Higher neutrophil counts before thrombolysis for cerebral ischemia predict worse outcomes

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ABSTRACT

Objective: To determine whether higher neutrophil counts before IV recombinant tissue plasminogen activator (rtPA) administration in ischemic stroke (IS) patients are associated with symptomatic intracerebral hemorrhages (sICH) and worse outcomes at 3 months.

Methods: Blood samples for leukocyte, neutrophil, and lymphocyte counts were drawn before IV rtPA administration in IS patients included in the cohorts of Lille and Helsinki. The primary endpoint was sICH (European Cooperative Acute Stroke-II definition). Secondary endpoints were death and excellent (modified Rankin Scale [mRS] score 0–1 or equal to prestroke mRS) and good (mRS score 0–2 or equal to prestroke mRS) outcomes at 3 months.

Results: We included 846 patients (median age 71 years; 50.8% men). The neutrophil count and neutrophil to lymphocyte ratio (NLR) were independently associated with the 4 endpoints: sICH (adjusted odds ratio [adjOR] for an increase of 1,000 neutrophils = 1.21 and adjOR 1.11, respectively), death (adjOR 1.16 and adjOR 1.08), and excellent (adjOR 0.87 and adjOR 0.85) and good (adjOR 0.86 and adjOR 0.91) outcomes. The total leukocyte count was not associated with any of the 4 endpoints. The best discriminating factor for sICH was NLR ≥ 4.80 (sensitivity 66.7%, specificity 71.3%, likelihood ratio 2.32). Patients with NLR ≥ 4.80 had a 3.71-fold increased risk for sICH (95% confidence interval adjOR: 1.97–6.98) compared to patients with NLR < 4.80 .

Conclusions: Higher neutrophil counts and NLR are independently associated with sICH and worse outcome at 3 months. The identification of mediators of this effect could provide new targets for neuroprotection in patients treated by rtPA. **Neurology® 2015;85:1408–1416**

GLOSSARY

adjOR = adjusted odds ratio; CI = confidence interval; ECASS = European Cooperative Acute Stroke Study; MMP-9 = matrix metalloproteinase-9; mRS = modified Rankin Scale; NIHSS = NIH Stroke Scale; NLR = neutrophil to lymphocyte ratio; rtPA = recombinant tissue plasminogen activator; sICH = symptomatic intracerebral hemorrhage.

IV recombinant tissue plasminogen activator (rtPA) given within 4.5 hours after the onset of symptoms increases survival without handicap and without dependency 3–6 months after ischemic stroke.^{e1–e5} Patients treated with rtPA have an increased risk of symptomatic intracerebral hemorrhage (sICH) when compared with placebo-treated patients, which does not hinder the overall benefit.^{e2–e5} Besides the already known predictors for sICH,^{e6} detection of new ones is worthwhile.

In animal studies, neutrophils play a role in sICH.^{1–4} In humans, independent of treatment by rtPA, high neutrophil counts are associated with infarct volume,⁵ stroke severity,⁶ and death.⁷ In SPECT studies, the accumulation of radio-labeled neutrophils correlates with infarct severity and growth and poor neurologic outcome.^{8,9} Recent studies suggested that the neutrophil to lymphocyte ratio (NLR) predicts short- and long-term outcome in stroke patients.^{10–13} However, the delay between stroke onset and blood sample collection varied from a few hours up to 1 week.^{5–7,10–13} Therefore, changes observed in neutrophil count and NLR could also be the consequence of the severity of stroke. Another limitation of these studies is the lack of information on thrombolytic therapy, or the inclusion of small numbers of patients treated by IV rtPA.

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Supplemental data
at Neurology.org

We aimed to test the hypothesis that higher neutrophil counts and NLR at baseline are associated with increased risks of sICH and worse outcomes at 3 months.

METHODS Setting. This study was conducted with data prospectively collected in 2 registries of patients treated with IV rtPA for cerebral ischemia in the stroke units of the Lille (France) and Helsinki (Finland) University Hospitals. These cohorts have been

described previously, as well as the local organizations and standard operating procedures in both centers.^{14–17}

Inclusion and exclusion criteria. In both centers, patients were treated according to the written institutional guidelines.^{14–17} The time window was extended from 3.0 hours to 4.5 hours after the results of the European Cooperative Acute Stroke Study (ECASS)–III.¹⁸ Patients with a discharge diagnosis of stroke mimic, or with a first blood sample taken after the bolus of rtPA, or with a lack of available information for the prestroke modified Rankin Scale (mRS),¹⁹ or without information on the mRS at 3 months, were excluded.

