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TITOLO TESI

Angiographic identification and role of endothelin-1 and nitric oxide in no-reflow phenomenon in patients with acute ischemic stroke from large vessels occlusion treated successfully with mechanical thrombectomy

Coordinatore: Ch.mo Prof. Gianpaolo Rossi
Supervisore: Ch.mo Prof. Danilo Toni

Dottorando: Ettore Nicolini

SUMMARY

ABSTRACT

Background. Futile recanalization (FR), defined as a 90-day mRS 3-6 despite successful recanalization, account for 29% to 60% of large vessel occlusion (LVO) acute ischemic stroke (AIS) treated with mechanical thrombectomy (MT). Failure of early neurological improvement (fENI) describes patients successfully recanalized but not clinically improving at 24-hours or at 7-days.

No-reflow phenomenon (NRP) is a possible cause of FR and fENI, described in animal models and myocardial infarction as deficient microvascular reperfusion. Evidence of NRP in AIS patients is scarce.

Proposed determinants of NRP include vasoactive agents such as Endothelin-1 (ET-1) and Nitric oxide (NO), possibly involved in microvascular disfunction of NRP.

Aim of our research was to define NRP in AIS from LVO of the anterior circulation treated with MT with successful recanalization.

STUDY 1.

Methods. We retrospectively analyzed 185 post-interventional digital subtraction angiographies (DSA) of anterior circulation LVO AIS patients treated with MT. We created a score, called modified capillary index score (mCIS), dividing middle cerebral artery territory in three segments. For each segment we gave 2 points if the capillary blush was present without any delay, 1 if delayed and 0 if absent. We used ROC curve to define mCIS≤3 as cut-off and marker of NRP. The primary endpoint was to identify a marker of NRP on post interventional DSA and to test whether this marker may predict FR and fENI. Secondary endpoint was to search a correlation between NRP, lesion volume and hemorrhagic transformation.

Results. NRP was present in 35.1% of patients. NRP predicted fENI at 24h (aOR2.617, 95%CI1.192–5.745, p=0.016) and at 7 days (aOR4.601, 95%CI1.636–12.936, p=0.004), but not FR. Moreover, NRP predicted hemorrhagic transformation (aOR2.444, 95%CI 1.266–4.717, p=0.008).

Discussion. NRP is poorly investigated in AIS. We identified an angiographic score able to identify NRP in AIS patients. Our angiographic marker was able to predict early outcome and hemorrhagic transformation of the ischemic lesion.

STUDY 2.

Methods. We prospectively enrolled 61 patients with AIS from LVO of the anterior circulation, successfully treated with MT. Patients were divided in groups according to the presence of NRP, as defined in study1. Peripheral venous blood samples were taken to dose ET-1 and NO at admission, after 24 and 48 hours. When technically possible, intracranial arterial blood samples, before and after recanalization, were taken. Primary endpoint was to test the association between ET-1 and NO levels and NRP. Secondary endpoint was to explore the association between ET-1 and NO levels and clinical outcome.

Results. NRP Patients showed lower pre-MT intracranial levels of NO (9.60 μM ±2.80 vs 18.58 μM ±5.92, p=0.004) but the association was not confirmed at logistic regression (aOR0.561, 95%CI 0.297–1.061, p=0.075). Mean peripheral NO levels at 48 hours were 20.46 μM ±7.08 in the NRP group and 14.00 μM ± 8.06 in the no-NRP group (p=0.084). fENI at 24h was associated to lower serum NO levels at 24h (aOR1.19, 95%CI 1.014–1.213, p=0.023).

Discussion. Our study tried to explore the role of ET-1 and NO in NRP. The trend to lower pre-MT levels of NO, might be the consequence of a reduced activity of the endothelial isoform of nitric oxide synthase (NOS). The increased values of NO at 24h in NRP patients might be due to the activation of the neuronal NOS. NO rather than ET-1 seems to have a major role in NRP.

Conclusion. Reperfusion of main arteries in AIS from LVO is not always sufficient to ensure clinical improvement, a possible cause might be deficient microvascular reperfusion. We identified a marker of NRP in AIS patients, which could represent a useful tool to study this poorly recognized condition. Furthermore, we outlined a possible role of NO in NRP.

Our data may contribute to future research studying NRP pathophysiology and possibly treatment.

Introduction

Futile recanalization after mechanical thrombectomy in acute ischemic stroke from large vessels occlusion

Reperfusion therapies have improved prognosis of acute ischemic stroke (AIS) patients. Albeit Intravenous thrombolysis (IVT) is an effective treatment, large vessels occlusions (LVOs) of cerebral circulation are poorly responsive to infusion of recombinant tissue plasminogen activator (rTPA). In particular, large thrombi seem to be resistant to rTPA penetration, probably due to an external layer composed of platelet and fibrin[1–3]. Large randomized controlled trials (RCTs) confirmed efficacy of endovascular treatment (EVT) in revascularization of anterior circulation LVOs, with outstanding clinical outcomes. According to subsequent metanalysis, mechanical thrombectomy (MT) provides high benefit to AIS patients with LVOs of anterior cerebral circulation with a number needed to treat of 3.6[4]. Nonetheless, from RCTs it emerged that up to 50% of patients treated with TM are bound to poor 90-day functional outcome, defined as a score between 3 and 6 at the modified Rankin Scale (mRS). Successful recanalization of occluded vessels, e.g. post procedural thrombolysis in cerebral infarction (TICI) score[5] ≥ 2b, represent a strong but not sufficient determinant of favorable outcome after EVT. Futile recanalization (FR) is defined when successful recanalization of a cerebral LVO does not lead to functional independence, i.e a 90-day mRS 0-2[6]. Large studies on MT reported rates of FR from 29% to 67%[4] when treatment is performed within 6 hours from symptoms onset, and from 31% to 35% and of cases when treatment is performed within 16-24 hours after the last time the patient was known to be well,[7,8]despite the selective inclusion criteria.

Looking at real life studies, in a large metanalysis including more than two thousand patients FR amounted to 48,7% of the population[9].

Variables not strictly related to acute stroke treatment, such as internist complications and motor rehabilitation, may have a relevant impact on 90-day functional outcome. Thus, some authors used an earlier outcome measure to detect direct clinical effect of the procedure. Early neurological improvement (ENI) is defined as improvement at the NIHSS at predefined times compared to baseline; generally, ENI is evaluated at 24 hours from procedure or at discharge and has emerged as reliable prognostic tool[10–12]. In the same way, absence of early improvement at NIHSS is termed failure of ENI (fENI), and hence fENI in patients with final TICI 2b-3 is an alternative definition of FR[13].

Some predictors of FR have been identified, in particular baseline stroke severity measured with NIHSS[14], pre-stroke functional status, age[14], female gender and early infarct signs at emergency computed tomography (CT), namely a low Alberta Stroke Program CT score (ASPECTS)[9,15]. Also, some cardiovascular (CV) risk factors seem to influence patients outcome despite successful procedure; indeed FR is more common in patients with diabetes mellitus, arterial hypertension, and atrial fibrillation[9]. The association between modifiable and not modifiable CV risk factors and worse prognosis after stroke is well established and reasonably due to higher burden of comorbidity and rates of complications. However, factors such as diabetes mellitus and arterial hypertension might also impair microvasculature reactivity and enhance the reperfusion injury with consequent clinical worsening. Notably, admission hyperglycaemia and higher systolic blood pressure are predictors of FR[9].

As to radiological features, leukoaraiosis[16] which is a marker of chronic microvascular subcortical damage, and large deep white matter ischemic lesions,[17] which cause more severe strokes, are associated with FR.

During AIS acute treatment many variables might differ from case to case, even when ending in a successful procedure. Latest evidence changed the concept of time in AIS which

was believed to be the main factor influencing outcome. This is actually not true for all cases, as patients are now classified as fast and low progressors according to the speed of damage progression before recanalization therapy[18]. Anyway, fast revascularization has shown to be still a key issue, as its benefit reduces the later the occluded vessel is reperfused. Both time before starting the procedure and delay of the procedure itself are important for clinically effective recanalization; indeed, longer onset-to-puncture and puncture-to-recanalization time resulted predictors of futile recanalization[9].

Some investigators have questioned the role of IVT for patients undergoing MT, but RCTs, retrospective studies and metanalysis on the argument could not demonstrate the non-inferiority of direct MT compared to bridging therapy[19]. For patients with successful recanalization, in the same way, IVT represents a predictor of good outcome (e.g. a negative predictor for FR)[9]. One hypothesis, which still needs confirmation from further studies, entails rTPA effect on microvascular occlusions not resolved by EVT.

Among the serum markers proposed as predictors of FR after EVT, some are involved in blood brain barrier rupture after ischemia, including metalloprotease-9 (MMP-9), tenascin and thioredoxin; high levels of these markers are associated with FR[20]. A disintegrin and metalloproteinase with a thrombospondin motif repeats 13 (ADAMTS 13), exerts its antithrombotic role through clavation multimeric Von Willebrand factor; lower levels of ADAMTS13 in peripheral blood predict poor functional outcome after successful MT[20,21]. ADAMTS13 gene deletion in mice was associated to larger infarct volumes and lower cortical blood flow after recanalization; indeed, the disintegrin might prevent thrombosis of cerebral microvasculature, namely the no-reflow phenomenon[22].

Till date the exact mechanisms underlying futile recanalization are not clear, though it may be the result of a combination of different factors [23]. Some of these factors include impaired cerebral autoregulation, large hypoperfusion volumes, poor collateral circulation, and microvascular compromise also known as the no-reflow phenomenon[24].

The No-reflow Phenomenon

No-reflow phenomenon (NRP) is defined as the absence of perfusion in the parenchymal microvasculature after an ischemic injury caused by occlusion of a large artery, even after restoration of patency in the main artery. As described, NRP is not specific of any one organ, as apart from the brain, it has also been reported to occur in the heart, kidneys and skin[25,26]. NRP was first described on rabbits' brain[27], in which experiments demonstrated the presence of blood elements entrapped within capillaries in the brain areas showing NRP phenomenon[27]. Later on, studies using "bloodless ischemia" models, e.g. ischemia preceded by blood wash-out, pointed to a causative role of both haematological components and ischemic damage to the microvascular unit[27].

Occlusion of microvasculature after reperfusion is possibly caused by microthrombi derived from the main occluding clot[28] or from in situ platelet activation as well[29]. Moreover, clotting cascade is further enhanced by the endothelial damage caused by thrombectomy itself[30].

As to microvascular unit disfunction, studies suggested that postischemic swelling in endothelial cells and abutting end feet of pericytes result in decreased luminal size within an hour after reperfusion, which impedes blood flow[31,32]. Indeed, after ischemia pericytes go in a state of "rigor" with consequent capillary constriction[33].

Concerning inflammation, studies in experimental mouse stroke models demonstrated that after ischemia, oxygen and nitrogen free radicals induced prolonged pericyte contraction of capillaries which then blocked flow of red blood cells[33]. Electron microscopy has shown that neutrophils contribute to capillary stalls after brain infarct;[34] in addition their depletion in animal models leads to reperfusion of stalled capillaries[34].

Furthermore, cortical spreading depression, which occurs in the ischemic penumbra, causes neurovascular uncoupling and cycles of hyperaemia and hypoperfusion, with elevations in

vasoconstrictive extracellular K+ concentration that contribute to the ongoing secondary damage during reflow [33,35].

Research question

Technical success of EVT is still not related to clinical benefit in a large proportion of patients. NRP is one possible explanation of FR in AIS. The occurrence of NRP has been proven in animal brain and in human heart, but there is still a lack of clinical evidence after AIS in humans.

Aim of this research was to define NRP in patients with AIS from LVO. The first study focused on a possible angiographic marker of NRP, through a retrospective analysis of post MT DSA to define brain capillary status after successful recanalization. From imaging analysis, we created a post-interventional angiographic score to clinically define NRP.

In the second study we tried to determine how ET1 and NO are involved in NRP in patients with AIS from LVO and successful recanalization.

STUDY 1 - Angiographic evaluation of no-reflow phenomenon in patients with acute ichemic stroke from large vessels occlusion successfully treated with mechanical thrombectomy

Introduction

No reflow phenomenon in clinical practice

Some authors tried to identify the NRP through MRI arterial spin labeling perfusion mapping[36]. In the study NRP was found just in one out of thirty-three patients and the authors conclude that NRP might be less frequent in human than expected. The authors assume as definition of NRP cerebral blood flow reduction ≥ 40% in anatomical region involved by ischemia on 24-hour MRI. When describing NRP Ames et al.[27] describe “mottled areas” of hypoperfusion which could be not evident in perfusion imaging due to its limited spatial resolution. In the abovementioned clinical study[36], one third of patients showed mild hypoperfusion, which could be a more sensitive marker of NRP.

The occlusion of microvasculature, as well as the entrapment of red blood cells in capillaries, probably occur immediately after reperfusion[25]. Nonetheless, after recanalitazion of an occluded artery an initial period of hyperperfusion and vasodilatation of intact vessels is evident, which might mask the concomitant mottled NRP on imaging[37]. A sensitive technique of imaging for capillary identification is needed to detect NRP. Concerning the timing of NRP identification, experimental data suggest that after around 5 hours from reperfusion an established no-reflow is evident.[37] But what occurs in vivo in microcirculation after this time interval is not known, and hence the NRP should be assessed within the first 6 hours after recanalization.

Other researchers found augmented microvascular resistances in patients with MCA occlusion by measuring the Gosling pulsatility index (PI) and the Pourcelot resistance index (RI) with transcranial Doppler and this finding was believed to be an instrumental marker of NRP.[38]

Angiographic evaluation of cerebral capillaries in patients with acute ischemic stroke from anterior cerebral circulation large vessels occlusion

Cerebral digital subtraction angiography (DSA) has a high spatial resolution

The capillary index score (CIS) is an angiographic index which evaluates parenchymal perfusion through retrograde flow on pre-interventional DSA in middle cerebral artery (MCA) occlusions[39]. CIS estimates the presence of collateral circulation before MT and is a strong predictor of functional independence[39].

