clot composition and fibrinolysis), endogenous neuroprotective mechanisms, neurological complications (edema, hemorrhagic transformation [HT]), systematic complications (infections), and many others.[6–11] Currently, there are limited treatment options available for the acute management of ischemic stroke, and not all patients are eligible for these medications and interventions. Despite extraordinary progress in reperfusion approaches, there have been few advances in other drug treatments for AIS.[12,13] Indeed, failure of translation from experimental models to human trials has slowed the drug development pipeline.[14,15] Using a clinical metric relevant to mechanisms of neurological change after AIS will permit informative reverse-translational approaches to identify potential drug targets.

There is accumulating evidence that the success of candidate drugs is greatly enhanced if the drug target is independently confirmed by human genetic data.[16,17] Thus, a clinical metric that captures mechanisms of AIS will permit genomic approaches that may inform potential drug targets. To be useful, such a metric would need to be validated by assessing it as follows: (1) ability to capture early mechanisms related to neurological change after AIS, (2) modification by acute therapies, (3) association with long-term outcomes, and (4) confirmation in independent cohorts. Here, we systematically validate a metric of early neurological instability, defined by the change in NIHSS from baseline (within 6 hours of stroke onset) to 24 hours (ΔNIHSS$_{6–24h}$).

The simplicity of this metric and the use of NIHSS as standard-of-care for patients with AIS[18] will permit large population-based observational studies to explore mechanisms underlying neurological change. It will also allow for investigation into whether a portion of ΔNIHSS$_{6–24h}$ is modulated by genetic mechanisms, which may then be leveraged to identify potential novel treatment targets. Indeed, ΔNIHSS$_{6–24h}$ is the principal phenotype for the GENISIS study (Genetics of Early Neurological Instability After Ischemic Stroke), which aims to use human genome-wide association studies (GWAS) to understand the biological underpinnings of early neurological fluctuation following AIS.

METHODS

GENISIS is an international collaboration currently recruiting patients from hospitals in 4 different locations (please see the Data Supplement); with a centralized repository maintained at Washington University. The study contains patient-level data from existing local registries and studies as well as sites participating in enrollment specifically for GENISIS. All sites are required to provide a minimum data set for participation in GENISIS. The data that support the findings of the current study are available from the corresponding authors upon reasonable request.

Inclusion and Exclusion Criteria for GENISIS

Adult patients with AIS (age≥18 years) with a measurable neurological deficit on the NIHSS within 6 hours of last known normal are eligible for inclusion. Potential ischemic stroke cases are identified during their hospital stay. All available inpatient data, including history, clinical exam, lab values, diagnostic tests, imaging, and discharge diagnosis, are used in the final determination of ischemic stroke. Patients who received endovascular thrombectomy, enrolled in treatment trials, or for whom consent and a blood sample cannot be obtained are excluded. If patients were ultimately determined to have a transient ischemic attack (either symptoms <1 hour and no lesion on neuroimaging or symptoms <24 hours if no follow-up neuroimaging

was obtained) or a stroke mimic, they were excluded from any planned analysis. The Institutional Review Boards at all participating sites approved patient enrollment and data sharing.

Phenotyping Methods

Data collected included acute treatment variables, baseline glucose, baseline stroke severity within 6 hours of last known normal using the NIHSS score ($NIHSS_{baseline}$), and follow-up NIHSS score ($NIHSS_{24hours}$) collected at 24 hours (±4 hours) from the baseline examination by the local study team. We calculated $\Delta NIHSS_{6-24h}$ as $NIHSS_{baseline}$–$NIHSS_{24hours}$. Stroke subtypes were classified according to TOAST (Trial of ORG 10172 in Acute Stroke Treatment) criteria.[19] Consistent with this classification scheme, undetermined was selected in the scenario where there was either no relevant mechanism identified or more than one relevant mechanism was present. All covariates were collected and aligned according to a data dictionary generated for the study, using NIH NINDS common data elements when available (https://www.commondataelements.ninds.nih.gov/). When follow-up imaging was available, HT was classified according to the European Cooperative Acute Stroke Study criteria, with centralized adjudication.[20] Ninety-day outcome was measured with a modified Rankin Scale (mRS) score. At Vall d'Hebron University Hospital, a subset of large vessel occlusion (LVO) stroke subjects underwent transcranial Doppler ultrasound examination at baseline, 2, and 6 hours after tPA (alteplase) bolus and then at 24 hours using 1-channel 2-MHz equipment, and also had 24-hour imaging.[21] Occlusions were defined according to the thrombolysis in brain ischemia grading system, and recanalization was present if end-diastolic flow velocities improved to normal or stenotic signals.[22] Due to the time period (2003–2009) during which these subjects were enrolled, thrombectomy was not standard-of-care and thus, none of these subjects with LVO underwent intervention.

