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MESTRADO INTEGRADO EM MEDICINA

Miguel Carvalho Neto de Queirós Pimenta

Anemia and its association with global disability outcomes in stroke patients who underwent reperfusion therapy

Anemia e a sua associação com prognóstico funcional em doentes com AVC isquémico submetidos a tratamento de reperfusão

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Faculdade de Medicina da Universidade do Porto, 29/03/2021

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DESIGNAÇÃO DA ÁREA DO PROJECTO

Medicina Clínica

TÍTULO DISSERTAÇÃO

Anemia and its association with global disability outcomes in stroke patients who underwent reperfusion therapy

ORIENTADOR

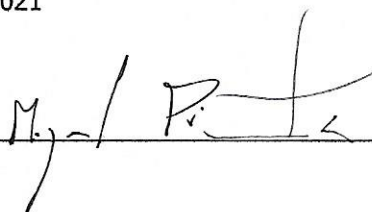Professor Doutor Pedro Miguel Araújo Campos de Castro

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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.	X
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, 29/03/2021

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Abstract

Ischemic strokes are one of the most important causes of mortality and mobility in the industrialized countries, including Portugal. In recent years, reperfusion therapies of ischemic stroke as improved and nowadays the use of thrombolysis and thrombectomy is indicated in a increasing number of patients. Anemia is a common pre-morbid condition in ischemic stroke patients, and it has a negative impact on the functional outcome and mortality of patients treated with a conservative approach. Its effect on patients subjected to reperfusion therapy still requires further study.

We studied the effect of anemia before and until 24 hours after reperfusion therapies on the prognosis of ischemic stroke patients.

We conducted a retrospective case-control analysis of ischemic stroke patients admitted to our comprehensive stroke centre and submitted to reperfusion therapy (thrombectomy and/or thrombolysis) between January and December of 2019

We reviewed 332 patients, of which 84 patients were excluded. We included 248 patients in the analysis with a median age of 76 years old, with 41.9% being male.

At baseline, the median Hb level was 13.5 mg/dL and after procedure there was a median drop of 0.8 mg/dL of Hb. Furthermore, according to the WHO criteria, 21.8% of the cohort presented with anemia, of which two thirds were mild. However, after reperfusion therapy, 41.9% of the cohort had anemia.

We found that the presence of anemia (before treatment and after treatment) in ischemic stroke patients was associated with a worse functional outcome and mortality rates.

Only patients submitted to EVT showed an association with worse functional outcome (p = 0,044) and patients submitted to IVT (p = 0,024) an association with worse survival. No statistically significant difference was found in the outcomes for any of the other treatment modalities, however there was a tendency towards worse functional and mortality outcomes in anemic patients in every treatment subgroup.

Our study demonstrates that the presence of anemia (both at admission and in the first 24 hours after the procedure) is associated with a worse degree of disability as well as to a higher rate of mortality in the first 3 months, however this association might be driven by other factors. Nevertheless, this might present as an interesting and important factor for further studies and for improvement in strategies of nationwide stroke prevention

Resumo

O acidente vascular cerebral (AVC) isquémico é uma das principais causas de morbilidade e mortalidade nos países industrializados, incluindo Portugal. Recentemente, as terapias de reperfusão melhoraram e atualmente a utilização de trombólise (IVT) e trombectomia (EVT) está indicada num número cada vez maior de doentes. A anemia é frequentemente observada como uma condição pré-mórbida dos doentes com AVC isquémico, apresentando um efeito negativo no prognóstico dos doentes com AVC isquémico tratados conservadoramente. No entanto, o seu efeito em doentes tratados com terapêuticas de reperfusão ainda permanece fracamente estudado.

Estudámos o efeito que a anemia pré-procedimento de reperfusão, assim como o desenvolvimento de anemia nas primeiras 24 horas pós-procedimento, terá no prognóstico de doentes com AVC isquémico.

Realizámos uma análise caso-controlo retrospetiva dos doentes com AVC isquémico submetidos a tratamentos de reperfusão (trombectomia e/ou trombólise) entre janeiro e dezembro de 2019.

Recolhemos dados de 332 doentes, dos quais 84 foram excluídos. Incluímos 248 pacientes na análise, com uma idade mediana de 76 anos, dos quais 41,9% eram do sexo masculino.

Demonstrámos que a presença de anemia (antes e após tratamento) está associada a um pior prognóstico funcional e a uma pior taxa de mortalidade nos doentes com AVC isquémico.

Apenas nos pacientes submetidos a trombectomia encontrámos uma associação com um pior prognóstico funcional (p = 0,044) e apenas nos pacientes submetidos a trombólise encontrámos uma associação com piores taxas de sobrevivência (p = 0,024). Nenhuma diferença estatisticamente significativa foi encontrada para os outcomes em qualquer um dos outros subgrupos de tratamento, no entanto houve uma tendência para um pior prognóstico funcional e para uma pior taxa de mortalidade em doentes anémicos em todos os grupos.

O nosso estudo demonstrou que a presença de anemia (tanto antes da admissão como nas primeiras 24 horas após o procedimento) está associada a um pior prognóstico funcional assim como a uma maior taxa de mortalidade nos primeiros 3 meses, no entanto esta associação pode ser devida a outros fatores. Ainda assim, este pode ser um fator importante para estudos futuros e para o melhoramento das estratégias de prevenção de AVC a nível nacional.

Title: Anemia and its association with global disability outcomes in stroke patients who underwent reperfusion therapy

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Introduction

The central factor in the pathophysiology of ischemic stroke is the impairment of oxygen and glucose supply due to vessel occlusion. (1) The brain receives 15% of resting cardiac output and, among all human organs, it is the least tolerant of ischemia.(2) After the infarct core is rapidly destroyed, the penumbra may still be salvaged, however survival of penumbra tissue depends on timely reperfusion and collateral perfusion.(1, 3) Currently two reperfusion techniques are indicated on selected cases of acute ischemic stroke (AIS): intravenous thrombolysis (IVT) and endovascular thrombectomy (EVT).(4)

Although collateral perfusion is paramount, it seems only logical that optimal oxygen-carrying capacity in the blood (strongly related to the hemoglobin level) might also be decisive for penumbral fate and neurons survival, as the brain tissue depends mainly on oxidative sources of energy.(1)

In fact, changes on hemoglobin (Hb) level are frequent and anemia is present on admission in up to 25% of patients with AIS.(5)

Two meta-analysis have shown that the presence of anemia is related to an increase in the mortality risk in patients with stroke but they have also presented notoriously asymmetrical

funnel plots, which raises some concern on whether these results might be influenced by publication bias.(6, 7) The outcome of many of these studies was mortality and not long-term disability, which suggests that this topic still deserves some debate and new analysis. Two studies have investigated the effect of anemia on outcome in thrombolysis-treated patients and only one study has looked into the association between anemia and both thrombectomy and thrombolysis-treated patients, concluding that anemia was an independent predictor for poor functional outcome and mortality in IVT-treated stroke patients. (1, 5, 8)

Previous studies evaluated the association between anemia on admission and development of anemia during hospital stay or up to 5 days after stroke, however AIS patients treated with IVT or EVT will have either been reperfused successfully or will have a definite infarct core by 24 hours after symptom onset.(9)

Therefore oxygen-transport capacity must be optimal mainly during the hyperacute and acute phase of AIS (first 24 hours).

When taking into consideration anemia and Hb levels, we must also consider other factors, including hemodilution and blood viscosity.

Besides, anemia might also be responsible for some indirect and less obvious effects which might as well play an important role: for instance, it has been found that anemic patients who underwent cardio-pulmonary bypass had an increased incidence of neurological injuries in the setting of a paradoxical cerebral hyperemia, as the increase in the cerebral blood flow was responsible for a concomitant increase in the risk of embolic events.(10)

So far, the optimum Hb level in acute ischemic stroke and the prognostic value of anemia in IVT and EVT treated patients remain unclear and definite guidelines are still lacking.(4)

The aim of this study is to study the impact of the presence of anemia in early neurological recovery as well as functional outcome of stroke patients treated with EVT and IVT.

Methods

Study Population

We included all ischemic stroke patients admitted to our comprehensive Stroke Center with ≥18 years of age with acute ischemic stroke of the anterior circulation who received intravenous thrombolysis or endovascular thrombectomy between January 2019 and December 2019. We excluded posterior circulation strokes, patients with active cancers, absent or inadequate imaging at 24 hours, and no follow-up data at 90 days. This study was approved by the local Ethics Committee.

Data collection and clinical variables

Demographic and clinical data, including vascular risk factors, previous medications, past medical history, signs of acute inflammatory response (Blood Pressure, temperature, heart rate and respiratory rate) in the first 24 hours after treatment were retrospectively collected. National Institute of Health Stroke Scale (NIHSS) scores were recorded at baseline and at 24 hours after recanalization. Stroke etiology was classified according to the Trial of Org 10172 in Acute Stroke Treatment scale (TOAST).

