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The role of endothelial dysfunction in the age-related poor prognosis of stroke patients undergoing endovascular treatment

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ABSTRACT

Background: Age is a well-established predictor of poor prognosis in patients with ischemic stroke (IS) undergoing endovascular treatment (EVT). The endothelium plays a central role in the pathophysiology of IS, and its function can be evaluated using various methods. This study aims to investigate the relationship between endothelial dysfunction, age, and clinical outcomes in IS patients treated with EVT

Methods: Patients with IS treated with EVT were included in the study. Ninety-day prognosis was evaluated using the modified Rankin Scale (mRS). Endothelial function was assessed through flow-mediated dilation (FMD) measured by brachial artery ultrasound, and by quantifying pre-EVT arterial serum levels of arginine metabolites, homoarginine (hArg), symmetric dimethylarginine (SDMA), and asymmetric dimethylarginine (ADMA), using ultra-performance liquid chromatography coupled with tandem mass spectrometry. Clinical, radiological, and EVT procedural data were recorded as independent variables. The outcome variable is good functional outcome by dichotomized mRS<2. Bivariate, multivariate, and mediation analyses were conducted to explore associations.

Results: A total of 150 stroke patients were included in the analysis (median age was 77 years and 50.7 % were female). Older patients exhibited poorer endothelial function, reflected by lower FMD values and altered arginine metabolite profiles. They also showed worse clinical outcomes at 90 days. Among the metabolites, SDMA emerged as an independent predictor of poor prognosis (OR = 1.343 per 0.1 umol/l increase, $p < 0.01$). Moreover, SDMA was identified as a mediator in the relationship between age and unfavorable outcomes following EVT

Conclusion: Endothelial dysfunction, as indicated by circulating biomarkers such as SDMA, may partially mediate the association between age and poorer outcomes in stroke patients treated with EVT.

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Introduction

Endovascular thrombectomy (EVT) has demonstrated high clinical efficacy in the treatment of acute ischemic stroke (IS) due to large vessel occlusion.[1] Despite technical success rates of recanalization exceeding 90 %, a substantial proportion of patients still experience poor outcomes that cannot be fully explained by stroke volume or time to treatment1. Especially, while EVT also benefits older patients, it is associated with lower rates of recanalization, which may in part be explained by more prevalent technical challenges such as vessel tortuosity or vascular atherostenotic disease, and their functional independence and mortality outcomes are significantly worse compared to younger individuals.[2,3]

The endothelium plays a central role in stroke pathophysiology. As a major source of nitric oxide (NO), it contributes to the regulation of vascular tone and resting cerebral blood flow (CBF).[4] Endothelial function can be assessed using a variety of methods. One common approach is the measurement of flow-mediated dilation (FMD) via brachial ultrasound, which provides an assessment of systemic endothelial function.[5] Additionally, several metabolites, including arginine derivatives such as homoarginine (hArg), symmetric dimethylarginine (SDMA), and asymmetric dimethylarginine (ADMA), have been identified as meaningful biomarkers.[6] Both ADMA and SDMA reduce NO bioavailability, whereas hArg not only serves as a substrate for nitric oxide synthase (NOS) but also increases the availability of arginine to the NOS enzyme by inhibiting arginase.[6]

The aims of the present study were to characterize endothelial function markers in IS patients treated with EVT, examine their relationship with age, and explore whether age-related poor prognosis could be partially explained by endothelial dysfunction in these patients.

Methods

The data supporting the study's findings are available from the corresponding author upon request.

Study population

We conducted a retrospective, observational cohort study using data from a prospective registry of acute IS patients treated with EVT at a tertiary stroke center. All patients included in the study were clinically managed according to the institutional protocol, based on current international stroke guidelines and clinical evidence at that time.

Inclusion criteria

Patients with disabling focal neurological deficit and AIS with demonstrated large or medium vessel occlusion in CT angiography (CTA). Concomitant ipsilateral extracranial carotid stenosis or occlusions were not excluded. Anterior and posterior circulation strokes were included.