Independent Analysis

To externally replicate our findings, we analyzed the publicly available dataset from the 2 NINDS-tPA trials (National Institute of Neurological Disorders and Stroke rt-PA)[23] for the influence of baseline NIHSS and change in NIHSS on 90-day outcome (Methods in the Data Supplement).[24] Since baseline NIHSS was recorded within 3 hours of symptom onset, the calculation of baseline NIHSS–NIHSS at 24 hours yielded a $\Delta NIHSS_{3-24h}$.

Statistical Analyses of Phenotype Data

Of the 5 TOAST categories (large artery atherosclerosis, cardioembolic, small vessel disease, undetermined, and other determined), other determined was not included in the analysis because of its rarity and heterogeneous nature.

All variables were tested for normalcy (see Methods in the Data Supplement). Univariate association of each variable with baseline NIHSS and $\Delta NIHSS_{6-24h}$ were performed with nonparametric Spearman ρ. To explore the role of TOAST, group differences were modeled with the Kruskal-Wallis test. Secondary analysis used the Dwass-Steel-Critchlow-Fligner test for pairwise comparison while controlling for family wise error.[25] Using a data-driven approach, only variables that met a threshold univariate *P* value of 0.20 were included in the stepwise multivariable linear regression analysis for association with either log-transformed baseline NIHSS or untransformed $\Delta NIHSS_{6-24h}$. Due to the retrospective approach, no a priori power analysis was conducted on univariate predictors. Collinearity and heteroscedasticity were checked for all variables included in the models (and in all subsequent modeling) using the variance inflation factor and residual plots, respectively. The false discovery rate was controlled using the Benjamini-Hochberg procedure to evaluate the resultant *P* values in the final models (and all subsequent modeling). The proportional amount of variance explained by a variable in multivariable analysis is presented by the partial eta-squared when traditional R^2 cannot be calculated. Raw *P* values are reported with a final error rate of 0.05 selected for significance.

While not required for inclusion in the study, a large proportion (90%) of the GENISIS cohort had 90-day mRS outcome data. A stepwise ordinal logistic regression analysis was performed for all categories of the mRS scale, but all significant models failed to maintain the proportional odds assumption. Therefore, a valid model was unable to be constructed. Thus, a stepwise logistic regression analysis was performed for favorable outcome (mRS score 0–2 versus 3–6) at 90 days.

To further characterize underlying causes of $\Delta NIHSS_{6-24h}$, recanalization and HT were examined in the LVO cohort. The cohort was divided into 6 groups, based on SD from the mean $\Delta NIHSS_{6-24h}$ of the GENISIS cohort: extreme worsening included subjects falling between −3 and −2 SD from the mean (group 1), whereas extreme improvement included those falling between +2 and +3 SD from the mean (group 6). Early sustained recanalization (ESR) was defined as recanalization within 2 hours after tPA administration with continued patency at 24 hours. These 6 groups were evaluated for differences in baseline characteristics with the Kruskal-Wallis test. Significant variables from univariate ordinal logistic regression analysis were used in stepwise ordinal logistic regression analysis. In addition to modeling odds ratios (OR) of better or worse outcomes, Fisher exact tests for both extreme improvement and extreme worsening were performed. Finally, analysis of variables associated with both $\Delta NIHSS_{6-24h}$ and favorable 90-day outcomes in the LVO cohort were performed in the same manner described above.

Similar to the analysis of the GENISIS cohort, subjects from the 2 NINDS-tPA studies were analyzed to confirm the association of baseline NIHSS and the change in NIHSS from baseline to 24 hours on favorable 90-day outcomes in this independent dataset. Given that the trial enrolled up to 3 hours from last known normal, the change form baseline to 24-hour NIHSS was defined as $\Delta NIHSS_{3-24h}$ to differentiate from the 6-hour enrollment window in GENISIS ($\Delta NIHSS_{6-24h}$). Placebo and tPA-treated cohorts were also analyzed separately to evaluate the differential influence on the respective contributions of baseline NIHSS and $\Delta NIHSS_{3-24h}$ to outcome between these 2 treatment arms.

All analyses were performed using SAS software version 9.4 (SAS Institute Inc, Cary, NC.).