Evidence of contrast medium stasis or absence of capillary blush, when injecting contrast medium distal to the occlusion before mechanical thrombectomy, is a sign of microcirculatory thrombosis and is associated with worse functional outcome.[40]

DSA allows a dynamic evaluation of cerebral circulation, through the detection of early or late appearance of arterial, capillary and venous phase. The evidence of any venous filling during the arterial phase is defined as early venous filling (EVF). EVF detected in post-recanalization DSA is a predictor of haemorrhagic transformation of brain infarct,[41,42] while in patients without intraparenchymal haemorrhage it is associated with better prognosis.[43] Authors speculate that EVF could be the evidence of a post-ischemic hyperaemic state known as luxury perfusion[44]. Other authors instead found EVF to be a sign of parenchymal injury[41,43].

Differently from TICI score, the assessment of reperfusion after myocardial infarction with the Thrombolysis in Myocardial Infarction (TIMI) score, evaluates microvascular perfusion rather than large vessel recanalization[45]. In cardiological setting, NRP is described as

substantial angiographical coronary flow reduction (TIMI≤2) after recanalization of coronary arteries, and is a predictor of poor outcome[46].

Delayed contrast washout in post-interventional angiographic images could represent a marker of NRP. However, a preliminary retrospective study of 53 patients did not show differences in terms of functional outcome related to the abovementioned angiographic sign[47].

Methods

Study population

This is a single center retrospective study on patients with acute ischemic stroke (AIS) treated with mechanical thrombectomy from January 2015 till June 2021 at Policlinico Umberto I University Hospital,. Patients were treated according to national and international guidelines on treatment of acute ischemic stroke[48]. All patients prospectively included in the database signed an informed consent according to the Italian regulation.

We included patients who had an occlusion of the anterior cerebral circulation, in particular: intracranial internal carotid artery (ICA) (C5-C6 segments), first segment of the middle cerebral artery (M1), second segment of the middle cerebral artery (M2), tandem occlusion (e.g. intracranial occlusion with ipsilateral extracranial ICA occlusion or stenosis ≥70%). Among these patients, we selected those in which a successful recanalization had been obtained, defined as a TICI score of 2b-3[5]. Patients without post interventional anterior-posterior DSA were excluded.

Primary and secondary Endpoints

The primary endpoint of our study was to identify a marker of NRP on post interventional digital subtraction angiography (DSA) and to test whether this marker may predict futile recanalization and fENI.

Secondary endpoint was to search a correlation between NRP on post interventional DSA, lesion volume and hemorrhagic transformation.

Operational definition of no-reflow

We first calculated the Capillary Index Score (CIS) on the pre-interventional angiographic images in order to evaluate collateral circulation according to the technique described by Firas Al-Ali et al [39]. Then, we studied post-interventional angiograms to look for NRP and we proposed a new score that we called modified CIS (mCIS) score. We used the anterior-posterior images and divided the ischemic territory into 3 equal segments, similarly to the calculation of CIS. For each segment, we gave 2 points if the capillary blush was present without any delay, 1 point if the capillary blush was present but was delayed as compared to the first appeared capillary blush or when possible, compared to anterior cerebral artery territory, and 0 points if there was no capillary blush. Hence, we obtained a score ranging from 0 to 6. (Fig.1.)

mCIS is a post-recanalization evaluation which considers not only the presence but also the delay of capillary blush in MCA territory, which should represent a marker of microvascular efficiency.

Each post-interventional DSA was independently scored by 2 experienced interventional neuroradiologist (MI and FB), They were blinded to all other information and then came to a unanimous agreement on the final score.

Data collection

For each patient we recorded age, sex and the presence of relevant cerebrovascular risk factors such as arterial hypertension, atrial fibrillation, current or past habit of cigarette smoking, diabetes, hypercholesterolemia, carotid atherosclerosis, history of AIS in the past 3 months, heart failure, coronary artery disease and presence of tumor. We collected the following clinical variables: NIHSS (baseline, post-intervention, at 24 hours and at 7 days or at discharge if the hospitalization was shorter than 7 days), mRS (pre-stroke and at 90-day, the latter evaluated by telephone interview of the patients or relatives), aetiology of stroke based on the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria[49], AIS presenting at wake-up, location of the large vessel occlusion, Alberta Stroke Program Early CT Score (ASPECTS) [50], presence and type of haemorrhagic transformation according to the European Cooperative Acute Stroke Study (ECASS) classification of intracerebral haemorrhage (ICH) following thrombolysis[51], presence of symptomatic ICH, defined as any ICH associated with an increase of ≥ 4 points on the NIHSS at 24 hours or leading to death[51], and the ischemic lesion volume which was calculated using the A*B*C/2 formula on post-interventional MRI images. Procedural variables included the following: time from stroke onset to recanalization, administration of IV rtPA before mechanical thrombectomy (i.e. bridging therapy), specific technique used during mechanical thrombectomy, total number of passes, TICI score and total duration of the procedure.

All data were collected by two neurologists specialized in cerebrovascular diseases (EN and MDM)

We used 90-day mRS and 24-hour and 7-day NIHSS as main outcome measures. We considered mRS 0-2 as good outcome, 0-1 as excellent outcome and patients with 90-day mRS of 3-6 were classified as FR. According to literature[13], we defined fENI at 24 hours and at 7 days as 24hour or 7-day NIHSS equal or higher than baseline NIHSS.

Statistical analysis

Continuous variables were presented as mean, median and standard deviation (SD), categorial variables as number and percentages, as appropriate. Student's t tests or Mann-Whitney U tests were applied for continuous data. Pearson's $\chi 2$ tests or Fisher's exact tests were used for categorical data as appropriate.

To determine the most accurate cut off of mCIS to define NRP, receiver operating characteristic (ROC) curve analyses were used, by dichotomizing patients for FR, 24-hour fENI and 7-day fENI. The value which was closest to the point with both maximum sensitivity and specificity was chosen as cut off.

Univariate analysis was applied to find clinical and radiological variables associated to FR and fENI. Predictive value of NRP as defined by dichotomized mCIS was tested using a logistic regression model including variables found significant at the univariate analysis.

Same analyses were repeated using hemorrhagic transformation, sICH and mortality as outcome measures.

All the analysis were repeated selecting the cases without collateral circulation before MT (CIS=0). As global ischemia, as described in animal models, is not applicable to human beings, we considered absence of collateral circulation as the best condition in human focal ischemia to generate NRP.

Probability values less than .05 were considered to be of statistical significance. All statistical analysis were performed using SPPS (V.26) ® (IBM) software.

Results

Clinical characteristics

We included 190 patients with AIS from LVO with successful recanalization after thrombectomy; 112 (68.9%) were females, mean age was 70.4 years (SD=14.6), 5 cases were excluded from analysis for missing data (table 1).

As to cerebrovascular risk factors, 142 (74.7%) patients had arterial hypertension, and 14 (17.9%) had diabetes. As regards AIS etiology, 112 (59.3%) patients had a cardioembolic event, 27 cases (14.3%) were classified as large artery atherosclerosis (LAA) and 12 (6.3%) as other determined etiology, while in 38 patients (20.1%) etiology remained undetermined.

Before stroke, 148 patients (78.3%) were completely independent and without symptoms (mRS 0), 19 (10.1%) had a mRS 1 and 14 (7.4%) a mRS 2, whereas just 8 (4.2%) had a pre-stroke mRS>2, but none was bedridden (mRS 5).

Our patients had moderate-severe stroke with a mean NIHSS at onset of 14, which decrease to 11 at 24 hours and to 7 at seven days.

Occlusion involved M1 segment in 90 (47.4%) cases, M2 in 51 patients (26.8%), intracranial ICA in 12 (6.3%) cases, while a tandem occlusion caused was present in 37 (19.5%) patients. Collateral status according to CIS was classified as: CIS 0 46 cases (25.3%), CIS 1 20 patients (11.0%), CIS 2 26 patients (14.3%), CIS 3 90 cases (49.5%). Mean Onset-to-recanalization time was 462.6 minutes ± 263.3 (7 hours and 42 minutes).

Concerning outcome measures, 71 (37.4%) patients had fENI at 24 hours and 30 (17.9%) patients had fENI at 7 days. At 90 days 86 patients (46.2%) were functionally independent (mRS 0-2), 68 (36.6%) reached an excellent outcome and 41 (22.3%) were dead.

At post MT neuroimaging, hemorrhagic transformation of any kind was detected in 83 (43.7%) cases, while 17 hemorrhages (8.9%) were classified as sICH.

Further characteristics of our cohort are reported in table 1.

Angiographic no-reflow phenomenon

ROC analysis revealed the following discriminating ability of mCIS for fENI at 24 hours (AUC 0.577, 95% CI 0.491-0.663), for fENI at 7 days (AUC 0.638, 95% CI 0.526-0.750) and for FR based on 90-day mRS (AUC 0.493, 95% CI 0.406 -0.577). (Fig.2)

The cut-off to define NRP was set at 3 points at the mCIS; 65 patients out of 185 analyzed (35.13%) were assigned mCIS≤3 and were considered as NRP cases.

Patients with NRP were 22/46 (47.8%) among the pre-interventional CIS 0, 7/20 (35.0%) among the CIS 1, 8/26 (30.7%) in the CIS 2 and 27/89 (30.3%) in the CIS 3, whereas the non NRP patients were respectively, 24/46 (52.2%),13/20 (65.0%), 18/26 (69.3%) and 62/89 (69.7%) CIS 3 (p=0.226). Distribution of mCIS score according to pre-interventional collateral status is reported in table 1B.

In NRP group mean age was 69.6 (SD 12.9, p=0.127), 33 patients (50.8%) were females. As to cardiovascular risk profile, 18 patients (27.7%) in the NRP group and 15 (12.5%) (p=0.015) in the good microvascular reperfusion group suffered from carotid atherosclerosis.

Primary Endpoint

Concerning clinical outcome, 32 patients in NRP group and 67 in the rest of the population had FR based on 90-day mRS (51.6% vs 56.3%, p=0.637). At early clinical evaluation, 31 NRP and 38 good microvascular reperfusion cases had fENI at 24 hours (47.7% vs 31.7%, p=0.039), while fENI at 7 days was found respectively in 15 and 13 patients (26.8% vs 12.1%, p=0.028) (Table 1 and Fig 3.). At multivariate analysis adjusted for age, sex, collateral status and variables influencing clinical outcome, NRP was associated with fENI both at 24 hours (aOR 2.825, 95% CI 1.265 – 6.308, p = 0.011) and at 7 days (aOR 2.814, 95% CI 1,018 - 7.776, p = 0.046) (Table 2.).

Considering just patients with CIS 0 on pre-interventional DSA, 14 patients in the NRP group and 14 controls had a 90-day mRS of 3-6 (66.7% vs 58.3%, p=0.331). As to early neurological outcome, fENI at 24 hours was found in 14 NRP cases and 8 controls (63.6% vs 33.3% p=0.040) (Table 3.), logistic regression model confirmed this association (aOR 4.430, 95% CI 1.148–17.095, p = 0.031) (Table 4.). At 7 days, 6 NRP cases and 4 patients with good microvascular reperfusion had fENI (40% vs 22.2%, p=0.448).

Secondary endpoint

Among patients showing NRP, 38 had hemorrhagic transformation of the ischemic lesion, whereas it occurred in 45 patients from the control group (58.5% vs 37.5%, p=0.008) (Table 1 and Fig 4.). At the multivariate analysis adjusted for age, sex, arterial hypertension, stroke severity and groin puncture-to-recanalization time, NRP predicted hemorrhagic transformation (aOR 2.444, 95% CI 1.266–4.717, p = 0.008) (Table 5.). Cerebral hemorrhage was symptomatic in 8 (12.3) and 9 (7.5) cases from the NRP and control group respectively (p=0.296).

Mean infarct volumes on MRI evaluation were 17 ml (SD 21.2) and 19 ml (SD 38.3) in the two groups (p=0.700) (Table 1.).

Association between clinical and radiological variables and clinical outcome

Patients with FR were older than patients who reached functional independence at 90 days with a mean age of 76.7 (SD 10.5) and 66.5 (SD 14.7) years respectively (p<0.0001). Arterial hypertension (85% vs 61.6%, p<0.0001) hypercholesterolemia (39%vs 24.4%, p=0.034 were more frequent in FR group. Stroke severity at any time was associated with 90-day functional outcome; in particular mean NIHSS was higher in FR group at onset (mean ± SD: 17 ± 5 vs 12 ± 7 p<0.0001), post TM (mean, SD 16 ± 6 vs 8, 6 p<0.0001), at 24h (mean ±

SD 15 ± 6 vs 5 ± 5 p<0.0001) and at 7 days (mean, SD 11 ± 7 vs 3, 3 p<0.0001). Similarly, functional independent patients showed smaller infarct volumes compared with FR (mean ± SD: 7.88 ml ± 19.40 vs 18.93 ± 40.72 p<0.0001). (Table 6.)

As to collateral status, CIS 3 was more frequent in mRS 0-2 group and CIS 0 in FR group (p=0.029). Hemorrhagic transformation and sICH were prevalent in FR group (56% vs 27.9% p<0.0001) (15% vs 1.2% p=0.001), respectively.

Patients who did not clinically improve at 24 hours suffered from hypertension (83.1 vs 69.7 p=0.040) and diabetes (25.4% vs 13.4% p=0.038), while history of tumor was less frequent in this group (11.3% vs 23.5% p=0.037). As to 90-day outcome, patients with fENI at 24h and at 7 days had higher mean post recanalization NIHSS (mean ± SD: 17 ± 7 vs 11 ± 7 p<0.001) (mean, SD: 15 ± 7 vs 11 ± 7 p=0.003) at 24 h (mean ± SD: 16 ± 7 vs 8 ± 7 p<0.001) (mean ± SD: 16 ± 7 vs 5 ± 5 p=0.003) and at 7 days (mean ± SD: 12 ± 8 vs 4 ± 4 p<0.001) (mean, SD: 16 ± 8 vs 5 ± 5 p=0.003).

