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Joana Isabel Afonso Neto

A Resposta Inflamatória Sistémica está ligada à severidade do Edema Cerebral e à Deterioração Neurológica após Recanalização no AVC isquémico

Systemic Inflammatory Response is Linked to the Severity of Cerebral Edema and Neurological Deterioration After Recanalization in Acute Ischemic Stroke

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Systemic Inflammatory Response is Linked to the Severity of Cerebral Edema and Neurological Deterioration After Recanalization in Acute Ischemic Stroke

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Faculdade de Medicina da Universidade do Porto, 18/02/2020

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Title: Systemic Inflammatory Response is Linked to the Severity of Cerebral Edema and Neurological Deterioration After Recanalization in Acute Ischemic Stroke

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Abstract

Background and Purpose: The mechanisms by which systemic inflammation worsens clinical outcome in ischemic stroke have not been still well explored. We hypothesized that the peripheral inflammatory response augments cerebral edema (CED) and it ultimately impends neurological recovery.

Methods: We analyzed consecutive patients with ischemic stroke of the anterior circulation submitted to intravenous thrombolysis (IVT) or endovascular treatment (EVT) during 2017 and 2018. We determined neutrophil-(NLR) and platelet-to-lymphocyte (PLR) ratios and the presence of Systemic Inflammatory Response Syndrome (SIRS). CED degrees were classified using the European Cooperative Acute Stroke Study (ECASS)-2 definition on CT scan at 24 hours. The clinical outcomes included early neurological deterioration (END) and functional dependence at 90 days. Ordinal and logistic regressions were used to predict the outcomes. Area Under the Curve (AUC) of Receiver Operating Characteristic curves were used to find the best cut-off values on continuous variables to predict outcomes.

Results: We included 376 patients; 67% received IVT and 61% EVT. Increasing values of NLR after recanalization were associated with higher degree of CED at 24 hours (adjusted odds ratio (aOR)=1.47, 95% Confidence Interval (CI)=1.18 – 1.82, $p<0.01$). Moreover, NLR was also significantly associated with END (aOR=1.61, CI=1.09 – 2.38, $p<0.05$) and poor functional status at 90 days (aOR=1.60, CI=1.24 – 2.07, $p<0.01$). PLR showed similar association but NLR >6.2 was the most accurate predictor of CED and clinical outcome (AUC ~0.7, $p<0.01$), even after adjustment to baseline severity and excluding cases of significant hemorrhagic transformation. SIRS was associated to CED severity but not clinical outcome.

Conclusions: Peripheral inflammation early after recanalization is associated with increasing severity of CED, neurological deterioration and ultimately, poor functional outcome. Easily

assessable pro-inflammatory indexes could be useful for patient stratification for future immunomodulation therapies.

Main Text

Introduction

Intravenous thrombolysis (IVT) and endovascular thrombectomy (EVT) are rapid recanalization therapies that revolutionized the treatment of acute ischemic stroke (AIS). However, the overall efficacy of IVT is limited (only 30% have better outcome at 3 months)[1] and about half of the patients submitted to EVT do not regain optimal functional status.[2] Possible causes for the lack of clinical benefit despite a successful restoration of cerebral blood flow are the dysfunction of blood-brain-barrier (BBB) and subsequent development of cerebral edema (CED), hemorrhagic transformation (HT), and infarct growth.[3, 4]

On this account, inflammation has been recognized as an important part of the pathophysiology of stroke.[5, 6] The neuronal cell death results in the release of humoral factors that elicit localized inflammation in the injured brain.[6] These factors trigger intracellular signaling receptors on both microglia and astrocytes that recruit peripheral immune cells, as neutrophils, which have been shown to contribute to BBB disruption.[2, 4]

Breakdown of the BBB occurs early after stroke and potentiates the infiltration of more peripheral leukocytes, which contributes to further injure of the brain tissue, by releasing proinflammatory cytokines, matrix metalloproteinases and reactive oxygen species as a result of this increased inflammatory status.[4, 6, 7]

The Systemic Inflammatory Response Syndrome (SIRS) has been the classical approach to define the presence of this increased inflammatory status. However, nowadays there are more

recognizable and easily assessed biomarkers that can stratify patients accordingly to the intensity of the peripheral inflammatory activity.[8] Specifically, Neutrophil-to-lymphocyte ratio (NLR) and Platelet-to-lymphocyte ratio (PLR) have been shown to be related to the severity of the disease and poor prognosis[5,6,8] and are very convenient to obtain from peripheral blood samples.

Despite the well documented effects of peripheral inflammation on cerebral infarction in animal models, the exact mechanism is still undetermined. One recent study suggested that this might be due to the increased risk of symptomatic hemorrhagic transformation (SICH)[2] which occurs in approximately 5% of the patients submitted to recanalization therapies.[9]

In this study, we analyze the association between systemic inflammatory markers, namely NLR, PLR and SIRS with de development of CED, HT and functional prognosis at 90 days after stroke, in patients treated with IVT or EVT.

Methods

Study Population

This retrospective study included all AIS patients that were ≥18 years of age, admitted to our Stroke Unit, and who received IVT or EVT between January 1, 2017 and December 31, 2018. We excluded vertebrobasilar strokes; patients on antibiotic or with infection 3 days before, and within 48 hours after admission (to reduce possible confounders for systemic inflammation); with major traumatic or surgery events 4 weeks before the hospital admission; with chronic inflammatory disease or under corticosteroids. This study was approved by the local Ethics Committee.

Data collection and clinical variables

The electronic medical records were reviewed. Demographic data and medical history, including vascular risk factors, medications, and previous cardiovascular disease were recorded. Blood pressure (BP) and serum glucose were recorded at admission. National Institutes of Health Stroke Scale (NIHSS) scores were obtained at baseline and after 24 hours from recanalization. Stroke type was classified by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) scale. We registered any infection diagnosed from admission until discharge from the stroke unit.

Inflammatory measures

Per standard protocol, every patient collects blood (on which are evaluated the total leukocytes, neutrophils, lymphocytes and platelet counts) after recanalization treatment, at day 1, at the Stroke Unit (within maximum of 16 hours after recanalization therapy). From these data we calculated the NLR and PLR by dividing the absolute number of neutrophils and platelets by the absolute number of lymphocytes, respectively.[10] We also determined if a patient fulfilled the criteria for SIRS within 24 hours after recanalization treatment, by having 2 out of 4 of the following items: temperature over 38º Celsius, heart rate over 90 beats/minute, respiratory rate over 20 cycles/minute, and leukocytosis, leukopenia or bandemia (leukocytes $>12.000/mm^3$, $<4.000/mm^3$ or bandemia $\geq 10\%$).[11] Per protocol in our unit, vital signs are recorded hourly in the electronic clinical registry.

Radiological measurements and endpoints

An experienced neurologist of the stroke team (PC) and others (MM, DF and JN), after tutoring and training, evaluated Computed Tomography (CT) scans in the electronic database performed at admission and repeated it after 24 hours. The admission CT was analyzed to calculate the Alberta Stroke Program Early Computed Tomography Score (ASPECTS)[12] that ranges from 0 (ischemic changes in all the middle cerebral artery territory) to 10 (no early ischemic changes). Location of the occlusion was determined from the admission CT angiography report. Recanalization of the affected vessel after thrombectomy was assessed using the Thrombolysis In Cerebral Infarction (TICI) scale[13] from procedure reports and imaging. The 24-hour CT scan was performed in a single machine (Siemens Somaton Emotion Duo, Erlangen, Germany) to determine infarct volume, CED and HT. Infarct volume was estimated by A×B×C/2 method.[14] CED and HT were classified using the European Cooperative Acute Stroke Study (ECASS)-2 definition.[15] The CED ranges from 0 (no edema) to 3 (edema with midline shift).[16] CED was the main radiological endpoint of this study. SICH was defined by any ECASS-2 class of intracerebral hemorrhage and worsening by ≥ 4 points on the NIHSS.[15]

Clinical endpoints

We assessed the functional outcome by dichotomization of the modified Rankin scale (mRS): 3-6 (dependent or dead) vs 0-2 (independent). An additional endpoint was used to evaluate the initial response to recanalization therapy: early neurological deterioration (END), defined as any increase in NIHSS at 24 hours from the baseline.

Statistical analysis

Normality of continuous variables was inferred by the Kolmorogov-Smirnov test. The baseline characteristics and inflammatory parameters of patients in outcome subgroups were compared in a univariate model using Chi-square/Fisher exact tests for categorical variables and Kruskal-Wallis/Mann-Whitney tests for continuous variables, as applicable. Bonferroni corrections were used to correct for multiple comparisons. The baseline variables independently associated with each outcome were selected by backward regression models applied to all of those associated with a $p<0.10$ in univariate analysis. These were later used for multivariate adjustment. The association between inflammatory parameters and CED was evaluated with ordinal shift analysis after verification of the assumption of common proportional odds across all CED degrees. The effect of the same inflammatory measures on clinical outcome was assessed by logistic regression generating the odds ratios (OR) and 95% confidence intervals (CI) for functional dependence (mRS 3 to 6 versus 0 to 2) at 90 days and END. Logistic regression models were also used to predict SICH. We also used the Akaike information criterion, a log-likelihood based test, to estimate the relative quality of the different statistical models to predict outcomes. We also determined the areas under the curve (AUC) of Receiver Operating Characteristic curves (ROC) to find relevant cut-off values on continuous variables to predict the outcomes. We used MedCalc® to compare different AUC. Graphs were designed in GraphPad Prim 8.0®.