RESULTS

A total of 2555 patients with AIS were available for analysis (Table 1). For the patients (N=1738) with reported last known normal and baseline NIHSS time/date, the median time to evaluation was 2.1 hours (interquartile range,

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Table 1. Baseline Variables and Univariate Analysis of $\Delta NIHSS_{24}$

Variable	Median (IQR) or %	Spearman ρ or directionality	$\Delta NIHSS_{24}$ *P* value
Age, y	74 (63–82)	−0.063	0.0015*†
Female	46.22%	NS	0.7704
African descent	6.93%	NS	0.1027†
Baseline NIHSS	9 (4–16)	0.298	<0.0001*†
IV tPA treatment	70.96%	tPA cohort has higher $\Delta NIHSS_{24}$	<0.0001*†
Atrial fibrillation	30.63%	NS	0.6936
Diabetes	27.36%	DM results in lower $\Delta NIHSS_{24}$	<0.0001*†
Antiplatelet use	40.98%	NS	0.8325
Statin use	34.48%	NS	0.6233
Baseline glucose, mmol/L	6.71 (5.72–8.31)	−0.090	<0.0001*†
Systolic blood pressure, mm Hg	154 (137–171)	−0.086	<0.0001*†
Diastolic blood pressure, mm Hg	81 (71–91)	−0.035	0.0849†
TOAST (N=2540)		Figure III in the Data Supplement	Figure III in the Data Supplement
LAA	379 (14.9%)		
CE	1070 (42.1%)		
UND	866 (34.1%)		
SVD	225 (8.9%)		

CE indicates cardioembolic; IQR, interquartile range; IV tPA, intravenous alteplase; LAA, large artery atherosclerosis; NIHSS, National Institutes of Health Stroke Scale; SVD, small vessel disease; TOAST, Trial of ORG 10172 in Acute Stroke Treatment (stroke etiology designations); and UND, undetermined.

*Variables that remain significant after Benjamini-Hochberg correction.

†Variables that were included in multivariable modeling.

1.2–3.0 hours; Figure I in the Data Supplement). Overall, 30% of subjects had follow-up imaging with minor differences in rates between tPA and non-tPA-treated subjects.

Baseline and 24-Hour Stroke Severity

Baseline NIHSS demonstrated a positive-skewed distribution with more patients having milder strokes, typical of a cross-sectional population of AIS (Figure 1A).[26–28] Median baseline NIHSS was 9 (interquartile range, 4–16). Across locations, there was variation in overall severity and age (Table I and Figure IIA in the Data Supplement).

TOAST was significantly associated with baseline NIHSS. More severe strokes fell into the large artery atherosclerosis and cardioembolic categories; milder strokes in the small vessel disease category, consistent with the findings of others (Figure IIB in the Data Supplement).[29,30] Except between large artery atherosclerosis and cardioembolic, mean NIHSS was significantly different when compared between TOAST designations. Univariate analysis (Table II in the Data Supplement) indicated that all variables but statin use should be included in multivariable modeling. As the distribution of baseline NIHSS violated normalcy testing, it was log-transformed for multivariable analysis. This analysis (Table III in the Data Supplement) revealed that stroke etiology explained a larger proportion of the log-transformed baseline NIHSS variance than all other variables combined (partial Eta-square of 0.0752 versus 0.0499). Other variables that independently influenced baseline NIHSS included location of enrollment, age, sex, atrial fibrillation, and baseline glucose.

NIHSS at 24 hours (Figure 1B) demonstrated a skew towards lower NIHSS with half the subjects scoring ≤4. Similar to baseline NIHSS, there were significant differences between all TOAST designations, except for large artery atherosclerosis and cardioembolic, for NIHSS at 24 hours (data not shown).

Change in Stroke Severity From Baseline to 24 Hours ($\Delta NIHSS_{6-24h}$)

In contrast to baseline NIHSS, $\Delta NIHSS_{6-24h}$ approximated a normal distribution (Figure 1C) which ranged from −34 (extreme deterioration) to +33 (extreme improvement) with a mean of +2.78 (SD 5.93).

