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# Predictors of Early Neurological Improvement and Its Relationship to Thrombolysis Treatment and Long-Term Outcome in the WAKE-UP Study

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**Keywords**
Early neurological improvement · Stroke · Outcome · Thrombolysis

**Abstract**
***Introduction:*** The aims of this study were to evaluate the relationship of clinical and imaging baseline factors and treatment on the occurrence of early neurological improvement (ENI) in the WAKE-UP trial of MRI-guided intravenous thrombolysis in unknown onset stroke and to examine the association of ENI with long-term favorable outcome in patients treated with intravenous thrombolysis. ***Methods:*** We analyzed data from all patients with at least moderate stroke severity, reflected by an initial National Institutes of Health Stroke Scale (NIHSS) score ≥4 randomized in the WAKE-UP trial. ENI was defined as a decrease in NIHSS of ≥8 or a decline to zero or 1 at 24 h after initial presentation to the hospital. Favorable outcome was defined as a modified Rankin Scale score of 0–1 at 90 days. We performed group comparison and multivariable analysis of baseline factors associated with ENI and performed mediation analysis to evaluate the effect of ENI on the relationship between intravenous thrombolysis and favorable outcome. ***Results:*** ENI occurred in 93 out of 384 patients (24.2%) and was more likely to occur in patients who received treatment with alteplase (62.4% vs. 46.0%, $p = 0.009$), had smaller acute diffusion-weighted imaging lesion volume (5.51 mL vs. 10.9 mL, $p \leq 0.001$), and less

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often large-vessel occlusion on initial MRI (7/93 [12.1%] versus 40/291 [29.9%], $p = 0.014$). In multivariable analysis, treatment with alteplase (OR 1.97, 95% confidence interval [CI] 0.954–1.100), lower baseline stroke volume (OR 0.965, 95% CI: 0.932–0.994), and shorter time from symptom recognition to treatment (OR 0.994, 95% CI: 0.989–0.999) were independently associated with ENI. Patients with ENI had higher rates of favorable outcome at 90-day follow-up (80.6% vs. 31.3%, $p \leq 0.001$). The occurrence of ENI significantly mediated the association of treatment with a good outcome, with ENI at 24 h explaining 39.4% (12.9–96%) of the treatment effect. ***Conclusion:*** Intravenous alteplase increases the odds of ENI in patients with at least moderate stroke severity, especially when given early. In patients with large-vessel occlusion, ENI is rarely observed without thrombectomy. ENI represents a good surrogate early marker of treatment effect as more than a third of good outcome at 90 days is explained by ENI at 24 h. 

## Introduction

Early neurological improvement (ENI) predicts a favorable long-term outcome in stroke patients after intravenous (i.v.) thrombolysis with alteplase [1–3] but is also observed in patients who do not receive acute reperfusion treatments [4, 5]. The patient's immediate response to acute i.v. treatment with thrombolysis is variable, with some patients improving dramatically, while others show persistent neurological deficits without improvement. Previous studies indicated an association of demographic and clinical baseline factors such as younger age [5–7], female sex [8, 9], lower serum glucose levels [6, 9, 10], and lower white blood cell count [11] with ENI. Imaging parameters such as smaller stroke volume [12, 13] and the absence of hemorrhagic transformation [14] were also observed to be associated with ENI. Identifying predictors of ENI is important in understanding outcome-related mechanisms in the first 24–48 h after stroke. ENI also correlates with better long-term functional outcome [3, 8, 9] and thus may be considered as a robust early surrogate marker of treatment response. The pathophysiological mechanisms behind the association of acute reperfusion treatment, early clinical response (i.e., ENI), and long-term functional outcome are currently under discussion, and ENI has been demonstrated in different subgroups [3, 5, 15]. We aimed to evaluate the influence of clinical and imaging baseline factors on the occurrence of ENI in patients randomized in the WAKE-UP trial of MRI-guided i.v. thrombolysis in unknown onset stroke. We further examined the association of ENI with favorable outcome in patients treated with i.v. thrombolysis.