Laboratory parameters

Admission and at 24h laboratory results of hemoglobin (Hb), hematocrit (Hct), white blood cell count and platelet count were collected retrospectively, as well as levels of vitamin B12, folates and ferritin in the first 24 hours.(11) The levels of Hb were recoded into a dichotomic variable (0 = absence of anemia and 1 = presence of anemia), following the WHO reference values for anemia for both male and female subjects (i.e. anemia in non-pregnant women and in men over 15 years of age corresponds to a value of Hb below 12 g/dL and 13 g/dL respectively).(19)

Radiological endpoints

Computed tomography (CT) was performed at admission and 24 hours after therapy. The Alberta Stroke Program Early Computed Tomography Score (ASPECTS) was calculated at admission.(12) Large-vessel occlusion was evaluated with the admission CT angiography. Recanalization was classified using the modified Thrombolysis In Cerebral Infarction scale (TICI).(13) The 24-hour CT scan was performed in a single machine (Siemens Somaton Emotion Duo, Erlangen, Germany) to determine the infarct volume and the presence of intracerebral hemorrhage. Infarct volume was estimated using the A×B×C/2 method.(14)

Clinical outcomes

We assessed the functional outcome by a dichotomization of the modified Rankin scale (mRS): 3-6 (dependent or dead) versus 0-2 (independent) at 90 days. We also evaluated the initial response to recanalization therapy with early neurological deterioration (END), defined as any increase in NIHSS at 24 hours from the baseline.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics® version 25. Significance was set at $p<0.05$. Testing for normality was performed with the Shapiro Wilk Test. We investigated associations between Hb and outcome measures using Hb as a continuous variable and as a

categorical variable distinguishing anemia and degrees of anemia as previous stated and according to WHO.

Continuous data were summarized as mean and standard deviation or median and interquartile range when appropriate. We used Chi-square test and Fisher's exact test for categorical variables where appropriate and the Mann–Whitney U-test for continuous variables.

Results

We reviewed 332 patients, of which 84 patients were excluded. We included 248 patients in the analysis. Baseline characteristics of the cohort are displayed on Table 1. The median age of the cohort was 76 years old, with 41.9% male.

Previous stroke/transient ischemic attack (TIA) and acute myocardial infarction (AMI) were present in 19.8% and 7.7% of the cohort, respectively. 32.7% of the cases were under antiplatelet therapy. Although atrial fibrillation (AF) was identified in 29.4% of patients, only 13.8% were already under anticoagulation therapy, showing the work still to be done in early identification of AF. Baseline median NIHSS was 15 and although around 44% of stroke was classified as cardioembolic, still around 28.6% had a negative complete evaluation.

At baseline, the median Hb level was 13.5 mg/dL and Hct level was 40.1% and after procedure there was a median drop of 0.8 mg/dL of Hb. Furthermore, according to the WHO criteria, 21.8% of the cohort presented with anemia, of which two thirds were mild. However, after reperfusion therapy, 41.9% of the cohort had anemia.

Baseline characteristics	Cohort N=248 (100%)	Thrombolysis N=84	EVT N =95	IVT+EVT N=69
Demographic and clinical characteristics				
Male, n (%)	104 (41.9)	41 (48.8)	31 (32.6)	32 (46.4)
Age – years, *median (IQR)*	76 (17)	76 (18)	76 (18)	76 (18)
Independent – n (%)	202 (81.5)	67 (79.8)	75 (78.9)	60 (87.0)
Hypertension, *n (%)*	190 (76.6)	68 (81.0)	72 (75.9)	50 (72.5)
Diabetes mellitus, *n (%)*	76 (30.6)	31 (36.9)	25 (26.3)	20 (29.0)
Dyslipidemia, *n (%)*	164 (66.1)	62 (73.8)	61 (64.2)	41 (59.4)
Atrial Fibrillation, *n (%)*	73 (29.4)	8 (9.5)	47 (49.5)	18 (26.1)
Previous Stroke/TIA, *n (%)*	49 (19.8)	15 (17.9)	23 (24.2)	11 (15.9)
Previous AMI, n (%)	19 (7.7)	7 (8.3)	7 (7.4)	5 (7.2)
Heart Failure, n (%)	41 (16.5)	8 (9.5)	22 (23.2)	11 (15.9)
Chronic hepatic disease, n (%)	5 (2.0)	0 (0)	3 (3.2)	2 (2.9)
Hypothyroidism, n (%)	12 (4.8)	4 (4.8)	5 (5.3)	3 (4.3)
Auto-immune disease, n (%)	6 (2.4)	0 (0)	4 (4.3)	2 (2.9)
Antiplatelet, *n (%)*	81 (32.7)	34 (40.5)	24 (25.3)	23 (33.3)
Anticoagulation, n (%)	34 (13.8)	2 (2.4)	30 (31.5)	2 (2.8)
GFR, in mg/dL, median (IQR)	81.5 (34.8)	80 (42.0)	80.5 (34.8)	85 (28.0)
Stroke characteristics				
Baseline NIHSS, *median (IQR)*	15 (12)	8 (12)	16 (9)	18 (12)
Glycemia – mg/dL, *median (IQR)*	130 (49)	132.5 (59)	129 (51)	133 (40)
Systolic BP admission - mmHg, mean (SD)	145 (24)	149 (24)	142 (33)	141 (25)
Diastolic BP admission – mmHg, mean (SD)	80 (15)	82 (14)	78 (16)	79 (23)
ASPECTS at admission, *median (IQR)*	10 (2)	10 (0)	9 (3)	9 (2)
Collateral Score, n (%)				
0	3 (1.6)	1 (1.6)	1 (1.3)	1 (1.9)
1	29 (15.3)	6 (9.8)	10 (13.3)	13 (24.5)
2	36 (19.0)	4 (6.6)	25 (33.3)	7 (13.2)
3	120 (63.5)	50 (82.0)	38 (50.7)	32 (60.4)
4	1 (0.5)	0 (0)	1 (1.3)	0 (0)
Etiology				
Cardioembolic, n (%)	109 (44)	16 (19.0)	60 (63.2)	33 (47.8)
Large Artery Atherosclerosis, n (%)	39 (15.7)	18 (21.4)	9 (9.5)	12 (17.4)
Small vessel disease, n (%)	4 (1.6)	4 (4.8)	0 (0)	0 (0)
Other (Carotid Dissection), n (%)	6 (2.4)	1 (1.2)	4 (4.2)	1 (1.4)
Incomplete evaluation, n (%)	15 (6.0)	4 (4.8)	6 (6.3)	5 (7.2)
Negative Evaluation, n (%)	71 (28.6)	38 (45.2)	16 (16.8)	17 (24.6)
Hb parameters				
Hb pre procedure – g/dL, median (IQR)	13.5 (2.3)	13.4 (2.4)	13.2 (2.4)	14.1 (2.08)
Hct pre procedure - %, median (IQR)	40.1 (6.0)	40.2 (5.9)	39.4 (6.0)	41.6 (6.3)
White Blood Cell Count in 10^9/L, median (IQR)	8.96 (4.28)	8.08 (3,83)	9.23 (4.73)	9.67 (4.85)
Platelet count in 10^9/L, median (IQR)	210 (86)	202 (86)	204 (97)	214 (79)
Hg post procedure – g/dL, median (IQR)	12.5 (2.0)	12.7 (1.8)	12.1 (2.3)	12.9 (2.2)
Hct post procedure - %, median (IQR)	37.4 (7.0)	38.9 (5.2)	36.3 (6.2)	38.0 (6.7)

Vitamin B12 deficiency, n (%)	21 (8.5)	7 (8.3)	6 (6.3)	8 (11.6)
Folate deficiency, n (%)	0 (0)	0 (0)	0 (0)	0 (0)
Iron Deficiency, n (%)	1 (0.4)	0 (0)	1 (1.1)	0 (0)
Delta Hg - g/dL, median (IQR)	- 0.8 (-1.27)	-0.8 (-1.3)	-0.9 (-1.1)	-0.85 (-1.45)
Delta Hct - %, median (IQR)	-2 (-3.5)	-1.7 (-3)	-2 (-3.9)	-2.15 (-3.77)
Anemia pre-procedures, n(%)	54 (21.8)	20 (23.8)	23 (24.2)	11 (15.9)
Anemia post procedures, n(%)	104 (41.9)	33 (39.3)	48 (50.5)	23 (33.3)
Anemia Degree pre procedure				
Mild n(%)	36 (14.5)	13 (15.5)	15 (15.8)	8 (11.6)
Moderate/Severe, n(%)	18 (7.3)	7 (8.3)	8 (8.4)	3 (4.3)
Anemia Degree post procedure				
Mild n(%)	65 (26.2)	23 (27.4)	28 (29.5)	14 (20.3)
Moderate/Severe,n (%)	39 (15.7)	10 (11.9)	20 (21.1)	9 (13.0)
Post-intervention characteristics				
mTICI 2b-3, n (%)	189 (78.8)	71 (84.5)	70 (73.7)	48 (69.6)
Presence of SIRS, n (%)	49 (19.8)	12 (14.3)	17 (17.9)	20 (30.8)
24 hours NIHSS, *median (IQR)*	10 (16)	4 (8)	14 (15)	13 (15)
Discharge/7 days NIHSS, median (IQR)	7 (16)	2 (6)	12.5 (17)	9.5 (14)
Infarct volume – ml, *median (IQR)*	15 (50)	1.3 (16)	25.8 (49.7)	29.9 (84.1)
sICH, n (%)	63 (25.4)	10 (11.9)	29 (30.5)	24 (34.8)
ICH Classification, n (%)				
H1	14 (5.6)	3 (3.6)	6 (6.3)	5 (7.2)
H2	37 (14.9)	4 (4.8)	21 (22.1)	12 (17.4)
PH1	8 (3.2)	2 (2.4)	1 (1.1)	5 (7.2)
PH2	4 (1.6)	1 (1.2)	1 (1.1)	2 (2.9)
Outcomes				
Early Neurological Deterioration, n (%)	73 (29.6)	16 (19.0)	35 (37.2)	22 (31.9)
Dependent at 3 months, n (%)	147 (59.3)	37 (44.0)	65 (68.4)	45 (65.2)
Dead at 3 months, n (%)	44 (17.7)	12 (14.3)	21 (22.1)	11 (15.9)

Table 1 Outcome and descriptive baseline statistics of 287 subjects (sorted by treatment)

Comparative analysis between anemic patients and non-anemic patients before and after treatment reperfusion was performed (Table 2) and the outcomes and represented in Figures 1, 2, 3 and 4). Anemic patients before and after reperfusion therapies had worse functional outcome at 3 months (p-value 0.005 and 0.002 respectively) and higher mortality at 3 months (p-value 0.002 and 0.022) respectively, despite similar rates of early neurological deterioration.

