U.PORTO

FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2019/2020

Francisca Isabel Barros Ferreira

The Impact of Very Short term Variability of Blood Pressure in Outcome after Successful Thrombectomy / Impacto da Variabilidade da Pressão Arterial de Muito Curto Prazo no Prognóstico após Trombectomia Mecânica

NOVEMBRO, 2019

Francisca Isabel Barros Ferreira

The Impact of Very Short term Variability of Blood Pressure in Outcome after Successful Thrombectomy / Impacto da Variabilidade da Pressão Arterial de Muito Curto Prazo no Prognóstico após Trombectomia Mecânica

Mestrado Integrado em Medicina

Área: Neurologia

Tipologia: Dissertação

Trabalho efetuado sob a Orientação de:

Doutor Pedro Miguel Araújo Campos Castro

E sob a Coorientação de:

Dr. Elsa Irene Peixoto Azevedo Silva

Trabalho organizado de acordo com as normas da revista:

STROKE

NOVEMBRO, 2019

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

UC Dissertação/Projeto (6º Ano) - DECLARAÇÃO DE INTEGRIDADE

Eu, Francisca Isabel Barros Ferreira, abaixo assinado, nº mecanográfico 201403302, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 19/11/2019

Assinatura conforme cartão de identificação:

Francisca Isabel Barros Ferreira

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

UC Dissertação/Projeto (6º Ano) – DECLARAÇÃO DE REPRODUÇÃO

NOME

Francisca Isabel Barros Ferreira

NÚMERO DE ESTUDANTE | E-MAIL

201403302 | franciscaibferreira@hotmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

3.2 Medicina Clínica

TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

The Impact of Very Short-Term Variability of Blood Pressure in Outcome after Successful Thrombectomy

ORIENTADOR

Pedro Miguel Araújo Campos Castro

COORIENTADOR (se aplicável)

Elsa Isabel Peixoto Azevedo Silva

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☒
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	☐

Faculdade de Medicina da Universidade do Porto, 19 / 11 / 2019

Assinatura conforme cartão de identificação: Francisca Isabel Barros Ferreira

Dedicatória

À minha família, por acreditar sempre em mim, por toda a paciência e por nunca medir esforços.

Aos meus amigos, por todo o companheirismo e por fazerem esta jornada valer sempre a pena.

Ao António, pelo apoio de todas as horas.

Title: The Impact of Very Short-term Variability of Blood Pressure in Outcome after Successful Thrombectomy

Authors:

Francisca Ferreira, MD, MSc

Department of Clinical Neurosciences and Mental Health, Faculty of Medicine, University of Porto, Porto, Portugal

franciscaibferreira@hotmail.com

Faheem Sheriff, MD

Department of Neurology, Brigham and Women's Hospital

fsheriff.md@gmail.com

Can Ozan Tan, PhD

Department of Neurology, Brigham and Women's Hospital Department of Physical Medicine and Rehabilitation, Harvard Medical School

Cerebrovascular Research Laboratory, Spaulding Rehabilitation Hospital

Department of Radiology, Massachusetts General Hospital

cotan@mgh.harvard.edu

Karen Li, MD

Department of Neurology, Brigham and Women's Hospital

KLI5@mgh.harvard.edu

Sarah LaRose Michaud

Department of Neurology, Brigham and Women's Hospital

slmichaud@bwh.harvard.edu

Henri Vaitkevicius, MD

Department of Neurology, Brigham and Women's Hospital

HVAIT@bwh.harvard.edu

Steven K. Feske, MD

Department of Neurology, Brigham and Women's Hospital

sfeske@bwh.harvard.edu

Elsa Azevedo, MD, PhD

Porto, Portugal

Department of Neurology, Centro Hospitalar Universitário São João, Faculty of Medicine of University of Porto, Porto, Portugal

eazvedo@med.up.pt

Pedro Castro, MD, PhD

Department of Neurology, Centro Hospitalar Universitário São João, Faculty of Medicine of University of Porto, Porto, Portugal

Portugal; pedromacc@med.up.pt

Corresponding author:

Pedro Castro MD, PhD Centro Hospitalar São João, Porto, Portugal;

Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal

Email: pedromacc@med.up.pt; telefone: +351 22 551 2100, fax: +351 22 502 5766

Cover title: blood pressure variability post thrombectomy

Tables: 3, Figures: 3

Supplemental material: Yes

Key-words: infarction, autonomic, ultrasound, transcranial Doppler, blood pressure

Subject terms: ultrasound, ischemic stroke, angiography, thrombectomy, blood pressure, autonomic

Word count: 8207

Abstract

Background and Purpose: We aim to determine if the very short-term blood pressure variability (BPV) after successful endovascular treatment of acute ischemic stroke has a relevant impact in the clinical outcome.

Methods: This is a prospective multicenter study with inclusion of consecutive AIS patients with occlusion of intracranial anterior circulation vessels who achieved successful recanalization (modified Treatment In Cerebral Ischemia grades 2b-3) after thrombectomy. Very short-term BPV was assessed by spectral analysis of spontaneous fluctuations of beat-to-beat systolic blood pressure values recorded by finger plethysmography with Finometer device. Outcomes included independence at 90 days (modified Rankin scale 0-2) and the initial clinical response to mechanical thrombectomy.

Results: We included 121 patients. Increased BPV at high frequencies (rapid oscillations) was independently associated with poor functional outcome at 90 days (normalized units, odds ratio (OR) = 0.56, 95% confidence interval (CI) 0.35 – 0.88, p=0.01; low/high frequency ratio OR = 1.38, CI 1.09 – 1.76, p<0.01) and early neurological recovery (normalized units, OR = 0.67, CI 0.46 – 0.98, p=0.04) in multivariate analysis.

Conclusions: The magnitude of rapid oscillations of blood pressure has a significant impact in early neurological recovery and late functional outcome of ischemic stroke patients after successful recanalization. Very short-term BPV can be assessed quickly throughout the post intervention period and potentially contribute to a more efficient blood pressure control in AIS patients submitted to endovascular treatment.

Main text

Introduction

Dysregulation of blood pressure (BP) is a common finding among patients with acute ischemic stroke (AIS) and carries relevant prognostic significance[1]. However, the optimal BP range to be maintained during the acute phase is still loosely defined in current guidelines. With the advent of modern endovascular treatment and the increased proportion of patients that are effectively revascularized it became more complex and urgent to revise the best approach for BP control. This is justified by the fact that the recanalization status modulates the association between BP and outcome.[2] It has been proposed that BP should be kept at lower values after successful recanalization but the between and within-subject variability of BP are still troublesome features.[3] An adequate supervision of the BP within the first hours is expected to maximize the benefits of acute revascularization by the prevention of major complications, such as hemorrhagic transformation, cerebral edema, or to avoid infarct growth.[1] We must acknowledge that approximately half of treated patients by endovascular thrombectomy fail to show clear clinical or functional benefits.[4] Whether or not a more tuned BP control could change this figures must be addressed.

Besides the standard measurements of BP, its time-varying behavior could be as important. From a pathophysiological perspective, major fluctuations in BP should be deleterious since cerebral autoregulation in globally impaired in AIS[5], more so in the affected territory.[6, 7] Therefore, to avoid large BP swings after revascularization should be as important as treating high BP levels. In this regard, BP variability (BPV) corresponds to BP fluctuations ranging from beat-to-beat, minute-to-minute, hour-to-hour oscillations, consisting of short-term BPV,

to periods as long as days to months, comprising long-term BPV.[8] Long-term BPV has showed additional prognostic value over the standard BP measurements for prediction of new cardiovascular events and increased mortality.[8] Visit-to-visit BPV is also associated with worse clinical outcomes after myocardial infarction and stroke.[9] However, long-term assessment is not suitable in the early stage of AIS. Interestingly, shorter assessments can have the same prognosis significance.[9-11] 24-hour BPV is also associated with functional outcome after AIS.[12, 13] But again, the shorter-term BPV still requires a minimum 24-h period, which limits its usefulness in AIS within the time window that has more potential to change patient's outcome.

Very short-term BPV can be assessed by spectral analysis within few minutes[14] which seems more adequate for BP monitoring in AIS but not studied so far. We aim to determine if the very short-term BPV, recorded over 5 minutes, is associated with early neurological recovery and long-term functional outcome in patients with AIS after successful endovascular treatment.

Methods

Patients and participating centers

This is a prospective multicenter study with inclusion of consecutive ischemic stroke patients selected for endovascular thrombectomy and achieved successful recanalization. Patients were recruited from the Brigham and Women's Hospital in Boston, MA, US and the Centro Hospitalar Universitário São João, Porto, Portugal, between September 1, 2017 and January 3, 2019. Eligible patients had a large vessel occlusion of the proximal middle cerebral artery

(M1 or M2 segment) and/or of the internal carotid artery (ICA) terminus, were ≥18 years of age, and had achieved grade 2b-3 according to the modified Thrombolysis in Cerebral Infarction scale (mTICI)[15] after thrombectomy or had spontaneous recanalization documented at acute angiographic study.

Clinical, laboratory and radiological assessment

Demographic data and medical history, including vascular risk factors, medications, and previous cardiovascular disease were recorded. Serum glucose was recorded at admission. National Institutes of Health Stroke Scale (NIHSS) score was obtained at baseline and after 24 hours from recanalization. Stroke type was classified by Trial of Org 10172 in Acute Stroke Treatment (TOAST) scale.

A member of the stroke team recorded the Alberta Stroke Program Early Computed Tomography Score (ASPECTS) for the admission noncontrast computed tomography (CT)[16] Initial CT angiography (CTA) was inspected to determine the location of vessel occlusion and collaterals grade.[17] The presence of hemorrhage[18] and infarct volumes calculated by the ABC/2 method were based on a head CT done approximately 24 hours after the procedure.[19] CT images at 24 hours were also reviewed for the presence of infarct lesions affecting selective brain areas involved with autonomic function control, also known as the central autonomic network[20, 21], namely the ventral medial prefrontal cortex, cingulate cortex, amygdala and its connection tracts (this includes anterior temporal area[22]), right anterior insular cortex and left posterior insular cortex.[21] We also considered midline shift at diencephalic level which affects paraventricular regions related to efferent sympathetic tracts.[20, 21] Examples of these areas are shown at Figure 2.

24-hour blood pressure monitoring

Per protocol, after thrombectomy, patients were admitted to the stroke unit where we started BP monitoring with standard brachial cuffs (EDAN iM80, Schenzhen, China) placed in the non-affected arm. This was done automatically every hour within the first 24 hours. We obtained averaged values for systolic and diastolic BP as well as their standard variation (SD).