Univariate analysis of consistently reported variables indicated that age, race (African descent), baseline NIHSS, tPA treatment, diabetes, baseline glucose, and blood pressure should be included in multivariable modeling (Table 1). Mean $\Delta NIHSS_{6-24h}$ did not differ across TOAST etiologies, except for the lower $\Delta NIHSS_{6-24h}$ in small vessel disease (Figure 2). In contrast to the influence on baseline NIHSS, multivariable analysis demonstrated that TOAST subtypes had the least significant influence on $\Delta NIHSS_{6-24h}$ (Table 2). Instead, baseline NIHSS, tPA treatment, age, and glucose were highly significant predictors of $\Delta NIHSS_{6-24h}$. Of these,

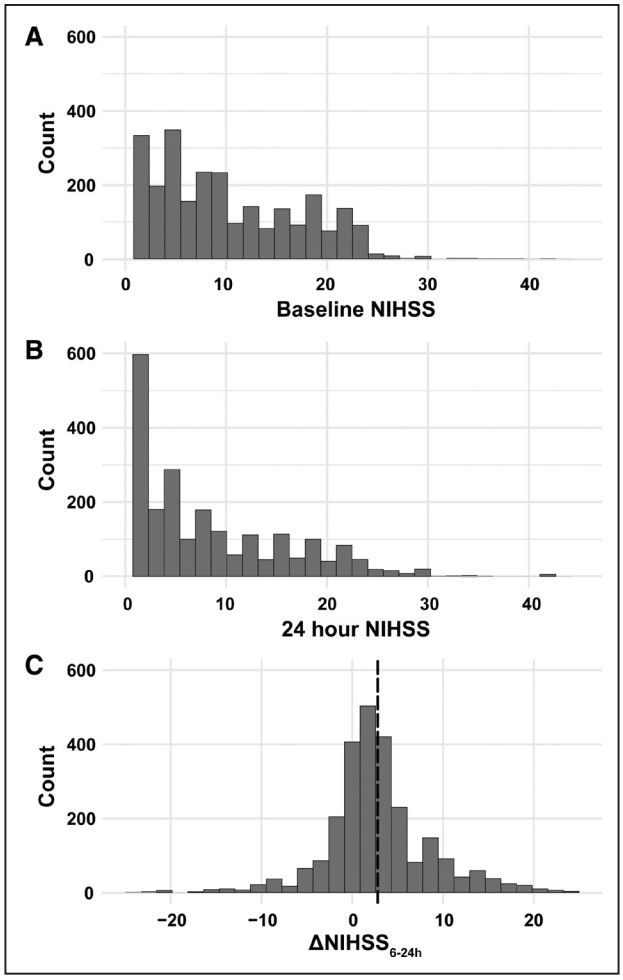

Figure 1. Frequency distribution histograms of baseline, 24-h, and change in score from NIHSS collected within 6 h of onset to follow-up NIHSS score collected at 24 h (+/− 4 h) from baseline examination ($\Delta NIHSS_{6-24h}$) in the GENISIS study (Genetics of Early Neurological Instability After Ischemic Stroke) cohort.

A, Distribution of baseline (collected within 6 h of last known normal) National Institutes of Health Stroke Scale scores (NIHSS). **B**, Distribution of the 24 h (±4 h) NIHSS scores. **C**, Distribution of $\Delta NIHSS_{6-24h}$ (calculated as baseline NIHSS−24 h NIHSS). Dotted line is the mean (2.78) of the distribution.

baseline NIHSS had the highest contribution of any variable to the model, likely due to greater variability of $\Delta NIHSS_{6-24h}$ at higher baseline NIHSS. Overall, this model accounted for only 14.9% of the variance seen in $\Delta NIHSS_{6-24h}$. Due to site differences for baseline NIHSS, a nested model was performed and did not differ significantly in terms of the covariates and explained variance in $\Delta NIHSS_{6-24h}$ (Methods and Table IV in the Data Supplement).

Association of $\Delta NIHSS_{6-24h}$ With Long-Term Outcome

To examine the association of $\Delta NIHSS_{6-24h}$ with long-term outcome, a model for 90-day favorable outcome

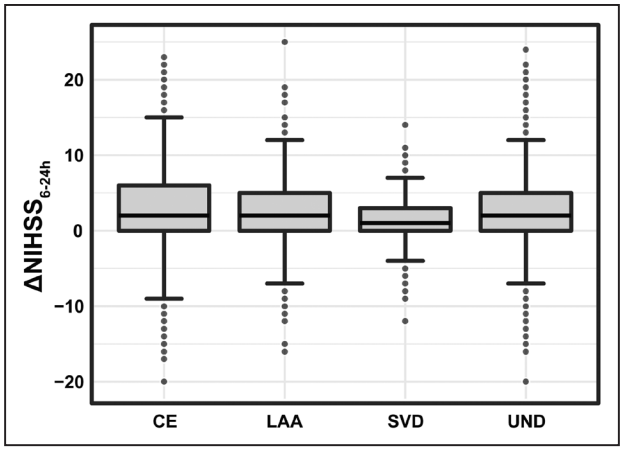

Figure 2. Frequency distribution of change in score from NIHSS collected within 6 h of onset to follow-up NIHSS score collected at 24 h (+/− 4 h) from baseline examination ($\Delta NIHSS_{6-24h}$) by TOAST (Trial of ORG 10172 in Acute Stroke Treatment) cause in the GENISIS study (Genetics of Early Neurological Instability After Ischemic Stroke) cohort.