## Methods

*Study Design*

For this exploratory post hoc analysis, we reviewed demographic, clinical, and MR imaging data on all patients randomized in WAKE-UP at baseline, at 24-h follow-up, and 90 days after stroke. WAKE-UP was a multicenter, randomized, double-blind, placebo-controlled clinical trial that established the MRI-guided efficacy and safety of alteplase treatment in patients with an acute stroke with unknown time of symptom onset [16]. Patients were included and underwent randomization if MR imaging revealed a mismatch between an acute ischemic lesion on DWI but no corresponding marked parenchymal hyperintensity on fluid-attenuated inversion recovery as a surrogate marker of lesion age. Patients were included if they had an mRS score of zero or 1 before the stroke. Patients who were planned to receive thrombectomy were excluded. Because ENI can meaningfully be considered only in patients with moderate to severe stroke, only patients with at least moderate neurological symptoms defined by a National Institutes of Health Stroke Scale (NIHSS) score ≥4 at baseline were included in this analysis.

*Baseline and Follow-Up Data*

Demographical, clinical, treatment, and MR imaging data were collected from the trial data base. Clinical data included NIHSS, vital parameters, laboratory parameters, medication intake, and preexisting comorbidities. MR imaging parameters included DWI lesion size at baseline, lesion location, the presence of large-vessel occlusion at baseline (occlusion of internal carotid artery, M1, M2, ACA or PCA), information on lesion growth from baseline to 24-h follow-up, new lesions at follow-up, and hemorrhagic transformation at 24-h follow-up. Favorable outcome was defined as a modified Rankin Scale score of 0–1 at 90-day follow-up.

*Definition of ENI*

NIHSS was recorded for patients at baseline and at 24 h. ENI was defined as a decrease in NIHSS of ≥8 or a decline to zero or 1 at 24 h after initial presentation to the hospital. To this date, there is no consensus on the definition of ENI, making meta-analyses impossible and hindering comparisons of results. Most studies used the delta between NIHSS at initial presentation versus at 24 h poststroke with cutoffs defining ENI ranging from ≥4 to ≥10 [5, 7, 8], while others defined ENI as a percentage change [17, 18] or reduction of NIHSS to 0–1 at 24 h [5, 7, 9]. We chose a cutoff of ≥8 and excluded patients with an initially low NIHSS, thus choosing a rather conservative definition to assure that our study represented a fraction with dramatic improvement.

*Statistical Analysis*

Statistical analysis was conducted using R [19]. We performed a group comparison of baseline variables between patients with and without ENI within the entire patient population. We performed logistic multivariable regression to identify predictors of the occurrence of ENI based on a model including factors from group comparison and review of the previous literature. The model