It is worth noting however that anemic patients before and after reperfusion therapies had a few differences. Anemic patients were older, were less often functionally independent, had higher rates of hypertension, diabetes mellitus, heart failure, lower glomerular filtration rate and a worse NIHSS at baseline. (p-value <0.05).

Anemic patients had similar ASPECTs and Collateral scores at admission, reperfusion successes (mTICI) and similar rates of hemorrhagic transformation (p-value >0.05).

Baseline characteristics	Cohort N=248 (100%)		p-value	Cohort N=248 (100%)		p-value
	Normal Hb pre-reperfusion N=166	Anemia pre-reperfusion N=54		Normal Hb pos-reperfusion N=126	Anemia pos-reperfusion N=104	
Demographic and clinical characteristics						
Male, n (%)	77 (44)	20 (37.0)	0.370	53 (42.1)	49 (47.1)	0.443
Age – years, *median (IQR)*	73 (19)	82 (12)	<0.001**	74 (17)	78 (17)	0.013*
Independent – n (%)	145 (87.3)	37 (68.5)	0.001**	114 (90.5)	75 (72.1)	<0.001**
Hypertension, *n (%)*	117 (70.5)	48 (88.9)	0.007*	91 (72.2)	87 (83.7)	0.039*
Diabetes mellitus, n (%)	46 (27.7)	20 (37.0)	0.194	32 (25.4)	40 (38.5)	0.033*
Dyslipidemia, *n (%)*	106 (63.9)	38 (70.4)	0.382	82 (65.1)	74 (71.2)	0.326
Atrial Fibrillation, n (%)	42 (25.3)	17 (31.5)	0.373	29 (23.0)	29 (27.9)	0.397
Previous Stroke/TIA, *n (%)*	38 (22.9)	7 (13.0)	0.116	27 (21.4)	20 (19.2)	0.681
Previous AMI, n (%)	14 (8.4)	3 (5.6)	0.491	11 (8.7)	5 (4.8)	0.245
Heart Failure, n (%)	19 (11.4)	15 (27.8)	0.004*	10 (7.9)	22 (21.2)	0.004**
Chronic hepatic disease, n (%)	4 (2.4)	2 (3.7)	0.253	2 (1.6)	1 (1.0)	0.692
Hypothyroidism, n (%)	8 (4.8)	4 (7.4)	0.440	3 (2.4)	9 (8.7)	0.028*
Auto-immune disease, n (%)	4 (2.4)	1 (1.9)	0.829	2 (1.6)	3 (2.9)	0.473
Antiplatelet, *n (%)*	55 (33.1)	18 (33.3)	0.978	40 (31.7)	34 (32.7)	0.878
Anticoagulation, n (%)	21 (12.6)	7 (13)	0.952	12 (9.5)	15 (14.4)	0.251
GFR, in mg/dL, median (IQR)	85 (29)	71.5 (42.5)	0.001**	85 (27)	79 (45)	0.013*
Stroke characteristics						
Baseline NIHSS, *median (IQR)*	13 (12)	17 (11)	0.044*	13 (13)	16 (11)	0.067
Glycemia – mg/dL, *median (IQR)*	128 (44)	133 (66.5)	0.716	126 (44.5)	136 (62)	0.330
Systolic BP admission - mmHg, mean (SD)	146 (25)	146 (22)	0.218	145 (25)	146 (25)	0.781
Diastolic BP admission – mmHg, mean (SD)	81 (16)	78 (14)	0.617	80 (15)	80 (15)	0.957

ASPECTS at admission, *median (IQR)*	10 (2)	10 (2)	0.868	10 (2)	10 (2)	0.940
Collateral Score, n (%)			0.866			0.724
0	1 (0.8)	1 (2.2)		2 (2.3)	1 (1.2)	
1	20 (15.5)	7 (15.6)		11 (12.8)	15 (17.4)	
2	25 (19.4)	7 (15.6)		14 (16.3)	15 (17.4)	
3	82 (63.6)	30 (66.7)		58 (67.4)	55 (64.0)	
4	1 (0.8)	0 (0)		1 (1.2)	0 (0)	
Etiology, n (%)			0.542			0.323
Cardioembolic, n (%)	68 (41.0)	26 (48.1)		49 (39.5)	46 (45.1)	
Large Artery Atherosclerosis, n (%)	27 (16.3)	10 (18.5)		20 (16.1)	19 (18.6)	
Small vessel disease, n (%)	4 (2.4)	0 (0)		3 (2.4)	1 (1.0)	
Other (Carotid Dissection), n (%)	6 (3.6)	0 (0)		5 (4.0)	1 (1.0)	
Incomplete evaluation, n (%)	9 (5.4)	3 (5.6)		10 (8.1)	3 (2.9)	
Negative Evaluation, n (%)	49 (29.5)	14 (25.9)		37 (29.8)	32 (31.4)	
Hb parameters						
Hb pre procedure – g/dL, median (IQR)	13.9 (1.7)	11.4 (1.04)	<0.001**	14.3 (2.0)	12.4 (2.3)	<0.001**
Hct pre procedure - %, median (IQR)	41.5 (4.1)	34.4 (3.37)	<0.001**	42.1 (7.5)	36.8 (6.0)	<0.001**
White Blood Cell Count in 10^9/L, median (IQR)	9.18 (4.29)	8.09 (4.45)	0.157	8.92 (4.12)	8.97 (4.74)	0.726
Platelet count in 10^9/L, median (IQR)	207 (82)	218 (112)	0.199	211 (84)	210 (96)	0.757
Hb post procedure – g/dL, median (IQR)	13.0 (1.9)	10.7 (2.2)	<0.001**	13.6 (1.7)	11.6 (1.5)	<0.001**
Hct post procedure - %, median (IQR)	39.1 (5.0)	31.6 (6.4)	<0.001**	40.6 (4.2)	34.7 (5.4)	<0.001**
Vitamin B12 deficiency, n (%)	13 (7.8)	6 (11.1)	0.468	10 (7.9)	10 (9.6)	0.552
Iron Deficiency, n (%)	0 (0)	0 (0)		1 (1.2)	0 (0)	0.379
Delta Hg - g/dL, median (IQR)	-0.9 (-1.3)	-0.7 (-1.1)	0.111	-0.6 (-1.1)	-1.2 (-1.1)	<0.001**
Delta Hct - %, median (IQR)	-2.1 (-3.5)	-1.8 (-3.6)	0.479	-1.4 (-3.0)	-3.4 (-3.2)	<0.001**
Anemia pre-procedure, n(%)				3 (2.4)	48 (46.2)	<0.001**
Anemia post procedures, n(%)	44 (26.5)	48 (88.9)	<0.001**			

Post-intervention characteristics						
mTICI 2b-3, n (%)	129 (77.7)	40 (76.9)	0.564	98 (77.8)	81 (77.9)	0.882
Presence of SIRS, n (%)	34 (20.5)	9 (16.7)	0.578	32 (25.4)	16 (15.4)	0.063
24 hours NIHSS, median (IQR)	8.0 (14)	13 (18)	0.063	8 (14)	11 (15)	0.061
Discharge/7 days NIHSS, median (IQR)	5.0 (15)	11 (19)	0.062	5 (15)	10 (16)	0.055
Infarct volume – ml, median (IQR)	11.2 (44.6)	15.6 (74.9)	0.139	11.5 (46.4)	16.6 (53.7)	0.242
Hemorrhagic transformation, n (%)	43 (25.9)	9 (16.7)	0.165	32 (25.4)	23 (22.1)	0.561
Hemorrhagic transformation Classification, n (%)			0.106			0.083
H1	11 (6.6)	1 (1.9)		7 (5.6)	6 (5.8)	
H2	26 (15.7)	4 (7.4)		22 (17.5)	11 (10.6)	
PH1	4 (2.4)	4 (7.4)		1 (0.8)	6 (5.8)	
PH2	2 (1.2)	0 (0)		2 (1.6)	0 (0)	
SICHS, n (%)						
Outcomes						
Early Neurological Deterioration, n (%)	47 (28.3)	17 (31.5)	0.656	38 (30.2)	28 (26.9)	0.621
Dependent at 3 months, n (%)	84 (50.6)	39 (72.2)	0.005*	60 (47.6)	71 (68.3)	0.002**
Dead at 3 months, n (%)	14 (8.4)	13 (24.1)	0.002**	12 (9.5)	21 (20.2)	0.022*