CE indicates cardioembolic; LAA, large artery atherosclerosis; SVD, small vessel disease; and UND, undetermined.

(mRS score 0–2 versus 3–6) was created. There were 2303 subjects with an available 90-day mRS. After univariate analysis (Table V in the Data Supplement), significant covariates were included in the model. Stepwise logistic regression demonstrated that baseline NIHSS and $\Delta NIHSS_{6-24h}$ had strong independent influences on outcome (Table 3). Baseline NIHSS had a partial correlation squared of 0.140, and $\Delta NIHSS_{6-24h}$ had a partial correlation squared of 0.085 (with respective ORs of 0.77 [95% CI, 0.75–0.79] and 1.24 [1.21–1.27]). Age, site, diabetes, and race (African descent) contributed to the final model. Imputation of missing 90-day mRS did not impact the final multivariable model (Methods, Results, Tables VI, VII, and VIII in the Data Supplement). These data indicate that baseline NIHSS and $\Delta NIHSS_{6-24h}$ had a significant and independent association with 90-day outcomes.

LVO Cohort: Role of Recanalization and HT

To determine if AIS mechanisms influence $\Delta NIHSS_{6-24h}$, we used a well-characterized subset of tPA-treated LVO patients from Vall d'Hebron University Hospital (n=248) to identify patients who experienced recanalization and HT. This subcohort was divided into 6 groups based on SD from the mean $\Delta NIHSS_{6-24h}$ (Methods and Figure III in the Data Supplement; −2 SD threshold was −9 and +2 SD was 15). Distribution of age and female sex was similar across groups; however, there were significant between groups differences in baseline NIHSS, $\Delta NIHSS_{6-24h}$, glucose, HT, parenchymal hematoma type 2 (PH2), and ESR (Table IXA in the Data Supplement). Using ordinal logistic regression multivariable models to evaluate the influence of these variables on early outcomes, the only variables that remained significant and

Table 2. Multivariable Analysis of $\Delta NIHSS_{24}$ (N=2346; R^2=0.149, Model *P*<0.0001)

Variable	Parameter estimate (95% CI)	Partial eta-square	*P* value
Baseline NIHSS	0.271 (0.236 to 0.306)	0.0892	<0.0001
IV tPA treatment	1.440 (0.909 to 1.970)	0.0120	<0.0001
Age (unit=10 y)	−0.495 (−0.676 to −0.313)	0.0121	<0.0001
Glucose (unit=1 mmol/L)	−0.199 (−0.275 to −0.124)	0.0114	<0.0001
Location of enrollment	NA	0.0055	0.0048
Systolic blood pressure (mm Hg)	−0.013 (−0.022 to −0.005)	0.0041	0.0020
TOAST	NA	0.0045	0.0144
LAA	−1.004 (−1.702 to −0.306)	NA	(0.0048)
CE	−0.611 (−1.140 to −0.082)	NA	(0.0235)
SVD	0.036 (−0.826 to 0.897)	NA	(0.935)
UND	Reference	NA	(NA)

CE indicates cardioembolic; IV tPA, intravenous alteplase; LAA, large artery atherosclerosis; N, number of subjects; NA, not applicable; NIHSS, National Institutes of Health Stroke Scale; R^2, R-squared (a statistical measure that represents the proportion of variance for a dependent variable explained by an independent variable or variables in a regression model); SVD, small vessel disease; TOAST, Trial of ORG 10172 in Acute Stroke Treatment (stroke etiology designations); and UND, undetermined.

did not violate the proportional odds were ESR and PH2 (for univariate results, Table IXB in the Data Supplement). Overall, subjects with ESR had a high likelihood of a better outcome (OR, 6.8 [4.0–11.5]) across the groups. The extreme improvement (group 6) versus the other combined groups showed an OR of 3.8 (1.3–11.4) for ESR. Subjects with a PH2 were more likely to have deterioration after the initial presentation (OR, 6.8 [2.0–22.7]). Extreme worsening (group 1) versus the other combined groups demonstrated an OR of 13.17 (2.1–81.5) for PH2.