Very short-term Blood Pressure by spectral analysis

Patients were monitored in supine position with continuous BP obtained by plethysmography (Finometer MIDI, FMS, The Netherlands), with the finger cuff placed in the non-affected arm within 72 hours from last-seen-well time. A 3-electrod electrocardiography II-derivation line was also co-registered for heart-beat detection. After stabilization, 5 minute recordings were used to analyze very short-term BPV, in frequency domain.[23] BPV was characterized by the power spectrum of beat-to-beat systolic BP values of successive normal RR intervals, in very-low (VLF; <0.04 H<), low (LF; 0.04–0.15 Hz) and high (HF; 0.15-0.40 Hz) frequency bands. Lower BPV power implies less variability of BP and different bands can harbor distinct physiological mechanisms. VLF power can have multiple influences either autonomic or circulating factors and it is less reliable because of the interference of white noise. [24] LF power is associated the arterial baroreceptor reflex, being determined by α-adrenergic sympathetic component of vasomotor function.[24] The HF component of BPV is mostly considered to be caused by mechanical effects of respiratory movements on cardiac

output, although β-adrenoceptor-mediated cardiac sympathetic function might play a role as well.[24, 25]

Clinical endpoints

The endpoints were functional outcome at 90 days, measured by modified Rankin scale score (mRS) dichotomized in subgroups 0-2 (independent) versus 3-6 (dependent or dead) and the initial response to mechanical thrombectomy: early neurological recovery at 24h, defined as a decline in NIHSS score of at least 4 points or a score of 0 or 1.

We received approval by the ethics committees of both institutions. Written informed consent was obtained from all participants/guardians.

Statistical Analysis

Normality of continuous variables was inferred by the Kolmogorov-Smirnov test. We used chi-square/Fisher exact tests for categorical variables and Student's t/Mann-Whitney tests for continuous variables to test differences between outcome groups. Bonferroni correction was used when categorical variables had more than one class. Logistic regression was used to generate the odds ratios (OR) and 95% confidence intervals (CI) of the BP parameters for functional independence (mRS 0 to 2 versus 3 to 6) at 90 days and early neurological recovery at 24 hours. We adjusted analyses for all outcomes with two multivariate models. In model 1, we adjusted to the independent variables associated with outcome in univariate analysis, i.e., age, NIHSS, Last-seen-well to canalization time, the ASPECTS, mTICI (Table 1). In model 2, we adjusted to variables associated with BP parameters, i.e., sex, tobacco,

heart failure, coagulant use, previous use of angiotensin conversion enzyme inhibitor or receptor blocker, respiratory frequency (Supplemental table 1).

We also studied the possible determinants of BP parameters variance. This was performed by linear regression with each independent variable and then those associated with a $P<0.1$ were included in multivariate models in a backward stepwise fashion to find those that were independently related to BP parameters (Supplemental Table 1). These were inserted in multivariate logistic regression model 2, as stated above. Box-Cox transformations of non-normally distributed continuous variables was used as in predictive models.

Considering that atrial fibrillation artificially increases BPV and interferes with spectral analysis, we pre-specified a subgroup analysis comparing patients with and without atrial fibrillation.

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

Data availability

Data and statistical syntax is available for research proposes and can be obtained by a reasonable request to the corresponding author.

Results

From September 1, 2017 through January 3, 2019, 151 patients fulfilled the inclusion criteria. Among these, 1 patient was excluded due to current severe infection causing hemodynamic instability, 3 due to poor quality signal recordings, and 18 due to unavailable monitoring within 72 hours. Therefore, we enrolled 121 patients (Figure 1). Patients' characteristics are

described in Table 1, as well as the comparison of baseline characteristics of patients based on outcome subgroups.

Standard Blood Pressure parameters

The univariate and multivariate analysis comparing BP parameters by outcome are presented in tables 1 and 2, respectively. Admission systolic and diastolic BP, before EVT, were not associated with outcome. However, patients with worse functional outcome at 90 days registered higher systolic BP values on average (128±16 vs 121±18, p=0.05) during the first 24 hours after intervention. After adjusting to other predictors of outcome, 24-hour averaged systolic BP maintained association with functional outcome at 90 days [adjusted OR (OR) = 0.97 (95% CI 0.95 – 1.00), p = 0.05], though in the limit of statistical significance. Variability of systolic BP during the same 24-hour period, expressed as SD, was not related to any outcome measure. Early neurological recovery was unrelated to standard BP measurements.

Very short-term BPV parameters

Very short-term BPV parameters were strongly associated with outcome. Absolute spectral power at HF range was significantly lower in patients presenting a faster neurological recovery [median (IQR) 4.0 (1.7 – 13.6) versus 7.7 (3.1 – 17.3), p<0.03] and those who were functionally independent at 90 days [3.8 (1.6 – 10.8) versus 8.8 (3.7 – 23.0), p<0.01). These associations were also significant in terms of relative units (normalized HF and LF/HF ratio). Spectral power of BP at LF and VLF ranges was not associated with the outcome measures. In multivariate models adjusting to baseline severity, higher HF relative spectral power

independently predicted poor functional outcome at 90 days, either expressed as normalized units (aOR=0.56, 0.35 – 0.88, p = 0.01) or as LH/HF ratio (aOR=1.38, 1.09 – 1.76, p < 0.01). Higher normalized HF spectral power also reduced the chance for an early neurological recovery (aOR= 0.67, 0.46 – 0.98, p = 0.04).

The subgroup analysis of patients with (n=54) and without (n=68) atrial fibrillation is shown in Table 3. Relative HF spectral power expressed as LH/HF ratio maintained significantly associated with functional outcome, while normalized HF spectral power remained statistically significant only in the subgroup of patients without atrial fibrillation.

Determinants of short-term BP variability

The relationship of baseline characteristics and BP parameters are presented in Supplemental Table 1. HF spectral power was increased in the presence of atrial fibrillation, in hypertensive patients especially if medicated with angiotensin-renin modulating drugs and by respiratory frequency. Tobacco use and heart failure were associated with increased LF/HF ratio but the relationships were weaker. Averaged systolic BP was not correlated with very short term BPV parameters.

Regarding the affection of brain areas related to autonomic control, normalized HF spectral power was significantly lower in patients with infarction of the left posterior insular cortex [median (IQR) 0.34 (0.21 – 0.52) versus 0.49 (0.30 – 0.67), p=0.02] and left amygdala [median (IQR) 0.33 (0.24 – 0.45) versus 0.48 (0.30 – 0.65), p= 0.03], resulting in higher LF/HF ratios in these patients.

Discussion and conclusions

Increased very short-term BPV was associated with poor functional outcome and lower probability of early neurological recovery in stroke patients with large vessel occlusion of the proximal MCA (M1/M2) or ICA and who achieved an mTICI score of 2b-3 following mechanical thrombectomy.

Effect of very short-term BPV on outcome

Very short-term BPV, particularly at high frequencies, was independently associated with functional outcome at 90 days and early neurological recovery, even after we adjusted to variables considered strong predictors of outcome in stroke, namely the baseline NIHSS, age, ASPECTS score and time to recanalization. Considering that all our patients had restoration of blood flow, this might be due to additional damage to the blood brain barrier caused by increased BP oscillations, thus contributing to increased reperfusion injury. Reperfusion injury is a deleterious phenomenon which antagonizes the beneficial effects of recanalization and can be explained by multiple functional and structural changes occurring after transient ischemia, leading to cell death even if successful restoration of cerebral blood flow is achieved.[26] These changes include increased metabolic rate, release of pro-inflammatory cytokines, degradation of extracellular matrix, release of free radicals and recruitment of leucocytes and microglia, which contribute to DNA damage, cytoskeletal degradation and increased blood-brain barrier dysfunction.[26] These effects are more prominent in infarcted areas but also occur in peri-infarcted regions, translating clinically into increased brain edema, infarct progression, intracranial hemorrhage, and neurologic worsening.[26] In fact, previous studies stated that higher BPV and elevated SBP could promote infarct growth,

increased vasogenic cerebral edema and increased hemorrhagic transformation, with deleterious effects in the blood vessels with impaired autoregulation.[3, 12, 13] This corroborates the possible association between higher BPV and increased reperfusion injury. High frequency oscillations, particularly, may be more hazardous because in this frequency range there is no active cerebral autoregulation. The latter is a protective mechanism that adjusts cerebral blood flow to fluctuations in BP and exerts its effects at frequencies <0.2 Hz[27] and perhaps event lower, close to or under 0.003 Hz.[28] *A priori*, we would expect to have an association of BPV with significant hemorrhagic transformation. However, we only registered 4 cases in this cohort, which do not explain the effect of BPV on outcome. Noteworthy, Cho et al also found a relationship of 24h-BPV and worse outcome but not with hemorrhagic transformation.[13] Moreover, we cannot document any significant association between BP parameters and the risk of significant hemorrhagic transformation in large studies on thrombolysis and endovascular treatment.[29, 30] Our results corroborate these findings and suggest that the deleterious effects of BPV might be more related to cerebral edema and dysfunction of blood-barrier which is too subtle to be detected with coarse methods such as CT. Had we included dedicated MRI studies in our patients, we could have identified other subclinical signs of blood-brain barrier dysfunction that could support our hypothesis.[26]

Most importantly, in our study, we were able to demonstrate that BPV can be assessed quickly (minutes) and still carries the same important prognostic significance, which has practical implications. This shows that it is possible to control not only BP levels but also to monitor its harmful fluctuations more closely in a simple and non-invasive manner.

Previous studies have already established an association with short-term and long-term BPV and functional outcome in acute ischemic stroke patients, suggesting the need to stabilize

BPV, in addition to controlling steady-state mean BP values.[8, 12] In our study, we were able to demonstrate that a fast assessment of BPV, in few minutes, carries the same prognostic significance. Contrary to previous studies, our measurement of BPV in very short-term period is more appropriate to monitor in AIS patients, especially in the first hours after revascularization.

Comparison with the prognostic yield of the standard measures of BP

Higher systolic BP in the post intervention period was significantly associated with functional outcome, which is consistent with previous findings correlating higher SBP with worse prognosis, most likely due to the harmful effects of higher pressure in recently reperfused vascular beds with failed autoregulation.[3, 13, 31] On the other hand, we did not find a significant association of outcome and 24h BPV. Cho et al[13] also studied BPV in AIS patients following endovascular treatment. They included 313 reperfused patients and, similarly to our results, 24h BPV was not significantly related to functional outcome, expressed as SD or covariation (the % of variability adjusted to individual average BP value). Only when more sophisticated dispersion measures were calculated, namely the successive variation of systolic BP, BPV was found to be increased in the poor outcome subgroup. Unlike SD, successive variations consider the order of BP values, alternating paterns of increases and decreases and correct for the decremental trend of BP in AIS. This reflects the fact that time-dependent changes of BP measure-to-measure are more significant that overall fluctuations of BP occurring in the whole 24h span. This gives credit to our findings that more rapid changes in BP are more deleterious to the ischemic area. Nevertheless, our results highlight the major advantage of very-short term BPV assessment by spectral analysis, that it can detect harmful patterns of BPV in small periods of time, around 5 minutes, more useful

in AIS settings and with greater potential to guide BP therapies. In practical terms, classical 24h BPV measurements cannot give the clinician the necessary information during this critical time period. Additionally, averaged systolic BP was not correlated with very short term BPV parameters which means that these two hemodynamic markers represent different aspects of BP physiology. Although much work is needed, it seems reasonable to conclude that it is advisable not only to keep BP low if patients achieve recanalization but also to avoid oscillations of BP in the same time period.