Procedures were shorter in the ENI group both at 24h and at 7 days (mean, SD: 64.11 min, 37.19 vs 76.55 min, 44.83 p=0.040) (mean, SD: 64.90 min, 38.28 vs 83.1 min, 45.20 p=0.024).

Finally, radiological outcomes were worse in patients with fENI: namely in fENI at 24h and 7 days groups, 42 (59.2%) (p=0.001) and 17 (56.7%) (p=0.055) patients respectively showed hemorrhagic transformation of any type as compared to 41 (34.5%) and 52 (37.7%) of ENI patients, while sICH was evident in 16 (22.5%) and 4 (13,3%) fENI at 24 hours and 7 days, as compared to 1 (0.8%) (p<0.0001) and 4 (2.9%) (p=0.015) ENI patients .

Discussion

AIS management has radically improved in last years, ensuring good prognosis to increasing number of patients. Unfortunately, futile recanalization may involve up to 60% of

cases[4]. As a consequence of multifactorial nature of AIS, understanding causes of poor outcome despite satisfying reperfusion of LVO represents one of the most complex current challenges for stroke physician and researchers.

Since long, animal models have shown how recanalization of large cerebral vessels does not always ensure parenchymal reperfusion. Indeed, after restoring blood flow in main arteries, microvascular occlusion may occur representing the NRP. NRP has been widely described in humans after acute myocardial infarction, but as yet it is not evident in patients with AIS. Some authors outlined indirect signs of NRP in human brain, nonetheless a direct sign of lack of microvascular reperfusion immediately after MT could help identifying candidates for treatments, combined with EVT, aimed at counteracting the NRP.

DSA owns the best resolution for small vessels brain and MT is the current gold standard for treatment of brain LVO in selected patients with AIS. We tried to identify a marker of NRP immediately after main artery revascularization in post interventional DSA, which is routinely performed at the end of every procedure. We created a score which considers not only the presence of parenchymal phase, e.g. capillary visualization, but its delay as well, considering it a marker of microvascular occlusion. Our score considers MCA territory parenchymal perfusion, similarly to the CIS used to evaluate collateral circulation before thrombectomy. We divided MCA territory in 3 portions, and for each portion we considered 3 possible gradings (0-2), depending on absence (0), delayed (1) or regular (2) appearance of parenchymal phase. Using ROC curve, our score showed to be able to discriminate clinical outcome, although with a weak evidence for 90-day functional independence, and a moderate one for early outcome at 24 hours and 7 days. On this basis we used a cut-off of 3 to define NRP in our population.

Angiographic NRP showed to be a predictor of fENI at 24 hours and at 7 days but not of FR defined on 90-day functional outcome. FRs are associated with various clinical variables including comorbidities such as diabetes, arterial hypertension, and AF, as outlined by

existing literature[9,14],and confirmed by our study, though in our population there was just a trend for higher AF prevalence in the FR group. Association between NRP and long-term functional outcome in AIS patients has never been reported. NIHSS represents an accurate evaluation of stroke severity and hence of parenchymal damage. Main aim of revascularization therapies in AIS is to save ischemic penumbra, in other words prevent extensive parenchymal damage. Given these assumptions, early clinical improvement represents a more direct marker of success of endovascular treatment, with lower influence of comorbidities and systemic complications as compared to 3-month functional independence. On the same line, new definitions of FR have been given, considering early neurological outcome rather than 90-day mRS[13]. Previous studies identified NRP through 24 hours perfusional neuroimaging[36], reporting a very low incidence of NRP, while in our population it was evident in about one third of the analyzed DSAs. Studies from animal models reported NRP as “mottled” and “patchy”[27], hence perfusional imaging might not own the high resolution needed to identify this not homogenous areas of parenchymal lack of perfusion. Moreover, DSA allows direct identification of capillaries and analysis of delay of blood flow for each of the three segments of MCA territory could be a more reliable imaging for NRP identification.

As outlined from the analysis of distribution of mCIS score according to pre-interventional collateral status (Table 1B.), different mCIS scores and mCIS≤3 were homogenously represented in all pre-TM CIS groups. Furthermore, multivariate analysis corrected for pre-interventional CIS confirmed the ability of our score to predict early outcome. As a matter of fact, our score seems to be a marker of NRP independently from pre-interventional collateral status.

NRP was first described in experimental condition of global ischemia[27], which requires a complete occlusion of all main arteries supplying cerebral parenchyma. As a matter of fact, collateral circulation even at low grades makes global ischemia just a virtual condition.

Anyway, focal ischemia in patients with poor collateral could be counted as the most comparable condition to global ischemia in AIS patients. Analysis of patients with poor collaterals (CIS 0) confirmed a strong predictive value of NRP, evaluated with mCIS, for fENI at 24 hours, which was not confirmed at 7 days, probably because of the small number of cases in this subanalysis.

In our study, presence of NRP predicted the development of post ischemic brain hemorrhage, both in the whole cohort of patients and in those with baseline poor collaterals. This finding is in contrast with evidence that early venous filling (EVF) is associated to higher probability of hemorrhagic transformation[41,52]. Differently from the study of Ohta et al.[41], our cohort of patients included only those with successful recanalization, while compared to patients included by Cartmel et al.[42], our series had smaller infarct volumes, so we cannot assume our data to be comparable to the abovementioned ones. We speculate that higher rates of cerebral hemorrhage in patients with NRP, might be the consequence of more extensive blood brain barrier rupture.

Eventually, among patients with NRP carotid atherosclerosis was more prevalent compared to the rest of the population. Endogenous factors, such as ET1 are supposed to be involved in NRP genesis through a vasoactive effect[53–55]. ET-1 release is promoted by shear stress[56], and we speculate that the higher shear stress condition bound to atherosclerotic plaques might stimulate ET-1 production and hence NRP occurrence after atherothrombotic AIS.

Our score, created to identify the NRP, needs for further studies to get an external validation in different populations. Nevertheless, our angiographic evaluation might represent a rapid and widely applicable method to detect lack of capillary perfusion after large brain vessels recanalization.

TABLES

Table 1. Clinical and radiological characteristics of the entire population and Univariate analysis for No-reflow phenomenon (A) and distribution of mCIS score according to pre-interventional collateral status (B)

	All (190)	Good microvascular reperfusion (mCIS>3) (120)	No-reflow phenomenon (mCIS≤3) (65)	p
Age mean ± SD (median)	71.9 ± 13.5 (75.0)	72.7 ± 13.7 (76.0)	69.6 ± 12.9 (72.0)	0.127
Sex				
Male N (%)	78 (41.1)	44 (36.7)	32 (49.2)	0.118
Female N (%)	112 (58.9)	76 (63.3)	33 (50.8)	
Hypertension N (%)	142 (74.7)	93 (77.5)	46 (70.8)	0.373
Diabetes N (%)	34 (17.9)	26 (21.7)	7 (10.8)	0.072
Smoking N (%)	36 (19.0)	25 (20.8)	10 (15.6)	0.436
Previous stroke or TIA N (%)	22 (11.6)	14 (11.7)	8 (12.3)	0.536
Heart failure N (%)	25 (13.2)	19 (15.8)	5 (7.8)	0.168
Coronary artery disease N (%)	19 (10.0)	9 (7.5)	10 (15.4)	0.127
Atrial fibrillation N (%)	93 (49.2)	58 (48.3)	31 (48.4)	1.00
Hypercolesterolemia N (%)	62 (32.6)	40 (33.3)	19 (29.2)	0.622
Carotid atherosclerosis N (%)	35 (18.5)	15 (12.5)	18 (27.7)	0.015
Tumor N (%)	36 (18.9)	24 (20)	10 (15.4)	0.552
mRS pre-stroke				
0 N (%)	148 (78.3)	90 (75)	54 (84.4)	0.119
1 N (%)	19 (10.1)	11 (9.2)	8 (12.5)	
2 N (%)	14 (7.4)	11 (9.2)	2 (3.1)	
3 N (%)	5 (2.6)	5 (4.2)	0	
4 N (%)	3 (1.6)	3 (2.5)	0	
Etiology (TOAST)				
Large-artery atherosclerosis N (%)	27 (14.3)	12 (10.1)	15 (23.1)	0.10
Cardioembolism N (%)	112 (59.3)	75 (63.0)	33 (50.8)	
Other determined etiology N (%)	12 (6.3)	7 (5.9)	5 (7.7)	

Undetermined etiology N (%)	**38 (20.1)**	**25 (21.0)**	**12 (18.5)**	
Wake-up Stroke N (%)	**52 (27.5)**	**33 (27.7)**	**19 (29.2)**	**0.865**
Baseline NIHSS mean ± SD (median)	**14.0 ± 6.0 (15.0)**	**14.1 ± 5.8 (15.0)**	**15.2 ± 6.9 (17.0)**	**0.248**
Post interventional NIHSS mean ± SD (median)	**12.0 ± 7.0 (12.0)**	**12.2 ± 6.7 (12.0)**	**13.4 ± 7.5 (14.0)**	**0.279**
24H NIHSS mean ± SD (median)	**11.0 ± 7.0 (10.0)**	**10.5 ± 7.0 (11.0)**	**11.1 ± 7.4 (9.5)**	**0.606**
7 days/Discharge NIHSS mean ± SD (median)	**7.0 ± 7.0 (5.0)**	**6.6 ± 6.7 (5.0)**	**7.1 ± 6.7 (5.0)**	**0.672**
Early outcome 24 hours				
Failure of Early Neurological Improvement N (%)	**71 (37,4)**	**38 (31.7)**	**31 (47.7)**	**0.039**
Early neurological improvement N (%)	**119 (62.6)**	**82 (68.3)**	**34 (52.3)**	
Early outcome 7 days				
Failure of Early Neurological Improvement N (%)	**30 (17.9)**	**13 (12.1)**	**15 (26.8)**	**0.028**
Early neurological improvement N (%)	**138 (82.1)**	**94 (87.9)**	**41 (73.2)**	
90 days functional outcome				
Bad outcome 3-6 N (%)	**100 (53.8)**	**67 (56.3)**	**32 (51.6)**	
Functional indipendence (mRS 0-2) N (%)	**86 (46.2)**	**52 (43.7)**	**30 (48.4)**	**0.637**
ExcellentOutcome (mRS 0-1) N (%)	**68 (36.6)**	**39 (32.8)**	**25 (40.3)**	**0.33**
Death at 90 days N (%)	**41 (22.3)**	**27 (22.2)**	**14 (23.3)**	**1.0**
Site of Occlusion				
M1 N (%)	**90 (47.4)**	**66 (55.0)**	**23 (35.4)**	
M2 N (%)	**51 (26.8)**	**29 (24.2)**	**18 (27.7)**	
Intracranial ICA N (%)	**12 (6.3)**	**5 (4.2)**	**7 (10.8)**	**0.040**
Tandem occlusion N (%)	**37 (19.5)**	**20 (16.7)**	**17 (26.2)**	
Direct MT N (%)	**102 (54.0)**	**65 (54.6)**	**37 (56.9)**	**0.877**

Bridging therapy N (%)	**87 (46.0)**	**54 (45.4)**	**28 (43.1)**	
Onset-to-recanalization Time (min) mean ± SD (median)	**462.6 ± 263.3 (362.5)**	**468.5 ± 278.2 (351.0)**	**456.0 ± 245.2 (376.0)**	**0.763**
Groin-to-recanalization Time (min) mean ± SD (median)	**68.7 ± 40.5 (61.0)**	**70.1 ± 38.7 (61.0)**	**69.9 ± 42.6 (63.0)**	**0.977**
CIS				
0 N (%)	**46 (25.3)**	**24 (20.5)**	**22 (34.4)**	**0.226**
1 (N %)	**20 (11.0)**	**13 (11.1)**	**7 (10.9)**	
2 N (%)	**26 (14.3)**	**18 (15.4)**	**8 (12.5)**	
3 N (%)	**90 (49.5)**	**62 (53.0)**	**27 (42.2)**	
TICI				
2b N (%)	**67 (35.3)**	**4 (34.2)**	**25 (38.5)**	**0.679**
2c N (%)	**16 (8.4)**	**9 (7.5)**	**3 (4.6)**	
3 N (%)	**107 (56.3)**	**70 (58.3)**	**37 (56.9)**	
N° of passages				
0 N (%)	**4 (2.1)**	**-**	**-**	**0.512**
1 N (%)	**87 (45.8)**	**52 (43.3)**	**34 (52.3)**	
2 N (%)	**58 (30.5)**	**41 (34.2)**	**17 (26.2)**	
3 N (%)	**24 (12.6)**	**17 (14.2)**	**7 (10.8)**	
4 N (%)	**14 (7.4)**	**9 (7.5)**	**5 (7.7)**	
5 N (%)	**3 (1.6)**	**1 (0.8)**	**2 (3.1)**	
Technique				
Thrombus aspiration N (%)	**109 (58.9)**	**69 (58.8)**	**40 (61.5)**	**0.818**
Stent retrieving N (%)	**13 (7.0)**	**8 (6.7)**	**5 (7.7)**	
combined technique N (%)	**63 (34.1)**	**42 (35.3)**	**2 (30.8)**	
Hemorragic Transformation N (%)	**83 (43.7)**	**45 (37.5)**	**38 (58.5)**	**0.008**
Infarct volume (ml) mean ± SD (median)	**18.2 ±33.1 (6.3)**	**19.1 ± 38.3 (6.6)**	**17.0 ± 21.2 (5.9)**	**0.700**
ECASS				
HI1 N (%)	**32 (38.1)**	**18 (39.1)**	**14 (36.8)**	**0.560**
HI2 N (%)	**15 (17.9)**	**6 (13.0)**	**9 (23.7)**	
PH1 N (%)	**15 (17.9)**	**8 (17.4)**	**7 (18.4)**	
PH2 N (%)	**22 (26.2)**	**14 (30.4)**	**8 (21.1)**	

sICH N (%)	**17 (8.9)**	**9 (7.5)**	**8 (12.3)**	**0.296**

A.

mCIS	**CIS**				**p**
	0	**1**	**2**	**3**	
2 N (%)	**1 (2.2)**	**0**	**0**	**3 (3.4)**	**0.014**
3 N (%)	**21 (45.7)**	**7 (35.0)**	**8 (30.8)**	**24 (27.0)**	
4 N (%)	**11 (23.9)**	**6 (30.0)**	**7 (26.9)**	**8 (9.0)**	
5 N (%)	**3 (6.5)**	**2 (10.0)**	**1 (3.8)**	**22 (24.7)**	
6 N (%)	**10 (21.7)**	**5 (25.0)**	**10 (38.5)**	**32 (36.0)**	

B.