Imaging. All patients underwent brain imaging in the emergency room, either a noncontrast CT scan or a MRI scan. All patients underwent another CT or MRI scan 22–36 hours after treatment, or earlier in case of clinical worsening.

Treatment administration. IV rtPA was administered according to the recommendations of the European Stroke Organisation¹⁸ (i.e., 0.9 mg/kg body weight, maximum 90 mg, 10% of the dose as a bolus, followed by a 60-minute infusion).

Clinical assessment. Stroke severity was assessed by the NIH Stroke Scale (NIHSS).¹⁸ The outcome at 3 months was assessed by the mRS.¹⁹ In both centers, physicians followed video training for the NIHSS and the mRS assessment, and were recertified every second year. All survivors were either seen at 3 months at the outpatient clinic or evaluated by a telephone interview.

We recorded (1) demographic data (sex, age); (2) vascular risk factors as already defined in both centers^{15,19} and related treatments; (3) medical history (myocardial infarction, atrial fibrillation, ischemic stroke, ongoing oral anticoagulant therapy, ongoing antiplatelet therapy, prestroke mRS¹⁹); (4) baseline vital signs (systolic and diastolic blood pressure); (5) baseline laboratory values (serum glucose concentration, C-reactive protein, hematocrit, baseline international normalized ratio or prothrombin time or activated partial thromboplastin time, platelet count, leukocytes, neutrophils, and lymphocytes); (6) characteristics of the thrombolytic procedure (onset-to-treatment time, considered as being of 4.5 hours for wake-up strokes or the delay since the patient was seen normal for the last time when shorter than 4.5 hours, onset-to-sample time, use of bridging therapy); and (7) sICH according to the ECASS-II definition,²⁰ which has been shown to be the most related to the outcome among all definitions of sICH.²¹ The ECASS-II definition of sICH was any hemorrhage with neurologic deterioration, as indicated by an NIHSS score that was higher by 4 points or more than the value at baseline or the lowest value in the first 7 days, or any hemorrhage leading to death.²⁰

Biological assessment. All patients underwent a blood sampling for total leukocyte, neutrophil, and lymphocyte count at admission, before the bolus of rtPA.

Endpoints. The primary endpoint was the occurrence of sICH.²⁰ Secondary endpoints evaluated after 3 months were (1) excellent outcome, defined as mRS of 0 or 1 (or equal to the prestroke mRS); (2) good outcome, defined as mRS 0 to 2 (or equal to the prestroke mRS); (3) death. Because of the heterogeneity in the second imaging assessment (CT or MRI scan), asymptomatic cerebral hemorrhages were not recorded.

Management of missing data. When a mRS was not available at 3 months, but was at a later time point, the 3-month value was assigned as the worst one between the mRS at discharge and the most recent one.

Statistical analyses. We used the Statistical Package for the Social Sciences 22 for Windows to determine median values, interquartile

Table 1 Baseline characteristics of the study population and bivariate comparison with patients excluded

	Included (n = 846)	Excluded (n = 201)	p Value
Demographic characteristics			
Male sex	430 (50.8)	105 (52.2)	0.719
Age ^a	71 (60–80)	67 (56–78)	0.002 ^b
Medical history			
Arterial hypertension	519 (61.3)	114 (56.7)	0.227
Diabetes mellitus	129 (15.2)	40 (19.9)	0.107
Hypercholesterolemia	357 (42.2)	81 (40.3)	0.624
Previous myocardial infarction	91 (10.8)	26 (12.9)	0.378
History of atrial fibrillation	171 (20.2)	43 (21.4)	0.709
Previous ischemic stroke	101 (11.9)	26 (12.9)	0.697
Ongoing oral anticoagulant therapy	51 (6.0)	9 (4.5)	0.395
Ongoing antiplatelet therapy	316 (37.4)	75 (37.3)	0.992
mRS 0–1 before stroke	742 (87.8)	161 (89.9)	0.564
Stroke characteristics			
Baseline NIH Stroke Scale ^a	10 (6–16)	11 (7–16)	0.263
Clinical and biological characteristics			
Baseline systolic blood pressure, mm Hg ^a	153 (140–170)	151 (139–165)	0.173
Baseline diastolic blood pressure, mm Hg ^a	81 (74–90)	80 (75–90)	0.262
Blood glucose concentration, mg/dL ^a	119 (105–142)	120 (107–140)	0.445
INR >1.6 or PT <50% or aPTT >40 s	7 (0.8)	2 (1.0)	0.686
Platelet count ×1,000/mm ^{3a}	223 (185–261)	228 (190–280)	0.108
Hematocrit, %	41 (38–43)	41 (38–43)	0.515
C-reactive protein, mg/L	4 (3–8)	5 (4–17)	0.493
Characteristic of the procedure			
Onset-to-treatment time, min ^a	145 (104–195)	140 (109–179)	0.320
Bridging therapy	56 (6.6)	11 (5.5)	0.550
Outcome measures			
sICH	54 (6.4)	11 (5.5)	0.628
Excellent outcome (mRS 0–1)	363 (42.9)	68 (36.0)	0.081
Good outcome (mRS 0–2)	486 (57.4)	102 (54.0)	0.383
Death (mRS 6)	101 (11.9)	27 (14.3)	0.376