Statistical significance was set at $p<0.05$. All statistical analyses were performed with IBM SPSS Statistics for Windows, version 25.

Results

We included 376 patients. The population's characteristics are shown in Table 1. Median (interquartile range) NIHSS at admission was 14 (7 – 19) and ASPECTS of 9 (7 – 10). Cardioembolic was the main etiology accounting for 48% of cases. Regarding recanalization therapies, 67% of patients were submitted to IVT and 61% received EVT. After recanalization, median NLR was 6.04 (3.01 – 10.03) and PLR was 175 (109 – 274). A total of 38 (10%) patients fulfilled criteria for SIRS within 24 hours after recanalization.

Inflammatory markers and cerebral edema

In our population, 180 patients (48%) had no detectable edema in CT scan performed in the first 24 hours, while 105 patients were classified as having edema grade CED 1 (28%), 62 as CED 2 (16%), and 27 as CED 3 (7%).

Baseline predictors of CED severity were occlusion site, baseline NIHSS, ASPECTS and glycemia at admission (Supplemental Table 1). Regarding the inflammatory parameters, both NLR and PLR were higher in patients with severe edema in a multivariate model adjusted to other predictors of CED (adjusted OR (aOR)=1.47, CI=1.18 – 1.82, p<0.01, and aOR=1.24, CI=1.01 – 1.53, p=0.04, respectively; Table 2). There was an association between the presence of SIRS and CED severity (aOR=2.35, CI=1.23 – 4.49, p=0.01) but this seems to be mainly due to altered white blood cell counts. Figure 1A depicts a violin plots to visualize the distribution of the NLR parameter and its probability density across each CED degree. NLR shows a step increase along the various degrees of CED severity. Figures 1B – F show the same violin plots separately for subgroups with and without endovascular treatment (B), infection (C) and subgroups of high and low onset-to-door time (D), NIHSS (E), and infarct volume (F).

Ordinal regression analysis shows no interaction between these factors on the relationship of NRL and CED (p>0.05).

Inflammatory markers and hemorrhagic transformation

In our population, 94 patients had hemorrhagic transformation. SICH occurred in 18 (5%) patients. NLR and PLR were good predictors of SICH (aOR=2.34, CI=1.37 – 4.04 and aOR=2.02, CI=1.20 – 3.41, respectively, p<0.05, Supplemental table 4). SIRS was not related to SICH. SICH was related to with higher severity of CED (Supplemental Figure 2).

Inflammatory markers and clinical outcome

END affected 93 (25%) patients and functional dependence at 90 days (mRS 3 – 6) was present in 203 (54%) patients. After backward logistic regression analysis the baseline characteristics that were independently associated with the END were age (median 79, p=0.02), glycemia (median 140mg/dL in patients with END versus 152mg/dL in patients with no END, p=0.02) and ASPECTS (p=0.02) at admission. Functional dependence at 90 days was associated with gender (female, p<0.01), age (median age 78, p<0.01), pre-morbid Rankin scale (>2 in 23% of dependent patients versus 5% of independent patients, p<0.01) and baseline NIHSS (>17, p<0.01) (Supplemental Table 3).

Both NLR and PLR in the first 24 hours of stroke were predictors of END (aOR=1.61, CI=1.09 – 2.38 and aOR=1.18, CI=0.92 – 1.53, respectively, p<0.05, Table 3) and poor functional outcome at 90 days (aOR=1.60, CI=1.24 – 2.07 and aOR=1.42, CI=1.10 – 1.82, respectively, p<0.05, Table 3). However, SIRS was not associated with clinical outcome after adjustment to baseline characteristics.

The more severe the CED degree was at 24 hours the higher the odds of a poor functional outcome at 90 days (p<0.01). This shift towards worse categories of mRS is depicted in Supplemental figure 1.

Final multivariate models

The prognostic accuracy and best cut-off values for the white blood cell derivate parameters was studied by ROC analysis whose results are shown in Figure 2. In general, the AUC were lower in the models that predict END (AUC ~0.6) than those that predict CED or functional outcome at 90 days (AUC ~0.7). NLR and absolute neutrophil counts were equally accurate to predict CED at 24 hours, but NLR was more accurate to predict poor functional outcome (AUC ~0.7, p<0.01). PLR showed the lowest performance among all markers.

The best cut-off values for NLR were 4.8, 5.1 and 8.5 for prediction of CED, END and functional outcome. We then dichotomized all cohort into groups of high (>6.2) and low (≤6.2) NLR based on the average value of those cut-offs. An NLR >6.2 was an independent predictor of CED (OR=2.56, CI=1.67 – 3.92), END (OR=2.44, CI=1.50 – 3.98) and poor functional outcome (OR=3.00, CI=1.96 – 4.59) even after adjustment to baseline severity, and exclusion of SICH, revascularization status, and infection (OR=1.97, CI=1.16 – 3.32; OR=1.90, CI=1.12 – 3.22; OR=2.37, CI=1.46 – 3.86, respectively) (Table 4).

Discussion and Conclusions

We found that higher neutrophil count, NLR and PLR were associated with END and poor neurological outcome. We advance knowledge by showing that these associations can explain the fact that higher systemic inflammation, reflected on higher PLR and NLR, after

recanalization is linked to the increasing severity of early development of CED at 24 hours. We also show that NLR stands out from other blood cell count derived indexes and from the classical definition of systemic inflammation as the most accurate and independent predictor of radiological and clinical outcome.

NLR and the development of CED

As showed by recent studies, disruption of the BBB takes place early after stroke and remains permeable, especially in the acute phase, possibly due to the inflammatory cascade.[4,6] This facilitates the infiltration of peripheral immune cells, such as neutrophils, to the injured brain. Neutrophils are then responsible for further exacerbating brain lesion, due to oxidative stress and BBB damage.[4] Pathologic CED and HT are two of the main complications of stroke and both result from increased BBB permeability.[6] It is also known that CED occurs between 24 to 48 hours after stroke. Song et al.,[6] conducted a meta-analysis that evaluated the significance of NLR in ischemic and hemorrhagic stroke. Higher NLR was associated with unfavorable outcome at 3 months and increased mortality. However, the precise mechanisms that intermediate the poor outcome were not elucidated. Our study further advances by showing that, besides SICH, one of the main culprits could be CED. NLR predicted CED at 24 hours, even after exclusion of SICH, being both related to poor outcome. Moreover, this association was maintained even after adjustment to baseline severity and the other relevant predictors of CED, namely glycemia on admission, ASPECTS and NIHSS scores, and occlusion site.[7] Particularly, NLR maintained significant and proportional associations with CED degree irrespectively of subgroups of high and low NIHSS, infarct volume, late versus early presentation to hospital, or whether the patient received endovascular treatment or not. More interestingly, patients who further developed infection 24 hours after admission did not alter their NLR *versus* CED relationship. Although the analysis is limited to the retrospective nature

of this study, it suggests that systemic inflammation is not just a mere reflection of stroke severity but has an important role in the pathophysiology of stroke, its complications and patients' outcome.

Small studies[17-19] have shown that pretreatment NLR has been associated with SICH and functional outcome at 90 days. However, in the recent paper by Semerano et al.,[2] no significant association was noted between SICH and admission leukocyte counts or NLR. In fact, NLR showed strongest association with SICH at day one. In line with these results, we collected posttreatment inflammatory parameters as these would probably correlate better with the outcomes we intended to measure.

NLR and Early Neurologic Deterioration

As discriminated in Table 4, NLR was an independently correlated with END, in patients who underwent successful revascularization therapy. However, this association lost its statistical significance probably due to the development of infection and SICH. This leads us to the fact that the variation of the neurological deficit, measured by NIHSS score, might not be the sole determinant between neutrophil activation, CED and long-term functional outcome. NIHSS score is mostly a representation of the neurological deficits cause by a focal brain lesion. There is increasing evidence that inflammation after stroke occurs and persists throughout the entire brain, promoting decline of global brain functions contributing to the patients' long-term neurological outcome.[4] We cannot exclude that an increased NLR might be an early phase marker of infection. In fact, there are studies indicating that NLR is a measure of systemic bacterial inflammation and has been used as a guide to prognosis in various circumstances.[2] However, the prediction of poor functional outcome at 90 days based on NLR was still significant in the subgroup that did not develop infection until discharge.