Table 2. Multivariate analysis of predictors of failure of early improvement at 24 hours and at 7 days

	fENI at 24 hours			fENI at 7 days		
	Adjusted OR	p	95% C.I.	Adjusted OR	p	95% C.I.
No-reflow phenomenon (mCIS≤3)	2.825	0.011	1.265 - 6.308	2.814	0.046	1,018 - 7.776
Age	0.975	0.184	0.939 - 1.012	1.001	0.983	0.950 - 1.054
Sex	0.727	0.469	0.307 - 1.721	1.197	0.759	0.380 - 3.776
Hypertension	1.625	0.376	0.555 - 4.762	0.359	0.199	0.075 - 1.714
Diabetes	2.905	0.036	1.071 - 7.883	0.414	0.166	0.119 - 1.441
Tumor	0.574	0.308	0.198 - 1.667	0.427	0.169	0.127 - 1.435
N° of passages		0.058				
1 passage	2.458	0.616	0.073 - 82.539	-	-	-
2 passages	0.881	0.944	0.026 - 30.108	-	-	-
3 passages	0.781	0.894	0.021 - 29.106	-	-	-
4 passages	0.472	0.690	0.012 - 18.812	-	-	-
Site of occlusion		0.144			0.254	
M1	1.291	0.654	0.423 - 3.941	0.872	0.857	0.197 - 3.860
M2	1.964	0.316	0.525 - 7.351	3.599	0.174	0.568 - 22.794
ICA	0.174	0.055	0.029 - 1.038	0.843	0.873	0.103 - 6.914
Hemorragic Transformation	2.917	0.008	1.330 - 6.397	0.372	0.064	0.130 - 1.061
Baseline NIHSS	1.077	0.024	1.010 - 1.149	0.925	0.082	0.847 - 1.010
CIS		0.885			0.030	
CIS(1)	1.437	0.542	0.448 - 4.614	10.230	0.005	1.992 - 52.546
CIS(2)	1.575	0.497	0.424 - 5.841	6.390	0.041	1,075 - 37.979
CIS(3)	1.228	0.736	0.372 - 4.062	7.564	0.013	1,523 - 37.561

Table 3.Univariate analysis according to No-reflow phenomenon in patients with CIS 0

CIS 0			
	Good microvascular reperfusion (mCIS>3) (24)	No-reflow phenomenon (mCIS≤3) (22)	p value
Age mean ± SD (median)	70.5 ± 15.0 (71.0)	71.0 ± 11.9 (74)	0.892
Sex			
Male N (%)	12 (50)	10 (45.5)	0.758
Female N (%)	12 (50)	12 (54.5)	
Early outcome at 24 hours			
Failure of Early Neurological Improvement N (%)	8 (33.3)	14 (63.6)	0,040
Early neurological improvement N (%)	16 (66.7)	8 (36.4)	
Early outcome at 7 days			
Failure of Early Neurological Improvement N (%)	4 (22.2)	6 (40.0)	0.448
Early neurological improvement N (%)	14 (77.8)	9 (60.0)	
Functional outcome			
mRS 3-6 N (%)	14 (58.3)	14 (66.7)	0.331
mRS 0-2 N (%)	10 (41.7)	7 (33.3)	
Excellent Outcome			
mRS 2-6 N (%)	18 (75)	16 (76.2)	0.926
mRS 0-1 N (%)	6 (25)	5 (23.8)	
Arterial hypertension N (%)	18 (75)	19 (86.4)	0.332
Diabetes N (%)	5 (20.8)	2 (9.1)	0.268
Smoker N (%)	8 (33.3)	5 (22.7)	0.425
History of stroke or TIA N (%)	2 (8.3)	2 (9.1)	0.927
Heart failure N (%)	7 (29.2)	3 (13.6)	0.202
Coronary artery disease N (%)	2 (8.3)	3 (13.6)	0.564
Atrial fibrillation N (%)	10 (41.7)	11 (50)	0.571
Hypercolesterolemia N (%)	10 (41.7)	8 (36.4)	0.713
Carotid atherosclerosis N (%)	4 (16.7)	9 (40.9)	0.068
History of tumor N (%)	4 (16.7)	3 (13.6)	0.775
mRS pre-stroke			

0 N (%)	17 (70.8)	20 (90.9)	
1 N (%)	4 (16.7)	2 (9.1)	
2 N (%)	1 (4.2)	0 (0)	0.429
3 N (%)	1 (4.2)	0 (0)	
4 N (%)	1 (4.2)	0 (0)	
TOAST			
Large-artery atherosclerosis N (%)	3 (13)	8 (36.4)	
Cardioembolism N (%)	14 (60.9)	9 (40.9)	0.327
Other determined etiology N (%)	1 (4.3)	1 (4.5)	
Undetermined etiology N (%)	5 (21.7)	4 (18.2)	
Wake up stroke N (%)	10 (41.7)	9 (40.9)	0.958
Baseline NIHSS mean ± SD (median)	17.0 ± 5.0 (18.0)	16 ± 6 (17.0)	0.944
Post Interventional NIHSS mean ± SD (median)	15.0 ± 7.0 (18.0)	16.0 ± 8.0 (17)	0.591
24 hours NIHSS mean ± SD (median)	12.0 ± 7.0 (11.0)	12.0 ± 8.0 (12.0)	0.866
7days/discharge NIHSS mean ± SD (median)	10.0 ± 8.0 (7.0)	8.0 ± 7.0 (7.0)	0.517
Location			
M1 N (%)	8 (33.3)	5 (22.7)	
M2 N (%)	0 (0)	1 (4.5)	0.534
Intracranial ICA N (%)	4 (16.7)	6 (27.3)	
Tandem occlusion N (%)	12 (50)	10 (45.5)	
Direct MT N (%)	15 (62.5)	11 (50)	0.393
Bridging therapy N (%)	9 (37.5)	11 (50)	
Onset-to-recanalization Time - Minutes mean ± SD (median)	577.08 ± 358.84 (404.0)	476.14 ± 267.40 (378.5)	0.289
Groin-to-recanalization - Minutes mean ± SD (median)	91.37 ± 54.47 (75.5)	72.54 ± 53.23 (62.5)	0.243
TICI			
2b N (%)	8 (33.3)	7 (31.8)	
2c N (%)	3 (12.5)	1 (4.5)	0.601
3 N (%)	13 (54.2)	14 (63.6)	
N of passages			
1 N (%)	8 (33.3)	9 (40.9)	
2 N (%)	8 (33.3)	8 (36.4)	
3 N (%)	3 (12.5)	2 (9.1)	0.651
4 N (%)	5 (20.8)	2 (9.1)	
5 N (%)	0 (0)	1 (4.5)	
Technique			

Thrombus aspiration N (%)	11 (45.8)	12 (54.5)	
Stent retrieving N (%)	3 (12.5)	2 (9.1)	0.827
combined technique N (%)	10 (41.7)	8 (36.4)	
	39.8, 14.5 (65.7)	21.5, 6.3 (22.7)	
Hemorragic Transformation N (%)	12 (50)	13 (59.1)	0.536
Infarct volume mean ± SD (median)	39.8 ± 65.7 (14.5)	21.5 ± 22.6 (6.3)	0.282
ECASS			
HI1 N (%)	1 (8.3)	3 (23.1)	
HI2 N (%)	2 (16.7)	2 (15.4)	0.217
PH1 N (%)	5 (41.7)	1 (7.7)	
PH2 N (%)	4 (33.3)	7 (53.8)	
sICH N (%)	3 (12.5)	7 (31.8)	0.113

Table 4. Multivariate analysis of predictors of failure of early neurological improvement at 24 hours in patients with CIS 0

	Adjusted OR	p-value	95% C.I.
NoReflow(1)	4.430	0.031	1.148 – 17.095
Age	0.964	0.297	0.899 – 1.033
Sex(1)	0.227	0.149	0.030 – 1.701
Hemorragic Transformation(1)	4.034	0.068	0.902 – 18.041
Baseline NIHSS	1.042	0.537	0.914 – 1.189

Table 5. Multivariate analysis of predictors of hemorrhagic Transformation.

	Adjusted OR	p-value	95% C.I.
NoReflow	2.444	0.008	1.266 – 4.717
Age	1.001	0.957	0.970 – 1.033
Sex	0.750	0.420	0.372 – 1.510
Baseline NIHSS	1.095	0.001	1.038 – 1.154
Arterial hypertension	0.801	0.632	0.323 – 1.988
Duration of procedure - Minutes	1.003	0.499	0.995 – 1.011

Table 6. Univariate analysis of clinical and radiological variables according to 90-day functional outcome

	90 days Funtional Outcome		
	Futile Recanalization (N 100)	Functional Indipendence (N 86)	p
Age mean ± SD (median)	76.7 ± 10.5 (78.0)	66.5 ± 14.7 (68.5)	<0.0001
Sex			
Male N (%)	35 (35)	40 (46.5)	0.111
Female N (%)	65 (65)	46 (53.5)	
Arterial hypertension N (%)	85 (85)	53 (61.6)	<0.0001
Diabetes N (%)	21 (21)	11 (12.8)	0.139
Smoker N (%)	15 (15)	19 (22.1)	0.212
History of stroke or TIA N (%)	12 (12)	8 (9.3)	0.554
Heart failure N (%)	16 (16)	7 (8.1)	0.104
Coronary artery disease N (%)	11 (11)	5 (5.8)	0.209
Atrial fibrillation N (%)	55 (55)	36 (41.9)	0.074
Hypercolesterolemia N (%)	39 (39)	21 (24.4)	0.034
Carotid atherosclerosis N (%)	19 (19.2)	14 (16.3)	0.606
History of tumor N (%)	18 (18)	17 (19.8)	0.758
mRS pre-stroke			
0 N (%)	68 (68)	79 (91.9)	0.001
1 N (%)	12 (12)	6 (7)	
2 N (%)	12 (12)	1 (1.2)	
3 N (%)	5 (5)	0 (0)	
4 N (%)	3 (3)	0 (0)	
Etiology (TOAST)			
Large-artery atherosclerosis N (%)	15 (15.2)	11 (12.8)	0.558
Cardioembolism N (%)	62 (62.6)	48 (55.8)	
Other determined etiology N (%)	5 (5.1)	7 (8.1)	
Undetermined etiology N (%)	17 (17.2)	20 (23.3)	
Wake-up Stroke N (%)	29 (29.3)	23 (26.7)	0.701
Baseline NIHSS mean ± SD (median)	17 ± 5 (18)	12 ± 7 (11)	<0.0001
Post interventional NIHSS mean ± SD (median)	16 ± 6 (16)	8 ± 6 (7)	<0.0001

24H NIHSS mean ± SD (median)	**14 ± 6 (15)**	**6 ± 5 (5)**	**<0.0001**
7 days/Discharge NIHSS mean ± SD (median)	**11 ± 7 (9)**	**3 ± 3 (2)**	**<0.0001**
Site of Occlusion			
M1 N (%)	**52 (52)**	**38 (44.2)**	**0.075**
M2 N (%)	**19 (19)**	**30 (34.9)**	
Intracranial ICA N (%)	**8 (8)**	**3 (3.5)**	
Tandem occlusion N (%)	**21 (21)**	**15 (17.4)**	
Direct MT N (%)	**57 (57.6)**	**42 (48.8)**	**0.235**
Bridging therapy N (%)	**42 (42.4)**	**44 (51.2)**	
Onset-to-recanalization Time - Minutes mean ± SD (median)	**467.78 ± 258.04 (382.5)**	**463.59 ± 274.49 (339.50)**	**0.915**
Groin-to-recnalization - Minutes mean ± SD (median)	**71.98 ± 40.93 (64.0)**	**65.86 ± 40.32 (55.50)**	**0.307**
Lesion volume mean ± SD (median)	**28.93 ± 40.72 (15.5)**	**7.88 ± 19.40(2.60)**	**<0.0001**
CIS			
0 N (%)	**28 (28.9)**	**17 (21)**	**0.029**
1 N (%)	**12 (12.4)**	**8 (9.9)**	
2 N (%)	**19 (19.6)**	**7 (8.6)**	
3 N (%)	**38 (39.2)**	**49 (60.5)**	
TICI			
2b N (%)	**37 (37)**	**28 (32.6)**	**0.633**
2c N (%)	**7 (7)**	**9 (10.5)**	
3 N (%)	**56 (56)**	**49 (57)**	
N° of passages			
0 N (%)	**1 (1)**	**3 (3.5)**	**0.165**
1 N (%)	**39 (39)**	**45 (52.3)**	
2 N (%)	**35 (35)**	**23 (26.7)**	
3 N (%)	**12 (12)**	**11 (12.8)**	
4 N (%)	**11 (11)**	**3 (3.5)**	
5 N (%)	**2 (2)**	**1 (1)**	
Technique			
Thrombus aspiration N (%)	**51 (51.5)**	**55 (67.1)**	**0.077**
Stent retrieving N (%)	**7 (7.1)**	**6 (7.3)**	
Combined technique N (%)	**41 (41.4)**	**21 (25.6)**	