Abbreviations: aPTT = activated partial thromboplastin time; INR = international normalized ratio; mRS = modified Rankin Scale; PT = prothrombin time; sICH = symptomatic intracerebral hemorrhage.

Unless specified, values are number of patients (%).

^aMedian (interquartile range).

^bSignificant.

Figure 1 Correlation between the onset-to-sample time and neutrophils, leukocytes, and neutrophil to lymphocyte ratio (NLR)

ranges, and percentages. We compared groups for categorical variables with the χ^2 test with Yates correction or Fisher exact test, and for continuous variables with the Mann-Whitney U test. We evaluated correlations between continuous variables with the Spearman test. p Values lower than 0.05 were considered significant.

According to a predetermined statistical analysis approach, we did the following:

- Compared baseline characteristics between patients included and not included.
- Correlated neutrophils, leukocytes, and NLR with the onset-to-sample time to evaluate the influence of delay on these variables.
- Compared neutrophils, leukocytes, and NLR between patients with and without sICH, excellent outcome, good outcome, and death.
- Compared outcomes among 4 groups of patients according to quartiles of neutrophils, leukocytes, and NLR.
- Performed stepwise forward conditional logistic regression analyses²² with sICH (classified 1 when present and 0 when absent), excellent outcome, good outcome, and death as dependent variables; the independent variables included in the analysis were selected from a bivariate analysis, with a 0.25 level as a screening criterion for the selection of candidate variables to increase the detection of associations²³; the variables neutrophil count (model 1) or leukocyte count (model 2) or NLR (model 3), baseline NIHSS, onset-to-treatment time, and age were forced into the model; correlations between independent variables were checked for possible collinearity between variables (defined as $\rho > 0.6$); adjusted odds ratios ($_{adj}$ ORs) and 95% confidence intervals (CIs) were calculated from the logistic regression analyses.
- Used receiver operating characteristic curves to determine the predictive values of the area under the curve and 95% CI, for the neutrophil count, the leukocyte count, and the NLR; for the biological value that better predicts outcome, we also determined the cutoff points that better distinguish presence and absence of the 4 endpoints; we considered the point at which the sum of specificity and sensitivity was the highest; we considered an area under the curve value of 0.70 or higher as indicating an acceptable discrimination.²⁴
- Performed logistic regression analyses with endpoints for which the discrimination was acceptable as dependent variables, and the same independent variables as in the previous model, except for values of neutrophils, leukocytes, and NLR that were dichotomized at high (i.e., at the cutoff value or above) or low (i.e., below the cutoff) value.

Standard protocol approvals, registration, and patient consents.

The stroke database of Lille was submitted to the Institutional Data Protection Board and to the Human Subject Protection Committee of Lille University Hospital. The study was considered as observational by the internal review board (Comité de protection des personnes Nord Ouest IV, March 1, 2010; Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de santé, no. 10.677, December 14, 2010). The Helsinki Stroke Thrombolysis Registry has been approved by the relevant authority as an observational study (§40, Dnro 33/2012). The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Patients or their relatives gave informed consent for the inclusion of their data in a registry, in accordance with the French and Finnish laws concerning research in humans.