PLR and SIRS versus NLR and outcome

This study directly compared the performance of PLR and SIRS with NLR which, to our knowledge, has not been done before.

The role of PLR in acute brain infarct was first studied in patients submitted to endovascular treatment, being related to a poor prognosis, rate of insufficient recanalization, size of infarcted area and burden of high-risk, more inflamed, plaques.[20] It has been shown that PLR is an inflammatory marker associated with prognosis in diverse arterial conditions.[21] This intimate relationship with the atherosclerotic etiology might explain the less robust, though significant, association between PLR and CED, when compared to NLR, that we found in our work, since this was one of the less represented etiologies (Table 1).

SIRS was related to CED but not to clinical outcomes. In fact, the former association is mainly due to abnormal leukocyte counts. There are several reasons for the underperformance of SIRS when compared to simple NLR and PLR. Many patients receive paracetamol as standard of care after thrombectomy which could minimize temperature increases. Also, some sedation is given during the procedure which, in addition to the central nervous system lesion, could alter the respiratory and heart rate patterns and diminish the influences of systemic inflammation.

Future Studies

This opens a new possibility to a novel therapeutic target: immunomodulation. In fact, there are some small trials with positive outcomes, such as the evaluation of natalizumab, fingolimod, and glyburide in AIS.[22-26] Additionally, new molecules responsible for the initiation and development of inflammation are being studied and tested as therapeutic targets.[4] However, it is still unknown which patients benefit the most from this therapy. We found

evidence that these cheap and easily assessable inflammatory markers, particularly NLR, could be useful tools to identify those patients. Larger and multicentric cohorts should confirm our results and provide refined cutoff values.

Limitations

On the other hand, this study has limitations that we must highlight. Firstly, we did a multivariate analysis to adjust for recorded baseline differences but there is still a potential for residual confounding due to factors not recorded among the baseline variables. Even so, we identified patients who were diagnosed with infections during inpatient care (potential confounder) and adjusted those on our multivariate analysis. We found that NLR and PLR were higher on those patients, but it did not change our results.

Secondly, we assessed the CED on the first 24 hours after admission and, knowing that it may develop later in the course, there might have been some cases of late onset CED that we missed. On the other hand, 24 hours is a long period of time for the edema to emerge and, in patients with both CED and hemorrhage, there is no certainty about the time relation between one another, as a cause or a consequence.

Thirdly, CED and HT were assessed using the CT-scan based scale, which is less sensitive for distinguishing early stages of edema from infarction than, for example, Magnetic Resonance Imaging (MRI), nevertheless, is the only validated and accessible method approved so far. Recently, Vorasayan et al.,[27] evaluated CED by quantifying the lesional net water uptake and, as this is a more sensitive tool, it would be interesting to use this method in future analysis. Also, there were four investigators evaluating the CT-scans which introduced interobserver variability on CED and HT classification. To minimize the classification bias, all four

investigators were trained and analyzed a pool of ten CT-scans obtaining maximum correlation on the classification scale.

Conclusions

Our study shows that systemic inflammation, measured by NLR and PLR, early after recanalization is associated, regardless of adjusted variables, with increasing severity of early CED, a mechanism by which stroke patients have END and ultimately, poor functional outcome. No specific treatments are currently approved to prevent or treat CED due to AIS, but the new evidence of this study highlights that immunomodulation might be a promising therapeutic strategy in selected patients based on easily assessable inflammatory indexes.

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Figure legends

Figure 1. Variations in mean normal score of NLR, in the different CED grade group (A), in subgroups with different endovascular treatment (B), with or without infection (C), with high (>140 minutes) or low (<=140 minutes) time of admission (D), with high (>10) or low (<=10) NIHSS (E) and with high (>=9 mL) or low (<9 mL) infarct volume (F).
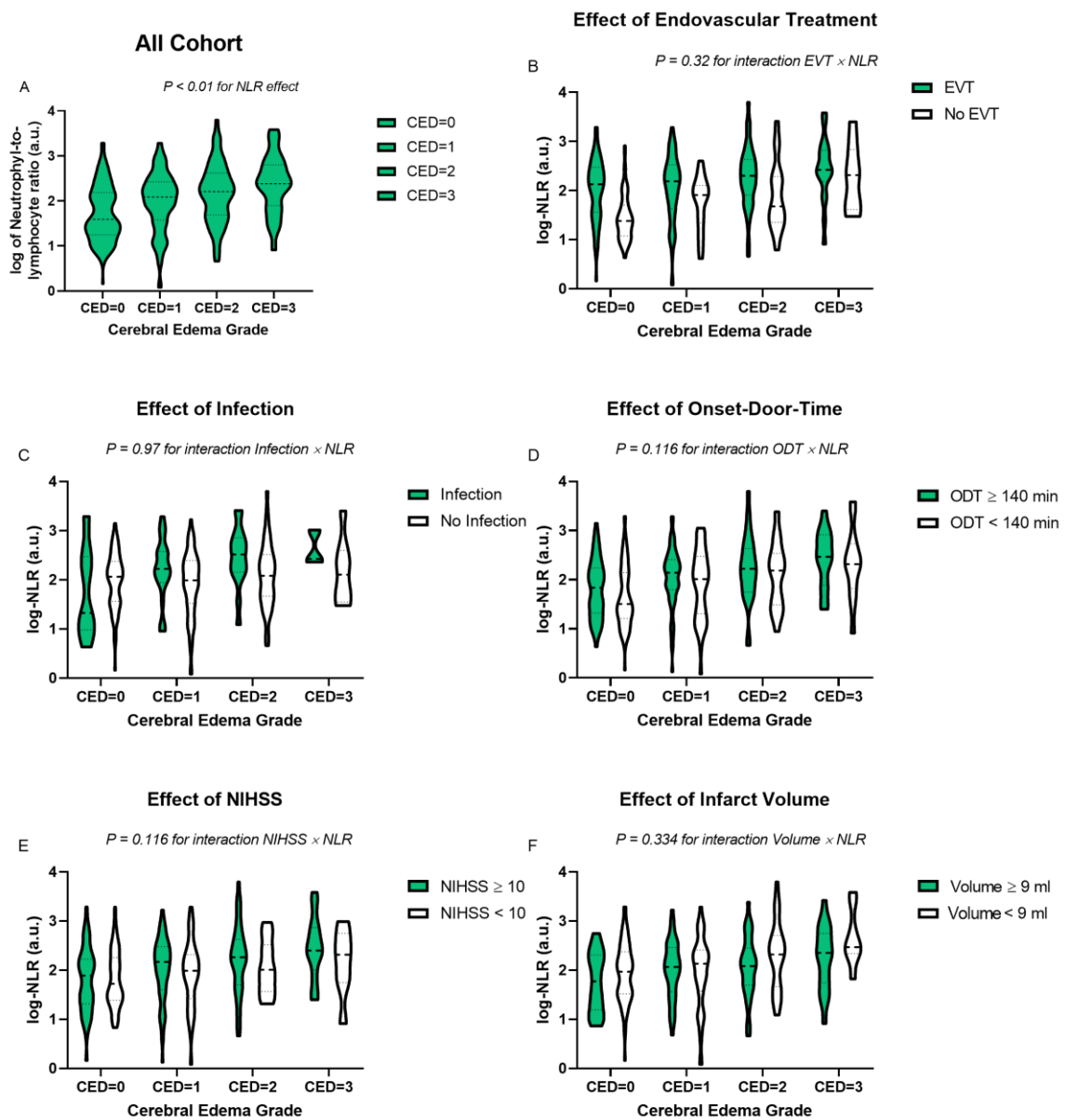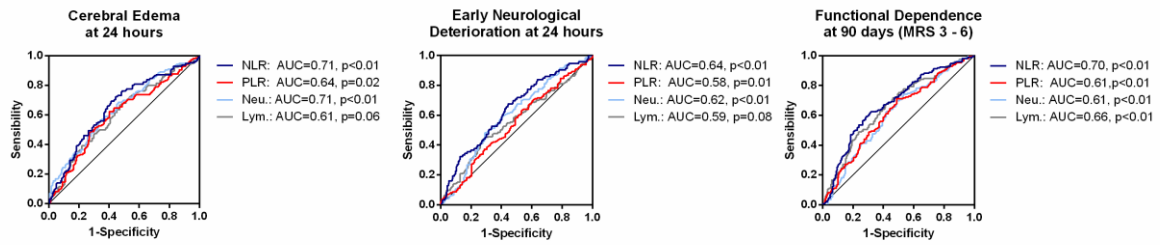
Figure 2. ROC curves generated for severe cerebral edema (CED=3) at 24-hour CT scan, END and functional dependence at 90 days (modified Rankin Scale 3 – 6) for the inflammatory markers NLR and PLR, absolute neutrophil and lymphocyte counts.

Tables

Table 1. Characteristics of the study cohort population.