Haemorrhagic transformation N (%)	56 (56)	24 (27.9)	<0.0001
ECASS			
HI1 N (%)	15 (26.8)	15 (62.5)	0.007
HI2 N (%)	10 (17.9)	4 (16.7)	
PH1 N (%)	11 (19.6)	4 (16.7)	
PH2 N (%)	20 (35.7)	1 (4.2)	
sICH N (%)	15 (15)	1 (1.2)	0.001

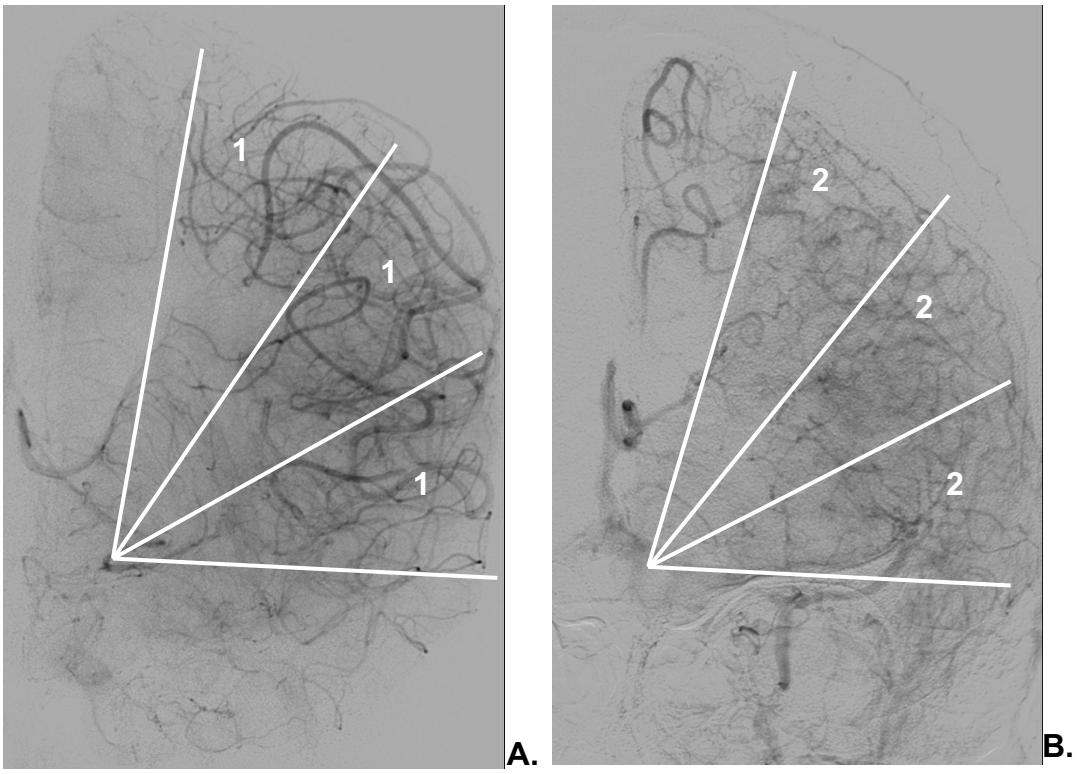
Table 7. Univariate analysis of clinical and radiological variables according to 24-hour and 7-day neurological outcome

	Early Outcome at 24 hours			Early Outcome at 7 days		
	Failure of Early Neurological Improvement	Early neurological improvement	p value	Early neurological improvement	Failure of Early Neurological Improvement	p value
Age mean ± SD (median)	73.7 ± 11.5 (75.0)	70.8 ± 14.4, (75.0)	0.142	71.3 ± 14.2, (75.0)	73.7 ± 10 (75.5)	0.366
	Sex					
Male N (%)	32 (45.1)	46 (38.7)	0.385	55 (39.9)	12 (40)	0.988
Female N (%)	39 (54.9)	73 (61.3)		83 (60.1)	18 (60)	
Arterial hypertension N (%)	59 (83.1)	83 (69.7)	0.040	99 (71.7)	25 (83.3)	0.191
Diabetes N (%)	18 (25.4)	16 (13.4)	0.038	21 (15.2)	8 (26.7)	0.133
Smoker N (%)	13 (18.6)	23 (19.3)	0.898	23 (16.7)	8 (26.7)	0.201
History of stroke or TIA N (%)	9 (12.7)	13 (10.9)	0.715	16 (11.6)	1 (3.3)	0.174
Heart failure N (%)	8 (11.4)	17 (14.3)	0.576	15 (10.9)	5 (16.7)	0.374
Coronary artery disease N (%)	11 (15.5)	8 (6.7)	0.051	9 (6.5)	5 (16.7)	0.068
Atrial fibrillation N (%)	38 (54.3)	55 (46.2)	0.284	66 (47.8)	16 (53.3)	0.584
Hypercholesterolemia N (%)	27 (38)	35 (29.4)	0.220	43 (31.2)	10 (33.3)	0.816
Carotid atherosclerosis N (%)	14 (19.7)	21 (17.8)	0.742	23 (16.8)	5 (16.7)	0.987

History of tumour N (%)	8 (11.3)	28 (23.5)	0.037	27 (19.6)	6 (20)	0.957
	Pre mRS					
0 N (%)	54 (77.1)	94 (79)	0.126	110 (79.7)	25 (83.3)	0.179
1 N (%)	5 (7.1)	14 (11.8)		14 (10.1)	1 (3.3)	
2 N (%)	5 (7.1)	9 (7.6)		11 (8)	1 (3.3)	
3 N (%)	3 (4.3)	2 (1.7)		2 (1.4)	2 (6.7)	
4 N (%)	3 (4.3)	0 (0)		1 (0.7)	1 (3.3)	
	Etiology (TOAST)					
Large-artery atherosclerosis N (%)	12 (16.9)	15 (12.7)	0.693	18 (13)	4 (13.3)	0.891
Cardioembolism N (%)	41 (57.7)	71 (60.2)		83 (60.1)	19 (63.3)	
Other determined etiology N (%)	3 (4.2)	9 (7.6)		10 (7.2)	1 (3.3)	
Undetermined etiology N (%)	15 (21.1)	23 (19.5)		27 (19.6)	6 (20)	
Baseline NIHSS mean ± SD (median)	14.0 ± 7.0 (15.0)	15 ± 6 (16)	0.318	14.0 ± 6.0 (15.0)	13.0 ± 7.0 (13.0)	0.449
Post interventional NIHSS mean ± SD (median)	16.0 ± 7.0 (17.0)	11.0 ± 7.0 (11.0)	<0.0001	11.0 ± 7 (11.0)	15.0 ± 7.0 (18.0)	0.003
24H NIHSS mean ± SD (median)	16.0 ± 7.0 (17.0)	8.0 ± 6.0 (6.0)	<0.0001	9.0 ± 6.0 (7.0)	16.0 ± 7.0 (18.0)	<0.0001
7 days/Discharge NIHSS mean ± SD (median)	12.0 ± 8.0 (12.0)	4.0 ± 4.0 (3.0)	<0.0001	5.0 ± 5.0 (4.0)	16.0 ± 8.0 (17.0)	<0.0001
Wake up N (%)	19 (26.8)	33 (28)	0.857	40 (29.2)	8 (26.7)	0.781
Direct MT N (%)	40 (56.3)	62 (52.5)	0.612	73 (53.3)	15 (50)	0.744
Bridging therapy N (%)	31 (43.7)	56 (47.5)		64 (46.7)	15 (50)	
Onset-to-recanalization Time - Minutes mean ± SD (median)	479.20 ± 216.28 (421.0)	452.65 ± 288.22 (324.0)	0.503	468.04 ± 283.93 (338.0)	481.73 ± 221.04 (469.5)	0.804
Groin-to-recanalization time - Minutes mean ± SD (median)	76.55 ± 44.83 (67.0)	64.11 ± 37.19 (57.0)	0.040	64.90 ± 38.29 (55.0)	83.1 ± 45.20 (73.0)	0.024
	TICI					
2b N (%)	26 (36.6)	41 (34.5)	0.952	45 (32.6)	13 (43.3)	0.534
2c N (%)	6 (8.5)	10 (8.4)		11 (8)	2 (6.7)	
3 N (%)	39 (54.9)	68 (57.1)		82 (59.4)	15 (50)	

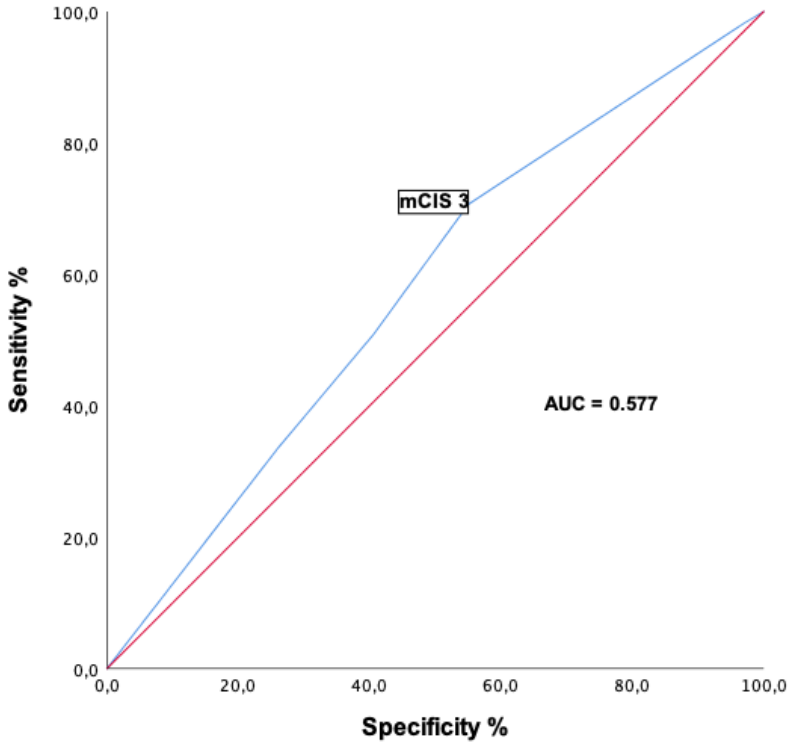
	N° of passages					
0 N (%)	1 (1.4)	3 (2.5)	0.023	3 (2.2)	1 (3.3)	0.155
1 N (%)	22 (31)	65 (54.6)		70 (50.7)	8 (26.7)	
2 N (%)	26 (36.6)	32 (26.9)		40 (29)	11 (36.7)	
3 N (%)	12 (16.9)	12 (10.1)		16 (11.6)	6 (20)	
4 N (%)	9 (12.7)	5 (4.2)		7 (5.1)	4 (13.3)	
5 N (%)	1 (1.4)	2 (1.7)		2 (1.4)	0 (0)	
	Technique					
Thrombus aspiration N (%)	30 (42.9)	79 (68.7)	0.002	86 (64.2)	12 (41.4)	0.072
Stent retrieving N (%)	6 (8.6)	7 (6.1)		10 (7.5)	3 (10.3)	
combined technique N (%)	34 (48.6)	29 (25.2)		38 (28.4)	14 (48.3)	
	Site of occlusion					
M1 N (%)	26 (36.6)	64 (53.8)	0.011	75 (54.3)	11 (36.7)	0.292
M2 N (%)	19 (26.8)	32 (26.9)		36 (26.1)	12 (40)	
Intracranial ICA N (%)	9 (12.7)	3 (2.5)		5 (3.6)	2 (6.7)	
Tandem occlusion N (%)	17 (23.9)	20 (16.8)		22 (15.9)	5 (16.7)	
Hemorragic Transformation N (%)	42 (59.2)	41 (34.5)	0.001	52 (37.7)	17 (56.7)	0.055
	ECASS					
HI1 N (%)	10 (23.8)	22 (52.4)	0.007	25 (48.1)	6 (35.3)	0.479
HI2 N (%)	6 (14.3)	9 (21.4)		10 (19.2)	2 (11.8)	
PH1 N (%)	9 (21.4)	6 (14.3)		8 (15.4)	5 (29.4)	
PH2 N (%)	17 (40.5)	5 (11.9)		9 (17.3)	4 (23.5)	
sICH N (%)	16 (22.5)	1 (0.8)	<0.0001	4 (2.9)	4 (13.3)	0.015
	CIS					
0 N (%)	22 (32.4)	24 (21.1)	0.388	23 (17.4)	10 (34.5)	0.077
1 N (%)	6 (8.8)	14 (12.3)		15 (11.4)	4 (13.8)	
2 N (%)	9 (13.2)	17 (14.9)		20 (15.2)	6 (20.7)	
3 N (%)	31 (45.6)	59 (51.8)		74 (56.1)	9 (31)	
Lesion Volume mL (cm3) mean ± SD (median)	28.71 ± 40.42 (16.25)	12.45 ± 26.84 (5.15)	0.002	14.58 ± 29.84 (5.40)	20.46 ± 22.39 (16.0)	0.335