To explore the combined effects of ESR and HT on $\Delta NIHSS_{6-24h}$, multivariable analysis was performed in the LVO cohort (Table X in the Data Supplement for univariate results). ESR accounted for the majority of explained variance (partial eta-square of 0.2136; parameter estimate 5.952 [4.526–7.377]) with PH2 providing an additional portion (partial eta-square of 0.061; parameter estimate −6.907 [−10.302 to −3.512]). None of the other variables remained in the multivariable model. Despite inclusion of recanalization and HT, the model explained only 27% of $\Delta NIHSS_{6-24h}$ variance.

In multivariable modeling of 90-day outcomes within the LVO cohort, neither PH2 nor ESR had a significant influence on favorable outcome despite meeting the univariate threshold (Table XI in the Data Supplement). While there was significant correlation between ESR and $\Delta NIHSS_{6-24h}$, as well as PH2 and $\Delta NIHSS_{6-24h}$, it was insufficient to violate the parameters for inclusion in the multivariable modeling of outcomes. For favorable outcome (n=241), only baseline NIHSS and $\Delta NIHSS_{6-24h}$ remained in the model, explaining 41.3% of the variance (with respective partial correlation squared and ORs of 0.136 and 0.74 [0.68–0.81] and 0.150 and 1.3 [1.2–1.4]).

Independent Analysis

Given the difference in study design, the distribution of baseline NIHSS (tPA/non-tPA) was different between

Table 3. Multivariable Analysis for Favorable Outcome (mRS Score 0–2) at 90 Days in GENISIS

Variable	Parameter estimate (95% CI)	Odds ratio (95% CI)	PR^2	*P* value
Baseline NIHSS	−0.2683 (−0.2950 to −0.2416)	0.77 (0.75 to 0.79)	0.140	<0.0001
$\Delta NIHSS_{6-24h}$	0.2145 (0.1871 to 0.2418)	1.24 (1.21 to 1.27)	0.085	<0.0001
Age	−0.0618 (−0.0720 to −0.0505)	0.94 (0.93 to 0.95)	0.045	<0.0001
Site				0.0002
Krakow	0.7308 (0.2893 to 1.1723)	3.32 (1.73 to 6.37)	0.003	0.0012
Helsinki	−0.3426 (−0.6066 to −0.0787)	1.13 (0.75 to 1.71)	0.002	0.0110
Spain	0.0796 (−0.1353 to 0.2945)	1.73 (1.21 to 2.47)	0.000	0.4678
St Louis, MO	Reference	NA	NA	NA
Diabetes	−0.2372 (−0.3666 to −0.1077)	0.62 (0.48 to 0.81)	0.004	0.0003
African descent	−0.4473 (−0.7185 to −0.1762)	0.41 (0.24 to 0.70)	0.003	0.0012

$\Delta NIHSS_{6-24h}$ indicates change in score from NIHSS collected within 6 hours of onset to follow-up NIHSS score collected at 24 hours (+/− 4 hours) from baseline examination; GENISIS, Genetics of Early Neurological Instability After Ischemic Stroke; mRS, modified Rankin Scale; NA, not applicable; NIHSS, National Institutes of Health Stroke Scale; and PR^2, partial correlation squared.

the GENISIS cohort and the 2 NINDS-tPA stroke trials (Figures IVA and IVB in the Data Supplement). Although there was a compression of ΔNIHSS distribution in the overall GENISIS population compared with the 2 NINDS-tPA stroke trials (Figures IVE and IVF in the Data Supplement), a comparison of the tPA-treated subjects in both GENISIS and NINDS revealed very similar distributions with a median change of 3. The difference in distributions was largely attributed to the non-tPA-treated patients in GENISIS who were fundamentally different from the NINDS control group (the non-tPA patients in GENISIS would have been excluded from the NINDS trials).

Despite the differences in study design and NIHSS distributions, analysis of data from the 2 NINDS-tPA stroke trials[23] (n=606) replicated the association of change in 24 hours from baseline NIHSS with 90-day outcome. For favorable outcome, $\Delta NIHSS_{3-24h}$ and baseline NIHSS accounted for similar portions of the variance (Table 4; partial correlation squared of 0.145 and 0.159, respectively) in the overall data set. This significant and independent contribution of both $\Delta NIHSS_{3-24h}$ and baseline NIHSS to long-term outcome was consistent when evaluating the tPA and placebo cohorts separately as well (Table 4).