Baseline characteristics	
Male, *n (%)*	181 (48)
Age, *median (IQR)*	75 (66 – 83)
Hypertension, *n (%)*	261 (69)
Diabetes mellitus, *n (%)*	116 (31)
Dyslipidemia, *n (%)*	217 (58)
Tobacco, *n (%)*	58 (15)
Atrial Fibrillation, *n (%)*	145 (39)
Prev. Stroke/TIA, *n (%)*	70 (21)
Prev. Myocardial Infarction, *n (%)*	47 (13)
Prev. Heart Failure, *n (%)*	37 (10)
Statin, *n (%)*	168 (50)
Antiplatelet, *n (%)*	112 (34)
Anticoagulant, *n (%)*	42 (13)
Stroke characteristics	
Baseline NIHSS, *median (IQR)*	14 (7 – 19)
Glycemia, *median (IQR)*	127 (110 – 162)
ASPECTS, *median (IQR)*	9 (7 – 10)

Stroke etiology	
Cardioembolic, *n (%)*	181 (48)
Large Artery Atherosclerosis, *n (%)*	47 (13)
Small vessel disease, *n (%)*	9 (2)
Undetermined, *n (%)*	124 (33)
Other (Carotid Dissection), *n (%)*	15 (4)
Occlusion site	
ICA, *n (%)*	47 (13)
M1, *n (%)*	180 (48)
M2, *n (%)*	74 (20)
Other, *n (%)*	75 (20)
IVT, *n (%)*	251 (67)
EVT, *n (%)*	230 (61)
Inflammatory indexes	
SIRS, *n (%)*	38 (10)
Temperature <36 or >38ºC, *n (%)*	32 (10)
Heart Rate >90 bpm, *n (%)*	89 (24)
Respiratory Frequency >20 cpm, *n (%)*	1 (0.3)
Leukopenia, Leukocytosis or bandemia, *n (%)*	87 (23)
Blood Cell Counts	

Neutrophils, *median (IQR)*	6.52 (4.60 – 8.65)
Lymphocytes, *median (IQR)*	1.15 (0.75 – 1.68)
Platelets, *median (IQR)*	201 (166 – 240)
NLR, *median (IQR)*	6.04 (3.01 – 10.03)
PLR, *median (IQR)*	175 (109 – 274)
Post-intervention characteristics	
24 hours NHISS, *median (IQR)*	7 (3 – 16)
Infarct volume, *median (IQR)*	8.19 (0.52 – 37.81)
Infection >24 hours until discharge, *n (%)*	59 (16)

Age is represented in years, glycemia (recorded at admission) in mg/dL, neutrophils, lymphocytes and platelets values in $x10^9$ cells/L and infarct volume is indicated in mL. Leukopenia is registered when leukocytes <4,000/mm3, leukocytosis >12,000/mm3 or bandemia ≥10%.

Abbreviations: Modified Rankin Scale (mRS), Transient Ischemic Attack (TIA), National Institutes of Health Stroke Scale (NIHSS), Alberta Stroke Program Early CT Score (ASPECTS), Thrombolysis in Brain Ischemia (TIBI), SIRS (Systemic Inflammatory Response Syndrome), NLR (Neutrophil-to-Lymphocyte Ratio), PLR (Platelet-to-Lymphocyte Ratio). Internal carotid artery (ICA); main trunk (M1) and its first-order branch (M2) of the middle cerebral artery, IVT (intravenous thrombolysis), EVT (endovascular thrombectomy).

Table 2. Relationship between the inflammatory parameters after revascularization treatment and the development of cerebral edema at 24 hours.

	CED degree by ECASS-2 definition				Univariate Model	Multivariate Model *		
	CED=0	CED=1	CED=2	CED=3	OR (CI)	OR (CI)	R^2	AIC
	n=180	n=105	n=62	n=27				
SIRS, *n (%)*	9 (5)	12 (11)	11 (18)	6 (21)	3.05 (1.65 – 5.62) [a]	2.35 (1.23 – 4.49) [a]	0.37	740
Temperature <36 or >38 ºC, *n (%)*	13 (7)	7 (7)	9 (14)	3 (11)	1.61 (0.83 – 3.13)	1.31 (0.65 – 2.64)	0.36	742
Heart Rate >90 bpm, *n (%)*	40 (22)	24 (23)	14 (23)	11 (39)	0.78 (0.50 – 1.20)	0.75 (0.47 – 1.21)	0.32	744
Respiratory Frequency >20 cpm, *n (%)*	1 (1)	0 (0)	0 (0)	0 (0)	NC	NC	NC	NC
Leukopenia, Leukocytosis or bandemia, *n (%)*	27 (15)	25 (24)	25 (41)	10 (36)	2.59 (1.67 – 4.04) [a]	1.87 (1.16 – 3.00) [a]	0.36	739

Neutrophils, *median (IQR)*	5.70 (4.01 – 7.65)	6.85 (5.10 – 8.70)	7.29 (5.03 – 10.82)	8.93 (6.65 – 11.23)	1.85 (1.51 – 2.28) [a]	1.53 (1.23 – 1.90) [a]	0.40	720
Lymphocytes, *median (IQR)*	1.31 (0.87 – 1.86)	1.01 (0.69 – 1.66)	1.05 (0.68 – 1.40)	0.90 (0.63 – 1.24)	0.69 (0.57 – 0.84)	0.84 (0.68 – 1.03)	0.36	736
Platelets, *median (IQR)*	202 (165 – 224)	198 (166 – 238)	197 (166 – 234)	215 (158 – 253)	1.02 (0.85 – 1.24)	1.12 (0.91 – 1.38)	0.23	756
NLR, *median (IQR)*	3.92 (2.49 – 7.96)	7.03 (3.83 – 10.25)	8.09 (4.43 – 12.70)	9.81 (5.68 – 15.45)	1.83 (1.49 – 2.24) [a]	1.47 (1.18 – 1.82) [a]	0.39	726
PLR, *median (IQR)*	150 (103 – 244)	195 (108 – 271)	199 (141 – 331)	241 (152 – 371)	1.43 (1.18 – 1.74) [a]	1.24 (1.01 – 1.53) [a]	0.37	735

[a] $p < 0.05$

* Multivariate Model: Adjusted for NIHSS, ASPECTS, Glycemia and occlusion level

Nagelkerke R^2 and Akaike Information criteria (AIC) are shown.

Neutrophils, lymphocytes and platelets values are registered in $x10^9$ cells/L. Leukopenia is registered when leukocytes <4,000/mm3, leukocytosis when >12,000/mm3 or bandemia ≥10%. NLR and PLR have no units.

Abbreviations: NC (non calculable), CED (Cerebral Edema), SIRS (Systemic Inflammatory Response Syndrome), NLR (Neutrophil-to-Lymphocyte Ratio), PLR (Platelet-to-Lymphocyte Ratio).

Table 3. Relationship between the inflammatory parameters after revascularization treatment and clinical outcome.

	Early Neurological Deterioration at 24 hours				Functional Dependence at 90 days			
	No	Yes	Univariate model	Multivariate model *	Yes	No	Univariate model	Multivariate model *
	n=277	n=93	OR (CI)	OR (CI)	n=203	n=171	OR (CI)	OR (CI)
SIRS, *n (%)*	26 (9)	12 (13)	1.45 (0.70 – 3.00)	1.12 (0.50 – 2.49)	27 (13)	11 (7)	2.23 (1.07 – 4.64)	1.86 (0.82 – 4.24)
Temperature <36 or >38 ºC, *n (%)*	26 (9)	5 (5)	2.10 (1.25 – 3.53)	0.53 (0.19 – 1.49) [b]	17 (8)	15 (9)	0.96 (0.46 – 1.97)	0.79 (0.35 – 1.80)
Heart Rate >90 bpm, *n (%)*	62 (22)	26 (28)	1.37 (0.80 – 2.33)	1.32 (0.75 – 2.34)	34 (20)	55 (27)	1.50 (0.92 – 2.44)	1.32 (0.75 – 2.34)
Respiratory Frequency >20 cpm, *n (%)*	1 (0.4)	0 (0)	NC	NC	0 (0)	1 (1)	NC	NC
Leukopenia, Leukocytosis or bandemia, *n (%)*	55 (20)	32 (34)	2.10 (1.23 – 3.53)	1.84 (1.05 – 3.24) [a, c]	61 (31)	24 (14)	2.64 (1.56 – 4.46)	2.38 (1.29 – 4.37) [a, g]