Source of very short-term oscillations

This study was not designed to determine the source of the harmful patterns of very-short term BPV, despite the interest of this topic regarding future trials for BP intervention in AIS. Both the mechanical effect of respiratory movements and sympathetic activity have been proposed to create most of the short-term BP oscillations.[24, 25, 32, 33] Autonomic dysfunction is common in patients with acute ischemic stroke, with a predominance of sympathetic activity.[34] This imbalance correlates with worse prognosis in these patients, due to its association with cardiac dysfunction, hyperglycaemia, immune depression, sleep disordered breathing, thrombotic events and malignant edema.[34] Low frequency oscillations are determined by α-adrenergic sympathetic vasomotor function, as part of the baroreceptor reflex, while high frequency oscillations derive from fluctuations in cardiac output caused by respiratory movements, mediated through β-adrenergic receptors.[24] Our findings correlating BPV, particularly at HF, could have a partial contribution from adrenergic sympathetic overactivity previously reported in this setting.[34]. At this regard, we found that patients with left posterior insula or left anterior temporal area (covering amygdala and is associated tracts) had lower HF power. These areas are involved in sympathetic modulation[21, 34] and if affected

could thus cause a depression of the sympathetic effects on the cardiovascular system with reduced HF power as shown in rats subjected to simultaneous α- plus β-blockade.[24] However, no single region was associated with increased HF BPV from which we conclude that fast BP fluctuations might be due to general autonomic dysregulation related to acute stress. We also found an association between respiratory frequency (RF) and HF oscillations, which was expected since RF is usually within the same frequency range and provides mechanical effects of ventilation in cardiovascular apparatus and intrathoracic pressure changes.[25] Nevertheless, respiratory frequency was not related to outcome measures (table 1), which does not support the idea that breathing pattern was primarily related to poor outcome and causing an increased HF BPV as a secondary epiphenomenon.

Some comorbidities were related to increased HF BPV like smoking, sex, heart failure, previous antihypertensive medication, especially those affecting angiotensin-renin system, but these didn't associate to outcome measures and BPV remained significantly associated to outcome after multivariate adjustment. We payed particular attention to the presence of atrial fibrillation. This condition is intrinsically associated with increased spectral power, which is due to the irregular heart rhythm and beat-to-beat changes in stroke filling times.[35] Despite this, atrial fibrillation was not related to outcome measures and subgroup analysis showed that the very-short term BPV, especially relative LF/HF ratio, was consistently related to functional outcome irrespective of this co-morbidity. It is remarkable that very short-term BPV seems to be useful in patients with atrial fibrillation, backing the idea that increased BPV oscillations are deleterious to the brain suffering from acute ischemia, irrespective of its source.

Limitations

Our study population was restricted to subgroup of patients with AIS that had a major intracranial occlusion and who achieved successful recanalization (mTICI 2b/3). Thus, our findings may not be generalized to all stroke patients.[31] We did not have direct measures of respiratory function, which could have permitted to explain if these were in fact the source of BP variability. Also, we did not perform MRI, and thus, may have missed smaller ischemic lesions, the more accurate calculation of the infarct volume or to be more ascertain of the involvement of autonomic areas. Nevertheless, we dichotomized stroke volume by median values instead of using this variable as a continuous variable to surpass this limitation.

Conclusions

The magnitude of rapid oscillations of blood pressure has significant impact in early neurological recovery and late functional outcome of ischemic stroke patients after successful recanalization. Very short-term BPV can be assessed quickly throughout the post intervention period and could contribute to a more efficient blood pressure control in AIS patients submitted to endovascular treatment.

Acknowledgments:

None

Sources of Funding:

None

Conflict(s)-of-Interest/Disclosure(s):

None

References

1. Leonardi-Bee J, Bath PM, Phillips SJ, Sandercock PAJS. Blood pressure and clinical outcomes in the international stroke trial. 2002;33:1315-1320
2. Martins AI, Sargento-Freitas J, Silva F, Jesus-Ribeiro J, Correia I, Gomes JP, et al. Recanalization modulates association between blood pressure and functional outcome in acute ischemic stroke. *Stroke*. 2016;47:1571-1576
3. Maier IL, Tsogkas I, Behme D, Bähr M, Knauth M, Psychogios M-N, et al. High systolic blood pressure after successful endovascular treatment affects early functional outcome in acute ischemic stroke. 2018;45:18-25
4. Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: A meta-analysis of individual patient data from five randomised trials. *Lancet*. 2016;387:1723-1731
5. Castro P, Azevedo E, Sorond F. Cerebral autoregulation in stroke. *Curr Atheroscler Rep*. 2018;20:37
6. Castro P, Serrador JM, Rocha I, Sorond F, Azevedo E. Efficacy of cerebral autoregulation in early ischemic stroke predicts smaller infarcts and better outcome. *Front Neurol*. 2017;8:113
7. Appleton JP, Sprigg N, Bath PMJS, neurology v. Blood pressure management in acute stroke. 2016;1:72-82
8. Parati G, Ochoa JE, Lombardi C, Bilo GJNRC. Assessment and management of blood-pressure variability. 2013;10:143
9. Soh M-S, Park J-S, Seo K-W, Yang H-M, Lim H-S, Choi B-J, et al. Visit-to-visit systolic blood pressure variability in patients with st-elevation myocardial infarction predicts long-term cardiovascular outcomes. 2019:1
10. Konstantinou K, Tsioufis K, Dimitriadis K, Mantzouranis M, Koumelli A, Fragoulis C, et al. Reduced blood pressure variability as a predictor of cardiac events after myocardial infarction: A 6 months follow-up study. 2018;36:e12
11. Hassan AKM, Abd–El Rahman H, Mohsen K, Dimitry SRJTJoCH. Impact of in-hospital blood pressure variability on cardiovascular outcomes in patients with acute coronary syndrome. 2017;19:1252-1259
12. Manning LS, Rothwell PM, Potter JF, Robinson TGJS. Prognostic significance of short-term blood pressure variability in acute stroke: Systematic review. 2015;46:2482-2490
13. Cho BH, Kim JT, Lee JS, Park MS, Kang KW, Choi KH, et al. Associations of various blood pressure parameters with functional outcomes after endovascular thrombectomy in acute ischaemic stroke. 2019;26:1019-1027
14. Parati G, Frattola A, Di Rienzo M, Castiglioni P, Mancia G. Broadband spectral analysis of blood pressure and heart rate variability in very elderly subjects. *Hypertension*. 1997;30:803-808

15. Higashida RT, Furlan AJ, Roberts H, Tomsick T, Connors B, Barr J, et al. Trial design and reporting standards for intra-arterial cerebral thrombolysis for acute ischemic stroke. *Stroke*. 2003;34:e109-137
16. Pexman JH, Barber PA, Hill MD, Sevick RJ, Demchuk AM, Hudon ME, et al. Use of the alberta stroke program early ct score (aspects) for assessing ct scans in patients with acute stroke. *AJNR Am J Neuroradiol*. 2001;22:1534-1542
17. Tan IYL, Demchuk AM, Hopyan J, Zhang L, Gladstone D, Wong K, et al. Ct angiography clot burden score and collateral score: Correlation with clinical and radiologic outcomes in acute middle cerebral artery infarct. *American Journal of Neuroradiology*. 2009;30:525
18. Fiorelli M, Bastianello S, von Kummer R, del Zoppo GJ, Larrue V, Lesaffre E, et al. Hemorrhagic transformation within 36 hours of a cerebral infarct: Relationships with early clinical deterioration and 3-month outcome in the european cooperative acute stroke study i (ecass i) cohort. *Stroke*. 1999;30:2280-2284
19. Sims JR, Gharai LR, Schaefer PW, Vangel M, Rosenthal ES, Lev MH, et al. Abc/2 for rapid clinical estimate of infarct, perfusion, and mismatch volumes. *Neurology*. 2009;72:2104-2110
20. BENARROCH EE. The central autonomic network: Functional organization, dysfunction, and perspective. *Mayo Clinic Proceedings*. 1993;68:988-1001
21. Sie J-H, Chen Y-H, Chang C-Y, Yen N-S, Chu W-C, Shiau Y-HJSr. Altered central autonomic network in baseball players: A resting-state fmri study. 2019;9
22. Nolte J. Drives and emotions: The hypothalamus and limbic system. *The human brain: An introduction to functional anatomy*. Elsevier; 2009:580-607.
23. Freitas J, Santos R, Azevedo E, Carvalho M, Boomsma F, Meiracker A, et al. Hemodynamic, autonomic and neurohormonal behaviour of familial amyloidotic polyneuropathy and neurally mediated syncope patients during supine and orthostatic stress. *Int J Cardiol*. 2007;116:242-248
24. Yoshimoto T, Eguchi K, Sakurai H, Ohmichi Y, Hashimoto T, Ohmichi M, et al. Frequency components of systolic blood pressure variability reflect vasomotor and cardiac sympathetic functions in conscious rats. 2011;61:373-383
25. Julien C, Zhang Z-Q, Cerutti C, Barres CJJotans. Hemodynamic analysis of arterial pressure oscillations in conscious rats. 1995;50:239-252
26. Choi JH, Pile-Spellman JJNC. Reperfusion changes after stroke and practical approaches for neuroprotection. 2018;28:663-682
27. Meel-van den Abeelen AS, van Beek AH, Slump CH, Panerai RB, Claassen JA. Transfer function analysis for the assessment of cerebral autoregulation using spontaneous oscillations in blood pressure and cerebral blood flow. *Med Eng Phys*. 2014;36:563-575
28. Hamner JW, Ishibashi K, Tan CO. Revisiting human cerebral blood flow responses to augmented blood pressure oscillations. *J Physiol*. 2019;597:1553-1564
29. Goyal N, Tsivgoulis G, Pandhi A, Chang JJ, Dillard K, Ishfaq MF, et al. Blood pressure levels post mechanical thrombectomy and outcomes in large vessel occlusion strokes. *Neurology*. 2017;89:540-547
30. Jiang S, Fei A, Peng Y, Zhang J, Lu YR, Wang HR, et al. Predictors of outcome and hemorrhage in patients undergoing endovascular therapy with solitaire stent for acute ischemic stroke. *PloS one*. 2015;10:e0144452
31. Martins AI, Sargento-Freitas J, Silva F, Jesus-Ribeiro J, Correia I, Gomes JP, et al. Recanalization modulates association between blood pressure and functional outcome in acute ischemic stroke. 2016;47:1571-1576