**Table 1.** Group comparison between patients with occurrence of ENI and patients without ENI at 24 h

| | ENI (*N* = 93) | No ENI (*N* = 291) | *p* value |
|---|---|---|---|
| Female, *N* (%) | 39 (41.9) | 106 (36.4) | 0.406 |
| Age (mean ± SD), years | 65.7 ± 10.8 | 66.1 ± 11.2 | 0.797 |
| Stroke and imaging specifics | | | |
| NIHSS score at baseline (median [IQR]) | 6 [5; 10] | 7 [5; 10] | 0.123 |
| Treatment with alteplase, *N* (%) | 58 (62.4%) | 134 (46%) | 0.009 |
| Time from last-seen-well to treatment (median [IQR]), min | 602 [515; 690] | 632 [490; 744] | 0.192 |
| Time from symptom recognition to treatment (median [IQR]), min | 170 [133; 231] | 190 [154; 230] | 0.054 |
| Stroke volume at baseline (mean ± SD) | 5.51 ± 8.56 | 10.9 ± 16.0 | <0.001 |
| FLAIR ratio (mean ± SD) | 1.07 ± 0.08 | 1.06 ± 0.09 | 0.133 |
| Large-vessel occlusion at baseline, *N* (%) | 11 (11.8%) | 71 (24.4%) | 0.015 |
| Lacunar infarct, *N* (%) | 19 (20.4%) | 55 (18.9%) | 0.861 |
| Vital parameters at baseline | | | |
| Systolic blood pressure (mean ± SD) | 152 ± 18.8 | 152 ± 21.9 | 0.883 |
| Diastolic blood pressure (mean ± SD) | 81.5 ± 12.8 | 83.0 ± 12.6 | 0.333 |
| Heart rate (mean ± SD) | 77.9 ± 17.7 | 77.1 ± 17.4 | 0.689 |
| Temperature (mean ± SD) | 36.6 ± 0.52 | 36.4 ± 0.55 | 0.064 |
| Weight (mean ± SD) | 77.7 ± 16.6 | 78.2 ± 15.6 | 0.769 |
| Cardiovascular risk factors and medication, *N* (%) | | | |
| Arterial hypertension | 44 (48.4) | 166 (57.2) | 0.172 |
| Atrial fibrillation | 11 (12.0) | 42 (14.7) | 0.621 |
| Hypercholesterolemia | 26 (29.2) | 108 (38.8) | 0.129 |
| Diabetes mellitus | 13 (14.0) | 52 (18.2) | 0.438 |
| Pre-treatment with platelet inhibitors | 37 (39.8) | 90 (30.9) | 0.146 |
| Pre-treatment with statins | 30 (32.3) | 87 (29.9) | 0.763 |
| Laboratory values | | | |
| Creatinine (mean ± SD) | 0.94 (0.26) | 0.95 (0.55) | 0.777 |
| Platelet count (mean ± SD) | 243 (67.3) | 235 (71.2) | 0.316 |
| Serum glucose (mean±SD) | 126 (47.7) | 133 (50.4) | 0.260 |
| White blood cell count (mean ± SD) | 7.98 (2.65) | 8.47 (3.15) | 0.149 |
| Follow-up results, *N* (%) | | | |
| Lesion growth at 24 h | 46/92 (50%) | 186/288 (64.6%) | 0.011 |
| Hemorrhagic transformation at 24 h | 14 (15.1%) | 87 (29.9%) | 0.007 |
| Type of hemorrhagic transformation* | | | |
| Hemorrhagic infarction type 1 | 6 (42.9%) | 33 (37.9%) | |
| Hemorrhagic infarction type 2 | 8 (57.1%) | 41 (47.1%) | |
| Parenchymatous hematoma type 1 | 0 | 13 (14.9%) | |
| New ischemic lesion at 24 h, *N* (%) | 28 (30.4) | 75 (26.0) | 0.490 |
| Favorable outcome at 90 days (mRS 0–1), *N* (%) | 75 (80.6) | 91 (31.3) | <0.001 |

ENI, early neurological improvement; SD, standard deviation; NIHSS, National Institute of Health Stroke Scale; IQR, interquartile range; FLAIR, fluid-attenuated inversion recovery; mRS, modified Rankin Scale. * According to the Heidelberg Bleeding Classification.

comprised treatment with alteplase, stroke severity (NIHSS), large-vessel occlusion, stroke volume, the presence of atrial fibrillation, prior statin treatment, blood pressure, glucose levels, leukocyte count, and time from symptom recognition to treatment [5, 7, 9–11, 20, 21]. Occurrence of ENI was examined as a moderator of the relationship between treatment with alteplase and good outcome at 90 days. Mediation analysis is a statistical method to examine a known relationship by exploring the underlying mechanism. It assumes that the relationship between an independent and a dependent variable is at least partially accounted for (or mediated by) a third variable. Mediation analysis was performed based on the principles defined by Baron and Kenny [22] and employing algorithms developed by Imai, Keele, and Tingley [23, 24] comprised in the R package "mediation" [25] that allow for estimation of causal mediation effects. We tested the significance of this indirect effect using bootstrapping procedures. Unstandardized indirect effects were computed for each of 500 bootstrapped samples, and the 95% confidence interval (CI) was computed by determining the indirect effects at the 2.5 and 97.5 percentiles. All tests were carried out with a two-sided alpha level of 5% without correction for multiple comparisons.