Treatment with EVT with or without previous administration of intravenous thrombolysis (IVT). Prior mRS<3 Baseline CT imaging including CTA and 24-hour follow-up CT scan to assess for HT after EVT. Between March 2018 and March 2020, patients were included consecutively in the study. However, from March 2020 to June 2020, recruitment continued, but on a non-consecutive basis due to the COVID-19 outbreak. Ninety-seven % of the sample was recruited before March 2020 and the COVID-19 outbreak.

Demographic and clinical variables

The following variables were included in the study: demographic data, pre-stroke functional status using the modified Rankin Scale (mRS), vascular risk factors, statin and antithrombotic treatment, baseline systolic blood pressure, baseline glycemia, stroke severity assessed with the National Institutes of Health Stroke Scale (NIHSS) on admission, intravenous thrombolysis (IVT) administration, the site of the large vessel occlusion on baseline CT angiography, The Alberta Stroke Program Early CT Score (ASPECTS) on baseline CT, EVT procedural data and times and the degree of reperfusion after EVT assessed by the modified Treatment in Cerebral Infarction scale,[7] with score 2b to 3 referring to successful reperfusion.

Clinical impact was assessed by the functional outcome at discharge and at 90 days, rated by a neurologist, either in person or through a telephone interview, using the mRS. Good clinical outcome (GCO) was defined as a mRS score of 0 to 2. Futile recanalization is defined as the successful reopening of the occluded artery (mTICI≥2b) without meaningful clinical improvement at 90 days (mRS from 3 to 6).

Ultrasound assessment of endothelial function: FMD measurement

Two investigators (JRA during the first year of recruitment and PR during the rest of the study time period) performed ultrasound assessment of systemic endothelial function within 72 h after the stroke event by measuring brachial artery FMD in response to reactive hyperemia. The assessment was conducted in keeping with the guidelines of the International Brachial Artery Reactivity Task Force and the Working Group of the European Society of Hypertension.[8] We used a high-resolution B-mode ultrasound (Philips CX50 by Philips ultrasound Andover, MA, USA) with a 7.5-MHz, linear-array transducer. In short, a longitudinal image was used to measure brachial artery diameter (first baseline value, d1). A blood pressure cuff was inflated on the upper arm to 300 mm Hg for 4 min and then deflated. 1 min later, a second longitudinal scan was obtained to calculate the brachial artery diameter (post-occlusion value, d2). FMD was expressed as the percentage of diameter variation (absolute diameter changes were also recorded, FMD $= d2 - d1/d1 \times 100$). Measurements were repeated twice and mean percentage values were obtained for the analysis.

Metabolites

Serum samples were obtained from arterial blood extracted immediately following femoral artery puncture for EVT. ADMA, SDMA and hArg, were quantified in samples and in conditioned cell culture media using an ultra-performance liquid chromatography–tandem mass spectrometry method as described in detail in Supplementary Methods. The laboratory performing and analyzing the mass spectrometric measurements was blinded to the clinical status of the patients.

The molar ratios of the arginine derivatives hArg/ADMA and hArg/SDMA have been suggested as potential biomarkers for predicting vascular phenotypes and risk, and these ratios were calculated and included in the study.[9,10]

We also included creatinine plasma levels and estimated glomerular filtration rate (eGFR) was calculated from creatinine levels using the 2021 CKD-EPI (CKD Epidemiology Collaboration) creatinine equation.[11]

Statistical analysis

The normality of data distribution was assessed using the Kolmogorov-Smirnov test. Baseline categorical variables are presented as frequencies and percentages (%), while continuous quantitative variables are expressed as means with standard deviations (SD) or medians with interquartile ranges (IQR), depending on their distribution. Baseline characteristics of the study groups were compared using the Chi-square test for categorical variables, the Student's T-test for quantitative variables with a normal distribution, and the Mann-Whitney U test for quantitative variables that did not follow a normal distribution, as well as for ordinal variables.

First, we compared elderly and younger patients in terms of demographic, clinical, radiological, prognostic, and endothelial function data. To do this, we calculated the median age of the study sample, and the population was dichotomized into two groups: patients aged ≤

median age and patients aged > median age. The relationships between endothelial function markers and risk factors were assessed using Pearson and Spearman correlation coefficients.