32. Pagani M, Lucini D, Rimoldi O, FURLAN R, Piazza S, Porta A, et al. Low and high frequency components of blood pressure variability. 1996;783:10-23
33. Stauss HMJC, pharmacology e, physiology. Identification of blood pressure control mechanisms by power spectral analysis. 2007;34:362-368
34. De Raedt S, De Vos A, De Keyser JJJotns. Autonomic dysfunction in acute ischemic stroke: An underexplored therapeutic area? 2015;348:24-34
35. Olbers J, Gille A, Ljungman P, Rosenqvist M, Östergren J, Witt NJBp. High beat-to-beat blood pressure variability in atrial fibrillation compared to sinus rhythm. 2018;27:249-255

Figure legends

Figure 1

Flow chart of the study's recruitment

Abbreviations: LOCF = last observation carried forward; mTICI = modified Thrombolysis in Cerebral Infarction; NICU = neurocritical care unit; TCD = transcranial Doppler

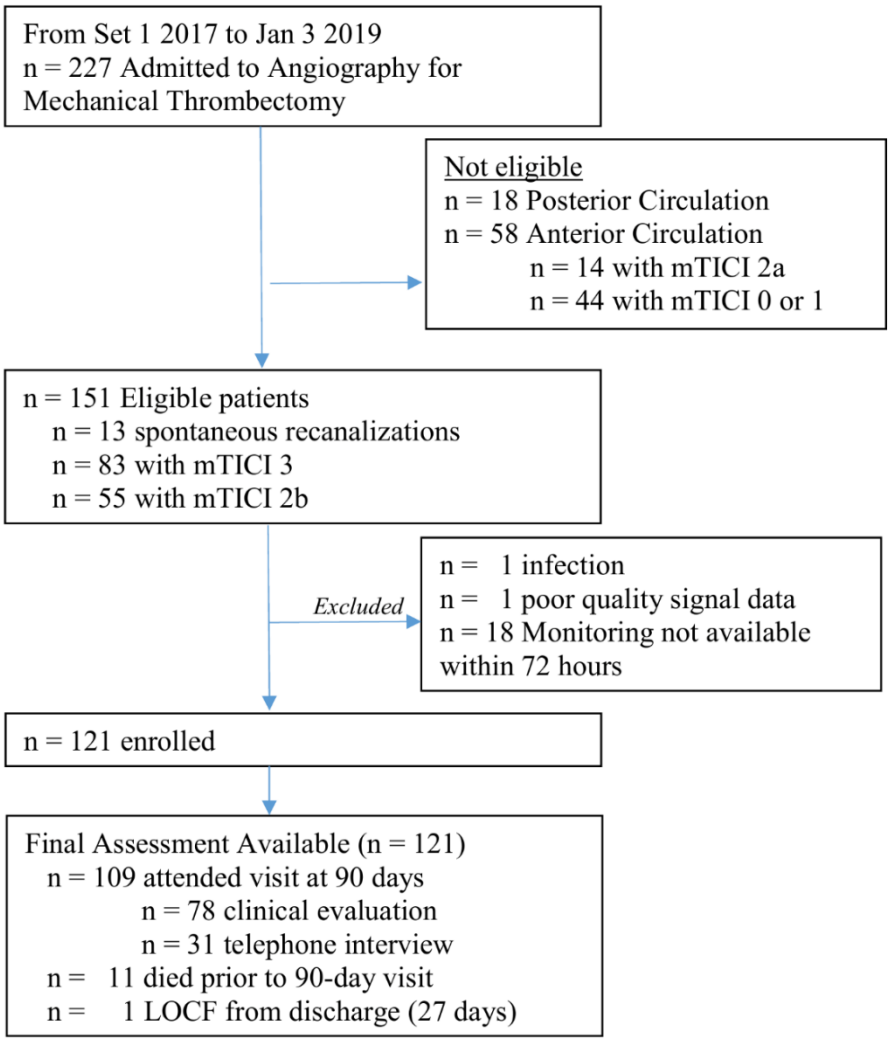


Figure 2

Template chart of strategic lesions affecting the central autonomic network areas studied in control 24-hour CT.

Figure legends: A – Right anterior insular cortex; B – Left posterior insular cortex; C – Frontobasal cortex; D – Amygdala efferent pathways; E – diencephalic midline shift; F – Cingulate cortex

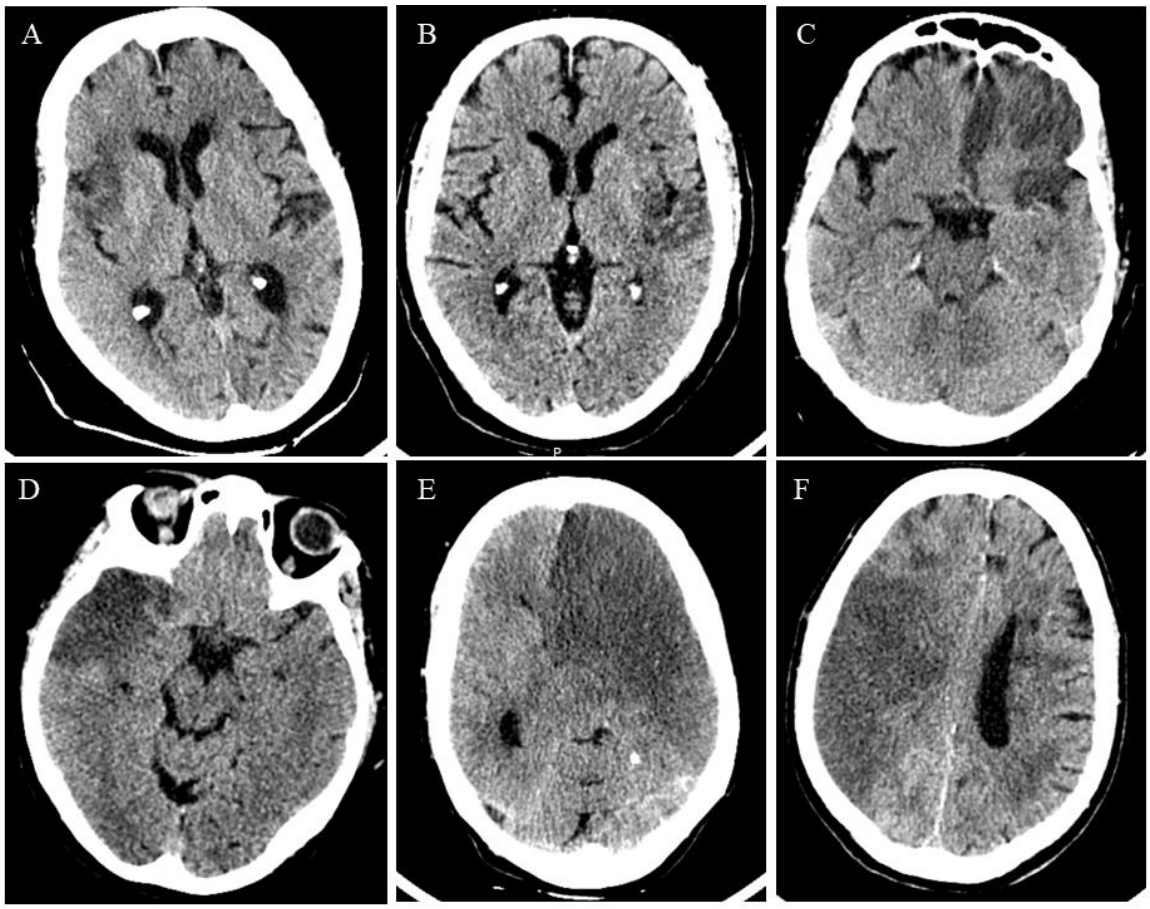
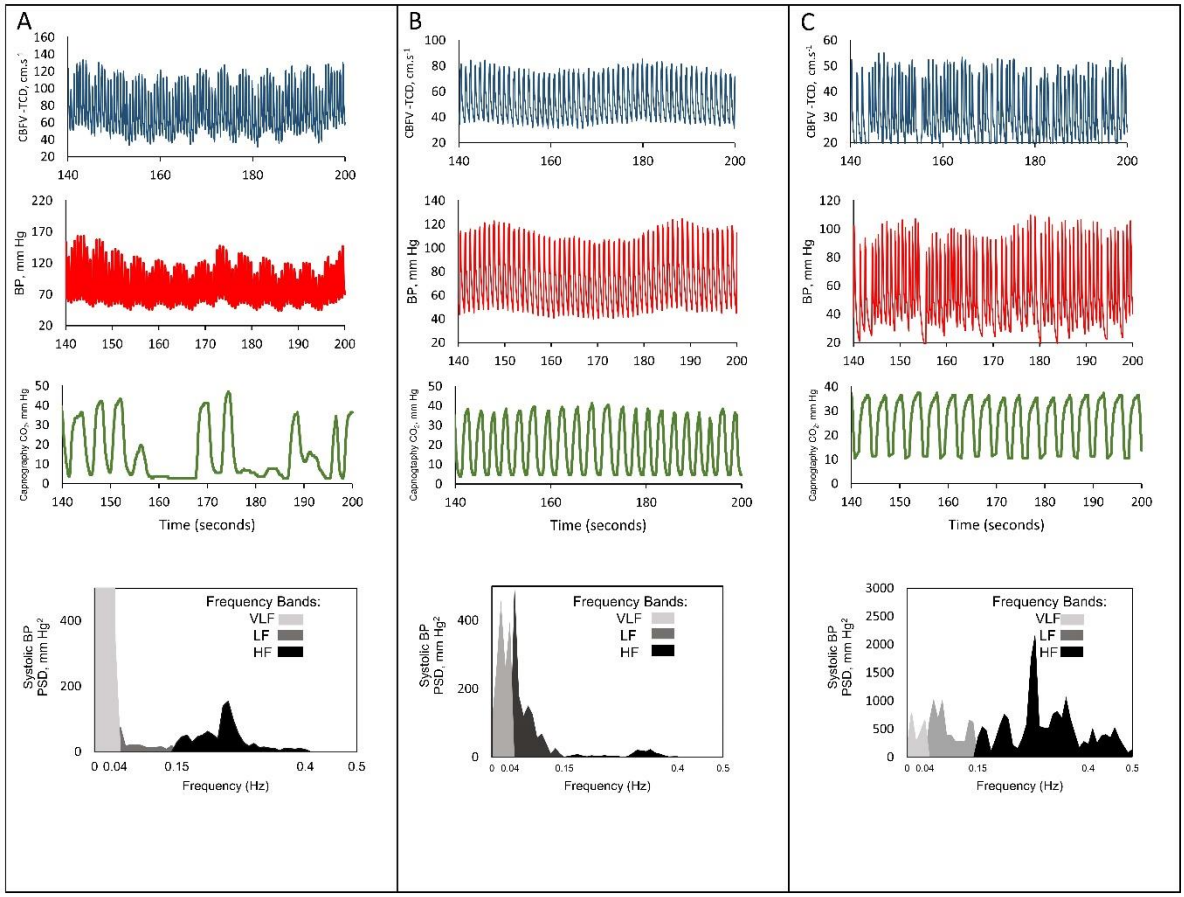


Figure 3

Exemplificative cases A, B and C of very-short term variability calculation. In all three cases we present the source recordings (only one minute is shown for graphical proposes) of continuous blood pressure (BP) obtained by Finometer MIDI (in red), cerebral blood flow velocity (CBFV) obtained by transcranial Doppler (TCD; blue) and expiratory carbon dioxide (CO_2) from capnography with nasal cannula (green). CBFV was not evaluated in the present study but is shown here for comparison with BP. At the bottom of each case the chart represents the spectral density of systolic BP across all frequency ranges with different shades accordingly standard frequency bands – very-low (VLF; light grey), low (LF; dark grey) and high (HF; black). Case A represents a patient with poor prognosis having the BPV peaking within HF range. This is also discernible in time domain by inspection of continuous blood pressure line (red) and the sinusoidal oscillation pattern of blood pressure according 3 times every 10 seconds (0.3 Hz). At this range, cerebral blood flow shows the same HF oscillatory pattern as in BP. Case B represents a patient with good prognosis that has the opposite pattern of increased LF over HF power of systolic BP spectrum. Case C represents a poor prognosis patient that has atrial fibrillation. Notice the remarkable beat-to-beat variation related to the co-morbidity causing spectral demodulation but still shows a peak at HF band as in Case A.