**Table 2.** Multivariate analysis of factors with independent influence on ENI as outcome

| Predictor | Odds ratio | 95% CI | | *p* value |
|---|---|---|---|---|
| | | lower | upper | |
| Intercept | 2.74 | 0.239 | 31.7 | 0.417 |
| NIHSS score | 1.030 | 0.954 | 1.100 | 0.483 |
| Large-vessel occlusion at baseline | 0.487 | 0.209 | 1.060 | 0.081 |
| Stroke volume per ml | 0.965 | 0.932 | 0.994 | 0.028 |
| Time from symptom recognition to treatment in minutes | 0.994 | 0.989 | 0.999 | 0.032 |
| Randomization to treatment with alteplase | 1.970 | 1.170 | 3.370 | 0.012 |
| Glucose | 0.996 | 0.989 | 1.000 | 0.144 |
| Blood pressure | 0.996 | 0.983 | 1.010 | 0.530 |
| Pre-treatment with statins | 1.210 | 0.681 | 2.120 | 0.509 |
| Atrial fibrillation | 0.814 | 0.344 | 1.779 | 0.618 |
| White blood cell count | 0.981 | 0.882 | 1.080 | 0.715 |

CI, confidence interval; NIHSS, National Institute of Health Stroke Scale.

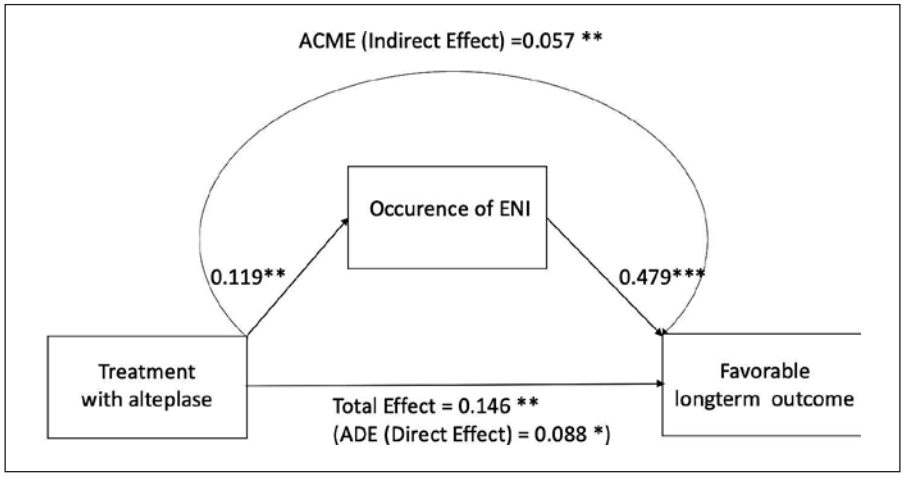


**Fig. 1.** Illustration of the regression coefficients for the association of treatment with alteplase as an independent predictor and occurrence of ENI as a mediator with favorable long-term outcome, showing significant effects for each relationship. The indirect effect, capturing the effect of treatment on outcome when accounting for the mediator (ACME) is also significant. The direct effect (ADE) is decreased when compared to the total effect (not accounting for the mediator) but remains significant, demonstrating a partial mediation. * $p$ value <0.05, ** $p$ value <0.01, *** $p$ value <0.001. ACME, average causal mediation effect.

## Results

*Patient Characteristics*

Of 503 patients randomized in WAKE-UP, 384 presented with a NIHSS ≥4 at baseline and were included in this analysis. ENI at 24 h was present in 24.2% ($n$ = 93) of patients (Table 1). Patients with ENI had more often been randomized to receive treatment with alteplase (58/93 [62.4%] vs. 134/291 [46.0%], $p$ = 0.009), had smaller acute diffusion-weighted imaging lesion volume (5.51 mL [±8.56] vs. 10.9 mL [±16.0], $p \geq 0.001$), and less often large-vessel occlusion on initial MRI (7/93 [12.1%] vs. 40/291 [29.9%], $p$ = 0.014).