Table 2 - Comparative analysis according to the presence of anemia at admission and in the first 24 hours
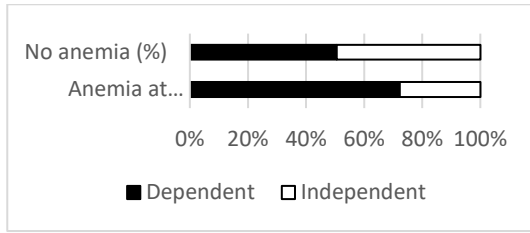
Figure 1 - Association between the functional outcome (independent defined as mRS 0-2) and the presence of anemia at admission.
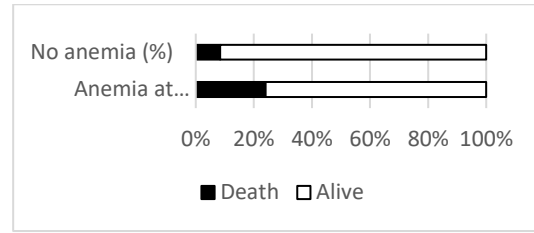
Figure 2 - Association between survival and the presence of anemia at admission.
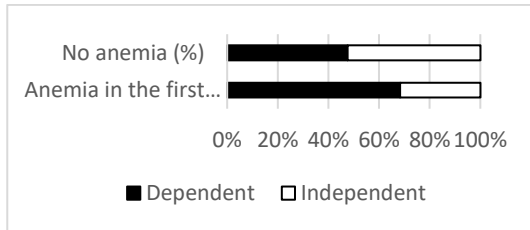

Figure 3 - Association between the functional outcome (independent defined as mRS 0-2) and the presence in the first 24 hours.
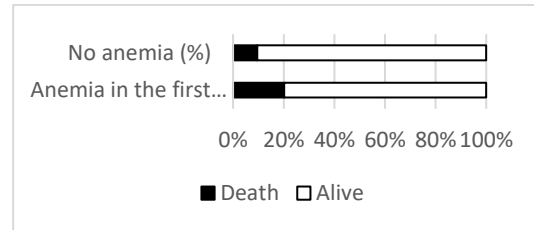

Figure 4 - Association between survival and the presence of anemia in the first 24 hours.

A similar analysis was applied to the differences between patients presenting with anemia at admission and those without anemia for each of the three treatment modalities (IVT, EVT and IVT plus EVT). The results are presented in Figure 5 and Figure 6. Only patients submitted to EVT showed an association with worse functional outcome (p = 0,044) and patients submitted to IVT (p = 0,024) associated with worse survival. No statistically significant difference was found in the outcomes for any of the other treatment modalities however there was a tendency towards worse functional and mortality outcomes in anemic patients in every treatment subgroup.
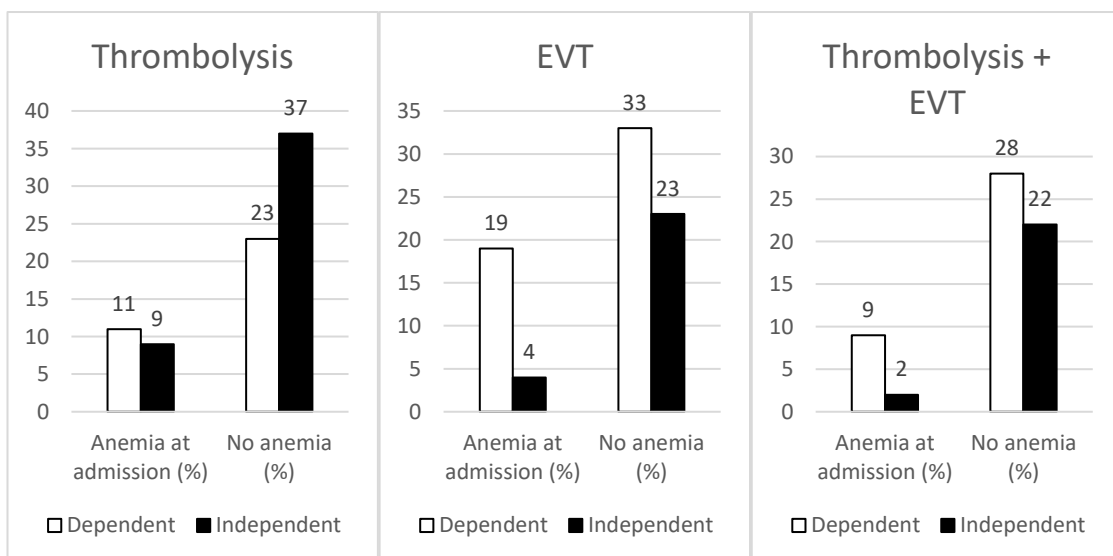

Figure 5 Relationship between the presence of anemia and functional outcome at 3 months for each of the treatment modalities. IVT – Intravascular thrombolysis. EVT- endovascular thrombectomy (p = 0,192, p = 0,044, p = 0,175)
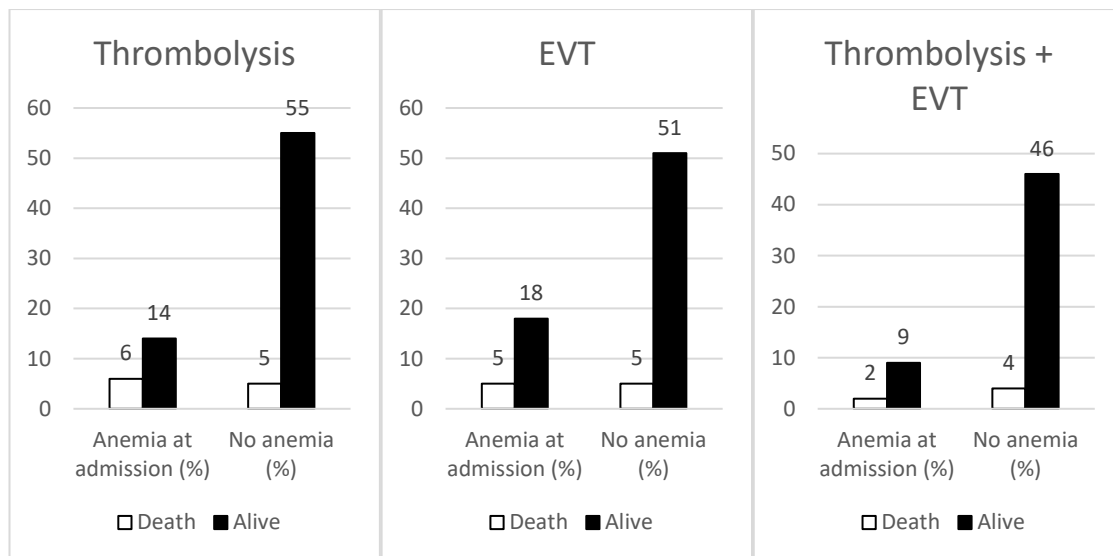
Figure 6 Relationship between the presence of anemia and mortality at 3 months for each of the treatment modalities. IVT – Intravascular thrombolysis. EVT- endovascular thrombectomy. (p = 0,024, p = 0,145, p = 0,294)

Discussion

We have found that the presence of anemia (both at admission and in the first 24 hours after the procedure) is associated with a worse degree of disability as well as to a higher rate of mortality in the first 3 months, however this association might be driven by other factors also present in anemic patients. The relationship between the level of Hb and the outcome in stroke patients had already been addressed and confirmed in previous studies (15-20), though the presence of a publication bias has already been reported in two different meta-analysis (6, 7). Only one recent multicenter study demonstrated a clear effect of anemia in outcome of thrombolysis patients.(5) Previous studies addressing other treatment modalities had clear limitations in its generability which supports the further need to address this topic in different contexts.

The rapid change in reperfusion techniques performed on ischemic stroke patients and the widening of treatment windows to perform them must be taken into consideration when reviewing the effects of anemia on outcomes. We also aimed at providing new insights on the role of anemia specifically for each treatment modality and, being the first study to address this topic specifically for patients who were managed with both IVT and EVT. When different treatment modalities were taken into consideration in the analysis only patients that underwent EVT maintained a statistically significant difference in the functional outcome in this specific group of patients (p = 0,044) and only patients in the IVT group had a statistically significant

difference in survival (p = 0,024). Further study of this specific group might provide important conclusions on this matter.

Although lacking statistical significance there is a slight tendency towards worse outcomes (defined either as dying or having a mRS score of less than 3) in patients with anemia when compared with the normal Hb levels counterpart, regardless of the treatment. Though all except two of these differences failed to be statistically significant, the homogeneity of results throughout the different subgroups suggests that it is likely that a bigger sample in further studies could come up with stronger evidence of the impact of anemia in different stroke settings.

The prevalence of anemia in our dataset of stroke patients (24,5% with a 95%CI 19,0-30,8) was different from previously reported estimates of prevalence of anemia in the general Portuguese population (19,9 % with a 95%CI of 19,0-20,8), though this difference was not statistically significant.(21) It is worth noting that, if we only consider the population of the north of Portugal (same region as CHSJ), the gap is even wider, as the prevalence of anemia was 17,9% (no CI provided). This indicates that the presence of anemia is especially prevalent in patients who present with a stroke, and it may potentiate other predictors of outcome not addressed in this study.