A
B
C
CBFV -TCD, cm.s-1
BP, mm Hg
Capnography CO2, mm Hg
Time (seconds)
Systolic BP PSD, mm Hg2
Frequency Bands:
VLF
LF
HF
Frequency (Hz)

Tables

Table 1 Demographic, clinical and radiological characteristics of all patients and the differences between functional outcome at 90 days and early neurological recovery subgroups.

	All (n = 121)	**Independent mRS 0-2 (n = 63)**	**Dependent mRS 3-6 (n = 58)**	**P value ***	**Early neuro recovery Yes (n = 59)**	**Early neuro recovery No (n = 58)**	**P value***
Age, years – mean (SD)	70 (14)	68 (14)	73 (13)	0.02[a]	70 (13)	70 (15)	0.84
Male sex – n (%)	60 (50)	31 (49)	29 (50)	0.93	33 (55)	27 (45)	0.27
Hypertension – n (%)	76 (63)	38 (60)	38 (66)	0.55	41 (69)	34 (59)	0.19
Diabetes mellitus – n (%)	29 (24)	16 (25)	13 (22)	0.70	16 (27)	12 (20)	0.39
Dyslipidemia – n (%)	70 (58)	35 (56)	35 (60)	0.59	38 (64)	31 (53)	0.20
Smoker – n (%)	17 (14)	11 (18)	6 (10)	0.26	6 (10)	11 (18)	0.19

Atrial Fibrillation – n (%)	53 (44)	26 (41)	27 (47)	0.56	26 (44)	26 (45)	1.00
Ischemic Heart Disease – n (%)	15 (12)	7 (11)	8 (14)	0.66	6 (10)	9 (15)	0.41
Heart Failure – n (%)	18 (15)	11 (18)	7 (12)	0.43	10 (17)	8 (14)	0.64
Chronic medication – n (%)							
Statin	58 (48)	30 (48)	28 (48)	0.94	32 (54)	25 (43)	0.20
Antiplatelet	33 (27)	17 (27)	16 (28)	0.94	15 (25)	18 (30)	0.54
Anticoagulant	27 (22)	11 (18)	16 (28)	0.18	18 (30)	8 (13)	0.03
Antihypertensive	76 (63)	38 (60)	38 (66)	0.55	38 (64)	37 (64)	0.85
Beta-blockers	45 (40)	27 (47)	18 (33)	0.15	18 (30)	26 (44)	0.10
Calcium-channel blockers	17 (15)	6 (10)	11 (20)	0.14	8 (14)	8 (14)	0.97
ACEI/ARB	43 (38)	20 (35)	23 (43)	0.38	22 (37)	20 (34)	0.75

Glucose, mg/dL – median (IQR)	131 (104 – 161)	123 (99 – 165)	140 (116 – 160)	0.22	137 (100 – 164)	130 (109 – 161)	0.96
Respiratory Rate, bpm – median (IQR)	17 (15 – 21)	18 (15 – 21)	17 (15-21)	0.71	18 (15 – 21)	16 (14 – 19)	0.22
TOAST Classification – n (%)				0.63			0.31
Large Artery Atherosclerosis	18 (15)	10 (16)	8 (14)		7 (12)	11 (18)	
Cardioembolism	58 (48)	28 (44)	30 (52)		29 (48)	28 (47)	
Undetermined	38 (31)	20 (32)	18 (31)		22 (37)	16 (27)	
Other (Carotid Dissection)	4 (3)	2 (3)	2 (3)		1 (2)	3 (5)	
Baseline NHISS – median (IQR)	15 (9 – 17)	10 (6 – 15)	16 (15 – 20)	<0.01[a]	15 (9 – 18)	15 (7 – 17)	0.63
Occlusion site – n (%)				0.89			0.52

Terminal ICA	16 (14)	8 (14)	8 (15)		6 (11)	10 (18)	
M1	84 (75)	43 (74)	41 (76)		44 (75)	39 (67)	
M2	12 (11)	7 (12)	5 (9)		6 (11)	6 (11)	
ASPECTS Score – median (IQR)	9 (7 – 10)	10 (7 – 10)	9 (7 – 10)	0.12	10 (8 – 10)	9 (7 – 10)	0.02[a]
Collateral grade – median (IQR)	2 (2 – 3)	3 (2 – 3)	2 (2 – 3)	0.36	3 (2 – 3)	2 (2 – 3)	0.35
IV Thrombolysis – n (%)	53 (44)	28 (44)	25 (43)	0.88	28 (47)	25 (42)	0.58
Grade 3 in mTICI scale – n (%)	79 (65)	38 (60)	41 (71)	0.23	43 (72)	35 (60)	0.13
Hemorrhage (PH1-PH2)	4 (3)	1 (2)	3 (5)	0.26	1 (2)	3 (5)	0.31
LSW to TPA time, minutes – median (IQR)	165 (115 – 241)	141 (107 – 225)	188 (138 – 258)	0.19	138 (105 – 206)	225 (157 – 250)	0.01

LSW to Recanalization time, minutes – median (IQR)	375 (297 – 494)	368 (289 – 481)	395 (327 – 571)	0.15	328 (249 – 450)	410 (333 – 595)	<0.01[a]
LSW to Finometer time, minutes – median (IQR)	1071 (623 – 2140)	1020 (610 – 1910)	1190 (646 – 2352)	0.44	1190 (611 – 2145)	1058 (667 – 2143)	0.65

Abbreviations: ACEI = Angiotensin Converting-Enzyme Inhibitor; ARB = Angiotensin Receptor Blocker; ASPECTS = The Alberta Stroke Program Early Computed Tomography Score (ASPECTS); BP = blood pressure; ICA = internal carotid artery; IQR = interquartile range; LSW = last-seen-well time; M1 = main trunk of the middle cerebral artery; M2 = first-order branch of the main trunk of the middle cerebral artery; mTICI = modified Thrombolysis in Cerebral Infarction; NHISS = Scores on the National Institutes of Health Stroke Scale; SD = standard deviation; TOAST = Trial of Org 10172 in Acute Stroke Treatment. Early neurological recovery defined as scoring a NIHSS of 0 or 1 or a drop of ≥8 points in NIHSS from baseline to 24 hours.

* P values are shown for differences between outcome subgroups obtained from chi-square test/ Fisher's exact test for categorical variables, or Student's t/Mann-Whitney test for continuous variables.

Multiple comparisons were adjusted by Bonferroni method (new P value was set to $P<0.05$ divided by the number of comparisons)

[a]Remained significantly associated ($p<0.05$) with the dependent variable after forward stepwise logistic regression.

Table 2 BP parameters among subgroups of functional outcome at 90 days and early neurological recovery

	All (n = 121)	**Independent mRS 0-2 (n = 63)**	**Dependent mRS 3-6 (n = 58)**	**P value ***	**Early neurological recovery Yes (n = 61)**	**Early neurological recovery No (n = 60)**	**P value ***
Admission							
Systolic BP, mm Hg – mean (SD)	143 (23)	140 (25)	145 (21)	0.23	146 (23)	141 (23)	0.29
Diastolic BP, mm Hg – mean (SD)	76 (19)	75 (23)	78 (14)	0.42	79 (16)	77 (13)	0.34
24-hour monitoring							
Averaged Systolic BP, mm	125 (17)	121 (18)	128 (16)	0.045	125 (18)	123 (17)	0.66

Hg – mean (SD)							
SD of Systolic BP, mm Hg – mean (SD)	13 (10 – 16)	13 (10 – 16)	14 (11 – 17)	0.14	13 (11 – 15)	14 (10 – 17)	0.25
Averaged Diastolic BP, mm Hg – mean (SD)	68 (12)	68 (13)	69 (11)	0.93	70 (13)	66 (10)	0.11
SD of Diastolic BP, mm Hg – mean (SD)	9 (7 – 11)	9 (7 – 11)	9 (8 – 12)	0.26	9 (7 – 11)	9 (8 – 12)	0.12
Very Short-term systolic BP variability (5 min)							

Total spectral power, mm Hg^2 – median (IQR)	47.7 (21.9 – 89.3)	45.5 (20.1 – 81.5)	51.3 (24.2 – 175)	0.28	46.2 (21.0 – 73.6)	51.3 (22.3 – 172)	0.28
VLF power, mm Hg^2 – median (IQR)	25.4 (11.6 – 48.0)	27.6 (11.3 – 44.5)	23.4 (12.8 – 52.3)	0.94	24.2 (11.6 – 44.3)	25.6 (10.9 – 59.5)	0.44
LF power, mm Hg^2 – median (IQR)	7.3 (3.7 – 20.2)	6.4 (3.6 – 20.2)	8.2 (3.8 – 22.1)	0.72	6.7 (3.6 – 19.2)	8.4 (3.6 – 21.4)	0.66
HF power, mm Hg^2 – median (IQR)	5.9 (2.6 – 16.6)	3.8 (1.6 – 10.8)	8.8 (3.7 – 23.0)	<0.01	4.0 (1.7 – 13.6)	7.7 (3.1 – 17.3)	0.03
LF spectral power, nu – mean (SD)	0.54 (0.23)	0.61 (0.24)	0.46 (0.19)	<0.01	0.58 (0.23)	0.50 (0.22)	0.03

HF spectral power, nu – mean (SD)	0.46 (0.23)	0.39 (0.24)	0.54 (0.19)	<0.01	0.42 (0.23)	0.50 (0.22)	0.03
LF/HF ratio – median (IQR)	1.11 (0.57 – 2.8)	1.91 (0.83 – 5.07)	0.82 (0.42 – 1.26)	<0.01	1.42 (0.72 – 3.84)	0.85 (0.49 – 2.07)	0.03

Abbreviations: BP = blood pressure; HF = High Frequency; IQR = interquartile range; LF = Low frequency; LF/HF ratio = ratio of low-high frequency power; nu = normalized units; SD = standard deviation; VLF = Very low frequency

Early neurological recovery defined as scoring a NIHSS of 0 or 1 or a drop of ≥8 points in NIHSS from baseline to 24 hours.