On follow-up imaging at 24 h, patients with ENI less often had lesion growth (46/92 [50%] vs. 186/288 [64.6%], $p$ = 0.011) and hemorrhagic transformation (14/93 [15.1%] vs. 87/291 (29.9%), $p$ = 0.007). More patients with ENI had a favorable outcome at 90-day follow-up (75/93 [80.6%] vs. 91/291 [31.3%], $p \leq 0.001$).

*Influence of Baseline Variables and Imaging Parameters on ENI*

In multivariable analysis, treatment with alteplase (OR 1.97, 95% CI 0.954–1.100), lower baseline stroke volume (OR 0.965, 95% CI 0.932–0.994), and shorter time from symptom recognition to treatment (OR 0.994, 95% CI 0.989–0.999) were independently associated with ENI (Table 2).

*Mediation Analysis*

The requirements for mediation analysis defined by Baron and Kenny [22] were fulfilled: regression analysis

**Table 3.** Mediation analysis

| | Estimate | 95% CI | | *p* value |
|---|---|---|---|---|
| | | lower | upper | |
| Total effect of treatment with alteplase (increase of probability for mRS ≤2 at 90 days) | 0.146 | 0.056 | 0.25 | 0.008 |
| Mediated effect explained by occurrence of ENI (ACME) | 0.057 | 0.017 | 0.10 | 0.004 |
| Direct effect explained by other factors not reflected in occurrence of ENI (ADE) | 0.088 | 0.005 | 0.19 | 0.044 |
| Proportion mediated by the occurrence of ENI | 39.4% | 13.4% | 90% | 0.004 |

MRS, modified Rankin Scale; ENI, early neurological improvement; ACME, average causal mediation effect; ADE average direct effect.

of the direct pathway and the mediation pathway showed significant association of treatment as an independent predictor and of ENI at 24 h as the mediator with good outcome (see Fig. 1). Furthermore, the coefficient of regression of the mediator on the independent predictor was significant; all effect metrics were significant at *p* value <0.01.

Mediation analysis revealed that the occurrence of ENI significantly mediated the association of treatment with good outcome (indirect effect/average causal mediation effect): the effect of treatment on good outcome at 90 days decreased when controlled for the mediating effect of ENI on good outcome but remained significant, demonstrating a partial mediation. 39.4% of the variance of the treatment effect was explained by the occurrence of ENI (Table 3).

## Discussion

In our analysis of predictors of ENI in patients randomized in the WAKE-UP trial of MRI-guided i.v. alteplase in unknown onset stroke, treatment with alteplase, shorter time to treatment, and smaller baseline stroke volumes, but no clinical baseline factors, were associated with ENI. In mediation analysis, 39% of the effect of treatment with alteplase on good outcome at 90 days was explained by the occurrence of ENI within 24 h.

The rate of 24.2% of patients with ENI in our cohort is in the lower range of results from previous studies using similar definitions of ENI [5, 7, 9, 20, 26]. This may result from differences between cohorts including the fact that half of the patients in our trial received placebo.

The occurrence of ENI in patients treated with i.v. thrombolysis has previously been associated with clinical factors such as age [5–7], normal baseline glucose levels [6, 9], female gender [8, 9], lower baseline NIHSS [6, 8], the absence of atrial fibrillation [7], lower leukocyte count [11], and pre-treatment with statins [21], as well as shorter time to treatment [5, 11]. Concerning imaging parameters, a higher DWI-ASPECTS at baseline [12, 13] was observed in patients with ENI treated with thrombolysis. Only few studies have examined cohorts with patients randomized to active treatment or placebo in this context. In the NINDS trial, only age and treatment with alteplase predicted the occurrence of ENI in the treatment group. In the placebo group, age, baseline NIHSS score, and glucose were predictors of ENI [5]. In a study analyzing data from patients with and without alteplase treatment, a nonsignificant association was found between complete neurological recovery and elevated blood pressure at admission [27]. This study however also included those patients with minor neurological deficits.