Neutrophils, *median (IQR)*	6.04 (4.28 – 8.25)	7.41 (5.85 – 9.89)	1.53 (1.20 – 1.96)	1.41 (1.09 – 1.84) [a, d]	7.08 (4.95 – 9.16)	5.93 (4.17 – 7.65)	1.44 (1.16 – 1.79)	1.34 (1.04 – 1.72) [a, h]
Lymphocytes, *median (IQR)*	1.21 (0.78 – 1.78)	0.98 (0.69 – 1.36)	0.74 (0.58 – 0.94)	0.85 (0.65 – 1.11)	0.96 (0.68 – 1.41)	1.37 (0.96 – 1.92)	0.56 (0.44 – 0.70)	0.66 (0.51 – 0.85) [a, i]
Platelets, *median (IQR)*	202 (168 – 240)	198 (157 – 239)	0.93 (0.73 – 1.18)	1.05 (0.81 – 1.35)	197 (161 – 234)	211 (171 – 248)	0.79 (0.64 – 0.97)	0.90 (0.70 – 1.16)
NLR, *median (IQR)*	5.50 (2.80 – 9.29)	8.23 (4.48 – 13.05)	1.62 (1.26 – 2.07)	1.61 (1.09 – 2.38) [a, e]	7.71 (4.36 – 11.74)	3.81 (2.34 – 7.96)	1.89 (1.50 – 2.39)	1.60 (1.24 – 2.07) [a, j]
PLR, *median (IQR)*	168 (105 – 261)	217 (133 – 338)	1.30 (1.02 – 1.65)	1.18 (0.92 – 1.53) [f]	212 (127 – 306)	153 (99 – 239)	1.53 (1.23 – 1.90)	1.42 (1.10 – 1.82) [a, k]

[a] $p < 0.05$; [b] $R^2 = 0.19$, AIC=443; [c] $R^2 = 0.20$, AIC=436; [d] $R^2 = 0.18$, AIC=443; [e] $R^2 = 0.20$, AIC=436;

[f] $R^2 = 0.17$, AIC=447; [f] $R^2 = 0.17$, AIC=447; [g] $R^2 = 0.34$, AIC=404; [h] $R^2 = 0.33$, AIC=408; [i] $R^2 = 0.35$, AIC=402; [j] $R^2 = 0.36$, AIC=399; [k] $R^2 = 0.34$, AIC=405.

* Multivariate Model: Adjusted for NIHSS, ASPECTS, Glycemia and occlusion level

Nagelkerke R^2 and Akaike Information criteria (AIC) were calculated.

Neutrophils, lymphocytes and platelets values are registered in $x10^9$ cells/L. Leukopenia is registered when leukocytes <4,000/mm3, leukocytosis when >12,000/mm3 or bandemia ≥10%. NLR and PLR have no units.

Abbreviations: NC (non calculable), Functional Dependence at 90 days YES (mRS 3–6), Functional Dependence at 90 days NO (mRS 0–2), SIRS (Systemic Inflammatory Response Syndrome), NLR (Neutrophil-to-Lymphocyte Ratio), PLR (Platelet-to-Lymphocyte Ratio).

Table 4. Final logistic regression models to predict cerebral edema and clinical outcome based on dichotomized NLR

|	Cerebral Edema at 24 hours		Early Neurological Deterioration at 24 hours		Functional Dependence at 90 days (mRS 3–6)	
	Univariate model OR (CI)	Multivariate model * OR (CI)	Univariate model OR (CI)	Multivariate model * OR (CI)	Univariate model OR (CI)	Multivariate model * OR (CI)
NLR >6.2 vs NLR ≤6.2 (a.u.)	2.56 (1.67 – 3.92)	1.97 (1.16 – 3.32)	2.44 (1.50 – 3.98)	1.90 (1.12 – 3.22)	3.00 (1.96 – 4.59)	2.37 (1.46 – 3.86)
All cohort n=376	2.70 (1.63 – 4.46) [a]	2.17 (1.62 – 3.98) [a]	0.59 (0.39 – 0.89) [b]	0.56 (0.49 – 1.18)	2.95 (1.93 – 4.50) [a]	2.32 (1.43 – 3.76) [a]
Without SICH n=358	2.66 (1.67 – 3.92) [a]	1.97 (1.16 – 3.32) [b]	0.64 (0.42 – 0.97) [b]	0.79 (0.49 – 1.28)	2.96 (1.97 – 4.57) [a]	2.28 (1.39 – 3.74) [a]
Without Infection [c] n=317	2.25 (1.41 – 3.50) [a]	1.86 (1.07 – 3.25) [b]	0.67 (0.39 – 1.17) [b]	0.98 (0.59 – 1.64)	2.30 (1.47 – 3.64) [a]	1.87 (1.10 – 3.19) [a]
Successfully Recanalized [d] n=322	2.45 (1.55 – 3.82) [b]	1.75 (1.02 – 3.06) [b]	0.72 (0.46 – 1.13)	0.70 (0.37 – 1.38)	1.87 (1.07 – 3.24) [a]	2.08 (1.12 – 3.87) [b]

[a] P<0.001, [b] P<0.05

[c] Infection developed after 24 hours from the revascularization procedure until discharge

[d] Included patients achieving TICI 2b-3 after thrombectomy and no vessel occlusion after at post therapy follow-up imaging (CTA or transcranial ultrasonography within 24 hours, per standard of care).

* Multivariate Model: Adjusted for NIHSS, ASPECTS, Glycemia and occlusion level

Supplemental Material

Supplemental Figure 1. Distribution of scores for disability on the modified Rankin scale at 3 months (mRS varies from 0 to 6 and higher scores indicate more severe disability) among patients with different degrees of CED at 24 hours (CED 0, none, to CED 3 with midline shift). The adjusted odds ratio (aOR) and confidence interval (CI) for each category of CED was calculated by ordinal regression analysis with CED=0 as reference: CED=1 aOR=1.91, CI=1.20 – 3.05; CED=2 aOR=4.54, CI=2.42 – 8.49; CED=3 aOR=8.76, CI=3.73 – 20.5.
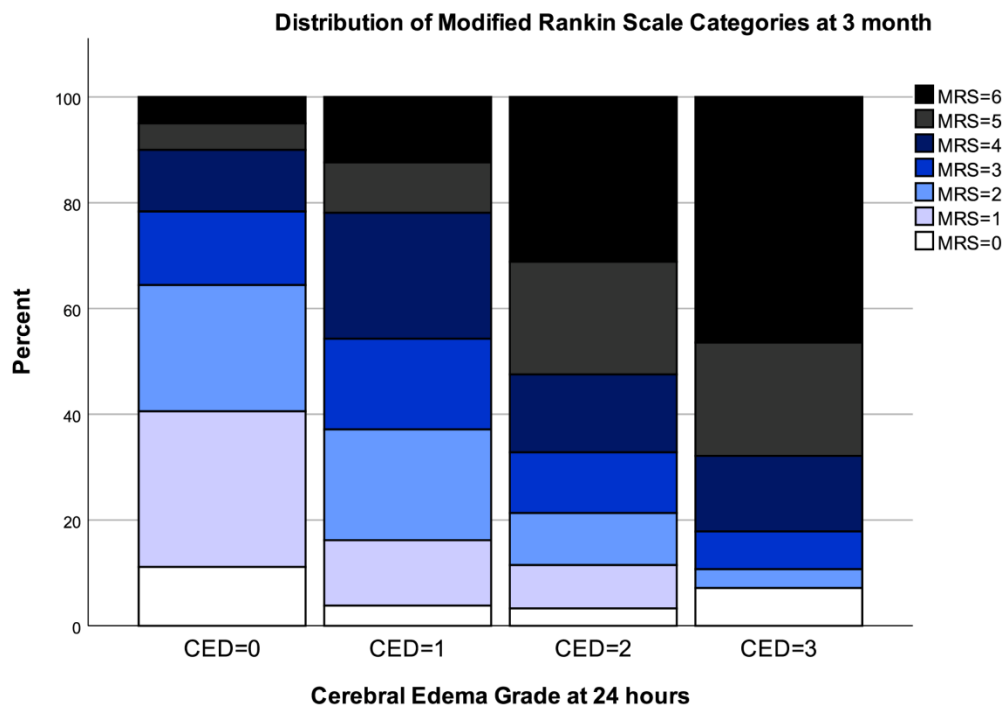
Supplemental Figure 2. Relationship between cerebral edema grades and the rate of symptomatic intracranial hemorrhage.