Second, the relationship between age, endothelial function, and functional outcome at 90 days was studied using univariable and multivariable ordinal regression analysis. A multivariable model was constructed, including variables that demonstrated a p-value < 0.05 in the bivariate analysis, with the mRS at 90 days as the dependent variable. Adjusted odds ratios (ORs) are presented with their corresponding 95 % confidence intervals.

Finally, mediation analysis based on the four-step procedure described by Baron and Kenny[12] was performed to assess the role of arginine metabolites and flow-mediated dilation (FMD) values as mediators (M1 and M2) of the relationship between age and clinical outcome at 90 days after stroke treated with EVT. The hypothesized mediation model is depicted in Fig. 1. Standard and bootstrapping methods (5000 samples) were used for confidence interval estimations.

Statistical analysis was conducted using SPSS statistics version 21 (USA) and the Jamovi 2.3.28.0 program (http://www.jamovi.org/). A p value of <0.05 was considered statistically significant. Listwise deletion was applied for missing data in the statistical analyses.

Informed consent

The study was approved by the local institutional Clinical Research Ethics Committee (Comité ético de investigación clínica del área sanitaria de Guipúzcoa). Written informed consent was obtained from all patients in the study or their relatives, who gave permission to include their data in the study database in accordance with the European and Spanish Personal Data Protection law.

Results

Patients

During the study period, a total of 232 EVT procedures were performed in 230 patients. Blood samples from 25 patients could not be analyzed due to technical processing issues, and blood was not obtained from 41 patients recruited during the COVID-19 pandemic months. Arginine derivatives could not be quantified in[10] patients due to inconclusive liquid chromatography measurements. Six patients were lost to follow-up and lacked outcome data at 90 days. As a result, a total of 150 stroke patients were included in this analysis.

The median age of the study population was 77 years (IQR = 69.0–84.8). The age distribution is shown in Fig. 2. Seventy-six patients were aged 77 years or younger, forming the "young" group, while 74 patients were older than 77 years, forming the "elderly" group."

Approximately half of the patients were female (50.7 %). The median NIHSS score on admission was [15] (IQR:8-20). A large proportion of patients (64.0 %) presented with anterior circulation large vessel occlusion. Cardioembolic was the most frequent stroke etiology, 56 % of patients.

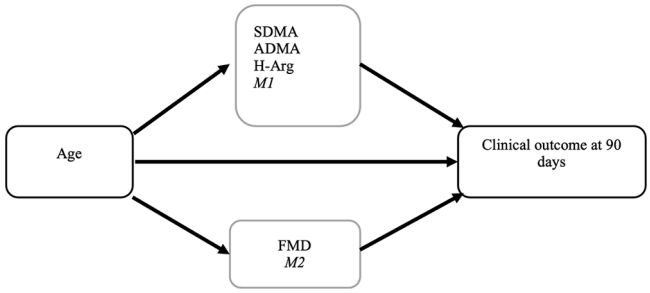


Fig. 1. Hypothetical mediation models with arginine metabolite levels (M1) and FMD (M2) as mediation variables, age as predictor variable and mRS scale score at 90 days as dependent variable.

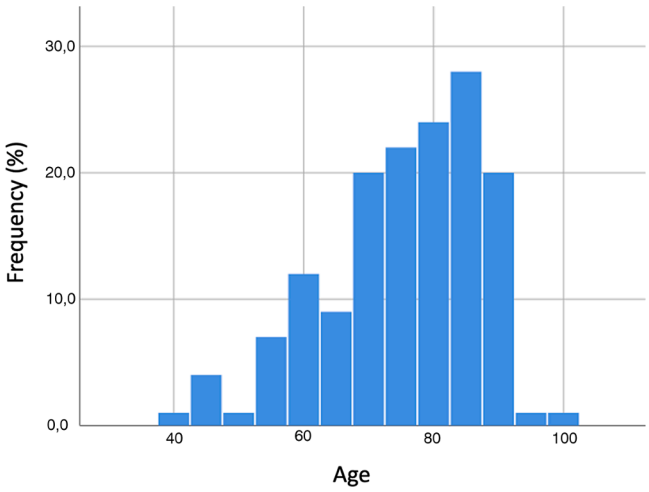


Fig. 2. Age distribution across the study patients.