Concerning imaging and treatment factors, our findings are in line with previous results. A smaller stroke volume at baseline has previously been associated with ENI [12, 28]. This finding highlights the importance of fast and advanced imaging even and especially in patients with unknown time of symptom onset to identify patients with large stroke volume who are therefore not likely to experience ENI to extend treatment options. Concerning treatment factors, the association of a shorter symptom onset to treatment times with ENI in our analysis confirms results from previous studies of cohorts with i.v. thrombolysis treatment [5, 11] and provides further evidence for the time dependency of the treatment effect of stroke thrombolysis. Remarkably, in our population, no clinical baseline or demographic factors had an independent influence on the occurrence of ENI. This can in part be attributed to the selection effect in this clinical trial population which excluded patients with previous disability.

In the WAKE UP trial, patients who were planned to receive mechanical thrombectomy were excluded from randomization, as thrombectomy was not established as a standard of care at the time. Our results show that ENI was less often achieved in patients with large-vessel

occlusion who did not receive thrombectomy, underlining the importance of additional reperfusion therapies. The influence of treatment with i.v. thrombolysis on positive short- and long-term outcomes has been widely established [3, 29]. Our results on the mediation effect of ENI on the relationship between treatment with alteplase and good long-term outcome further elevate the importance of promoting ENI and point toward ENI as a potential surrogate marker for favorable long-term outcome. Identifying patients who will most likely not experience ENI could potentially become a useful tool to select patients to be evaluated for further recanalization therapy options such as thrombectomy, add-on antithrombotic drugs, and intra-arterial therapies [30, 31]. It furthermore points toward the need for further research into factors with predictive value for ENI.

This study has several limitations. There is no consensus on the definition of ENI, implying that comparisons between studies are limited. The setting of a randomized clinical trial with the use of MRI to select limits the generalizability of the results.

To summarize, in the randomized controlled WAKE-UP trial, treatment with alteplase was associated with ENI within 24 h, especially when given early. Contrary to previous works, we did not find clinical baseline characteristics as potentially modifiable factors to promote ENI in acute stroke patients, but ENI was less frequently observed with larger DWI lesion volumes. Moreover, ENI is rarely observed in patients with large-vessel occlusion without thrombectomy. ENI represents a good surrogate early marker of treatment effect as more than a third of good outcome at 90 days is explained by ENI at 24 h.

## Statement of Ethics

Study protocols and procedures conformed to the Declaration of Helsinki and were reviewed and approved by the local Ethics Committee (Ethics Committee of the Physicians Chamber Hamburg, Germany; approval Number MC-039/16). Written informed consent was obtained from patients or their legal representatives to participate in the study or waived, when patients lacked the capacity to give consent with no available proxy or the patients died before consent could be obtained.

## Conflict of Interest Statement

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## Author Contributions

Marlene Heinze and Götz Thomalla conceived and designed the study. Marlene Heinze analyzed and interpreted the data and wrote the first draft of the manuscript. Bastian Cheng, Tae-Hee Cho, Martin Ebinger, Matthias Endres, Jochen B Fiebach, Jens Fiehler, Josep Puig, Robin Lemmens, Vincent Thijs, Keith W Muir, Norbert Nighoghossian, Alina Königsberg, Märit Jensen, Ewgenia Barow, Iris Lettow, Salvador Pedraza, Claus Ziegler Simonsen, and Christian Gerloff acquired and curated data and critically revised the manuscript.

## Data Availability Statement

The full trial protocol and the statistical analysis plan of WAKE-UP have already been published along with the main trial publication [16]. Individual patients' data, after de-identification, will be shared with the Virtual International Stroke Trials Archive (VISTA) and be accessible for researchers who provide a methodologically sound proposal according to the VISTA rules (http://www.virtualtrialsarchives.org/vista/). Further inquiries can be directed to the corresponding author.

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