FIGURES



A. **B.**

Figure 1. Post-interventional DSA showing on parenchymal phase (A) delayed contrast washout in all the 3 segments of MCA territory (mCIS 3) and (B) regular contrast washout (mCIS 6) (DSA=digital subtraction angiography; MCA= middle cerebral artery)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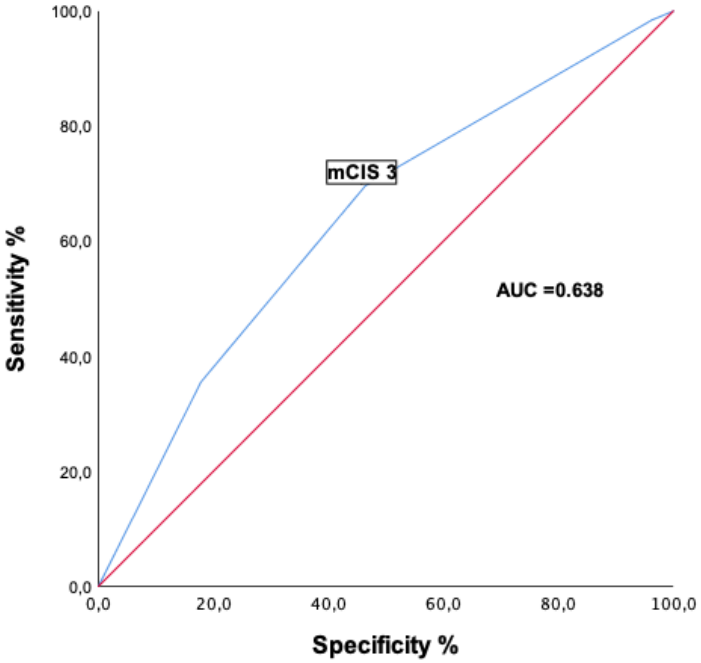
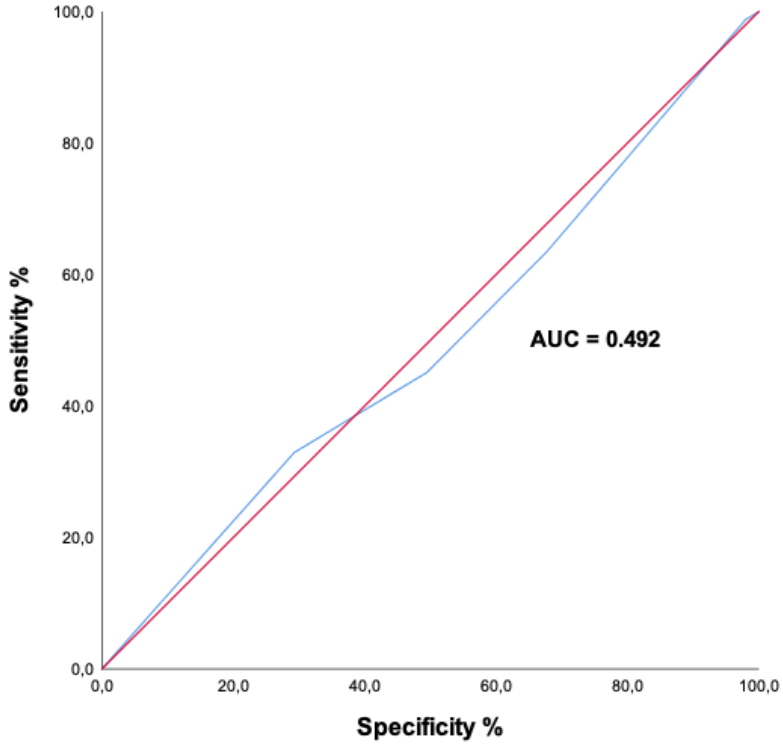
60,0
40,0
20,0
0,0
Sensitivity %
mCIS 3
AUC = 0.577
0,0
20,0
40,0
60,0
80,0
100,0
Specificity %

A.

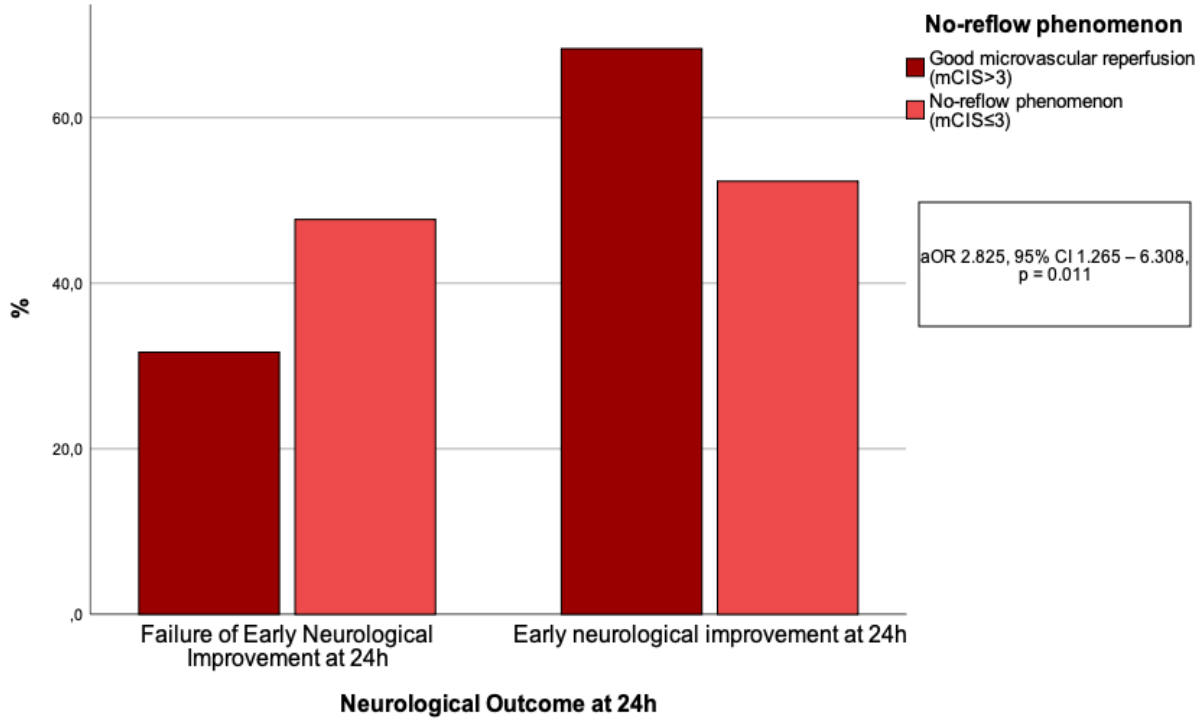

100,0
80,0
60,0
40,0
20,0
0,0
Sensitivity %
mCIS 3
AUC =0.638
0,0
20,0
40,0
60,0
80,0
100,0
Specificity %

B.

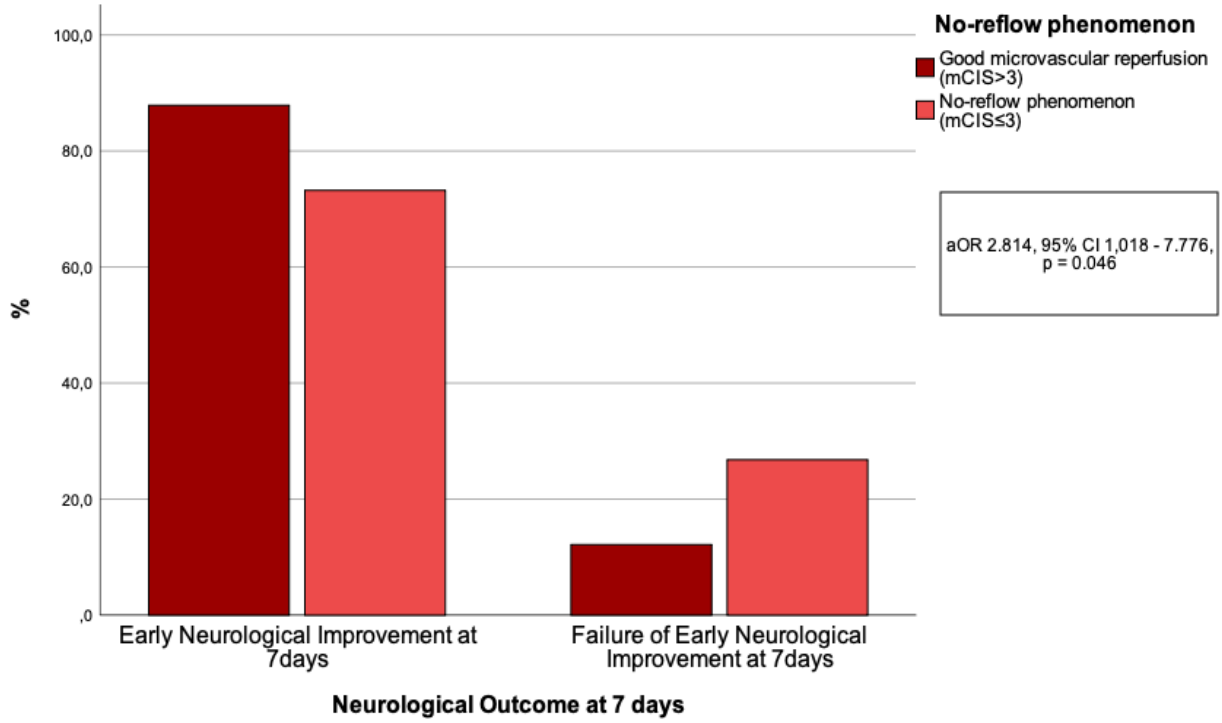


C.

Figure 2. ROC curves of post-interventional angiographic mCIS for discriminating (A) fENI at 24 hours, (B) at 7 days and (C) FR at 90 days. (fENI= failure of early neurological improvement; FR= futile recanalization). mCIS 3 for fENI at 24 hours: sensitivity 70.7%, specificity 55.1%. mCIS 3 for fENI at 7 days: sensitivity 69.9%, specificity 46.4%



A.



B.

Figure 3. Angiographic no-reflow phenomenon and fENI (A) at 24 hours and (B) at 7 days. (fENI= failure of early neurological improvement)

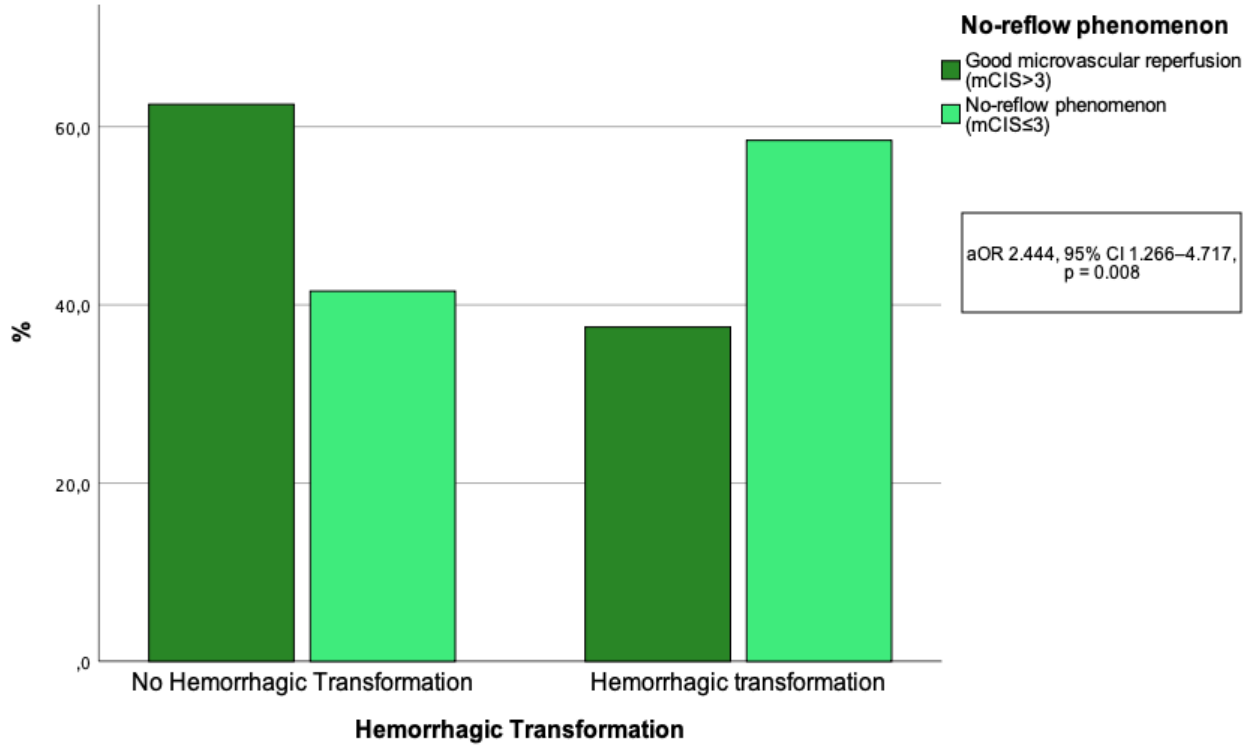


Figure 4. Angiographic no-reflow phenomenon and hemorrhagic transformation of the ischemic lesion.

STUDY 2 – The role of Endothelin-1 (ET-1) and Nitric Oxide (NO) in no-reflow phenomenon in patients with acute ischemic stroke from large vessels occlusion

Introduction

Endothelin-1 in acute ischemic stroke

Endothelin-1 (ET-1) is an endogen peptide produced mainly by endothelial cells, most known for its vasoconstrictor effect[57]. Both physical and chemical stimuli induce ET-1 over expression. Shear stress on vessel walls affect vascular tone through varying production of ET-1 and nitric oxide (NO),[56] while hypoxia causes ET-1 precursor mRNA transcription thanks to genes sensitive to oxygen concentration. Other factors inducing ET-1 production are vasoactive agents such as angiotensin II, or augmentation of thrombin concentration and transforming growth factor-β (TGF-β)[58].

The two main receptors for ET-1 activity are ET_A and ET_B, that are both G protein coupled receptors (GPCR). When binding ET_A receptor (ET_AR), ET-1 induce vasoconstriction[57], while binding to ET_B receptor (ET_BR) leads to vasodilatation through NO release. Actually, two classes of ET_BR exists ET_{B1} and ET_{B2}, the latter would induce vasoconstriction in smooth muscular cells (SMC)[59]. ET1 receptors are expressed in endothelial cells and in SMC in all human organs: ET_AR are prevalent in heart, great vessels, pulmonary arteries and in less extent in central nervous system (CNS), whereas ET_BR are prominent in CNS and brain cortex. Namely, brain little parenchymal arteries, when undamaged, express just ET_AR[60], and hence ET-1 induces vasoconstriction. Conversely, according to studies on animal

models, in stress conditions such as AIS, ET_BR are upregulated, and ET-1 induces vasodilatation[61,62].