RESULTS General features. Out of 1,047 patients, we recruited 846 patients who met inclusion criteria for this study, after exclusion of 26 patients with stroke mimics, 144 patients whose blood samples were taken

Table 2 Unadjusted comparison of neutrophil count ($\times 1,000/\text{mm}^3$), leukocyte count ($\times 1,000/\text{mm}^3$), and neutrophil to lymphocyte ratio in each outcome category

	sICH	Excellent outcome			Good outcome			Death		
		Present	Absent	p Value	Present	Absent	p Value	Present	Absent	p Value
Neutrophil count	7.2 (5.2-9.2)	5.3 (4.0-7.1)	5.9 (4.5-8.0)	<0.001 ^a	5.0 (3.8-6.3)	6.1 (4.7-8.3)	<0.001 ^a	7.0 (4.7-9.3)	5.2 (4.0-7.0)	<0.001 ^a
Leukocyte count	8.0 (6.9-9.0)	7.8 (6.3-9.6)	8.0 (6.4-9.6)	0.755	7.6 (6.3-9.4)	8.0 (6.4-9.8)	0.056	8.4 (7.0-10.3)	7.7 (6.3-9.5)	0.015 ^a
NLR	6.1 (3.1-11.5)	3.0 (2.0-5.1)	4.0 (2.3-6.8)	<0.001 ^a	2.7 (1.9-4.1)	4.4 (2.4-7.4)	<0.001 ^a	6.1 (2.6-11.1)	3.0 (2.0-5.0)	<0.001 ^a

Abbreviations: NLR = neutrophil to lymphocyte ratio; sICH = symptomatic intracerebral hemorrhage. Values are median (interquartile range) and statistics were performed with the Mann-Whitney U test. Outcomes were evaluated at 3 months except for symptomatic intracerebral hemorrhage. See text for definitions.
^a Significant.

during thrombolysis, 34 patients with missing data (prestroke mRS in 22 patients and 3-month mRS in 12), or any combination of these exclusion criteria.

Main baseline characteristics and outcomes. The baseline characteristics and outcomes of the study population, and differences with patients excluded, are detailed in table 1. Among 846 patients, 54 (6.4%) presented sICH, 101 (11.9%) died, 363 (42.9%) presented an excellent outcome, and 486 (57.4%) presented a good outcome at 3 months.

Influence of the onset-to-sample time on the neutrophils, leukocytes, and NLR. Increasing onset-to-sample time was correlated with increasing neutrophil count ($\rho = 0.128$; $p < 0.001$), increasing leukocyte count ($\rho = 0.117$; $p = 0.001$), and increasing NLR ($\rho = 0.182$; $p < 0.001$) (figure 1).

Unadjusted association of neutrophils, leukocytes, and NLR with the outcome measures. The unadjusted comparisons of neutrophil count, leukocyte count, and NLR according to the outcome measures are detailed in table 2. The neutrophil count was higher in patients who developed sICH ($p < 0.001$), or who died within 3 months ($p < 0.001$), and lower in patients with mRS 0–1 at 3 months ($p < 0.001$) and mRS 0–2 at 3 months ($p < 0.001$). The leukocyte count was higher in patients who died at 3 months ($p = 0.015$) and tended to be lower in patients with mRS 0–1 ($p = 0.086$) and mRS 0–2 ($p = 0.056$) at 3 months. The NLR was higher in patients who developed sICH ($p < 0.001$) or died within 3 months ($p < 0.001$) and lower in patients with excellent ($p < 0.001$) or good ($p < 0.001$) outcomes at 3 months.

The unadjusted comparison of the 4 groups of patients divided by quartiles according to neutrophil count or NLR showed more sICH and deaths and less excellent and good outcomes in higher quartiles for neutrophils and NLR. Higher leukocyte counts were associated with death but not with the other outcome measures (figure 2).

Adjusted association of neutrophil count, leukocyte count, and NLR with outcomes. After adjustment, patients who developed sICH or who died had higher neutrophil count and NLR, and patients with excellent or good outcomes had lower neutrophil count and NLR. The leukocyte count was not independently associated with any of the 4 outcome measures. The results of the logistic regression analyses are detailed in table e-1 on the *Neurology*[®] Web site at Neurology.org.