There are several limitations associated with our study. Due to the retrospective observational design of this study there are several limitations inherent to the design and generalization of conclusions must be taken carefully. Another weakness of this study is that the division of anemic and non anemic groups only took into consideration cut-offs defined for the general population and not for stroke patients. Furthermore, it is possible that high levels of Hb, even in the normal range, could also be deleterious in a stroke context.(22, 23) The existence of a true parabolic relationship between Hb levels and the outcome of stroke patients, rather than a linear one, might therefore be responsible for a underestimation of the anemia effect on our results.

In fact, it is plausible to assume a parabolic relationship between these two factors when we take into consideration the complex interplay between blood parameters like the hematocrit and the blood rheology. The hematocrit (Hct) is the volume percentage (expressed as vol%) of erythrocytes in the blood. The mean value is typically 0.45 in men (reference values, 0.42–0.52) and 0.40 in women (reference values, 0.37–0.47). Hematocrit is one of the major determinants of blood viscosity along with red blood cell deformability, and has been proved to be an independent risk factor for cerebrovascular disorders in a few clinical studies(24).

Also, it has been hypothesized that the hemodilution of blood following the use of blood-free solutions in fluid resuscitation might optimize blood rheology (25), which clearly shows that the relationship between anemia and stroke outcome might not be a simple one, but rather a complex interplay between the benefits of having a high level of oxygen transporting Hb and the detrimental effect of having high values of hematocrit.

Finally, it is also important to point out that our study was conducted retrospectively making use of data that was collected for clinical management purposes. A prospective homogenous study would be needed to confirm our findings. Conducting a regression analysis with bigger data might also be important for clarifying if the relationship we found is truly owed to the presence anemia on its own or if it might be better explained by the presence of other independent factors.

Nonetheless, this might present as an interesting and important factor for further studies and for improvement in strategies of nationwide stroke prevention.

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DIRECÇÃO CLÍNICA

17 / 6 / 2020

SÃO JOÃO

PEDIDO DE AUTORIZAÇÃO

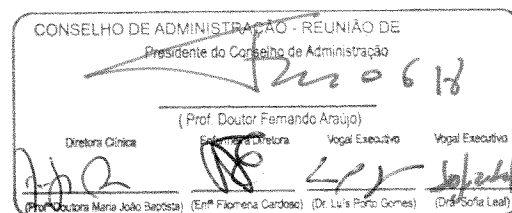## Realização de Investigação

Exmo. Senhor Presidente do Conselho de Administração
do Centro Hospitalar de São João

CONSELHO DE ADMINISTRAÇÃO - REUNIÃO DE
Presidente do Conselho de Administração

(Prof. Doutor Fernando Araújo)

Diretora Clínica   Vogal Executivo   Vogal Executivo

(Prof.ª Doutora Maria João Baptista) (Enf.ª Filomena Cardoso) (Dr. Luís Porto Gomes) (Dr.ª Sofia Leal)

Nome do Investigador Principal:

Miguel Carvalho Neto de Queirós Pimenta

Título da Investigação:

Anemia e a sua associação com prognóstico funcional em doentes com
AVC isquémico submetidos a tratamento de reperfusão

Pretendendo realizar no(s) Serviço(s) de:

Neurologia
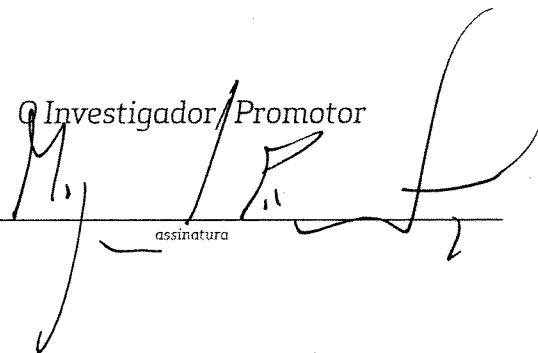
a investigação em epígrafe, solicito a V. Exa., na qualidade de Investigador/Promotor, autorização para a sua efetivação.

Para o efeito, anexo toda a documentação referida no dossier da Comissão de Ética do Centro Hospitalar de São João/ Faculdade de Medicina da Universidade do Porto respeitante à investigação, à qual enderecei pedido de apreciação e parecer.
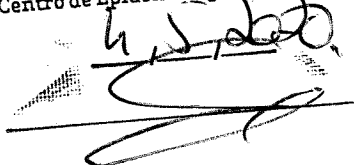
Com os melhores cumprimentos.

O Investigador/Promotor

Porto, 13 de outubro de 2019.

_____
assinatura

Centro Hospitalar São João
Centro de Epidemiologia Hospitalar

CES-IM005-0

Título do Projeto: Anemia e a sua associação com prognóstico funcional em doentes com AVC isquémico submetidos a tratamento de reperfusão

Nome do Investigador Principal: Miguel Carvalho Neto de Queirós Pimenta, aluno do Mestrado Integrado em Medicina da FMUP

Onde decorre o Estudo: No Serviço de Neurologia do CHUSJ. Apresentou declaração da Prof.ª Doutora Elsa Azevedo.

Objectivos do Estudo:
Verificar o efeito que a anemia pré-procedimento de reperfusão, assim como o desenvolvimento de anemia precoce pós-procedimento terá no prognóstico de doentes com AVC isquémico submetidos a tratamento de reperfusão, com enfoque no tamanho do enfarte estabelecido e no edema perilesional precoce associado. Estudo realizado no âmbito do Mestrado Integrado em Medicina da FMUP, sob orientação do Prof. Doutor Pedro Miguel Araújo Campos de Castro.

Conceção e Pertinência do estudo:
Estudo retrospetivo, que inclui todos os doentes com AVC isquémico submetidos a tratamento de reperfusão no ano de 2019. Espera-se com esta análise obter nova informação sobre a influência destes indicadores no prognóstico do AVC isquémico, assim como possíveis mecanismos subjacentes de modo a estabelecer possíveis novos alvos terapêuticos em futuros ensaios clínicos.
Os dados a recolher são os seguintes: dados sociodemográficos (idade, género); apresentação e evolução clínica; presença de fatores de risco cardiovasculares; valores dos estudos analíticos efetuados; dados de vigilância clínica; folhas terapêuticas; relatórios e imagens de exames imagiológicos realizados; registo de consultas externas.

Benefício/risco: Não aplicável

Confidencialidade dos dados:
Os pacientes serão codificados, de forma a garantir o anonimato.
Apresentou um pedido de reutilização de registos clínicos para Investigação e Desenvolvimento ao RAI.

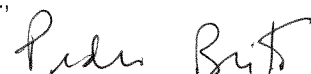**Respeito pela liberdade e autonomia do sujeito de ensaio:** Não aplicável
Curriculum **do investigador**: Adequado à investigação.
Data previsível da conclusão do estudo: fevereiro de 2021

Conclusão: Proponho um parecer favorável à realização deste projeto de investigação.

Porto, 19 de março de 2020

O Relator da CE,

U.PORTO
FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

SÃO JOÃO

Questionário para submissão de Investigação

Exmo. Sr. Presidente da Comissão de Ética do Centro Hospitalar de São João/
Faculdade de Medicina da Universidade do Porto,

Pretendendo realizar a investigação infracitada, solicito a V. Exa., na qualidade de Investigador, a sua apreciação e a elaboração do respetivo parecer. Para o efeito, anexo toda a documentação requerida.

IDENTIFICAÇÃO DO ESTUDO

Título da investigação: Anemia e a sua associação com prognóstico funcional em doentes com AVC isquémico submetidos a tratamen

Nome do investigador: Miguel Carvalho Neto de Queirós Pimenta

Endereço eletrónico: miguel.97.pimenta@gmail.com   Contacto telefónico: 911517498

Caracterização da investigação:

[X] Estudo retrospetivo   [ ] Estudo observacional   [ ] Estudo prospetivo

[ ] Inquérito   [ ] Outro. Qual? _____

Tipo de investigação:

[ ] Com intervenção   [X] Sem intervenção

Formação do investigador em boas práticas clínicas *(GCP)*: [ ] Sim   [X] Não

Promotor *(se aplicável)*: _____

Nome do orientador de dissertação/tese *(se aplicável)*: Pedro Miguel Araújo Campos de Castro

Endereço eletrónico: pedromacc@gmail.com

Local/locais onde se realiza a investigação: Serviço de Neurologia do Centro Hospitalar de São João

Data prevista para início: 1 / 10 / 2019   Data prevista para o término: 29 / 02 / 2021

PROTOCOLO DO ESTUDO

Síntese dos objetivos:

Pretendemos verificar o efeito que a anemia pré-procedimento de reperfusão, assim como o desenvolvimento de anemia precoce pós-procedimento terá no prognóstico de doentes com AVC isquémico submetidos a tratamento de reperfusão, com enfoque no tamanho do enfarte estabelecido e no edema perilesional precoce associado.

Fundamentação ética (ganhos em conhecimento/inovação; ponderação benefícios/riscos):

Espera-se com esta análise obter nova informação sobre a influência destes indicadores no prognóstico do AVC isquémico, assim como possíveis mecanismos subjacentes de modo a estabelecer possíveis novos alvos terapêuticas em futuros ensaios clínicos.