Percent of SICH per CED category at 24 hours
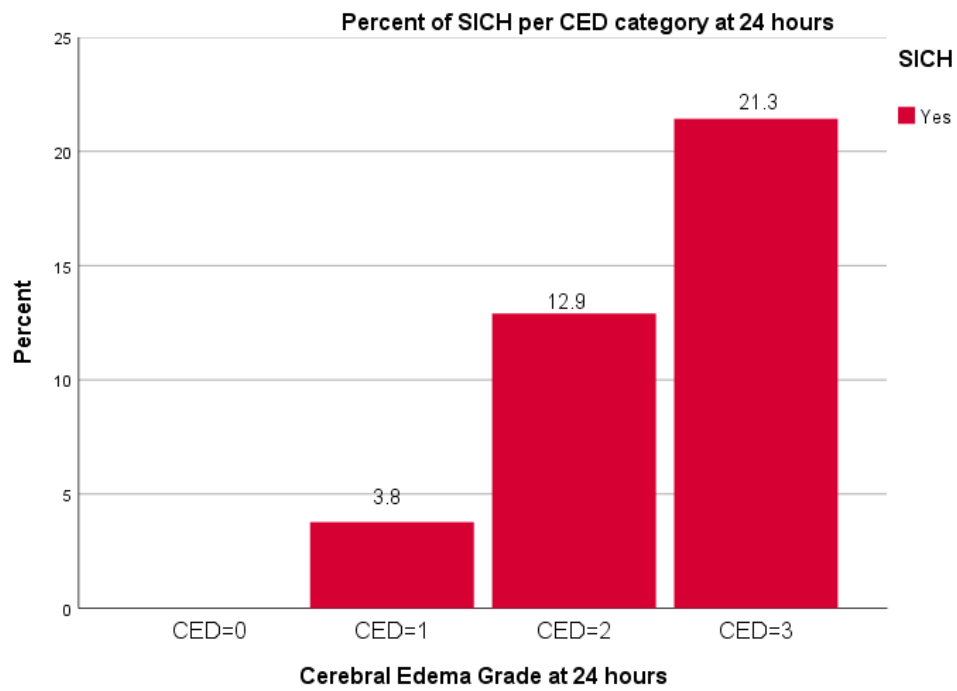
Supplemental Table 1. Relationship between cerebral edema grade and patients' baseline characteristics.

	CED 0	CED 1	CED 2	CED 3	P value†
	n=180	n=105	n=62	n=27	
Male sex, *n (%)*	91 (51)	45 (42)	33 (53)	12 (43)	0.43
Age, *median (IQR)*	75 (65 – 82)	75 (68 – 83)	74 (64 – 84)	82 (65 – 88)	0.46
Hypertension, *n (%)*	120 (67)	76 (72)	45 (73)	20 (71)	0.74
Diabetes mellitus, *n (%)*	57 (32)	33 (31)	11 (18)	15 (54)	<0.01
Dyslipidemia, *n (%)*	105 (58)	68 (64)	33 (53)	11 (39)	0.10
Tobacco, *n (%)*	25 (14)	15 (14)	12 (19)	6 (21)	0.58
Atrial Fibrillation, *n (%)*	62 (34)	44 (42)	28 (45)	11 (39)	0.42
Prev. Stroke/TIA, *n (%)*	40 (25)	16 (17)	10 (20)	4 (14)	0.34
Prev. Myocardial Infarction, *n (%)*	22 (12)	12 (11)	8 (13)	5 (18)	0.83
Prev. Heart Failure, *n (%)*	14 (8)	14 (13)	4 (7)	5 (18)	0.17
Chronic medication					
Statin, *n (%)*	81 (51)	56 (59)	20 (39)	11 (39)	0.08
Antiplatelet, *n (%)*	55 (34)	30 (32)	15 (29)	12 (43)	0.64
Anticoagulant, *n (%)*	18 (11)	15 (16)	6 (12)	3 (11)	0.74

Baseline NHISS, *median (IQR)*	9 (6 – 16)	16 (11 – 19)	16 (14 – 21)	19 (14 – 21)	<0.01
Glycemia, *median (IQR)*	124 (104 – 153)	130 (110 – 172)	131 (110 – 161)	166 (128 – 198)	<0.01
ASPECTS, *median (IQR)*	10 (9 – 10)	9 (7 – 10)	8 (6 – 9)	7 (6 – 9)	<0.01
Etiology ‡					0.53
Cardioembolic, *n (%)*	82 (46)	51 (48)	34 (55)	14 (50)	
Large Artery Atherosclerosis, *n (%)*	18 (10)	2 (2)	8 (13)	4 (14)	
Small vessel disease, *n (%)*	7 (4)	17 (16)	0 (0)	0 (0)	
Undetermined, *n (%)*	63 (35)	32 (30)	20 (32)	9 (32)	
Other (Carotid Dissection), *n (%)*	10 (6)	4 (4)	0 (0)	1 (4)	
Occlusion site ‡					<0.01
ICA, *n (%)*	19 (11)	10 (9)	12 (19)	6 (21)	
M1, *n (%)*	66 (37)	61 (23)	38 (61)	15 (54)	
M2, *n (%)*	38 (21)	24 (23)	8 (13)	4 (14)	
Other, *n (%)*	57 (32)	11 (10)	4 (7)	3 (11)	
IVT, *n (%)*	128 (71)	65 (61)	36 (58)	22 (79)	0.08
EVT, *n (%)*	86 (48)	81 (76)	44 (71)	19 (68)	<0.01

†P values of Mann-Whitney or Chi-square as appropriate

‡ Categorical variables with >2 levels were compared after Bonferroni correction

Age is represented in years, glycemia in mg/dL and infarct volume is indicated in mL.

Abbreviations: Modified Rankin Scale (mRS), Transient Ischemic Attack (TIA), National Institutes of Health Stroke Scale (NIHSS), Alberta Stroke Program Early CT Score (ASPECTS), Thrombolysis in Brain Ischemia (TIBI), Thrombolysis in cerebral infarction (TICI). Internal carotid artery (ICA); main trunk (M1) and its first-order branch (M2) of the middle cerebral artery, IVT (intravenous thrombolysis), EVT (endovascular thrombectomy).

Supplemental Table 2. Relationship between the baseline characteristics and the development of symptomatic intracranial hemorrhage at 24 hours.

	Symptomatic intracranial hemorrhage at 24h		P value†
	Yes	No	
	n=18	n=358	
Male, *n (%)*	8 (44)	173 (48)	0.75
Age, *median (IQR)*	73 (65 – 85)	75 (66 – 83)	0.69
Hypertension, *n (%)*	15 (83)	246 (69)	0.19
Diabetes mellitus, *n (%)*	7 (39)	109 (30)	0.45
Dyslipidemia, *n (%)*	9 (50)	208 (58)	0.50
Tobacco, *n (%)*	53 (15)	5 (28)	0.14
Atrial Fibrillation, *n (%)*	139 (39)	6 (33)	0.64
Prev. Stroke/TIA, *n (%)*	69 (22)	1 (6)	0.14
Prev. Myocardial Infarction, *n (%)*	2 (11)	45 (13)	0.86
Prev. Heart Failure, *n (%)*	1 (6)	36 (10)	0.53
Chronic medication			
Statin, *n (%)*	6 (38)	162 (51)	0.29
Antiplatelet, *n (%)*	6 (38)	106 (33)	0.73
Anticoagulant, *n (%)*	1 (6)	41 (13)	0.43
Baseline NHISS, *median (IQR)*	16 (10 – 20)	14 (7 – 19)	0.31
Glycemia, *median (IQR)*	133 (106 – 170)	127 (110 – 161)	0.76

ASPECTS, *median (IQR)*	7 (6 – 9)	9 (8 – 10)	0.02
Etiology ‡			0.51
Cardioembolic, *n (%)*	7 (39)	174 (49)	
Large Artery Atherosclerosis, *n (%)*	0 (0)	9 (2)	
Small vessel disease, *n (%)*	1 (6)	46 (13)	
Undetermined, *n (%)*	9 (50)	115 (32)	
Other (Carotid Dissection), *n (%)*	1 (6)	14 (4)	
Occlusion site ‡			0.47
ICA, *n (%)*	3 (17)	44 (12)	
M1, *n (%)*	10 (56)	170 (48)	
M2, *n (%)*	4 (22)	70 (20)	
Other, *n (%)*	1 (6)	74 (21)	
IVT, *n (%)*	11 (61)	240 (67)	0.60
EVT, *n (%)*	12 (67)	218 (61)	0.62

†P values of Mann-Whitney or Chi-square, as appropriate.

‡ Categorical variables with >2 levels were compared after Bonferroni correction

Age is represented in years, glycemia in mg/dL and infarct volume is indicated in mL.

Abbreviations: Modified Rankin Scale (mRS), Transient Ischemic Attack (TIA), National Institutes of Health Stroke Scale (NIHSS), Alberta Stroke Program Early CT Score (ASPECTS), Thrombolysis in Brain Ischemia (TIBI), Thrombolysis in cerebral infarction (TICI). Internal carotid artery (ICA); main trunk (M1) and its first-order branch (M2) of the middle cerebral artery, IVT (intravenous thrombolysis), EVT (endovascular thrombectomy).

Supplemental Table 3. Relationship between the baseline characteristics and clinical outcome.