ET-1 is involved in different neurological diseases. In subarachnoid hemorrhage ET-1 increase was observed in patients developing delayed clinical vasospasm.[63,64]

Data on role of ET-1 in AIS are conflicting. High ET-1 plasmatic level in acute phase (24 hours) of AIS have been reported[65–68,] but other studies disagreed with these findings [69,70]. Some authors found high ET-1 in AIS just in cerebrospinal fluid[69].

As to clinical evidence of ET-1 in AIS, according to some studies elevated serum levels directly correlate with most severe strokes[65,66,71] and worse early prognosis[72]. High serum and CSF ET-1 was described in association with larger infarcts and severe brain edema[68,69,73] as well. Authors speculated that ET-1 could impair blood brain barrier and promote edema enlargement[73,74].

The precise trigger for ET-1 increase in brain infarct is not known. Possibly ET-1 release might be the vascular response to ischemia related stress[65], or, as an alternative, ET-1 might be overexpressed by glial cells of the damaged parenchyma. Release of ET-1 from endothelium in response to hypoxia and thrombin[75] is, indeed, well established[65].

Animal models showed upregulation of ET_BR in SMC after ischemia with a consequent vasoconstriction, inhibited by ET_BR antagonists[76–78]. Otherwise, augmented ET_AR was not constantly reported[77,78].

A metanalysis of animal studies on the therapeutic role of ET_AR antagonist in AIS, highlighted better outcomes and reduced infarct volume. [76] Proposed mechanisms include preservation of ischemic penumbra through vasodilatation and reduction of blood brain barrier disfunction. Another hypothesis to explain the positive effect of ET_AR antagonist, involves the ET-1 role on microcirculation during cerebral post ischemic hypoperfusion, observed in animal experiments after restoring circulation, in other terms on the NRP[53–55].

One experimental study on mice showed how ET1 infusion impairs cerebral autoregulation through its action on SMC and endothelial cells; this was not observed on resting cerebral blood flow (CBF), but just after whisker stimulation, which mimics hyperemia induced by neural activity [79]. In detail, CBF impairment was inhibited by ET_AR antagonist and did not depend on oxidative stress, but rather on a Rho-associated protein kinase (ROCK) activity[79]. Through ROCK activity ET1 suppresses NO production on endothelial cells. Globally, this study showed that ET1 worsens ischemic damage disrupting the delicate equilibrium between CBF and energy demand[79]

Nitric oxide (NO) in acute ischemic stroke

Nitric oxide (NO) is an inhorganic gas, commonly considered toxic, first recognized in 1980 as a signaling molecule involved in vascular smooth cells relaxation and hence vasodilatation[80]. Later, NO has emerged as a fundamental endogenous factor not only in maintaining vascular tone, but also, in inflammatory response reduction, regulation of thrombotic-thrombolytic balance and cell growth regulation[81].

NO is produced by three different isoforms of the enzyme nitric oxide synthase (NOS) which synthetizes NO and citrulline from L-arginine and oxygen[82] in endothelial cells, neurons, glia, and macrophages[81,83]. Endothelial NOS (eNOS) is present in vascular endothelium and choroidal plexus and plays a protective role through inhibition of platelet aggregation, reduction of leukocyte endothelial adhesion and promotion of vasodilation and hence blood flow maintenance[83–85]. Neuronal NOS (nNOS) activity is promoted by calcium released by ischemic neurons [86], and is mainly evident after flow is restored in AIS[87]. Differently from the constitutive NOS isoforms, the inducible form of NOS (iNOS) is calcium independent and its transcription is stimulated by inflammatory cytokines and growth factors; hence, iNOS mediated NO production is more sustained and continues till the enzyme is degraded[88].

NO role in ischemic stroke is controversial. Indeed, after cerebral arteries occlusion NO production decreases rapidly, to further increase after recanalization[89]. nNOS is involved in parenchymal injury after ischemia; in animal models, inhibition, or depletion of nNOS causes reduction of the extent of necrosis in both core and ischemic penumbra and of apoptosis in penumbra[87,90]. On the other hand, eNOS knockout mice showed larger infarcts after brain ischemia and smaller ischemic penumbra;91 probably eNOS, compared to the other isoforms, might play a major role on cerebral flow during AIS[92]. Moreover, NO production might contribute to reperfusion injury through oxidative stress, which does not seem to depend on nNOS activity but rather on eNOS[93]. In pathological conditions, such as brain ischemia, lack of tetrahydrobiopterin and of L-ariginine leads to production of both NO and superoxide by eNOS[94,95]. Furthemore, NO and superoxide can further react generating peroxynitrite, an oxidant agent even more powerful than NO and superoxide themselves[93]. At the same time, during AIS, iNOS activation provides more NO as substrate for peroxynitrite formation contributing to reperfusion oxidative injury[96].

Furthermore, NO inhibits ET-1 production acting on endothelin converting enzyme-1 (ECE-1) and Nuclear factor κB (NF-kB), with consequent reduction of ET-1 mediated vasoactive action[97,98]. In coronary arteries NO is able to revert vasospastic action of ET-1.[99,100]

Several studies tested efficacy of transdermal glyceryl trinitrate (TGT), an NO donor in acute ischemic stroke, with the aim of blood pressure management, improving cerebral perfusion and clinical outcome[101–104], but though lowering blood pressure, clinical efficacy was not evident[105]. Interestingly, one study outlined that TGT mediated blood pressure lowering did not affect cerebral blood flow[103].

NO serum dosage is extremely difficult because of its short half-life (6 seconds). To get over this problem, nitrites and nitrates, which are stable metabolites of NO, are measured for clinical purposes. Namely, nitrite ion is widely accepted as a marker of NO blood levels.

Methods

Aim of the Study

Primary endpoint of the study was to investigate the role of ET-1 and NO in NRP in patients with acute ischemic stroke from large vessels occlusion who were treated with MT and reached a successful recanalization. In particular, we tried to detect whether higher peripheral and intracranial levels of ET-1 and lower levels of NO are related to cerebral microvascular disfunction which causes NRP despite successful brain main artery recanalization. Samples were taken at pre-specified time points before and after the recanalization. To detect NRP in our patients we used the post-interventional angiographic score tested in the previous study.

As secondary endpoint, we tried to investigate the association between ET-1 and NO blood levels and early neurological outcome.

Study Population

We enrolled patients older than 18 years old admitted to Policlinico Umberto I University hospital for AIS from LVO of the anterior cerebral circulation (i.e. intracranial internal carotid artery (ICA) (C5-C6 segments), first segment of the middle cerebral artery (M1), second segment of the middle cerebral artery (M2), tandem occlusion that is intracranial occlusion with ipsilateral ICA occlusion or stenosis ≥70%) who underwent endovascular revascularization and who signed informed consent to be included in the study.

Patients who did not reach a successful recanalization, defined as a TICI score of 2b-3[5], were excluded from the study after the procedure.

Endothelin-1, nitrites, and nitrates serum level detection

Study specific peripheral venous blood samples were taken from each patient to dose ET-1 and nitrites and nitrates at hospital admission before endovascular procedure, after 24 hours and after 48 hours. When technically possible, intracranial arterial blood samples, during MT before and after main artery recanalization, were taken as well.

All blood samples were stored at 4°C for no more than 12 hours and then stored in freezer at -80°C. ET-1 and nitrites and nitrates were measured in serum by ELISA performed between 30 and 120 days from collection.

Operational definition of no-reflow

We first calculated the Capillary Index Score (CIS) on the pre-interventional angiographic images in order to evaluate collateral circulation according to the technique described by Firas Al-Ali et al [39]. Then, we defined NRP as previously descrided in study 1, using the mCIS score. In particular, on post interventional DSA anterior-posterior images of the MCA territory were acquired and divided into 3 equal segments. For each segment, we gave 2 points if the capillary blush was present without any delay, 1 point if the capillary blush was present but was delayed as compared to the first appeared capillary blush or when possible, compared to anterior cerebral artery territory, and 0 points if there was no capillary blush present thus allowing a range of 0-6 for each patient.

A cut-off of mCIS score ≤ 3 to define NRP presence.

Each post-interventional DSA was independently scored by 2 experienced interventional neuroradiologist (MI and FB), They were blinded to all other information and then came to a unanimous agreement on the final score.

Data collection

For each patient we recorded age, sex and the presence of relevant cerebrovascular risk factors such as arterial hypertension, atrial fibrillation, current or past habit of cigarette smoking, diabetes, hypercholesterolemia, carotid atherosclerosis, history of AIS in the past 3 months, heart failure, coronary artery disease and presence of tumor. We collected the following clinical variables: NIHSS (baseline, post-intervention, at 24 hours and at 7 days or at discharge if the hospitalization was shorter than 7 days), mRS (pre-stroke and at 90 days, the latter evaluated by telephone interview of the patients or relatives), aetiology of stroke based on the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria[49], AIS presenting at wake-up, location of the large vessel occlusion, Alberta Stroke Program Early CT Score (ASPECTS) [50], presence and type of haemorrhagic transformation according to the European Cooperative Acute Stroke Study (ECASS) classification of intracerebral haemorrhage (ICH) following thrombolysis[51], presence of symptomatic ICH, defined as any ICH associated with an increase of ≥ 4 points on the NIHSS at 24 hours or leading to death[51], and the ischemic lesion volume which was calculated using the A*B*C/2 formula on post-interventional MRI images. Procedural variables included the following: time from stroke onset to recanalization, administration of IV rtPA before mechanical thrombectomy (i.e. bridging therapy), specific technique used during mechanical thrombectomy, total number of passes, TICI score and total duration of the procedure.

Blood pressure was recorded at hospital admission before MT, after 24 hours and at 48 hours.

All data were collected by two neurologists specialized in cerebrovascular diseases (EN and MDM)

We used 90-day mRS and 24-hour NIHSS as clinical outcome measures. We considered mRS 0-2 as good outcome, 0-1 as excellent outcome and patients with 90-day mRS of 3-6 were classified as FR. Given the earliest data from literature[13], we used two early neurological outcome measure as well, hence we defined fENI at 24 hours and at 7 days as 24h or 7 days NIHSS equal or higher than baseline NIHSS.

Statistical analysis

Continuous variables were presented as mean, median and standard deviation (SD), categorial variables as number and percentages, as appropriate. Student's t tests or Mann-Whitney U tests were applied for continuous data. Pearson's $\chi 2$ tests or Fisher's exact tests were conducted for categorical data as appropriate.

Univariate analysis was applied test the association between mean ET-1 and NO levels at any time point and angiographic NRP. Pearson's correlation was used to test the association between ET-1 and NO values at any time and continuous variables such as NIHSS, systolic BP, diastolic BP and mean BP and infarct volume.

Predictive value of ET-1 and NO levels for NRP was tested using a logistic regression model including variables found significant at the univariate analysis.

Same analyses were repeated using FR, fENI at 24h and at 7days as outcome measures.

Probability values less than .05 were considered to be of statistical significance. All statistical analysis were performed using SPPS (V.26) ® (IBM) software.

Results

Descriptive analysis

From January 2021 we enrolled 61 patients with AIS from LVO of the anterior cerebral circulation, mean age was 74.1± 11.2 years old and 32 (52.5%) were females. Most patients were affected by arterial hypertension (78.7%), atrial fibrillation (49.2%), hypercholesterolemia (34.4%) and diabetes (21.3%). Cardioembolic etiology was the most common cause of stroke (69.7%) followed by large artery atherosclerosis (16.4%) and undetermined etiology (16.4%), while in 4 cases (6.6%) stroke was consequent to other determined causes. Mean NIHSS in the entire population was 15 ± 6 at admission, 9 ± 7 at 24 hours, and 6 ± 6 at 7 days. 48 patients (78.7%) showed a neurological improvement after 24 hours and 54 (88.5%) after 7 days. As to 90-day functional outcome 28 (45.9%) patients reached functional independence (mRS 0-2) and 24 (39.3% an excellent outcome (mRS 0-1), while 8 patients (13.1%) were dead at 90 days.

LVOs gained successful recanalization after a mean time of 483.2 minutes (8 hours and 3 minutes) from symptom onset, and mean interventional time was 81.8 minutes (1 hours and 21 minutes). Concerning radiological outcome, 27 (46.6%) patients showed hemorrhagic transformation of the ischemic lesion, but just 2 (3.6%) were classified as sICH. Mean infarct volume was 17.2 ± 32.9 ml. Further features of the whole study population are reported in table 8.

Clinical characteristic of patients showing angiographic no-reflow phenomenon

In this cohort, 17 patients (27.8%) were classified as having NRP (mCIS≤3), after analyzing post-interventional DSA. Mean age was 73.2 y.o (SD 9.8) in NRP group and

74.5 y.o. (SD 11.8) in the rest of the population (p=0.273), females were respectively 41.2% and 56.8% in the two groups (p=0.793).

As to cardiovascular risk factors, patients with NRP showed more frequently carotid atherosclerosis (47.1% vs 20.5% p=0.038) and coronary artery disease (29.4% vs 9.1% p=0.045). In patients with mCIS≤3, stroke were clinically more severe at hospital admission (NIHSS, mean ± SD: 19 ± 5 vs 13 ± 6 p=0.001) and immediately after MT (NIHSS, mean SD: 16 ± 7 vs 11 ± 7 p=0.018). Ten (22.7%) patients with good microvascular reperfusion and 3 (17.6%) patients with NRP did not clinically improve at 24-hour examination (p=0.664), while at 7 days fENI was reported in 39 (88.6%) and 15 (88.2%) patients respectively (p=0.965). At 90 days, 9 patients (52.9%) in the NRP and 19 (43.2%) in the rest of the population had an mRS 0-2 (p=0.493). Patients with good collateral status before thrombectomy (CIS 3) were prevalent among patients not showing NRP (72.1% vs 47.1% p=0.026).

On the control neuroimaging, 10 infarcts (66.7%) in the NRP had an hemorrhagic transformation compared to 17 (39.5%) in the rest of the population (p=0.07). Infarcts were larger if post-recanalization microvascular impairment was evident (mean ± SD: 39.9 ml ± 18.1 vs 8.8 ml ± 57.2 p=0.002).