CES-IM007-0

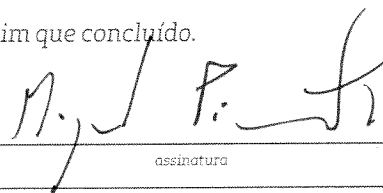## LISTA DE DOCUMENTOS ANEXOS

- [X] Pedido de autorização ao Presidente do Conselho de Administração do Centro Hospitalar de São João (se aplicável)
- [ ] Pedido de autorização à Diretora da Faculdade de Medicina da Universidade do Porto (se aplicável)
- [X] Protocolo do estudo
- [X] Declaração do Diretor de Serviço onde decorre o estudo
  (sendo um estudo na área de enfermagem deve anexar também a concordância da chefia de enfermagem)
- [ ] Profissional de ligação
- [X] Informação dos orientadores
- [ ] Informação ao participante
- [ ] Modelo de consentimento
- [ ] Instrumentos a utilizar (inquéritos, questionários, escalas, p.ex.): _____
- [X] Curriculum Vitae abreviado (máx. 3 páginas)
- [ ] Protocolo financeiro
- [ ] Outros: _____

COMPROMISSO DE HONRA E DECLARAÇÃO DE INTERESSES

Declaro por minha honra que as informações prestadas neste questionário são verdadeiras. Mais declaro que, durante o estudo, serão respeitadas as recomendações constantes da Declaração de Helsínquia (1960 e respetivas emendas), e da Organização Mundial da Saúde, Convenção de Oviedo e das "Boas Práticas Clínicas" (GCP/ICH) no que se refere à experimentação que envolve seres humanos. Aceito, também, a recomendação da CES de que o recrutamento para este estudo se fará junto de doentes que não tenham participado em outro estudo, nos últimos três meses. Comprometo-me a entregar à CES o relatório final da investigação, assim que concluído.

Porto, __13__ de ___outubro___ de __2019__

Nome legível: __Miguel Carvalho Neto de Queirós Pimenta__

assinatura

Pedido de Reutilização de Registos Clínicos para Investigação e Desenvolvimento (I&D)

SÃO JOÃO

Exmo. Senhor
Responsável pelo Acesso à Informação
(Artigo 9.º da Lei n.º 26/2016, de 22 de agosto)
Dr. Rui de Vasconcellos Guimarães

AUTORIZADO

SÃO JOÃO

29/04/20

---

1. Identificação do(s) Investigador(es) Preenchimento Obrigatório

1.1. Investigador Principal
Nome **Miguel Carvalho Neto de Queirós Pimenta**
Contacto telefónico | 9 | 1 | 1 | 5 | 1 | 7 | 4 | 9 | 8 | | | |
Endereço eletrónico   miguel.97.pimenta @ gmail.com

1.2. Investigador(es) Associado(s)
Número Total: | 5 |
Nome **Pedro Miguel Araújo Campos Castro**
Contacto telefónico | 9 | 3 | 1 | 7 | 2 | 5 | 1 | 8 | 1 | ? | | |
Endereço eletrónico   pedromacc @ gmail.com

Nome **Rafael Azevedo Dias**
Contacto telefónico | 9 | 3 | 5 | 8 | 9 | 6 | 5 | 4 | 4 | | | |
Endereço eletrónico   rafael27dias @ gmail.com

Nome **Ana Luísa Aires da Silva**
Contacto telefónico | 9 | 1 | 6 | 9 | 5 | 8 | 0 | 3 | 5 | | | |
Endereço eletrónico   ana.aires.mail @ gmail.com

1.3. Afiliação Institucional do Investigador Principal
1.3.1. Grupo Profissional
- [ ] Médico(a)
- [ ] Enfermeiro(a)
- [ ] Docente
- [x] Estudante
- [ ] Outro. Qual?_____

1.3.2. Documento de identificação pessoal ou profissional
- [x] Cartão de Cidadão
- [ ] Bilhete de Identidade
- [ ] Célula Profissional
- [ ] Cartão de Docente
- [ ] Cartão de Estudante
- [ ] Outro. Qual?_____

Número de Documento | 1 | 4 | 9 | 4 | 9 | 5 | 0 | 1 | | | | |

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2. Enquadramento e Identificação do Trabalho de Investigação e Desenvolvimento Preenchimento Obrigatório

2.1. Enquadramento da investigação
- [x] Trabalho académico de investigação e desenvolvimento:
  - [ ] Não conferidor de grau
  - [x] Conferidor de grau: [ ] Licenciatura [x] Mestrado [ ] Doutoramento
- [x] Projeto de investigação e desenvolvimento

Gabinete
de Apoio ao RAI
Recebido em
29/04/20
A funcionária

RAI-IM002-0

3. Observações

4. Aceitação dos Termos e Condições da Reutilização

Cumulativamente com as obrigações decorrentes da lei já citada *(n.º 2 e 3 do artigo 21 e o n.º 1 e 2 do artigo 12, ambos da Lei n.º 26/2016, de 22 de agosto)* ao submeter o presente pedido concordo e fico ainda vinculado aos seguintes termos e condições:

- Comprometo-me a manter confidencial toda a informação à qual vou ter acesso;
- Não vou elaborar registos, susceptíveis de identificar ou tornar identificável a identidade das pessoas a quem os mesmos dizem respeito;
- Não vou elaborar, nem ficar na posse, de cópias de bases de dados utilizadas na recolha de informação;
- Comprometo-me a obter junto da Comissão Nacional de Proteção de Dados (CNPD) as necessárias autorizações, para eventuais bases de dados que venha a conceber e utilizar no âmbito da presente investigação;
- Comprometo-me a devolver ao Centro Hospitalar de São João, na pessoa do seu Diretor Clínico, as bases de dados e o resultado da investigação;
- Comprometo-me a ocultar os elementos de identificação da(s) pessoa(s) a quem os registos digam respeito, em futuras e eventuais publicações de resultados;
- Comprometo-me a consultar os processos clínicos nas instalações que me forem indicadas para o efeito;
- Comprometo-me a obter os necessários pareceres, quer da Comissão de Ética do Hospital, quer do Centro de Epidemiologia Hospitalar, sempre que necessário;
- Comprometo-me a citar as fontes sempre que publicitar o trabalho de investigação independentemente de requerer a Certidão de Reutilização (DAta REuse Certificate for Research – DARE);
- Tomei conhecimento, que a violação de qualquer dos compromissos aqui assumidos, resultará no apuramento de responsabilidades disciplinares, civis e penais e ainda, à impossibilidade futura de aceder a informação de saúde para fins de investigação.

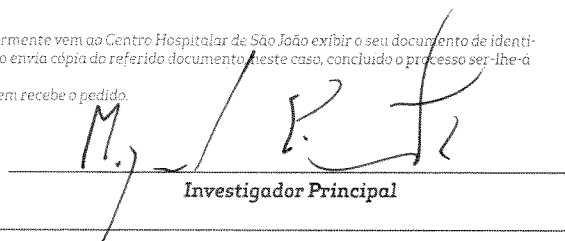## 5. Decisão do investigador sobre requerer a DAta REuse Certificate for Research – DARE *Preenchimento Obrigatório*

[X] Pretendo desde já requerer a Certidão de Reutilização (DARE) cujo sentido, valor e significado consultei em *http://portal-chsj.min-saude.pt/pages/710.*

[ ] Não pretendo requerer a Certidão de Reutilização (DARE) cujo sentido, valor e significado consultei em *http://portal-chsj.min-saude.pt/pages/710.*

6. Assinatura

Nota 1: Se o presente pedido for submetido eletronicamente ou faz assinatura digital qualificada; ou posteriormente vem ao Centro Hospitalar de São João exibir o seu documento de identificação pessoal; ou no âmbito do seu espaço de liberdade e como manifestação expressa do seu consentimento envia cópia do referido documento, neste caso, concluído o processo ser-lhe-á devolvida ou eliminada a cópia do documento de identificação pessoal, conforme as indicações que dê.
Nota 2: Se o presente pedido for entregue presencialmente, assina e exibe o documento de identificação a quem recebe o pedido.

Data | 2 | 0 | 1 | 9 | - | 1 | 0 | - | 1 | 3 |

_____
Investigador Principal

SUBMETER

STROBE Statement—Checklist of items that should be included in reports of *case-control studies*

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	4
		(*b*) For matched studies, give matching criteria and the number of controls per case	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-5
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	12
		(*c*) Explain how missing data were addressed	-
		(*d*) If applicable, explain how matching of cases and controls was addressed	-
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	4
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5
		(b) Indicate number of participants with missing data for each variable of interest	7-8
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	10-11

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
		(*b*) Report category boundaries when continuous variables were categorized	4-5
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12

Discussion

Key results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	15

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	-

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Transcriptions and Explanations:

1 (a) "To test our hypothesis, we conducted a retrospective case-control analysis"

1 (b) "We found the presence anemia (before treatment and after treatment) to have a statistically significant association with the development of worse functional outcomes and mortality rates, and this relationship was also present in every treatment subgroup analysis - though only statistically significant for the functional outcome in the thrombectomy subgroup and for the mortality rate in the thrombolysis subgroup)."