	Early Neurological Deterioration			Functional Dependence at 90 days		
	Yes	No	P value ‡	Yes	No	P value †
Male sex, *n (%)*	38 (41)	139 (50)	0.13	88 (43)	92 (54)	<0.01
Age, *median (IQR)*	79 (68 – 86)	75 (65 – 82)	0.02	78 (69 – 85)	71 (61 – 81)	<0.01
Hypertension, *n (%)*	66 (71)	27 (29)	0.83	149 (73)	111 (65)	0.10
Diabetes mellitus, *n (%)*	34 (37)	59 (63)	0.20	68 (33)	48 (28)	0.28
Dyslipidemia, *n (%)*	53 (57)	161 (58)	0.88	119 (59)	97 (57)	0.39
Tobacco, *n (%)*	13 (14)	45 (16)	0.61	24 (12)	34 (20)	0.04
Atrial Fibrillation, *n (%)*	41 (44)	102 (37)	0.20	90 (44)	53 (31)	<0.01
Prev. Stroke/TIA, *n (%)*	22 (28)	48 (19)	0.10	37 (21)	32 (21)	0.56
Prev. Myocardial Infarction, *n (%)*	13 (14)	34 (12)	0.66	32 (16)	15 (9)	0.38
Prev. Heart Failure, *n (%)*	7 (8)	30 (11)	0.36	25 (12)	12 (7)	0.05
Chronic medication						
Statin, *n (%)*	35 (40)	130 (52)	0.23	87 (49)	80 (52)	0.90

Antiplatelet, *n (%)*	24 (30)	87 (35)	0.47	59 (33)	53 (35)	0.44
Anticoagulant, *n (%)*	14 (18)	27 (11)	0.10	26 (14)	15 (10)	0.01
Baseline NHISS, *median (IQR)*	15 (7 – 18)	14 (8 – 19)	0.63	17 (12 – 20)	9 (6 – 15)	<0.01
Glycemia, *median (IQR)*	140 (110 – 177)	152 (108 – 158)	0.02	133 (110 – 173)	123 (104 – 152)	0.02
ASPECTS, *median (IQR)*	9 (7 – 10)	9 (8 – 10)	0.02	9 (7 – 10)	10 (8 – 10)	0.04
Etiology ‡			0.40			0.02
Cardioembolic, *n (%)*	46 (50)	133 (48)		105 (52)	74 (43)	
Large Artery Atherosclerosis, *n (%)*	0 (0)	9 (3)		25 (12)	22 (13)	
Small vessel disease, *n (%)*	12 (13)	34 (12)		3 (2)	6 (4)	
Undetermined, *n (%)*	27 (29)	95 (34)		61 (30)	63 (37)	
Other (Carotid Dissection), *n (%)*	8 (8)	7 (2)		9 (4)	6 (3)	
Occlusion site ‡			0.08			<0.01
ICA, *n (%)*	16 (17)	30 (11)		31 (15)	16 (9)	
M1, *n (%)*	48 (52)	129 (46)		104 (51)	75 (44)	

M2, *n (%)*	18 (19)	56 (20)		39 (19)	34 (20)	
Other, *n (%)*	11 (12)	63 (23)		29 (14)	46 (37)	
IVT, *n (%)*	59 (63)	190 (68)	0.38	131 (64)	120 (70)	0.25
EVT, *n (%)*	167 (60)	60 (64)	0.45	133 (65)	95 (56)	<0.01

†P values of Mann-Whitney or Chi-square as appropriate

‡ Categorical variables with >2 levels were compared after Bonferroni correction

Age is represented in years, glycemia in mg/dL and infarct volume is indicated in mL.

Abbreviations: Functional Dependence at 90 days YES (mRS 3 – 6), Functional Dependence at 90 days NO (mRS 0 – 2), Transient Ischemic Attack (TIA), National Institutes of Health Stroke Scale (NIHSS), Alberta Stroke Program Early CT Score (ASPECTS), Thrombolysis in Brain Ischemia (TIBI). Internal carotid artery (ICA); main trunk (M1) and its first-order branch (M2) of the middle cerebral artery, IVT (intravenous thrombolysis), EVT (endovascular thrombectomy).

Supplemental Table 4. Relationship between the inflammatory indexes and the development of symptomatic intracranial hemorrhage at 24 hours.

	Symptomatic intracranial hemorrhage (ECASS II Criteria)		Univariate Model	Multivariate Model *	R^2	AIC
	No	Yes	OR (CI)	OR (CI)		
	n=358	n=18				
SIRS, *n (%)*	36 (10)	2 (11)	1.11 (0.25 – 5.03)	0.99 (0.21 – 4.64)	0.05	138
Temperature <36 or >38 ºC, *n (%)*	32 (9)	0 (0)	NC	NC	NC	NC
Heart Rate >90 bpm, *n (%)*	84 (24)	5 (26)	1.24 (0.43 – 3.60)	1.35 (0.44 – 3.85)	0.05	138
Respiratory Frequency >20 cpm, *n (%)*	1 (0.4)	0 (0)	NC	NC	NC	NC
Leukopenia, Leukocytosis or bandemia, *n (%)*	80 (22)	7 (39)	2.19 (0.82 – 5.83)	2.00 (0.72 – 5.56)	0.06	136
Neutrophils, *median (IQR)*	6.45 (4.57 – 8.48)	9.04 (6.15 – 11.39)	1.75 (1.07 – 2.86)	1.71 (1.02 – 2.87) [a]	0.08	134
Lymphocytes, *median (IQR)*	1.17 (0.77 – 1.81)	0.73 (0.51 – 1.05)	0.46 (0.27 – 0.76)	0.50 (0.30 – 0.84) [a]	0.11	131
Platelets, *median (IQR)*	201 (166 – 240)	204 (167 – 240)	1.01 (0.63 – 1.63)	1.06 (0.65 – 1.72)	0.42	138

NLR, *median (IQR)*	5.92 (2.97 – 9.62)	12.08 (6.94 – 17.35)	2.52 (1.50 – 4.34)	2.34 (1.37 – 4.04) [a]	0.14	129
PLR, *median (IQR)*	171 (107 – 266)	336 (219 – 393)	1.47 (1.16 – 1.88)	2.02 (1,20 – 3.41) [a]	0.11	131

[a] p<0.05

* Multivariate Model=Adjusted for NIHSST0, ASPECTS, Glycemia and occlusion level

Nagelkerke R^2 and Akaike Information criteria (AIC) are shown.

Neutrophils, lymphocytes and platelets values are registered in $x10^9$ cells/L. Leukopenia is registered when leukocytes <4,000/mm3, leukocytosis >12,000/mm3 or bandemia ≥10%.

Abbreviations: NC (non calculable), SIRS (Systemic Inflammatory Response Syndrome), NLR (Neutrophil-to-Lymphocyte Ratio), PLR (Platelet-to-Lymphocyte Ratio).

Attachments

Manuscript Formatting for Stroke

Only Microsoft Word files will be accepted for review.

Manuscripts must be double-spaced, including references, figure legends, and tables.

We recommend using Times New Roman 12-point font.

Leave 1-inch margins on all sides. Number every page, beginning with the abstract page, including tables, figure legends, and figures.

Manuscripts should be presented in the following sequence:

- Title page
- Abstract
- Text, including Introduction, Methods, Results, Discussion and Summary/Conclusions
- Acknowledgments
- Sources of Funding
- Conflict(s)-of-Interest/Disclosure(s)
- References
- Figure Legends
- Tables
- Figures
- Visual Abstract (ONLY for Basic Science Articles)
- Online Supplement

Cite each reference in the text in numerical order and list in the References section. In text, reference numbers may be repeated but not omitted. Do not duplicate references either in text or in the reference list.

Cite each figure and table in the text in numerical order.

Upload one copy of any in-press article that is cited in the references, if applicable.

Upload one copy of any abstracts published or submitted for publication, if applicable.

Use SI units of measure in all manuscripts. For example, molar (M) should be changed to mol/L; mg/dL to mmol/L; and cm to mm. Units of measure previously reported as percentages (e.g., hematocrit) are expressed as a decimal fraction. Measurements currently not converted to SI units in biomedical applications are blood and oxygen pressures, enzyme activity, H+ concentration, temperature, and volume. The SI unit should be used in text, followed by the conventionally used measurement in parentheses. Conversions should be made by the author before the manuscript is submitted for peer review.

Provide $US dollar equivalents if you include other currency amounts in the manuscript.

Please provide sex-specific and/or racial/ethnic-specific data, when appropriate, in describing outcomes of epidemiologic analyses or clinical trials; or specifically state that no sex-based or racial/ethnic-based differences were present. See the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals for more details.

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Standards for Preclinical Studies of Stroke Therapy" (*Stroke*. 2016;47:2435-2438) for more information.

Please review the correct usage of the terms "sex" and "gender." "'Gender' refers to a person's self-representation...or how that person is responded to by social institutions on the basis of the person's gender presentation. 'Gender' is rooted in biology and shaped by environment and experience;" "sex" describes a class of "living things as male or female according to their reproductive organs and functions assigned by chromosomal compliment" (AMA 10th ed. 2007: p 395. Please use the terms appropriately.