* P values are shown for differences between outcome subgroups obtained from chi-square test/ Fisher's exact test for categorical variables, or Student's t/Mann-Whitney test for continuous variables.

Table 3 Univariate and multivariate logistic regression analysis for predicting outcome from BP parameters

	Outcome measures	mRS 0 – 2 at 90 days – n (%)			Early neurological recovery at 24 hours – n (%)		
		Univariate Model	*Multivariate Model 1	†Multivariate Model 2	Univariate Model	*Multivariate Model 1	†Multivariate Model 2
		Unadjusted Odds ratio (CI 95%); P value	Adjusted Odds ratio (CI 95%); P value	Adjusted Odds ratio (CI 95%); P value	Unadjusted Odds ratio (CI 95%); P value	Adjusted Odds ratio (CI 95%); P value	Adjusted Odds ratio (CI 95%); P value
All (n=121)							
	24-h averaged Systolic BP, mm Hg	0.98 (0.96 – 1.00); p = 0.05	0.97 (0.95 – 1.00); p = 0.05	0.98 (0.96– 1.00); p = 0.11	1.0 (0.98 – 1.02); p = 0.89	1.00 (0.98 – 1.04); p = 0.90	0.97 (0.98– 1.01); p = 0.44
	HF spectral power, mm Hg^2	0.56 (0.33 – 0.964); p = 0.04	0.63 (0.34 – 1.18); p = 0.15	0.60 (0.35 – 1.015); p = 0.07	0.93 (0.60 – 1.44); p = 0.74	0.88 (0.56 – 1.37); p = 0.57	0.72 (0.44 – 1.20); p = 0.21

	HF spectral power, nu	0.50 (0.33 – 0.74); p <0.01	0.56 (0.35 – 0.88); p = 0.01	0.40 (0.24– 0.68); p = 0.01	0.69 (0.48 – 0.996); p = 0.047	0.67 (0.46 – 0.98); p = 0.04	0.61 (0.38 – 0.97); p = 0.04
	LF/HF ratio	1.45 (1.15 – 1.84) p < 0.01	1.38 (1.09 – 1.76) p < 0.01	1.92 (1.32 – 2.78) p = 0.01	0.99 (0.95 – 1.02) p = 0.47	0.98 (0.95 – 1.02) p = 0.39	0.99 (0.90 – 1.08) p = 0.75
No AF							
	24-h averaged Systolic BP, mm Hg	0.95 (0.92 – 0.987); p < 0.01	0.94 (0.89 – 0.99); p = 0.01	0.96 (0.92– 0.995); p = 0.03	1.01 (0.98 – 1.04); p = 0.75	1.00 (0.97 – 1.04); p = 0.83	0.99 (0.97– 1.03); p = 0.97
	HF spectral power, mm Hg^2	0.001 (0.00 – 0.15); p < 0.01	0.001 (0.00 – 0.38); p = 0.02	0.001 (0.00 – 0.33); p = 0.02	0.91 (0.22 – 3.85); p = 0.90	0.73 (0.17 – 3.20); p = 0.68	1.34 (0.29 – 6.54); p = 0.69
	HF spectral power, nu	0.37 (0.21 – 0.67); p < 0.01	0.47 (0.25 – 0.88); p = 0.02	0.30 (0.14– 0.63); p = 0.02	0.63 (0.39 – 1.03); p = 0.07	0.65 (0.39 – 1.08); p = 0.10	0.55 (0.29– 1.01); p = 0.07
	LF/HF ratio	1.44 (1.10 – 1.88)	1.35 (1.04 – 1.75)	1.90 (1.25 – 2.89)	0.99 (0.95 – 1.02)	0.98 (0.95 – 1.02)	1.21 (0.93 – 1.58)

		p < 0.01	p = 0.02	p = 0.03	p = 0.52	p = 0.40	p = 0.15
AF							
	24-h averaged Systolic BP, mm Hg	1.00 (0.97 – 1.04); p = 0.83	1.00 (0.96 – 1.04); p = 0.99	1.01 (0.97– 1.05); p = 0.73	1.00 (0.97 – 1.03); p = 0.90	0.99 (0.96 – 1.03); p = 0.67	0.98 (0.93 – 1.02); p = 0.25
	HF spectral power, mm Hg^2	0.66 (0.39 – 1.12); p = 0.12	0.67 (0.35 – 1.27); p = 0.22	0.60 (0.34 – 1.08); p = 0.08	0.92 (0.55 – 1.52); p = 0.74	0.85 (0.51 – 1.44); p = 0.56	0.62 (0.33 – 1.20); p = 0.15
	HF spectral power, nu	0.69 (0.37 – 1.29); p = 0.25	0.65 (0.31 – 1.34); p = 0.24	0.43 (0.18 – 1.02); p = 0.06	0.73 (0.39 – 1.37); p = 0.33	0.67 (0.34 – 1.31); p = 0.24	0.71 (0.32– 1.56); p = 0.39
	LF/HF ratio	1.59 (0.93 – 2.70) p = 0.09	2.19 (1.07 – 4.49) p = 0.03	2.52 (1.15 – 5.55) p = 0.02	0.97 (0.87 – 1.08) p = 0.60	0.99 (0.88 – 1.12) p = 0.90	0.99 (0.87 – 1.12) p = 0.82

Abbreviations: AF = Atrial fibrillation; CI = confidence interval; nu = normalized units; HF and LF = High and low frequencies;

Early neurological recovery defined as scoring a NIHSS of 0 or 1 or a drop of ≥4 points in NIHSS from baseline to 24 hours.

* Multivariate model 1 = adjusted to predictors of outcome – age, NIHSS, Last-seen-well to canalization time (hours), the Alberta Stroke Program Early Computed Tomography Score (ASPECTS), modified Thrombolysis in Cerebral Infarction (mTICI).

† Multivariate model 2 = adjusted to variables significantly associated with the BP parameters (Supplemental Table 1) – sex, tobacco, heart failure, coagulant use, previous use of angiotensin conversion enzyme inhibitor or receptor blocker, respiratory frequency.

Odds ratio, CI and P values were obtained by logistic regression analysis to predict outcome

Table 4 BP parameter differences accordingly to the affected selective regions of the brain related to central autonomic network and infarct volume

		24-h Averaged systolic BP, mm Hg Mean (SD)	P value *	HF spectral power, mm Hg^2 Median (IQR)	P value	HF spectral power, nu Median (IQR)	P value *	LF/HF ratio Median (IQR)	P value *
Right vs Left hemisphere infarct	Yes n=58	126.4 (16.7)	0.25	6.24 (2.62 – 17.3)	0.42	0.47 (0.28 – 0.64)	0.91	1.14 (0.57 – 2.58)	0.91
	No N=49	122.5 (18.0)		5.44 (1.82 – 13.6)		0.48 (0.24 – 0.64)		1.10 (0.56 – 3.21)	
Insular cortex (any side)	Yes n=62	123.4 (15.9)	0.43	5.26 (2.55 – 14.3)	0.71	0.46 (0.21 – 0.64)	0.40	1.16 (0.57 – 3.67)	0.40

	No N=44	126.1 (19.4)		6.84 (2.69 – 17.3)		0.49 (0.33 – 0.66)		1.06 (0.52 – 2.01)	
Right Anterior Insular cortex	Yes n=33	127.6 (17.2)	0.23	5.24 (2.62 – 20.5)	0.88	0.48 (0.20 – 0.71)	0.54	1.07 (0.40 – 3.91)	0.54
	No N=73	123.2 (17.4)		6.77 (2.51 – 17.3)		0.46 (0.30 – 0.62)		1.16 (0.61 – 2.35)	
Left Posterior Insular cortex	Yes n=19	120.8 (12.6)	0.31	4.79 (1.82 – 13.7)	0.40	0.34 (0.21 – 0.52)	**0.02**	1.93 (0.93 – 3.75)	**0.02**
	No N=87	125.4 (18.2)		6.77 (2.61 – 17.3)		0.49 (0.30 – 0.67)		1.03 (0.49 – 2.31)	
Cingulate Cortex	Yes n=5	135.0 (12.2)	0.17	5.24 (1.20 – 43.4)	0.93	0.34 (0.10 – 0.65)	0.14	1.96 (0.54 – 8.99)	0.14
	No N=101	124.0 (17.5)		6.24 (2.61 – 14.2)		0.48 (0.30 – 0.64)		1.09 (0.57 – 2.39)	
Right Amygdala	Yes n=15	128.7 (19.0)	0.33	6.77 (1.84 – 21.4)	0.93	0.48 (0.26 – 0.64)	0.83	1.09 (0.57 – 2.85)	0.83
	No N=91	123.9 (17.1)		5.89 (2.60 – 17.3)		0.47 (0.27 – 0.65)		1.13 (0.55 – 2.71)	

Left Amygdala	Yes n=11	126.3 (12.2)	0.73	10.3 (3.41 – 14.2)	0.46	0.33 (0.24 – 0.45)	**0.03**	2.02 (1.21 – 3.19)	**0.03**
	No N=95	124.4 (17.9)		5.62 (2.36 – 17.4)		0.48 (0.30 – 0.65)		1.07 (0.53 – 2.35)	
Frontobasal cortex	Yes n=20	129.0 (14.6)	0.21	8.76 (2.63 – 25.5)	0.37	0.45 (0.20 – 0.64)	0.65	1.22 (0.57 – 4.09)	0.65
	No N=86	123.5 (17.9)		5.79 (2.17 – 14.2)		0.48 (0.30 – 0.64)		1.10 (0.55 – 2.32)	
Diencephalic midline shift	Yes n=4	136.8 (13.4)	0.15	5.24 (1.95 – 79.5)	0.75	0.42 (0.10 – 0.68)	0.52	1.37 (0.48 – 8.99)	0.52
	No N=102	124.1 (17.4)		6.24 (2.60 – 15.1)		0.48 (0.29 – 0.64)		1.10 (0.57 – 2.49)	
Stroke volume, ml †	<=11.7 N=56	123.5 (18.2)	0.48	7.19 (1.59 – 25.1)	0.99	0.47 (0.21 – 0.64)	0.88	1.14 (0.56 – 3.82)	0.88
	>11.8 N=51	125.8 (16.4)		5.62 (2.98 – 12.0)		0.48 (0.32 – 0.63)		1.10 (0.58 – 2.11)	