EVT was performed with a mean OTG puncture time of 244.5 minutes (sd: 195), which also corresponds to the time of blood sample collection. IVT was administered to 28 % of patients. Successful recanalization (TICI 2b–3) was achieved in 90 % of cases, with a median of two thrombectomy passes.

The median ASPECTS was 9 (IQR: 7–10), indicating relatively preserved brain tissue. At 90 days, 44.9 % of patients (out of 149 with available follow-up data) achieved a GCO. However, radiological hemorrhagic transformation occurred in 34.9 % and symptomatic hemorrhagic transformation in 17.9 % of cases and futile recanalization was observed in 52.6 % of patients (Table 1).

Mean plasma levels of hArg, ADMA, and SDMA were 2.32 ± 1.27 μmol/L, 0.53 ± 0.15 μmol/L, and 0.21 ± 0.12 μmol/L, respectively. The mean hArg/SDMA and hArg/ADMA ratios were 13.49 ± 9.39 and 4.39 ± 1.95, respectively.

Arginine derivatives showed significant correlations with one another but not with FMD (Supplementary Table 1). Correlation analysis between endothelial markers and stroke risk factors revealed associations with several demographic and analytical variables (Supplementary Table 2).

Comparison between younger and older patients

When comparing elderly and younger patients, the proportion of women was significantly higher in the elderly group (60.8 % vs. 40.8 %, p = 0.014). The prevalence of comorbidities and stroke severity did not differ significantly between the groups. Recanalization rates and procedural times were comparable between groups.

However, older patients exhibited significantly worse outcomes, with lower rates of GCO both at discharge (24.6 % vs. 46.6 %, p = 0.006) and at 90 days (31.1 % vs. 57.9 %, p = 0.001), as well as significantly higher rates of futile recanalization (67.2 % vs. 38.2 %, p = 0.001) (Table 1).

Regarding endothelial function, mean FMD was significantly lower in elderly patients (2.98 % vs. 5.07 %, p = 0.002), and prerecanalization SDMA levels were higher in the elderly group (0.233 μmol/L vs. 0.186 μmol/L, p = 0.018). Consequently, the hArg/SDMA ratio was significantly lower among elderly patients (10.92 vs. 15.99, p = 0.001). No significant differences were observed in hArg or ADMA levels between the two groups (Table 1).

Association between age, endothelial function markers and clinical outcome after stroke treated with EVT.

Ordinal regression analysis identified several factors independently

Table 1
Baseline demographics, clinical, neuroimaging, procedural and outcome data in the entire study population and bivariate analysis between younger and older patients.