Confidence intervals should be reported instead of P values for estimated parameters, such as odds ratios and relative risks; P values should be reported only for relevant analytic tests. Authors are encouraged to avoid the pitfalls associated with the misuse of P values as measures of significance. Please refer to "The ASA's Statement on p-Values: Context, Process, and Purpose." *The American Statistician.* 2016.70;2: 129-133. http://dx.doi.org/10.1080/00031305.2016.1154108.

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Consult the AMA Manual of Style: A Guide for Authors and Editors, 10th ed, Oxford: Oxford University Press; 2007, for style.

Consult current issues for additional guidance on format.

Title Page

The first page of the manuscript should be the title page. This page must include:

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Total number of tables and figures, e.g., Tables 2; Figures 3.

3 to 7 key words for use as indexing terms. Consider using terms found in the Medical Subject Headings (MeSH) database.

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Abstract

Do not cite references in the abstract.

Limit use of acronyms and abbreviations.

Be concise (**300** words, maximum).

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The abstract should have the following headings:

Background and Purpose (description of rationale for study)

Methods (brief description of methods)

Results (presentation of significant results)

Conclusions (succinct statement of data interpretation)

When applicable, include a fifth heading: "Clinical Trial Registration" Please list the URL, as well as the Unique Identifier, for the publicly accessible website on which the trial is registered. If the trial is not registered, please indicate the reason in the heading.

- Example 1: Clinical Trial Registration-URL: http://www.clinicaltrials.gov. Unique identifier: NCT00123456.

- Example 2: Clinical Trial Registration-URL: http://www.controlled-trials.com. Unique identifier: ISRCTN70000879.

- Example 3: Clinical Trial Registration-URL: http://www.chictr.org. Unique identifier: ChiCTR-RCH-14004884.

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Text

The following are typical main headings: Materials and Methods, Results, Discussion, and Summary.

Abbreviations must be defined at first mention in the text, tables, and figures.

Introduction: This section should briefly introduce the context of the results to be presented and should duplicate what is contained elsewhere in the manuscript

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For any apparatuses used in Methods, the complete names of manufacturers must be supplied.

For human subjects or patients, describe their characteristics.

For animals used in experiments, state the species, strain, number used, and other pertinent descriptive characteristics.

When describing surgical procedures on animals, identify the preanesthetic and anesthetic agents used, and state the amount or concentration and the route and frequency of administration for each. The use of paralytic agents, such as curare or succinylcholine, is not an acceptable substitute for anesthetics.

For other invasive procedures on animals, report the analgesic or tranquilizing drugs used. If none were used, provide justification for such exclusion.

Manuscripts that describe studies on humans must include a statement indicating if ethics approval was obtained from the local institutional review board and if written informed consent was obtained from patients or if the board waived the need for patient consent.

Manuscripts involving animals must indicate that the study was approved by an institutional animal care and use committee.

Reports of studies on both animals and humans must indicate that the procedures followed were in accordance with institutional guidelines.

All drugs should be referred to by their generic names rather than trade names. The generic chemical identification of all investigational drugs must be provided.

A statistical subsection must be provided at the end of the Methods section describing the statistical methodology employed for the data presented in the manuscript.

The Methods section should provide essential information related to the conduct of the study presented in the manuscript. For methodology previously published by the authors, the prior publication should be referenced and a copy of the paper provided to the reviewers, if necessary.

The Methods section should only contain material that is absolutely necessary for comprehension of the results section. Additional (more detailed) methods can be provided as a data supplement.

Prevention of bias is important for experimental stroke research (see Macleod et al, *Stroke*.2009;40:e50-e52). For studies where the primary objective is the preclinical testing of therapies, the following checklist items must be adhered to and clearly documented in the manuscript:

Animals: Species, strains and sources must be defined. For genetically modified animals, wildtype controls including background and back-crossing must be defined.

Statistics and sample size: Specific statistical methods must be defined, including parametric versus nonparametric and multigroup analyses, and sample size powering based on expected variances and differences between groups.

Inclusions and exclusions: Specific criteria for inclusions and exclusions must be specified. For example, only animals where blood flow reductions fall below a certain threshold are included. Or only animals with a certain degree of neurological deficits are included. Once animals are randomized (see below), all excluded animals must be reported, including explicit presentation of mortality rates.

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point assessments must be performed by investigators blinded to the groups for which each animal is assigned.

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Results:

This section should succinctly report the results of experimental studies and clinical research or clinical series/observations.

Confidence intervals should be reported instead of P values for estimated parameters, such as odds ratios and relative risks; P values should be reported only for relevant analytic tests. Authors are encouraged to avoid the pitfalls associated with the misuse of P values as measures of significance. Please refer to "The ASA's Statement on p-Values: Context, Process, and Purpose." *The American Statistician.* 2016.70;2: 129-133. http://dx.doi.org/10.1080/00031305.2016.1154108.

Discussion:

This section should not reiterate the results but put the results in appropriate context regarding relevant literature and the importance of new observations contained in the manuscript.

Summary/Conclusions:

A brief paragraph summarizing the results and their importance may be included but is not required.

Acknowledgments

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Accuracy of reference data is the author's responsibility. Verify all entries against original sources, especially journal titles, inclusive page numbers, publication dates, accents, diacritical marks, and spelling in languages other than English.

Do not list the month/issue/day (the number in parentheses) in the reference.

References with more than 6 authors should list the first 6 authors followed by et al.

Cite references in numerical order according to first mention in text.

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Example References:

Print journal reference: Mistry EA, Mistry AM, Nakawah MO, Chitale RV, James RF, Volpi JJ, et al. Mechanical Thrombectomy Outcomes With and Without Intravenous Thrombolysis in Stroke Patients: A Meta-Analysis. *Stroke.* 2017;48:2450-2456.

Online journal references: Muller CJ, Alonso A, Forster J, Vock DM, Zhang Y, Gottesman RF, et al. Stroke Incidence and Survival in American Indians, Blacks, and Whites: The Strong Heart Study and Atherosclerosis Risk in Communities Study. *J Am Heart Assoc.* 2019;8:e010229.

Li J, Liu J, Liu M, Zhang S, Hao Z, Zhang J, et al. Closure versus medical therapy for preventing recurrent stroke in patients with patent foramen ovale and a history of cryptogenic stroke or transient ischemic attack. *Cochrane Database Syst Rev.?* 2015; 9: CD009938.

Publish-Ahead-of-Print reference: Mossavar-Rahmani Y, Kamensky V, Manson JE, Silver B, Rapp SR, Haring B, et al. Artificially Sweetened Beverages and Stroke, Coronary Heart Disease, and All-Cause Mortality in the Women's Health Initiative. [published online February 14, 2019]. *Stroke.* 2019.

https://www.ahajournals.org/doi/10.1161/STROKEAHA.118.023100. Accessed February 15, 2019.

Book reference: Schermerhorn ML et al. Carotid Artery Stenting. Fischer JE, Bland KI, Callery MP, eds. In: Mastery of Surgery. Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2007.

Website reference: Stroke Death Rates, Hispanics Age 65+. Quick Maps of Heart Disease and Stroke. National Center for Chronic Disease Prevention and Health Promotion, Division for Heart Disease and Stroke Prevention. https://www.cdc.gov/dhdsp/maps/national_maps/stroke65_hispanics.htm. Accessed July 26, 2019.

Web sites generally follow this format: Author names (if any). Title of information or page. Name of website. URL. Publication date (if any). Access date.

Software reference: StataCorp. Stata statistical software: Release 12. College Station, TX: StataCorp LP; 2011.

Conference Proceeding:Author(s) Name(s). Title of Paper/Poster. Paper/Poster presented at: Name of Conference; Month Dates, Year; City, State. URL [link]. Accessed Month Day, Year.

Government bulletin: Author. Title of bulletin. Place of publication: Name of issuing department or agency; publication date. Page numbers (if any). Publication number (if any). Series number (if any).

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Tables should be used to present comparisons of large amounts of data at a glance. Tables with only 1 or 2 rows of data should be incorporated into the text.

Tables should be as compact as possible. Avoid unnecessary rows and columns.

Use indenting within the stub column to indicate subgroups. Do not use bold, shading, rules, etc.

Tables should not contain vertically merged cells; horizontally merged cells are permitted when necessary in the heading row.

Internal headings are not permitted outside of the stub column. If internal headings are required, the table should be split into 2 tables.

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Units of measure should be in the heading row or stub column rather than the body of the table whenever possible.

Indicate footnotes in the table in this order: *,†, ‡, §, | |, #, * * . Follow AMA 9th edition for footnote styles.

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Use the largest font size possible without distorting the figures. Text for super- or subscripts should be no smaller than 6 points.

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Any abbreviations or symbols used in the figures must be defined in the figure or figure legend.

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The supplement should be single-spaced.

If citations are made in the Supplemental Material, the supplement must contain its own independent Reference Section with references numbered sequentially, beginning with reference 1, even if some of these references duplicate those in the print version.

Number supplementary figures and tables as Figure I, Figure II, Table I, Table II, etc.

Place the supplemental figure legend underneath the corresponding figure.

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