2 "Anemia is a common pre-morbid condition in ischemic stroke patients, and it has a negative impact on the prognosis of patients treated with a conservative approach. Its effect on patients subjected to reperfusion therapy, which aims at rescuing ischemic cerebral tissue, still requires further study."

3 "Thus, we planned to study the effect of both pre-procedure and early onset post-procedure anemia on the prognosis of ischemic stroke patients subjected to reperfusion therapy."

4 "We reviewed 332 patients, of which 84 patients were excluded. We included 248 patients in the analysis. Baseline characteristics of the cohort are displayed on Table 1."

5 "We included all ischemic stroke patients admitted to our comprehensive Stroke Center with ≥18 years of age with acute ischemic stroke of the anterior circulation who received intravenous thrombolysis or endovascular thrombectomy between January 2019 and December 2019."

6 (a) "We excluded posterior circulation strokes, patients with active cancers, absent or inadequate imaging at 24 hours, and no follow-up data at 90 days."

6 (b) Not a matching study

7 "We assessed the functional outcome by a dichotomization of the modified Rankin scale (mRS): 3-6 (dependent or dead) versus 0-2 (independent) at 90 days. We also evaluated the initial response to recanalization therapy with early neurological recovery (ENR), defined as any decrease in NIHSS at 24 hours from the baseline."

8 "Demographic and clinical data, including vascular risk factors, previous medications, past medical history, signs of acute inflammatory response (Blood Pressure, temperature, heart rate and respiratory rate) in the first 24 hours after treatment were retrospectively collected."; "Demographic and clinical data, including vascular risk factors, previous medications, past medical history, signs of acute inflammatory response (Blood Pressure, temperature, heart rate and respiratory rate) in the first 24 hours after treatment were retrospectively collected."; "Admission and at 24h laboratory results of hemoglobin (Hb), hematocrit (Hct), white blood cell count and platelet count were collected retrospectively, as well as levels of vitamin B12, folates and ferritin in the first 24 hours."~

9 "We excluded posterior circulation strokes, patients with active cancers, absent or inadequate imaging at 24 hours, and no follow-up data at 90 days."

10 "We included all ischemic stroke patients admitted to our comprehensive Stroke Center with ≥18 years of age with acute ischemic stroke of the anterior circulation who received intravenous thrombolysis or endovascular thrombectomy between January 2019 and December 2019."

11 "We investigated associations between Hb and outcome measures using Hb as a continuous variable and as a categorical variable distinguishing anemia and degrees of anemia as previous stated and according to WHO."

12 (a) "Testing for normality was performed with the Shapiro Wilk Test. Continuous data were summarized as mean and standard deviation or median and interquartile range when appropriate. We used Chi-square test and Fisher's exact test for categorical variables where appropriate and the Mann–Whitney U-test for continuous variables. The association between anemia and each outcome was estimated by calculating odds ratios (OR) with 95% confidence intervals (95% CI), using binary logistic regression models."

12 (b) "A similar analysis was applied to the differences between patients presenting with anemia at admission and those without anemia for each of the three treatment modalities (IVT, EVT and IVT plus EVT)."

12 (c) Missing data were excluded from the analysis.

12 (d) Not applicable, no matching analysis was conducted.

12 (e) Not applicable, no sensitive analysis was conducted.

13 (a) "We reviewed 332 patients, of which 84 patients were excluded. We included 248 patients in the analysis."

13 (b) "We excluded posterior circulation strokes, patients with active cancers, absent or inadequate imaging at 24 hours, and no follow-up data at 90 days."

13 (c) No flow diagram was presented.

14 (a) "We included 248 patients in the analysis. Baseline characteristics of the cohort are displayed on Table 1. The median age of the cohort was 76 years old, with 41.9% male."

14 (b) Table 1 (no missing data presented)

15 Table 2

16 (a) "Anemic patients before and after reperfusion therapies had worse functional outcome at 3 months (p-value 0.005 and 0.002 respectively) and higher mortality at 3 months (p-value 0.002 and 0.022) respectively, despite similar rates of early neurological deterioration."

16 (b) "We assessed the functional outcome by a dichotomization of the modified Rankin scale (mRS): 3-6 (dependent or dead) versus 0-2 (independent) at 90 days"; "The levels of Hb were recoded into a dichotomic variable (0 = absence of anemia and 1 = presence of anemia), following the WHO reference values for anemia for both male and female subjects"

16 (c) Not conducted.

17 "A similar analysis was applied to the differences between patients presenting with anemia at admission and those without anemia for each of the three treatment modalities (IVT, EVT and IVT plus EVT)."

18 "Although lacking statistical significance there is a slight tendency towards worse outcomes (defined either as dying or having a mRS score of less than 3) in patients with anemia when compared with the normal Hb levels counterpart, regardless of the treatment."

19 "Due to the retrospective observational design of this study there are several limitations inherent to the design and generalization of conclusions must be taken carefully. Another weakness of this study is that the division of anemic and non anemic groups only took into consideration cut-offs defined for the general population and not for stroke patients."; "The existence of a true parabolic relationship between Hb levels and the outcome of stroke patients, rather than a linear one, might therefore be responsible for a underestimation of the anemia effect on our results."; "A prospective homogenous study would be needed to confirm our findings. Conducting a regression analysis with bigger data might also be important for clarifying if the relationship we found is truly owed to the presence anemia on its own or if it might be better explained by the presence of other independent factors."

20 "We have found that the presence of anemia (both at admission and in the first 24 hours after the procedure) is associated with a worse degree of disability as well as to a higher rate of mortality in the first 3 months, however this association might be driven by other factors also present in anemic patients."

21 "A prospective homogenous study would be needed to confirm our findings. Conducting a regression analysis with bigger data might also be important for clarifying if the relationship we found is truly owed to the presence anemia on its own or if it might be better explained by the presence of other independent factors."

22 There were no sources of funding

INSTRUCTIONS FOR AUTHORS

Sections:

1. GENERAL

Authors should kindly note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.

Once the submission materials have been prepared in accordance with the Author Guidelines, manuscripts should be submitted online at https://mc.manuscriptcentral.com/eurjneurol

Click here for more details on how to use **ScholarOne**.

For help with submissions, please contact Ms. Adlin Neefa at: **eurjneurol@wiley.com**

Author's Checklist

Read the checklist with care. Manuscripts not consistent with the checklist may be unsubmitted for correction.

General

- Have the manuscript written or proof-read by a person proficient in English. Details of Wiley recommended English language editing services can be found here
- Make sure the manuscript complies with the maximum word count.
- Enter all authors' names and affiliations in the submission process. Please contact the office if you have any difficulties doing so – your manuscript will be unsubmitted if any authors cannot be reached by email. If possible, authors should provide an institutional address.
- Supply all information requested in the Ethical Requirements section.
- When submitting a revision, provide two revised versions: one with the changes highlighted, the other without highlights. Delete all previous versions of the manuscript.
- Submit the manuscript text file as editable file, such as .doc or .docx (not as .pdf).

Report pages and references

- Limit your acknowledgement to max. 50 words.

- Report IRB approval, informed consent and trial registration in the Methods section of your manuscript. Provide a conflict of interest statement and disclose your sources of funding at the end, if applicable.
- Use AMA Style referencing.
- Make sure each citation is present in the reference list and vice versa.

Artwork and tables

- Upload images of sufficient resolution (300 dpi for photographs, 600 dpi for line art). Photoshop or IrfanView (free to download on the Web) may be used to produce high resolution figures. In their final size, figures need to fit the width of a single column of text, (88 mm) or, if necessary, the full page width (184 mm). The final size of the printed figure cannot exceed 230 x 184 mm including captions. Lettering should be no less than 2 mm in height in the final printed figure, should be in proportion to the overall dimensions of the figure and be consistent between figures. Where several figures are mounted together they should be squared accurately and separated by about 5 mm. All of the figures in such a group should have approximately the same contrast values.
- Indicate approximate positions of tables and figures in the text
- Submit the text of the manuscript, including table and figure captions, as one file and each figure/table as a separate file.
- Submit your tables as editable files, such as .doc, .docx, .xls or .xlsx files, not as scanned or image files.

Data protection:

By submitting a manuscript to or reviewing for this publication, your name, email address, and affiliation, and other contact details the publication might require, will be used for the regular operations of the publication, including, when necessary, sharing with the publisher (Wiley) and partners for production and publication. The publication and the publisher recognize the importance of protecting the personal information collected from users in the operation of these services and have practices in place to ensure that steps are taken to maintain the security, integrity, and privacy of the personal data collected and processed. You can learn more at https://authorservices.wiley.com/statements/data-protection-policy.html.

Preprint policy:

European Journal of Neurology (EJoN) will consider for review articles previously available as preprints. Authors may also post the submitted version of a manuscript to a preprint server at any time. Authors are requested to update any pre-publication versions with a link to the final published article. Please find the full Wiley preprint policy here.

2. AIMS AND SCOPE

The *European Journal of Neurology* publishes the highest quality peer-reviewed original research articles, reviews – preferably systematic reviews, invited or unsolicited – , EAN guidelines and position papers on pioneering efforts and innovative studies in the medical management of all neurological conditions such as dementia, stroke, epilepsy, headache, multiple sclerosis, movement disorders, neuromuscular and infectious diseases.