	Total	Young (≤77)	Elderly (>77)	p
n	150	76	74	
Demographics and vascular risk factors				
Age, mean (sd)	75.4 (11.7)	66.4 (9.23)	84.7 (4.37)	**<0.001**
Age, median (IQR)	77 (69-84.8)	69 (61-73)	85 (82-88)	**<0.001**
Female sex, *n*(%)	76 (50.7)	31(40.8)	45 (60.8)	**0.014**
High blood pressure, *n* (%)	97 (64.7)	44(57.9)	53 (71.6)	0.089
Diabetes mellitus, *n*(%)	32 (21.3)	19(25.0)	13 (17.6)	0.32
Dyslipidemia, *n*(%)	68 (45.3)	38 (50.0)	30 (40.5)	0.25
Atrial fibrillation, *n* (%)	48 (32.0)	22 (28.9)	26 (35.1)	0.48
Prior mRS, median (IQR)	1 (0-1)	0 (0-1)	1 (1-2)	**<0.001***
Clinical stroke data				
NIHSS on admission, median (IQR)	15 (8-20)	13 (8-19)	16 (11-20)	0.16*
Anterior large vessel occlusion (TICA or MCA1)	96 (64.0)	47 (61.8)	49 (66.2)	0.613
Site of occlussion, *n* (%)				0.081
TICA	16 (10.7)	6 (7.9)	10 (13.5)	
MCA1	80 (53.3)	41 (53.9)	39 (52.7)	
MCA2	39 (26.0)	24 (31.6)	15 (20.3)	
PCA	3 (2.0)	2 (2.6)	1 (1.3)	
Basilar artery	7 (4.7)	1 (1.3)	6 (8.1)	
Extracranial vertebral artery	5 (3.3)	2 (2.6)	3 (4.0)	
SBP (mmHg), mean (sd)	152.0 (26.7)	148.8 (25.8)	155.3 (27.3)	0.144
DBP (mmHg), mean (sd)	80.5 (15.3)	81.9 (15.0)	78.8 (15.6)	0.216
Baseline glycemia (mg/dl), mean (SD)	132.4 (32.0)	133.1 (36.4)	131.6 (27.0)	0.783
Creatinine (mg/dl), mean (sd)	0.98 (0.38)	0.91 (0.43)	1.05 (0.32)	0.028
eGFR (mL/min/1.73m^2), mean (sd)	81.6 (21.4)	90.9 (21.3)	72.0 (16.9)	<0-001
Stroke etiology, *n* (%)				0.044
Large artery atheroesclerosis	31 (20.7)	18 (23.7)	13 (17.6)	
Cardioembolic	84 (56)	35 (46.0)	49 (66.2)	
Other determined etiology	3 (2.0)	3 (3.9)	0 (0)	
Undetermined	32 (21.3)	20 (26.4)	12 (16.2)	
Procedural data				
Intravenous thrombolysis, *n* (%)	42 (28)	20 (26.3)	22 (29.7)	0.64
Onset to groin time (minutes), mean (sd)	244.5 (195.0)	243.3 (155.7)	245.7 (214.4)	0.94
Groin to recanalization time (minutes), mean (sd)	54.5 (39.7)	53.7 (41.4)	55.3 (38.3)	0.81
Onset to recanalization (minutes), mean (sd)	299.0 (192.5)	297.0 (168.96)	301 (214.7)	0.90
Succesful recanalization (TICI2b-3), *n*(%)	135 (90.0)	68 (89.5)	67 (90.5)	1
Number of passes, median (IQR)	2 (1-3)	1.5(1-2)	2 (1-3)	0.068
ASPECTS, median (IQR)	9 (7-10)	9 (7-10)	9 (7-10)	0.90
Outcome data				
Good outcome at discharge (mRS<3), *n* (%)	51 (35.9)	34 (46.6)	17 (24.6)	**0.006**
Death during hospitalization, *n* (%)	16 (10.7)	6 (7.9)	10 (13.5)	0.265
Good outcome at 90 days (mRS<3), *n* (%) (*n* = 149)	67 (44.9)	44 (57.9)	23 (31.1)	**0.001**
Hemorrhagic transformation, *n* (%)	52 (34.9)	28 (37.3)	24 (32.4)	0.53
Symptomatic hemorrhagic transformation, *n*(%)	25 (17.9)	12 (15.8)	13 (17.6)	0.705
Futile recanalization, *n* (%) (*n* = 135)	71 (52.6)	26 (38.2)	45 (67.2)	**0.001**
Endothelial function				
FMD (%), mean (SD)	4.04 (3.81)	5.071 (4.529)	2.979 (2.516)	**0.002**
SDMA (μmol/L), mean (sd)	0.21 (0.122)	0.186 (0.108)	0.233 (0.130)	**0.018**
ADMA (μmol/L), mean (sd)	0.53 (0.153)	0.513 (0.158)	0.547 (0.146)	0.170
hArg (μmol/L), mean (sd)	2.32 (1.272)	2.437 (1.247)	2.188 (1.295)	0.239
hArg/SDMA ratio, mean (sd)	13.49 (9.39)	15.990 (11.243))	10.917 (6.086)	**0.001**
hArg/ADMA ratio, mean (sd)	4.398 (1.95)	4.813 (2.045)	3.971 (1.771)	**0.009**

ASPECTS: Alberta Stroke Program Early CT Score; IQR: interquartile range; MCA: middle cerebral artery; TICA: terminal internal carotid artery; mRS: modified Rankin Scale; NIHSS: National Institutes of Health Stroke Scale; SBP: systolic blood pressure; TICI: Thrombolysis in Cerebral Infarction scale; FMD: flow mediated dilation; hArg: homoarginine; SDMA: symmetric dymethilarginine; ADMA: asymmetric dymethilarginine. Statistics: $\chi 2$ test for categorical variables, Student's t- test for continuous normal variables; Mann-Whitney *U* test for continuous non-normal and ordinal variables (*).