Predictive values of neutrophil count, leukocyte count, and NLR for the 4 endpoints. The receiver operating characteristic curves and the area under the curve values for neutrophil count, leukocyte count, and NLR with respect to primary and secondary endpoints are

Figure 2 Comparison of outcomes between patients divided per quartiles for neutrophil count, leukocyte count, and neutrophil to lymphocyte ratio (NLR)

Comparison of outcomes between patients from the lowest (Q1) to the highest (Q4) quartiles for neutrophil count, leukocyte count, and NLR. See text for definitions. sICH = symptomatic intracerebral hemorrhage.

shown in figure 3. The best predictive values for the 4 outcome measures were found with the NLR. The only value for an acceptable discrimination was found with NLR for the prediction of sICH, a value of 4.80 or more being a predictor of sICH (sensitivity 66.7%; specificity 71.3%; likelihood ratio 2.32). The same analyses performed separately in patients treated within 3 hours and beyond found similar results for NLR (area under the curve within 3 hours = 0.72, 95% CI 0.59–0.85; area under the curve beyond 3 hours = 0.73, 95% CI 0.65–0.82). However, 93 of 250 patients (37.2%) with $\text{NLR} \geq 4.80$ were treated beyond 3 hours, while 167 of 554 patients (30.1%) with $\text{NLR} < 4.80$ were treated within 3 hours ($p = 0.048$).

Predictive value of NLR (dichotomized) for the occurrence of sICH. The logistic regression analysis with sICH as dependent variable, and a dichotomized value of NLR, found that a NLR of 4.80 or more (adjOR 3.71; 95% CI 1.97–6.98, $p < 0.001$), male

sex (adjOR 2.67; 95% CI 1.39–5.10, $p = 0.003$), baseline NIHSS score (adjOR for 1-point increase 1.06; 95% CI 1.01–1.11, $p = 0.019$), and age (adjOR for 1-year increase 1.04; 95% CI 1.02–1.07, $p = 0.002$) were independently associated with a higher risk of sICH. In patients with a $\text{NLR} \geq 4.8$, the incidence of sICH was 13.6% (34/250), while in patients with $\text{NLR} < 4.8$, it was 3.1% (17/553) ($p < 0.001$).

DISCUSSION Our study has shown that (1) baseline neutrophil count and NLR were independently associated with increased risks of sICH and death, and decreased probability of excellent or good outcome; (2) the best discriminating variable for the occurrence of sICH was a NLR value of 4.80 or more, associated with 3.7-fold increased risk for sICH in patients with ischemic stroke treated by IV rtPA; (3) the leukocyte count was not independently associated with any of the 4 outcome measures.

Figure 3 Predictive values of neutrophil count, leukocyte count, and neutrophil to lymphocyte ratio (NLR) for the 4 endpoints

Receiver operating characteristic curves for the 4 endpoints. (A) Area under the curve for symptomatic intracerebral hemorrhage (sICH) 0.68, confidence interval (CI) 0.60–0.76 for neutrophils; 0.51, CI 0.44–0.58 for leukocytes; and 0.73, CI 0.66–0.80 for NLR. (B) Area under the curve for death 0.66, CI 0.59–0.72 for neutrophils; 0.56, CI 0.49–0.62 for leukocytes; and 0.68, CI 0.62–0.75 for NLR. (C) Area under the curve for excellent outcomes 0.63, CI 0.59–0.66 for neutrophils; 0.52, CI 0.48–0.56 for leukocytes; and 0.66, CI 0.62–0.70 for NLR. (D) Area under the curve for good outcomes 0.64, CI 0.60–0.68 for neutrophils; 0.53, CI 0.49–0.57 for leukocytes; and 0.67, CI 0.63–0.71 for NLR.

The strengths of our study are that data were prospectively collected in 2 different countries, in homogeneous groups of patients treated by IV rtPA in routine practice. Blood samples were drawn strictly before the bolus of rtPA, meaning that the study was conducted in the ideal conditions to evaluate the influence of neutrophils at baseline on the outcome. In previous studies, blood samples were collected later, sometimes several days after stroke onset. Another strength is that the overall profile of patients included in the analysis is representative of patients treated with rtPA for ischemic stroke according to demographic characteristics, baseline findings, medical history, and vascular risk factors.²⁵

Besides the usual limitations of all studies based on registries (study not planned before data collection, retrospective analysis with possible missing data, small differences between centers), the most important limitation in our study is the lack of information about preexisting infections and infections and fever occurring during the first days, which have been shown to be associated with worse outcomes in patients treated or not with rtPA.¹⁹ However, even in subgroups of patients with worse outcomes, the absolute values for neutrophil and leukocyte counts remained within a normal range for most patients and the observed differences were between normal values. Another limitation is that neutrophil counts could

also have been influenced by the severity of stroke, or other intercurrent complications. Although the main results remained similar between patients recruited within 3 hours and beyond, we cannot exclude that neutrophil count rose quickly after stroke onset, as suggested by the correlation between the onset-to-sample time and neutrophils, leukocytes, and NLR, and the highest proportion of patients with $\text{NLR} \geq 4.80$ in those treated beyond 3 hours.