The *European Journal of Neurology*, the official journal of the European Academy of Neurology is an international peer-reviewed publication that covers the whole field of neurology, with a good balance between subspecialties. Basic science papers are welcome if they help with the understanding of an important clinical issue, and will be published together with an editorial commentary written by a clinician. The target audience consists of neurologists who are non-specialised in their clinical activity, and of those who have a specific field of interest and want to be kept informed about the latest neurological innovations and what is of interest to neurologists working in other subspecialities of neurology. Therefore, a priority will be given, by the editors, to articles of interest for most neurologists – irrespective of their subspecialty and

practice – and to manuscripts covering aspects at the crossroads between different subspecialties. Manuscripts that are of interest only for a few highly specialised neurologists are more suitable for specialised journals.

The journal is aimed at an international audience of clinicians and basic researchers in neurology. The journal uses the most advanced production methods to ensure rapid publication and welcomes submission of papers from all parts of the world.

3. MANUSCRIPT CATEGORIES AND REQUIREMENTS

The *European Journal of Neurology* publishes papers in neurology and related areas in the following categories:

- Original Articles
- Review Articles
- Short Communications
- Letter to the Editors
- Case reports
- Guidelines
- Editorials
- Commentaries

Original Papers: max. 3500 words including Introduction, Method, Results and Discussion. A maximum of eight figures or tables and fifty references are allowed. Additional figures may be submitted as supplementary material for publication online only at the discretion of the editor. This supplementary material must be clearly labelled as supplementary on the title page and in citations throughout the manuscript and must be uploaded as a separate file type.

Review Articles: max. 5000 words including Introduction, Method, Results and Discussion. A maximum of eight figures or tables and fifty references are allowed. Systematic reviews are preferred. Additional figures may be submitted as supplementary material for publication online only at the discretion of the editor. This supplementary material must be clearly labelled as supplementary on the title page and in citations throughout the manuscript and must be uploaded as a separate file type.

Short Communications: max. 1500 words, including only Introduction, Method, Results and Discussion. Two tables or figures are allowed and a maximum of fifteen references.

Letters to the Editor: max. 500 words, including a maximum of five references. Letters express views about articles published in *EJoN* or present ideas or findings of scientific interest that do not constitute original research. Letters to the Editor are published online only.

Case reports: max. 750 words. One figure or table is allowed and a maximum of five references. Acceptance of case reports is very limited, and only reports that make a major clinico-pathological or educational contribution or suggest a significant change of diagnosis or therapy will be accepted.

Guidelines/ Consensus Statements/ Position Papers: max. 6000 words, 8 tables or figures and 100 references. Word count does not include title page, abstract, conflicts of interest, references and legends of tables and figures. Guidelines are prepared by task forces appointed by EAN and reviewed by the EAN Scientific Committee. Consensus statements do not meet criteria for EAN guidelines but are produced by an EAN panel or a subspecialty society. Position papers are produced by a group of individuals who do not represent officially a society.

Editorials: max. 1000 words,1 table or figure and 10 references. Editorials are invited by the Editors, at their discretion, to comment on timely and important topics. Editorials express the authors personal views and do not represent the opinions of *EJoN* or the EAN.

Commentary: max. 500 words. A maximum of five references are allowed, including the article to which the commentary is associated. Commentaries are opinionated, subjective pieces by one or more experts on a topic or publication and are invited by the Editors.

EJoN publishes supplements on topics of general interest. All manuscripts submitted for inclusion in a supplement are peer-reviewed in the same way as unsolicited submissions.

4. PREPARING THE SUBMISSION

Parts of the Manuscript

The manuscript should be submitted in separate files: main text file; figures.

Main Text File

Each manuscript should have a title page with the following information:

1. A short informative title containing the major key words. The title should not contain abbreviations (see Wiley's best practice SEO tips);
2. The full names of the authors;
3. The author's institutional affiliations where the work was conducted.
4. E-mail data of the corresponding author for correspondence, proofs and reprint requests.
5. Total word count of the manuscript including title page, references, and structured abstract;
6. A short running title of less than 40 characters;
7. Up to five keywords.

Figures and supporting information should be supplied as separate files.

Authorship

Please refer to the journal's Authorship policy in the Editorial Policies and Ethical Considerations section for details on author listing eligibility.

Author Contributions

For all articles, the journal mandates the CRediT (Contribution Roles Taxonomy), for more information please see Author Services.

Acknowledgments

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section in the manuscript. Such people include, for instance, technicians, statisticians and translators.
 Financial and material support should also be mentioned. if you had funding for any kind for your study, indicate the source under 'Funding'. Also indicate if you had no funding.
Thanks to anonymous reviewers are not appropriate.

Conflict of Interest Statement

Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the 'Conflict of Interest' section in the Editorial Policies and Ethical Considerations section below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Abstract

Please provide a structured abstract (Background, Methods, Results, Conclusions) of up to 250 words. Letters to the Editor do not have an abstract.

Keywords

Please provide five keywords. Keywords should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser list at www.nlm.nih.gov/mesh.

Main Text

The journal uses British/US spelling; however, authors may submit using either option, as spelling of accepted papers is converted during the production process.

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If a method or tool is introduced in the study, including software, questionnaires, and scales, the author should state the license this is available under and any requirement for permission for use. If an existing method or tool is used in the research, the authors are responsible for checking the license and obtaining the permission. If permission was required, a statement confirming permission should be included in the Methods and Materials section. Authors who wish you reuse previous methods from their own research should make sure to cite their own publications.

Please give the full name of the institutional review board or ethics committee that approved your research protocol. If no ethics board was needed, explain why it was not needed. Indicate that the subjects or their next-on-kin gave informed consent for participation. If you submit personal information or identifiable images, indicate that you had the person's permission for it. If your study is a registered trial, give the name of trial register.

Ethical Standards

Studies using human or animal subjects should include an explicit statement identifying the Institution or Review Committee which approved the study, and preferably providing the permit number given. Editors reserve the right to reject papers if there is doubt whether appropriate procedures were followed.

(i) Studies involving human subjects
When human subjects are used, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject, and that the study conforms with World Medical Association Declaration of Helsinki published on the website of the **Journal of American Medical Association**. In addition, the name of the institutional review board (or appropriate review committee) that approved the study should be given and their approval should be explicitly stated.

(ii) Studies involving experimental animals
When experimental animals are used, specify the species, strain, sex, age, supplier, and numbers of animals used in total and for individual experimental conditions. The species should be identified in the Title or Abstract. If genetically modified mice were used, the Standards for the publication of mouse mutant studies (see Crusio et al., **Genes, Brain and Behavior** (2009) 8:1–4) should be followed. Please provide detailed and full strain and sub-strain information and use the correct nomenclature.

The Materials and Methods section must briefly but explicitly state measures which were taken to minimize pain or discomfort, e.g. type and dose of anesthetic used and peri-operative care, and how the number of animals used was minimized. Experiments should be carried out in accordance with the **Council Directive 2010/63EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes** or with the Guidelines laid down by the NIH in the US regarding the care and use of animals for experimental procedures. In addition, the name of the Animal Use and Care Committee or Institution that approved the study should also be given and their approval should be explicitly stated. Work using animals that does not conform to these standards would not be repeatable in Europe or the USA and so falls outside of the scope of the journal.

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References

All references should be numbered consecutively in order of appearance and should be as complete as possible. In text citations should be superscript numbers. Journal titles are abbreviated; abbreviations may be found in the following: MEDLINE, Index Medicus, or CalTech Library.

Submissions are not required to reflect the precise reference formatting of the journal (use of italics, bold etc.), however it is important that all key elements of each reference are included. Please see below for examples of reference content requirements.

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1. King VM, Armstrong DM, Apps R, Trott JR. Numerical aspects of pontine, lateral reticular, and inferior olivary projections to two paravermal cortical zones of the cat cerebellum. J Comp Neurol 1998;390:537-551.

Book:

2. Voet D, Voet JG.Biochemistry.. New York: John Wiley & Sons; 1990. 1223 p.

Please note that journal title abbreviations should conform to the practices of Chemical Abstracts.

For more information about AMA reference style AMA Manual of Style.

Endnotes

Endnotes should be placed as a list at the end of the paper only, not at the foot of each page. They should be numbered in the list and referred to in the text with consecutive, superscript Arabic numerals. Keep endnotes brief; they should contain only short comments tangential to the main argument of the paper.

Footnotes

Footnotes should be placed as a list at the end of the paper only, not at the foot of each page. They should be numbered in the list and referred to in the text with consecutive, superscript Arabic numerals. Keep footnotes brief; they should contain only short comments tangential to the main argument of the paper and should not include references.

Tables

Tables should be self-contained and complement, not duplicate, information contained in the text. They should be supplied as editable files, not pasted as images. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

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Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement.

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Although authors are encouraged to send the highest-quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are accepted.

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The journal's table of contents will be presented in graphical form with a brief abstract.

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This journal has the option for authors to embed rich media (i.e. video and audio) within their final article. These files should be submitted with the manuscript files online, using either the "Embedded Video" or "Embedded Audio" file designation. If the video/audio includes dialogue, a transcript should be included as a separate file. **The combined manuscript files, including video, audio, tables, figures, and text must not exceed 350 MB.** For full guidance on accepted file types and resolution please see here.

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Guidelines on Publishing and Research Ethics in Journal Articles

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