associated with clinical outcomes at 90 days following IS treated with EVT. Age (OR = 1.056, $p < 0.001$), a history of hypertension (OR = 2.597, $p = 0.009$) and NIHSS score on admission (OR = 1.115, $p < 0.001$) were all associated with poorer outcomes. In contrast, successful recanalization (OR = 0.0.093, $p < 0.001$) and administration of intravenous thrombolysis (OR = 0.485, $p = 0.044$) were independently associated with more favorable outcomes. Among endothelial function markers, SDMA was identified as an independent predictor of poor functional outcome at 90 days (OR =1.343 per 0.1 umol/l increase, $p = 0.047$) (Table 2).

Mediation effects analysis of endothelial function markers in the relationship between age and clinical outcome

Mediation analyses were conducted as previously described, considering SDMA, ADMA, hArg, hArg/SDMA, hArg/ADMA, and FMD as potential mediators in the relationship between age and 90-day clinical outcomes.

Among these, only SDMA met the necessary assumptions for mediation analysis. Significant correlations were found, and the analysis revealed that prerecanalization SDMA levels significantly mediated the association between age and 90-day outcomes, with an indirect effect of 0.0068 (95 % CI: 0.0015–0.156) with standard mediation method and of 0.00671 (95 % CI: 0.00017-0.0140) with bootstrapping technique woth 5000 resamples for mediation. The total effect of age on the mRS at 90 days was 0.0584 (95 % CI: 0.0339–0.829), with SDMA accounting for 11.6 % of the mediation in this relationship (Fig. 3).

Discussion

The main finding of this study is that the endothelial biomarker SDMA is an independent predictor of poor clinical outcome at 90 days and significantly mediates the association between age and prognosis. These results suggest that endothelial dysfunction may contribute to the poorer response to EVT observed in older patients compared to their younger counterparts.

SDMA is a methylated arginine derivative produced by all nucleated cells as a by-product of protein metabolism and primarily eliminated by the kidneys.[13] Unlike its isomer ADMA, which directly inhibits NOS, SDMA impairs NO production indirectly by limiting the cellular availability of l-arginine, the substrate for NOS 6. Biologically, SDMA has been associated with pro-inflammatory activation, oxidative stress, reduced NO bioavailability, and high-density lipoprotein (HDL) dysfunction.[14]

In the context of stroke due to large vessel occlusion, elevated SDMA levels may contribute to increased cytotoxic injury, disruption of the blood–brain barrier, or impaired collateral circulation, among other

Table 2
Univariate and multivariate ordinal regression analysis of factors influencing clinical outcome at 90 days after stroke treated with EVT.

	Univariate OR (95 %CI)	p	Adjusted OR (95 % CI)	Adjusted p-value
Age (per year)	1.07 (1.04-1.09)	**<0.001**	1.056 (1.019-1.096)	**<0.001**
Female sex	1.28 (0.73-2.26)	0.385		
High blood pressure	2.82 (1.53-5.25)	**<0.001**	2.597 (1.268-5.38)	**0.009**
Diabetes mellitus	1.66 (0.82-3.42)	0.163		
Dyslipidemia	1.65 (0.93-2.94)	0.086		
Atrial fibrillation	1.21 (0.66-2.22)	0.544		
Creatinine (per mg/dl)	2.08 (1.02-4.63)	0.053		
Renal function (eGFR) (per point)	0.407 (0.22-0.743)	**0.004**	1.068(0.454-2.498)	0.879
Prior mRS (per point)	3.46 (1.76-6.93)	**<0.001**	2.205 (0.989-4.960)	0.054
NIHSS on admission (per point)	1.11 (1.06-1.16)	**<0.001**	1.115 (1.059-1.177)	**<0.001**
Procedural data				
Intravenous thrombolysis	0.51 (0.27-0.96)	**0.039**	0.485 (0.239-0.977)	**0.044**
Onset to recanalization (per minute)	1 (0.99-1.00)	0.246		
Succesful recanalization (TICI2b-3)	0.259 (0.09-0.68)	**0.007**	0.093 (0.029-0.278)	**<0.001**
>2 passes	1.97 (0.94-4.18)	0.075		
Endothelial biomarkers				
FMD	0.96 (0.88-1.05)	0.397		
SDMA (per 0.1 μmol/L increase)	1.62 (1.26-2.11)	**<0.001**	1.343 (1.004-1.801)	**0.047**
ADMA (μmol/L)	1.44 (0.22-9.45)	0.704		
hArg (μmol/L)	0.85 (0.67-1.09)	0.201		
hArg/SDMA ratio	0.96 (0.92-0.99)	**0.012**	1.003 (0.960-1.043)	0.877
hArg/ADMA ratio	0.88 (0.76-1.03)	0.115		