DISCUSSION

We demonstrate that neurological deficit, as measured by NIHSS, is dynamic in the first 24 hours after ischemic stroke onset. This neurological change, captured by $\Delta NIHSS_{6-24h}$, has a statistically significant association with 90-day outcome; an association which is independent not only of baseline NIHSS but also of the presumed stroke cause (TOAST). A variety of mechanisms are likely to contribute to $\Delta NIHSS_{6-24h}$. In a subcohort of tPA-treated LVO patients, ESR and severe HT (PH2) were associated with extreme degrees of neurological improvement or deterioration, respectively. The addition of these covariates in a model of $\Delta NIHSS_{6-24h}$ increased the amount of explained variance within the LVO cohort but still left a majority of variance unexplained. Thus, $\Delta NIHSS_{6-24h}$ captures mechanisms related to early neurological change after AIS, thereby creating a significant association with long-term outcomes.

The association of $\Delta NIHSS_{6-24h}$ with long-term outcome in the GENISIS cohort was independent of early baseline stroke severity. Indeed, some of the factors that influence each of these metrics are also independent. TOAST designation had a strong influence on baseline NIHSS, accounting for 7.5% of the variance—the highest of all baseline variables—but had little influence on $\Delta NIHSS_{6-24h}$, accounting for <0.5%. These findings are consistent with previous studies[7,29] and indicate that stroke cause has a major influence on initial stroke severity, but limited influence on the processes that account for early neurological deterioration or improvement.

Both HT and lack of recanalization have been described as significant factors in neurological worsening.[5,7] Previous studies have also demonstrated that early neurological improvement has consistently been associated with successful reperfusion.[5,31,32] A recent multicenter study showed that early tPA induced recanalization in patients with LVO significantly improved both early and long-term outcomes.[33] While this holds true in our analysis of the LVO cohort, recanalization and HT explain a minority of variance in $\Delta NIHSS_{6-24h}$, leaving the majority of variance unexplained and suggesting that there are other contributing factors that are being captured by $\Delta NIHSS_{6-24h}$. In addition, since neither ESR nor PH2 remained in the

Table 4. Multivariable Analysis for Favorable Outcome (mRS Score 0–2) at 90 Days in NINDS

Variable	Parameter estimate (95% CI)	Odds ratio (95% CI)	PR²	*P* value*
Baseline NIHSS†	−0.3227 (−0.3767 to −0.2687)	0.72 (0.69 to 0.76)	0.159	<0.0001
tPA cohort	−0.3234 (−0.4015 to −0.2453)	0.72 (0.67 to 0.78)	0.151	<0.0001
Placebo cohort	−0.3422 (0.2548 to 0.4295)	0.72 (0.66 to 0.78)	0.158	<0.0001
$\Delta NIHSS_{3-24h}$†	0.3342 (0.2758 to 0.3927)	1.4 (1.3 to 1.5)	0.145	<0.0001
tPA cohort	0.3351 (0.2527 to 0.4175)	1.4 (1.3 to 1.5)	0.145	<0.0001
Placebo cohort	0.3422 (0.2548 to 0.4295)	1.4 (1.3 to 1.5)	0.137	<0.0001
Atrial fibrillation†	−0.4713 (−0.8122 to −0.1303)	0.39 (0.20 to 0.77)	0.006	0.0067
tPA cohort	−0.5268 (−0.9745 to −0.0790)	0.35 (0.14 to 0.85)	0.008	0.0211
Placebo cohort	−0.6650 (−1.1594 to −0.1705)	0.26 (0.10 to 0.71)	0.012	0.0084
Diabetes	NA	NA	NA	NA
tPA cohort	−0.5707 (−1.0087 to −0.1328)	0.32 (0.13 to 0.77)	0.011	0.0106
Age†	−0.0271 (−0.0499 to −0.0043)	0.97 (0.95 to 1.0)	0.004	0.0199

mRS indicates modified Rankin Scale; NA, not applicable; NIHSS, National Institutes of Health Stroke Scale; PR², partial correlation squared; and tPA, alteplase.

*Table only includes variables that remain significant after Benjamini-Hochberg correction.

†Results for overall NINDS data are presented while the results of the tPA and placebo treatment arms, respectively, are presented below each relevant variable.

model for favorable outcome, it appears that they play an indirect role through $\Delta NIHSS_{6-24h}$. Similar to this finding, it was recently reported that the effect of endovascular treatment on 90-day mRS is in large part mediated by early changes in NIHSS.[34] Moreover, HT and ESR develop in a minority of patients, supporting the suggestion that other mechanisms are involved in the determination of $\Delta NIHSS_{6-24h}$.