Abbreviations: BP = blood pressure; CI = confidence interval; HF and LF = high and low frequency components of very short-term BP variability; nu = normalized units;

† Stroke volume was assessed at 24-hour CT scan and dichotomized by median value

Supplementary Data

Supplemental Table I Linear regression analysis exploring the relationship of baseline characteristics and relevant BP parameters

	24-h Averaged systolic BP, mm Hg		HF spectral power, mm Hg^2		HF spectral power, nu		LF/HF ratio	
	Beta (CI 95%)	P value	Beta (CI 95%)	P value	Beta (CI 95%)	P value	Beta (CI 95%)	P value
Age, years – mean (SD)	0.18 (-0.07 – 0.43)	0.15	0.007 (-0.006–0.02)	0.29	0.01 (-0.002 – 0.03)	0.09	-0.003 (-0.02 – 0.01)	0.61
Male sex – n (%)	**-7.61 (-14.1 - -1.10)**	**0.02[a]**	-0.12 (-0.48 – 0.25)	0.53	-0.18 (-0.55 – 0.19)	0.33	-0.09 (-0.45 – 0.28)	0.64
Prev. Stroke/TIA – n (%)	5.30 (-3.56 – 14.2)	0.24	0.09 (-0.42 – 0.59)	0.74	0.06 (-0.44 – 0.57)	0.80	-0.14 (-0.65 – 0.36)	0.57
Ischemic Heart Disease – n (%)	8.0 (-2.10 – 18.1)	0.12	0.04 (-0.52 – 0.60)	0.88	-0.003 (-0.56 – 0.55)	0.99	0.08 (-0.48 – 0.64)	0.78
Heart Failure – n (%)	-5.04 (-14.1 – 4.04)	0.27	0.10 (-0.42 – 0.62)	0.72	0.03 (-0.49 – 0.54)	0.92	**0.52 (0.01 – 1.03)**	**0.048**
Atrial Fibrillation – n (%)	3.59 (-3.06 – 10.2)	0.29	**0.79 (0.44 – 1.13)**	**<0.01[a]**	**0.57 (0.21 – 0.92)**	**<0.01[a]**	-0.19 (-0.56 – 0.18)	0.32
Hypertension – n (%)	6.15 (-0.77 – 13.1)	0.08	0.30 (-0.08 – 0.68)	0.12	**0.53 (0.16 – 0.89)**	**<0.01**	0.13 (-0.25 – 0.51)	0.50

Diabetes mellitus – n (%)	7.34 (-0.66 – 15.3)	0.07	0.28 (-0.15 – 0.71)	0.20	0.29 (-0.14 – 0.71)	0.19	0.31 (-0.11 – 0.74)	0.15
Dyslipidemia – n (%)	1.95 (-4.78 – 8.68)	0.57	-0.02 (-0.39 – 0.35)	0.92	-0.01 (-0.39 – 0.36)	0.94	0.13 (-0.24 – 0.50)	0.49
Obesity – n (%)	5.5 (-3.35 – 14.4)	0.22	0.17 (-0.33 – 0.67)	0.51	-0.04 (-0.54 – 0.47)	0.89	-0.14 (-0.64 – 0.37)	0.59
Smoker – n (%)	-5.60 (-15.4 – 4.22)	0.26	-0.42 (-0.94 – 0.10)	0.12	-0.02 (-0.55 – 0.51)	0.94	**0.57 (0.05 – 1.09)**	**0.03[a]**
Chronic medication – n (%)								
Statin	0.18 (-6.49 – 6.85)	0.96	0.02 (-0.35 – 0.39)	0.91	0.01 (-0.36 – 0.38)	0.95	0.15 (-0.22 – 0.51)	0.43
Antiplatelet	-0.95 (-8.45 – 6.55)	0.80	-0.08 (-0.49 – 0.33)	0.70	0.03 (-0.38 – 0.44)	0.89	0.28 (-0.13 – 0.69)	0.17
Anticoagulant	**8.57 (0.97 – 16.2)**	**0.02[a]**	**0.79 (0.37 – 1.20)**	**<0.01[a]**	0.08 (-0.36 – 0.52)	0.71	-0.16 (-0.60 – 0.29)	0.49
Antihypertensive	-0.33 (-7.39 – 6.73)	0.93	0.16 (-0.22 – 0.54)	0.42	**0.47 (0.10 – 0.84)**	**0.01**	0.13 (-0.25 – 0.51)	0.49
Beta-blocker	2.55 (-4.21 – 9.31)	0.46	0.30 (-0.10 – 0.69)	0.14	0.10 (-0.28 – 0.48)	0.60	0.05 (-0.07 – 0.17)	0.40
ACEI	4.32 (-2.46 – 11.2)	0.21	**0.40 (0.003 – 0.80)**	**0.048**	**0.78 (0.42 – 1.14)**	**<0.01[a]**	-0.02 (-0.14 – 0.10)	0.76
Calcium-channel Blocker	1.40 (-7.72 – 10.5)	0.76	0.36 (-0.18 – 0.91)	0.19	**0.73 (0.23 – 1.23)**	**<0.01**	-0.10 (-0.26 – 0.07)	0.24
24-h Averaged systolic BP, mm Hg			-0.002 (-0.01 – 0.01)	0.63	-0.003 (-0.01 – 0.01)	0.44	0.004 (-0.004 – 0.01)	0.36

Diastolic BP, mmHg – mean (SD)	0.05 (-0.12 – 0.23)	0.056	0.00 (-0.01 – 0.01)	1.00	-0.004 (-0.01 – 0.01)	0.45	0.01 (-0.01 – 0.02)	0.33
Glucose, mg	0.05 (-0.03 – 0.12)	0.20	0.001 (-0.003 – 0.01)	0.63	**0.004 (0.00 – 0.01)**	**0.03**	-0.002 (-0.01 – 0.003)	0.44
Respiratory Frequency, cycles/minute	-0.08 (-0.80 – 0.64)	0.83	-0.02 (-0.06 – 0.02)	0.43	**-0.06 (-0.10 - -0.02)**	**<0.01[a]**	-0.01 (-0.05 – 0.03)	0.67
TOAST Classification–n (%)	-0.10 (-0.09 – 0.29)	0.30	0.00 (-0.01 – 0.01)	0.97	$-1.5x10^{-5}$ (-0.01 – 0.01)	0.95	0.00 (-0.01 - -0.01)	0.93
NHISS baseline – median (IQR)	-0.02 (-0.57 – 0.52)	0.93	0.02 (-0.01 – 0.04)	0.12	**0.04 (0.01 – 0.07)**	**0.02**	-0.02 (-0.05 – 0.01)	0.18
Occlusion site – n (%)	5.42 (-1.15 – 12.0)	0.11	-0.07 (-0.46 – 0.33)	0.74	-0.13 (-0.51 – 0.24)	0.49	0.01 (-0.11 – 0.12)	0.93
ASPECTS Score–median (IQR)	0.25 (-1.73 – 2.23)	0.80	0.07 (-0.04 – 0.18)	0.23	0.02 (-0.09 – 0.13)	0.69	0.04 (-0.07 – 0.15)	0.45
Collateral grade	-4.01 (-8.74 – 0.72)	0.10	-0.28 (-0.62 – 0.05)	0.10	-0.20 (-0.47 – 0.07)	0.14	0.04 (-0.01 – 0.08)	0.11
IV Thrombolysis – n (%)	**-6.89 (-13.7 – -0.12)**	**0.046**	-0.08 (-0.45 – 0.29)	0.68	-0.01 (-0.38 – 0.36)	0.95	0.14 (-0.23 – 0.51)	0.45
Grade 3 mTICI scale – n (%)	6.42 (-0.44 – 13.3)	0.07	0.25 (-0.13 – 0.63)	0.20	0.06 (-0.33 – 0.45)	0.76	0.09 (-0.30 – 0.48)	0.65
Hemorrhage (PH1-PH2) – n (%)	9.68 (-10.4 – 29.8)	0.34	-0.24 (-1.27 – 0.78)	0.64	-0.17 (-1.19 – 0.86)	0.75	-0.12 (-1.15 – 0.91)	0.82

LSW to TPA, hours – median (IQR)	0.02 (-0.003 – 0.04)	0.10	0.00 (-0.001 – 0.001)	0.79	0.00 (0.00 – 0.001)	0.26	0.00 (0.00 – 0.001)	0.95
LSW to Rec., hours – median (IQR)	0.002 (-0.01 – 0.01)	0.58	0.00 (0.00 – 0.001)	0.95	0.00 (-0.001 – 0.00)	0.79	0.00 (0.00 – 0.001)	0.86
Gt5LSW to TCD, hours – median (IQR)	0.00 (-0.002 – 0.004)	0.41	0.00 (0.00 – 0.001)	0.28	0.00 (0.00 – 0.001)	0.62	0.00 (0.00 – 0.001)	0.58

Abbreviations: ACEI = Angiotensin Converting-Enzyme Inhibitor; ARB = Angiotensin Receptor Blocker; ASPECTS = The Alberta Stroke Program Early Computed Tomography Score (ASPECTS); BP = blood pressure; ICA = internal carotid artery; IQR = interquartile range; LSW = last-seen-well time; M1 = main trunk of the middle cerebral artery; M2 = first-order branch of the main trunk of the middle cerebral artery; mTICI = modified Thrombolysis in Cerebral Infarction; NHISS = Scores on the National Institutes of Health Stroke Scale; nu = normalized units; SD = standard deviation; HF and LF = High and low frequency powers of BP; TOAST = Trial of Org 10172 in Acute Stroke

Beta coefficients (95% CI) and P values were calculated by univariate linear regression for BP parameters with each brain are parameter as independent variables. The values of $P<0.05$ are highlighted in bold.

[a]Remained significantly associated ($p<0.05$) after forward stepwise multivariate logistic regression.

ANEXO

Normas de Publicação - STROKE

Manuscript Formatting

- 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.
- Basic Science submissions: Authors are required to submit an online checklist requesting reporting of randomization procedures, blinding, a priori definition of

inclusion and exclusion, etc. After selecting a Basic Science article type in the submision system, the form becomes avialable to complete as part of the submission process. If the manuscript is accepted, the form will be published as supplementary material. See Reporting 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.
- Authorship Responsibility and Copyright Transfer Agreement Forms (and Licensing Agreements for Original Contributions) are ONLINE ONLY. Forms will be required PRIOR to resubmission, or if the manuscript has only one version (e.g., a letter to the editor) after acceptance. Each author will be sent an email containing a link to the form at the appropriate time.
- 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:
- Full title of the article, limited to 120 characters.
- Authors’ names, highest academic degree earned by each, authors’ affiliations
- Name and complete address for the corresponding author, and address for reprints if different from address for correspondence. Please also include any study group or collaboration in the author list, i.e., “ . . .Last Author, on behalf of the Stroke Study Group”
- Email address and telephone for the corresponding author.
- **Optional**: *Stroke* posts about its published articles on Facebook and Twitter. If you would like us to include an author or department social media handle in our posts, please provide it on the title page.
- Cover title (total characters must not exceed 50, including spaces) to be typeset on the top of the journal page.
- 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.
- Subject Terms for use as search terms across online journals article collections database. Please select from the Journal Subject Terms List.