Our study suggests that higher neutrophil counts are associated with worse outcomes, regardless of the reason for neutrophil increase. Although our study was not designed to understand the underlying mechanism, if a causal relationship exists, this effect could be mediated by the release of matrix metalloproteinase-9 (MMP-9), as suggested in animal²⁶ and human^{27,28} studies. A study on the prethrombolysis and 24-hour post-thrombolysis variations of each MMP and tissue inhibitors of metalloproteinase levels measured in ischemic stroke patients treated by IV rtPA showed that, after adjusting for major clinical determinants, only MMP-9 variation proved independently associated with death or sICH.²⁹ At the acute phase of experimental stroke, the administration of granulocyte colony-stimulating factor associated with rtPA increases the risk of hemorrhage.³⁰ This finding could be explained by an early proliferation of neutrophils due to granulocyte colony-stimulating factor, or a release of MMP-9 by neutrophils under the effect of rtPA.³⁰

The best predictor of sICH was the NLR, a value ≥ 4.8 indicating a 3.7-fold increased risk for sICH. This is the first time this has been shown in patients treated by IV rtPA for ischemic stroke. In previous studies^{10–13} in which patients did not receive rtPA or where this information was missing, the NLR was associated with death. In 2 of them a threshold of 5.67¹⁰ and 4.1¹² indicated an increased risk of death. Another study in patients treated by endovascular therapy found that a $\text{NLR} \geq 5.9$ before intervention might predict a poor outcome, defined as mRS of 4–6 at 3 months (attempting not to eliminate patients who could benefit from endovascular therapy) and death, while a $\text{NLR} \leq 3.2$ predicted functional independence.¹³ These studies did not specifically evaluate sICH and the cause of death was not reported. The NLR represents the balance between the neutrophils, i.e., the active inflammatory component, and the lymphocytes, i.e., the regulatory and protective component.³¹ Therefore, higher NLR represents higher levels of inflammation.

Our results need to be replicated in an independent cohort of patients. Even if they are confirmed, we cannot recommend the use of the NLR in routine practice for the selection of patients to be treated with rtPA, because it will increase delays in all patients, for a not yet proven benefit in few of them. It might be of

interest in a few difficult cases only, as part of risk-benefit judgment and probably as part of recently developed scores.^{32–36}

The next step is to determine whether there is a causal association between neutrophils and outcome, and what mediates this effect. Studies of biomarkers (e.g., MMP-9, chemotaxis, inflammation, tissue necrosis) collected before thrombolysis at a large scale, and the kinetics of these markers, are needed.^{37,38} Another important direction for research is to determine the underlying molecular mechanism. Although it has not been proven in human studies that a reduction in the level of neutrophils improves outcome, animal studies have shown that a pharmacologic neutropenia induced by vinblastine and by RP3-anti-rat neutrophils monoclonal antibodies is associated in rats with prevention of endothelial dysfunction after experimental cerebral ischemia.³⁹

Higher neutrophil counts and NLR are independently associated with sICH and worse outcome at 3 months. Identification of mediators of this effect could help identify new targets for neuroprotection in patients treated by rtPA.

AUTHOR CONTRIBUTIONS

Dr. Maestrini collected data, analyzed and interpreted data, performed the literature search, conceptualized the study, and drafted the manuscript. Dr. Strbian collected and interpreted data, conceptualized the study, and revised the manuscript. Dr. Gautier conceptualized the study and revised the manuscript. Dr. Haapaniemi collected data and revised the manuscript. Dr. Moulin collected data and revised the manuscript. Dr. Sairanen collected data and revised the manuscript. Dr. Dequatre-Ponchelle collected data and revised the manuscript. Dr. Sibolt collected data and revised the manuscript. Dr. Cordonnier interpreted data, conceptualized the study and revised the manuscript. Dr. Melkas collected data and revised the manuscript. Dr. Leys collected data, designed the study, analyzed and interpreted all data, drafted the manuscript, and conceptualized the study. Dr. Tatlisumak interpreted data, conceptualized the study, and revised the manuscript. Dr. Bordet designed the study, conceptualized the study, and revised the manuscript.

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DISCLOSURE

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