The impact of early neurological change on long-term outcome was further supported in an independent secondary analysis of the 2 NINDS-tPA trials,[23] in which baseline NIHSS was measured within 3 hours of stroke onset (in contrast to within 6 hours in GENISIS). The influence of $\Delta NIHSS_{3-24h}$ on 90-day mRS was larger than that observed in our study. It is likely that dynamic changes in early neurological function are time-dependent, with a higher likelihood of change earlier in the ischemic course, similar to the degree to which brain tissue in the penumbra remains salvageable is highly time-dependent.[35] Therefore, it is not surprising that ΔNIHSS values measured between 3 hours of onset and 24 hours have greater association with outcome than ΔNIHSS between 6 and 24 hours. Furthermore, the independent influence of ΔNIHSS seen in both GENISIS and the NINDS studies suggests that early interventions (such as reperfusion) that alter ΔNIHSS could have a lasting impact on long-term outcomes. Thus, ΔNIHSS is not only an important early metric that is associated with long-term outcome but also a phenotype for discovering additional mechanisms to influence the neurological changes that occur during that window.

For the purposes of the GENISIS study, we have chosen to focus on arithmetic NIHSS change as opposed to percentile or normalized change. While a recent publication has indicated that percentile change of NIHSS has better predictive capabilities for 3-month outcomes than arithmetic change,[36] our goal is to use a quantitative phenotype for deterioration/improvement in large-scale genetic studies to identify potential modifiers of early neurological changes. Indeed, $\Delta NIHSS_{6-24h}$ is the primary quantitative phenotype for the GENISIS study, which aims to identify novel mechanisms involved in early neurological change using unbiased approaches (such as GWAS). For GWAS, there are major advantages for a quantitative phenotype that has a normal distribution, which makes statistical analysis more manageable.

This study has several strengths, including the large sample size, involving patients in real-world standard-of-care practice, careful documentation of NIHSS at standardized time intervals after stroke onset, and replication in an independent cohort of patients enrolled in a randomized controlled trial. There are also several limitations to the study. Due to the pragmatic design of the study with the intent to generate a large collection of subjects for the purposes of a GWAS, a minimum number of variables were required for inclusion. As such we are unable to provide an estimation of bias in recruitment (ie, potential selection and bidirectional ascertainment bias) or an assessment of intrasite and intersite reliability for assessment of NIHSS and mRS, lack serial NIHSS post-tPA within the 24-hour window to analyze for potential interval censoring between baseline and the 24-hour NIHSS, and do not have potential confounders (such as participation in rehabilitative therapies, recurrent stroke, or infections) that might have occurred after discharge and influenced 90-day outcome. It is likely that the phenotype, $\Delta NIHSS_{6-24h}$, when combined with GWAS will capture only early mechanisms involved in AIS. Later epochs of NIHSS change (eg, ΔNIHSS 24–72 hours or ΔNIHSS 72 hours–90 days) might capture subsequent independent mechanisms that are not captured in the earlier time period. In part because of differences in the availability of certain variables, several of the analyses were performed on smaller cohorts of the GENISIS population, in some cases limiting the generalizability of conclusions. Another limitation is that our AIS population is biased towards severe strokes because inclusion is limited to patients that present within 6 hours of stroke onset. While the median baseline NIHSS is higher in GENISIS than that seen in unselected cross-sectional studies, it is well known that earlier presenters tend to have higher NIHSS scores.[27,28,37,38] Thus, the GENISIS cohort is consistent with the real-world population that is amenable to acute interventions and, therefore, relevant to our study. A final limitation is that the current study excludes patients treated with thrombectomy. In the early years of recruitment, this was a small proportion of patients presenting within 6 hours. However, as the number of patients eligible for this treatment grows, future studies will incorporate this potentially informative population.

CONCLUSIONS

In conclusion, this study demonstrates that neurological deficits are unstable in the first 24 hours after stroke onset. This dynamic change, defined by $\Delta NIHSS_{6-24h}$, is influenced by mechanisms such as recanalization and severe hemorrhage as well as potential other mechanisms. In turn, $\Delta NIHSS_{6-24h}$ is significantly associated with long-term outcomes. Therefore, $\Delta NIHSS_{6-24h}$ will be a valuable quantitative phenotype in large genetic studies of patients with AIS, to identify novel mechanisms involved in neurological change after stroke that are relevant to human disease.

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