- Specify the number of words in the whole document on your title page, e.g., Word Count: 4896. Word count should include all parts of the manuscript (i.e., title page, abstract, text, acknowledgments, sources of funding, disclosures, references, figure legends, tables, and appendices intended for print publication). Over-length manuscripts will NOT be accepted for publication. See the Costs to Authors above.

Abstract

- Do not cite references in the abstract.
- Limit use of acronyms and abbreviations.
- Be concise (**300** words, maximum).
- December 2015: For authors following the PRISMA guideline, please use the journal abstract headings detailed below.
- 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.
    - Example 4: Clinical Trial Registration-This trial was not registered because enrollment began prior to July 1, 2005.

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

Methods:

- **Please ensure that your manuscript adheres to the AHA Journals' implementation of the Transparency and Openness Promotion (TOP) Guidelines (available online at http://www.ahajournals.org/content/TOP-guidelines). This means adding a sentence about data availability to the beginning of the Methods section.**
- 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.
- Randomization, allocation concealment and blinding: All animals must be randomized. Investigators responsible for surgical procedures or drug treatments must be blinded. End point assessments must be performed by investigators blinded to the groups for which each animal is assigned.
- Any submitted meta-analyses should follow the PRISMA or MOOSE guidelines. The authors must clearly state in the Methods section which guideline was followed. If you

use the PRISMA guidelines, please include a copy of the PRISMA checklist as a related manuscript file (not for publication) and include a flow diagram in your manuscript or supplemental data. The authors should use journal formatting for abstracts. Details on PRISMA guidelines can be found here http://www.prisma-statement.org. Details on MOOSE can be found via the EQUATOR Network.

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

The acknowledgments section lists all substantive contributions of individuals. Author contributions may be listed in the Acknowledgments section. Authors should obtain written, signed permission from all *non-author* individuals listed in the "Acknowledgments" section of the manuscript, because readers may infer their endorsement of data and conclusions. These permissions must be provided to the Editorial Office. Please see the Acknowledgment Permission Form. The corresponding author must mark the following statement on the ONLINE ONLY Copyright Transfer Agreement form or Licensing Agreement, certifying that (1) all persons who have made substantial contributions in the manuscript (e.g., data collection, analysis, or writing or editing assistance), but who do not fulfill authorship criteria, are named with their specific contributions in the Acknowledgments section of the manuscript; (2) all persons named in the Acknowledgments section have provided the corresponding author with written permission to be named in the manuscript; and (3) if an Acknowledgments section is not included, no other persons have made substantial contributions to this manuscript.

Sources of Funding

Authors must list all sources of research support relevant to the manuscript in this location. All grant funding agency abbreviations should be completely spelled out, with the exception of the NIH. Note that funding should be listed separately from disclosures.

Disclosures

Authors must state disclosures in the manuscript text prior to first review and provide disclosures online when submitting a revision or upon request after acceptance. Disclosures stated in the text must match the online disclosures. If you have no disclosures, please state "Disclosures: None" in the manuscript text before the references. Conflicts of interest pertain to relationships with pharmaceutical companies, biomedical device manufacturers, or other corporations whose products or services are related to the subject matter of the article. Such relationships include, but are not limited to, employment by an industrial concern, ownership of stock, membership on a standing advisory council or committee, being on the board of directors, or being publicly associated with the company or its products. Other areas of real or perceived conflict of interest could include receiving honoraria or consulting fees or receiving grants or funds from such corporations or individuals representing such corporations. The corresponding author should collect Conflict of Interest information from all co-authors before submitting a manuscript online.

References

- 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.
- Personal communications, unpublished observations, and submitted manuscripts must be cited in the text, not in the references, as "([name(s)], unpublished data, 2017)"
- References must be from a full-length publication in a peer-reviewed journal.
- Abstracts may be cited only if they are the sole source and must be identified in the references as "Abstract"
- "In-press" citations must have been accepted for publication and the name of the journal or book publisher included. Please provide a copy of any potentially overlapping manuscript that has been submitted to another journal or is in press or published elsewhere.

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).

Database reference: CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute; 1996. Updated March 29, 1996.

Figure Legends

Provide figure legends on a separate page of the manuscript.

Permission is required for all images that are reused or adapted from another source. To obtain permission, please follow the instructions provided by the copyright holder or listed in the license agreement. This includes Creative Commons material; please refer to http://creativecommons.org/licenses for more information about properly crediting Creative Commons sources. Follow the copyright holder or licensor's requirements for credit attributions and provide them in the figure legend. If no language is provided in the permission letter, use the following sample: Reprinted from Lin et al,[19] with permission from Science Publishing. Copyright 2016, American Science Society.

Tables

- Each table must be typed on a separate sheet and double-spaced, if possible. The table number should be Arabic, followed by a period and a brief informative title.
- Use the same size type as in text.
- Tables should be cell-based (i.e., constructed using Microsoft Word tables or Excel). Do not use tabs or hard returns. Do not supply tables as graphics.
- 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.
- No internal shading is permitted.
- 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.
- Permission is required for all tables that are reused or adapted from another source. To obtain permission, please follow the instructions provided by the copyright holder or listed in the license agreement. Follow the copyright holder or licensor's requirements for credit attributions and provide them in the table footnote. If no language is provided in the permission letter, use the following sample: Reprinted from Roberts et al,[14] with permission from Smith Publishing. Copyright 2015, American Society of Medical Research.

Figures

- The combined total number of figures and tables is limited to 6 (3 for Brief Reports). Each figure may contain up to 4 panels (i.e., parts A to D) and must conform to the requirements for figures described below.
- Authors should be pleased with the figure submission quality before submission. We recommend that you print the figure at its final publication size to check the quality.
- Figures should be submitted as high-resolution TIFF or EPS files. PowerPoint files are discouraged because elements within the figure (such as axis labels) may shift location or drop out during conversion. Further, do not create figures in Powerpoint

because even if you convert to a different file type, the resolution will be too low for publication. JPEG, Word, PPT, and Excel files should not be used. See **Artwork and Table Guidelines** (PDF) for instructions for creating high-quality digital art.

- Figures should be supplied at the highest resolution possible for optimal clarity. Color figures should be at least 300 dpi; halftones, 600 dpi; and line art, 1200 dpi.
- Figures should be submitted at the final publication size. Please note that most figures will be sized at 1 column wide. Dimensions for figures are:

- 1 column: 3.25 inches wide (8 cm or 19.5 picas)
- 2 columns: 6.80 inches wide (17.272 cm or 40.8 picas)

- Color figures should be in RGB (red/green/blue) mode. If a figure is supplied in CMYK (cyan/magenta/yellow/black) mode, there may be a shift in the appearance of colors, especially fluorescents. Figures that will appear in black and white should be submitted in black and white.
- For line and bar graphs and pie charts, ensure that the colors/lines/symbols used for the different sets of data are easily distinguishable. Hair lines are hard to reproduce as are lines that are too thick, as they may make it hard to distinguish between the coordinates.
- Graphs and charts should have a white background.
- Labels for panels should be uppercase letters (A, B, C, D) in boldface Arial or Helvetica.
- Multipart figures may have no more than 4 panels (i.e., A, B, C, D).
- Multipart figures may be set at 2 columns across the page and should be laid out horizontally if appropriate.
- Use the same font (typeface) throughout the figure. Sans serif fonts, such as Arial and Helvetica, work best.
- Use the largest font size possible without distorting the figures. Text for super- or subscripts should be no smaller than 6 points.
- Whenever possible, all text within a figure should be the same size. If this is not possible, the font size should vary by no more than 2 points.
- Label units of measure consistently with the text and legend. Follow the AMA for unit abbreviations.
- Incorporate figure keys into the legend rather than including them as part of the figure whenever possible.
- Avoid heading/Title on the figure. Title information should be included in the figure legends.
- Any abbreviations or symbols used in the figures must be defined in the figure or figure legend.
- Follow AMA 9th edition for footnote style in legends.
- If the figure is reprinted/adapted from another source, please provide a permission letter and include the source in the legend as noted above.
- Supply a scale bar with photomicrographs.
- Authors are responsible for the cost of printing color illustrations. Authors are also responsible for obtaining from the copyright holder permission to reproduce previously published artwork.
- See AMA, 10th edition, Section 4.2 for more information on figures.

Visual Abstract (ONLY for Basic Science Articles)

The intent of the visual abstract is to provide readers with a succinct summary of the study in a form that facilitates its dissemination in presentations. It can be submitted at any time, but is an absolute requirement for revision submissions of Basic Science submissions.

- A single figure panel/diagram/cartoon.
- **Emphasize the new findings in the paper and clinical implications.**
- Size: The submitted document should be no larger than 18 cm (7 inches) square.
- Font: Prefer a san serif font that is no less than 12 point. Use the largest font size possible without distorting the figure.
- Do include a legend of no more than 50-100 words.
- Do not include data items; all content should be graphical.
- Please upload as Supplemental Material as a JPG file format. **October 1, 2018: Due to a change in vendor, we now require a JPG instead of a PDF for the Visual Abstract.** This is separate from the single Supplemental PDF containing additional manuscript content noted below.

Supplemental Material

- This optional section provides an opportunity for authors to present supporting materials to the manuscript. The manuscript appears both in the print version and online, whereas Supplemental material are independent from the manuscript and appear only online in the format submitted by the authors. Supplemental material undergoes peer review and must be submitted simultaneously with original submissions.
- Any collaborators who need to be cross-referenced in PubMed should be listed either as authors or, for study groups, in the main manuscript file as an Appendix. This information is included in the word count. If contributors do not need to be listed as authors or cross-referenced in PubMed, then they may be included in a PDF Supplemental Material File.
- The guidelines below should be used for supplemental material:
- Material to be published as an online only supplement should be uploaded online as a single PDF. An exception to this would be if the online supplement is a video file or an Excel file that contains too much material (e.g., hundreds of rows and columns that cross muliple pages) to convert to PDF and still be easily readable.
- The supplemental material should have a title page with the label of SUPPLEMENTAL MATERIAL above the title. The supplemental material to be included in this PDF is as follows: Supplemental Methods, Supplemental Tables, Supplemental Figures and Figure Legends, and Supplemental References. If applicable, the legends for the Video files should also be included in this PDF.
- 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.
- When referring to online-only material in the print version of the manuscript, use the phrase “please see https://www.ahajournals.org/journal/str”
- Supplemental Material appears only online and will not appear in reprints of the article. The Editorial Office is not responsible for converting files to PDF.