FMD: flow mediated dilation; IQR: interquartile range; mRS: modified Rankin Scale; NIHSS: National Institutes of Health Stroke Scale; TICI: Thrombolysis in Cerebral Infartion scale; hArg: homoarginine; SDMA: symmetric dymethilarginine; ADMA: asymmetric dymethilarginine.eGFR: stimate glomerular filtration rate.



Fig. 3. Results of the proposed mediation models.

pathophysiological mechanisms. Indeed, SDMA has previously been associated with poor outcomes after ischemic stroke, including higher mRS scores at 90 days,[15]increased rates of cardiovascular events and mortality within 30 days of stroke,[16] and higher long-term mortality.[17] However, none of these studies have specifically investigated the role of SDMA in the current context of stroke care, where recanalization therapies such as EVT play a central role. This study uniquely focuses on patients undergoing EVT and provides valuable data on SDMA levels measured during the very early phase of stroke, prior to recanalization, with a mean time from symptom onset to blood sampling of 244.5 minutes (SD = 195), approximately four hours. While SDMA is known to rise within the first six hours and peak at 24 hours after an acute event,[18] we found no significant correlation between time from stroke onset and SDMA levels in our cohort ($r = 0.091$, $p = 0.287$; Supplementary Table 1). Therefore, we cannot conclude whether SDMA levels fluctuate within this very early window post-stroke onset.

The finding that SDMA, but not hArg or ADMA, correlates with clinical outcome after stroke could be explained by differences in their biological roles, clearance mechanisms, and vascular impacts, particularly in relation to renal function, endothelial health, and NO metabolism: SDMA does not inhibit NOS directly but it interferes with l-arginine transport into cells; SDMA is more related to inflammatory function and the effect of SDMA might be more chronic and systemic, aligning better with long-term vascular dysfunction. Therefore, and even if we cannot fully discard methodological factors contributing to this, biological explanations could also explain a specific role of SDMA over the other arginine derivatives.

Systemic endothelial function, as assessed by FMD and SDMA, was significantly worse in the elderly group in this study. Endothelial cell senescence is a pathophysiological process marked by structural and functional alterations in the endothelium, leading to increased intercellular permeability, arterial stiffness, impaired angiogenesis and vascular repair, and dysregulation of vasodilatory and vasoconstrictive responses. Notably, infusion of the nitric oxide synthase inhibitor l-NMMA (NG-monomethyl-l-arginine) has been shown to significantly reduce CBF and increase cerebrovascular resistance in elderly individuals, while having no significant effect in younger participants—indicating that CBF regulation in older adults may rely more heavily on the integrity of the NO pathway.[19]

Many therapeutic strategies under investigation for stroke focus on or involve the NO-endothelium pathway, with varying results. The present study contributes to a more nuanced understanding of the role of the NO-endothelium pathway in the context of recanalization therapies and could ultimately facilitate the design of better-targeted treatment strategies. This study has several limitations. First, it was a single-center study with a relatively small sample size, which may limit the generalizability of the findings.

Second, while we attempted to adjust for relevant covariates, it is possible that important confounders were omitted from the multivariable analysis. In this regard, SDMA levels have previously been associated with renal function, time from stroke onset, and infarct size. In our cohort, no significant correlation was found between SDMA and time from stroke onset ($\rho = 0.048$, $p = 0.577$) or infarct size, as estimated by NIHSS on admission ($\rho = 0.093$, $p = 0.276$). Regarding renal function, SDMA was significantly correlated with creatinine levels ($\rho = 0.465$, $p < 0.001$) and eGFR ($\rho = -0.495$, $p < 0.001$ (Supplementary Table 1); eGFR, but not creatinine levels, correlated with mRS but lost significance in the multivariant models while SDMA's association remained significant suggesting an independent role of SDMA on stroke prognosis. (Table 2), However, we acknowledge that the correlation between renal function and SDMA is strong as shown by this and other published studies and we should be cautious with our conclusions due to the small sample size and observational nature of this study.

Furthermore, SDMA correlates strongly with age and renal function ($p < 0.001$), but its lack of association with hypertension ($p = 0.080$) seems paradoxical given the endothelial pathophysiology. We believe

that given the p-value for the SDMA hypertension association approached conventional significance thresholds ($p = 0.08$), this paradoxical finding might be explained by terms of statistical power in a relatively small sample size observational study and that a true underlying association between SDMA and hypertension cannot be denied.

In addition to this, hArg correlated with sex in this study (supplementary Table 2) and mean hArg levels were higher in males (2.61 (sd=1.3) vs 2.03 (sd=1.18), $p = 0.005$. Multiple large population-based studies have consistently demonstrated that plasma hArg concentrations are higher in men than in women. The underlying mechanisms for these differences are not fully elucidated but may involve hormonal, genetic, and metabolic factors.[20,21] In the setting of our study, women are more represented in the elderly group but we found no correlation between sex and GCO (Table 2) and when adjusting for sex, hArg did not correlate with mRS at 90 days either, adjusted OR= 0.883 (95 %CI 0.688-1.13), $p = 0.324$.

Unfortunately, and may in part be due to the emergency setting of this condition, toxic habits (tabacco and alcohol consumption) were not well recorded in this registry, only 50 % of patients had this information available and most of them belonged to the younger group, where more attention is paid to these risk factors. Therefore, we thought that including these variables with the missing values could lead to bias and concluded not to include them in our models.

Thirth, FMD ultrasound studies were performed by two different investigators but interoperator variability in FMD measurements was not assessed, and this would strengthen the methodological reliability of the FMD measurement protocol, And fourth, blood samples were collected directly from the arterial access immediately after groin puncture, which may limit the reproducibility of these findings in studies utilizing venous sampling.

In conclusion, this study highlights the involvement of the SDMA metabolite and the NO-endothelium pathway in the poorer outcomes observed in elderly patients with IS treated with EVT. These findings offer new insights into age-related vascular vulnerability in the context of acute stroke and suggest potential therapeutic targets. Given the growing proportion of elderly individuals among stroke patients, further research is warranted to explore endothelial-based interventions aimed at improving outcomes in this high-risk population.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author used ChatGPT in order to improve readability and language of the manuscript. After using this tool, the author reviewed and edited the content as needed and take full responsibility for the content of the published article.

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CRediT authorship contribution statement

Patricia de la Riva: Writing – original draft, Project administration, Methodology, Conceptualization. **Jon Rodríguez-Antigüedad:** Methodology, Investigation. **Juan Marta-Enguita:** Writing – review & editing, Methodology, Formal analysis. **Virginia Gómez:** Investigation. **Gorka Arenaza:** Investigation. **Ana Gorostidi:** Investigation. **Noemi Díez:** Investigation. **Ana de Arce:** Investigation. **Eñaut Garmendia:** Investigation. **Eider Zumeta:** Investigation. **Jose Angel Larrea:** Investigation. **Alberto Bergareche:** Writing – review & editing, Supervision, Conceptualization. **Adolfo López de Munain Arregui:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Patricia de la Riva reports financial support was provided by Basque Government Department of Health. Patricia de la Riva reports a relationship with Basque Government Department of Health that includes: funding grants. Patricia de la Riva reports a relationship with Daiichi Sankyo Inc that includes: travel reimbursement. Patricia de la Riva reports a relationship with Spanish Society of Neurology that includes: speaking and lecture fees. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jstrokecerebrovasdis.2025.108412.

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