Association between no-reflow phenomenon and ET-1 and NO in peripheral and intracranial arterial blood

Mean ET-1 peripheral blood levels in NRP group and in patients with good microvascular reperfusion were respectively 15.55 pg/ml ± 19.65 and 13.92 pg/ml ± 7.20 at baseline (p=0.464), 22.26 pg/ml ± 10.37 and 17.55 pg/ml ± 7.13 at 24 hours (p=0.255) and, 14.95 pg/ml ± 3.90 and 17.03 pg/ml ± 6.94 at 48 hours (p=0.337).

Concerning intracranial arterial samples, before MT ET-1 mean values were 19.75 pg/ml ± 5.98 in patients with mCIS ≤3 and 16.40 pg/ml ± 3.01 in mCIS>3 cases (p=0.294). After the procedure, mean ET-1 was 22.01pg/ml ±8.92 in the first group and 22.79 ± 7.62 in the second one (p=0.886).

As to venous NO in NRP and in good reperfusion group, mean levels were respectively 41.20 μM ± 15.4 and 44.71 μM ± 19.65 (p=0.558) at admission, 17.71 μM ± 10.99 and 19.77 μM ±11.30 (p=0.644) at 24 hours and 20.46 μM ±7.08 and 14.00 μM ± 8.06 (p=0.084) at 48 hours.

Before recanalization, mean NO in intracranial arterial blood was 9.60 μM ± 2.80 in the NRP group and 18.58 μM ±5.92 in patients with good microvascular reperfusion (p=0.004) (Table 9 and Fig 7), while after MT it was 23.17 μM ± 8.91 and 19.52 μM ± 7.85 in (p=0.516), respectively. Multivariate analysis did not show any association between intracranial NO and NRP (aOR 0.561, 95%CI 0.297 – 1.061, p=0.075) (Table 10.).

Association between ET-1 and NO and outcome measures

Patients who clinically improved after 24 hours showed a mean serum ET-1 at baseline of 15.37 pg/ml ± 6.88 compared to 10.90 ± 5.54 in the fENI group (p=0.061); values at 24 hours were 19.43 pg/ml ± 8.35 and 15.67 pg/ml ± 6.08 (p=0.125) and at 48 hours 16.64 pg/ml ± 7.10 and 16.65 pg/ml ± 5.18 (p=0.995), respectively.

Concerning 7-day outcome, mean serum ET1 levels at admission, at 24h and at 48 hours in fENI and ENI groups were respectively: 12.80 pg/ml ± 5.48 vs 14.64 pg/ml 7.04 (p=0.448), 13.31 pg/ml ± 5.25 vs 19.14 pg/ml ± 8.02 (p=0.068) and 17.64 pg/ml ± 4.13 vs 16.50 pg/ml ± 6.79 (p=0.657).

NO values in fENI and ENI patients at at 24 hours were as follows at the three time points: 37.47 μM ± 16.89 vs 45.56 μM ± 18.74 (p=0.237); 12.82 μM ± 8.91 vs 21.71 μM ± 11.03

(p=0.014) (Table 11 and Fig 8.); 14.63 μM ±7.70 vs 15.48 μM ± 8.56 (p=0.784). The association between NO levels at 24 hours and fENI was confirmed by logistic regression model adjusted for age, sex, stroke severity and mean blood pressure at T1 (aOR 1.19, 95% CI 1.014–1.213, p = 0.023) (Table 12.).

Cases in 7-day fENI and ENI groups showed the following NO mean levels in peripheral blood: at T0 37.62 μM ± 15.57 vs 44.82 μM ± 18.91 (p=0.343); at T1 11.80 μM ± 12.64 vs 20.40 μM ± 10.70 (p=0.208); at T2 15.57 μM ± 9.80 vs 16.50 μM ± 6.79 (p=0.941).

For none of the patients in the 7-day and 24 hours fENI groups intracranial arterial samples could be taken.

Differences of ET-1 and NO between 90-day functional outcome groups and according on hemorrhagic transformation are reported in table 13.

Pearson's correlation model showed an association between stroke severity at admission and ET-1 levels at 24 hours (r=0.374 p=0.015) and intracranial NO levels before thrombectomy (r=-0.733 p=0.004) (Table 14.).

Discussion

The NRP is well determined in animals[27,31–34] and after myocardial infarct[46], and probably it is in part responsible for clinical failure of revascularization therapies in AIS[24,106].

The precise determinant of the post-recanalization microvascular impairment in human brain is not well established, though several vasoactive and prothrombotic mechanisms have been proposed[32–34,53–55].

Our study tried to explore the role of two vasoactive agents ET-1 and NO in NRP, radiologically determined as a microvasculature impairment on post-interventional DSA. Albeit not confirmed at the logistic regression model, we found a trend for lower values of NO in intracranial arterial blood before TM, in patients showing NRP. After the procedure, intracranial levels of NO were not different between the two groups. Our finding is in line with evidence from animal models, indicating that eNOS knockout mice show larger infarct volumes;[91] indeed, in our population, patients with microvascular impairment after main artery recanalization showed larger infarct volumes as well. Endothelial NO, thanks to its vasoactive effect, owns a protective role on microvascular reperfusion after AIS[92]. Pearson's correlation model outlined a not statistically significant inverse correlation between pre-TM NO levels and infarct volume (r= -0.449 p=0.143). Unfortunately, intracranial samples were scarce in our cohort, hence confirmation of this association in larger cohort is needed.

As a matter of fact, NO might influence both parenchymal capillaries and pial small arteries, in other words it might affect collateral status before flow restoration, as well as NRP. In our patients showing NRP, bad collateral status (CIS 0) was more frequent compared to the rest of the population, but still about 80% of the NRP cases had a CIS≥2. Larger cohorts are needed to explore the effect of pre-interventional collateral status on NRP.

The incremental trend of intracranial NO after main artery patency restoration was evident as well in animal models[89]**.** In this setting, production of NO might involve nNOS[87], rather than eNOS, in response to calcium released by ischemic neurons[86]. At 48 hours patients with NRP showed a trend toward higher NO levels, we suppose that this augmentation might be due to larger infarcts observed in this population. Namely, extensive parenchymal damage accounts for higher calcium release by ischemic neurons, which is, finally, a stimulus for nNOS activity[86,87].

Concerning clinical outcome, patients not improving after 24 hours had lower T1 mean values of NO, as detected in peripheral blood samples. This could be a sign of enhanced consumption of NO to form superoxide and peroxynitrite, which contribute to oxidative stress during brain ischemia and hence worse parenchymal damage. NO contribution to oxidative stress is mainly due to nNOS and iNOS, which, in condition of deficit of substrate for enzymatic reactions, contribute to this harmful cascade[94–96].

We could not find any difference for ET-1 levels in our groups at any time. Authors reported high ET-1 levels in larger infarcts, explanations include ET-1 related capillaries vasocontriction, determining more extensive damage, and a possible role of ET-1 promoting blood brain barrier disfunction[53,54,60]. In our patients, ET-1 showed high levels in both NRP and no NRP groups during the first 24 hours (Fig.5), which is in line with other previous reports of ET-1 higher serum levels in AIS during the first 24-48 hours[65–67]. Hence, association between ET-1 and worse outcome or extent of ischemic damage is still controversial and debated[70].

Notably no differences were found in peripheral blood levels of NO nor of ET-1. We speculate that local production of these factor might be more important in determining parenchymal reperfusion.

Our data outline a possible role of NO in promoting microvascular reperfusion after cerebral artery recanalization in AIS, but scarce number of intracranial samples was a great limitation of our study. Larger cohorts are needed to confirm our results which might contribute to future research on pre-interventional treatments focused on capillary vasoactive agents.

Tables

Table 8. Clinical and radiological characteristics of the entire population and Univariate analysis for No-reflow phenomenon

	All (N 61)	Good microvascular reperfusion (mCIS>3) (N 44)	No-reflow phenomenon (mCIS≤3) (N 17)	p
Age mean ± SD (median)	74.1 ± 11.2 (78.0)	74.5, ± 11.8 (78.0)	73.1 ± 9.8 (75.0)	0.660
Sex				
Male N (%)	29 (47.5)	19.0 (43.2)	10 (58.8)	0.273
Female N (%)	32 (52.5)	25.0 (56.8)	7 (41.2)	
Hypertension N (%)	48 (78.7)	35.0 (79.5)	13 (76.5)	0.793
Diabetes N (%)	13 (21.3)	10.0 (22.7)	3 (17.6)	0.664
Smoking N (%)	11 (18.0)	8.0 (18.2)	3 (17.6)	0.961
Previous stroke or TIA N (%)	6 (9.8)	4.0 (9.1)	5 (29.4)	0.753
Heart failure N (%)	9 (14.8)	7.0 (15.9)	2 (11.8)	0.682
Coronary artery disease N (%)	9 (14.8)	4.0 (9.1)	5 (29.4)	0.045
Atrial fibrillation N (%)	30 (49.2)	22.0 (50.0)	8 (47.1)	0.837
Hypercolesterolemia N (%)	21 (34.4)	14.0 (31.8)	7 (41.2)	0.490
Carotid atherosclerosis N (%)	17 (27.9)	9.0 (20.5)	8 (47.1)	0.038
Tumor N (%)	10 (16.4)	7.0 (15.9)	3 (17.6)	0.869
mRS pre-stroke				
0 N (%)	51 (83.6)	37.0 (84.1)	14 (82.4)	0.240
1 N (%)	4 (6.6)	2.0 (4.5)	2 (11.8)	
2 N (%)	4 (6.6)	4.0 (9.1)	0	
3 N (%)	1 (1.6)	0 0	1 (5.9)	
4 N (%)	1 (1.6)	1.0 (2.3)	0	
Etiology (TOAST)				
Large-artery atherosclerosis N (%)	10 (16.4)	6.0 (13.6)	4 (23.5)	0.784
Cardioembolism N (%)	37 (60.7)	27.0 (61.4)	10 (58.8)	
Other determined etiology N (%)	4 (6.6)	3.0 (6.8)	1 (5.9)	

Undetermined etiology N (%)	**10 (16.4)**	**8.0 (18.2)**	**2 (11.8)**	
Wake-up Stroke N (%)	**24 (38.7)**	**17.0 (38.6)**	**7 (41.2)**	**0.856**
Baseline NIHSS mean ± SD (median)	**15.0 ± 6.0 (15.0)**	**13.0 ± 6.0 (15.0)**	**19.0 ± 5.0 (20.0)**	**0.001**
Post interventional NIHSS mean ± SD (median)	**12.0 ± 7.0 (12.0)**	**11.0 ± 7.0 (11.0)**	**16.0 ± 7.0 (17.0)**	**0.018**
24H NIHSS mean ± SD (median)	**9.0 ± 7.0 (8.0)**	**9.0 ± 7.0 (7.0)**	**11.0 ± 5.0 (10.0)**	**0.287**
7 days/Discharge NIHSS mean ± SD (median)	**6.0 ± 6.0 (5.0)**	**6.0 ± 7.0 (4.0)**	**7.0 ± 4.0 (7.0)**	**0.532**
Early outcome 24 hours				
Failure of Early Neurological Improvement N (%)	**13 (21.3)**	**10.0 (22.7)**	**3 (17.6)**	**0.664**
Early neurological improvement N (%)	**48 (78.7)**	**34.0 (77.3)**	**14 (82.4)**	
Early outcome 7 days				
Failure of Early Neurological Improvement N (%)	**7 (11.5)**	**39.0 (88.6)**	**15 (88.2)**	**0.965**
Early neurological improvement N (%)	**54 (88.5)**	**5.0 (11.4)**	**2 (11.8)**	
90 days functional outcome				
Bad outcome 3-6 N (%)	**33 (54.1)**	**25 (56.8)**	**8 (47.1)**	**0.493**
Functional indipendence (mRS 0-2) N (%)	**28 (45.9)**	**19 (43.2)**	**9 (52.9)**	
ExcellentOutcome (mRS 0-1) N (%)	**24 (39.3)**	**17 (38.6)**	**7 (41.2)**	**0.856**
Death at 90 days N (%)	**8 (13.1)**	**6 (13.6)**	**2 (11.8)**	**0.846**
Site of Occlusion				
M1 N (%)	**32 (54.2)**	**22,0 (51.2)**	**10 (62.5)**	**0.289**
M2 N (%)	**16 (27.1)**	**14.0 (32.6)**	**2 (12.5)**	
Intracranial ICA N (%)	**0**	**0**	**0**	
Tandem occlusion N (%)	**11 (18.6)**	**7.0 (16.3)**	**4 (25.0)**	
Direct MT N (%)	**37 (60.7)**	**25.0 (56.8)**	**12 (70.6)**	**0.324**

Bridging therapy N (%)	**24 (39.3)**	**19.0 (43.2)**	**5 (29.4)**	
Onset-to-recanalization Time (min) mean ± SD (median)	**483.2 ± 293.0 (340.0)**	**483.9 ± 268.2 (350.0)**	**481.4 ± 320.5 (315.5)**	**0.878**
Groin-to-recanalization Time mean ± SD (median)	**81.8 ± 47.5 (71.0)**	**82.8 ± 51.7 (70.0)**	**79.4 ± 35.3 (72.0)**	**0.808**
CIS				
0 N (%)	**7 (11.7)**	**3 (7.0)**	**4 (23.5)**	**0.026**
1 (N %)	**5 (8.3)**	**5 (11.6)**	**0**	
2 N (%)	**9 (15.0)**	**4 (9.3)**	**5 (29.4)**	
3 N (%)	**39 (65.0)**	**31 (72.1)**	**8 (47.1)**	
TICI				
2b N (%)	**18 (31.0)**	**13.0 (31.0)**	**5 (31.3)**	**0.973**
2c N (%)	**3 (5.2)**	**1.0 (4.8)**	**1 (6.3)**	
3 N (%)	**37 (63.8)**	**27.0 (64.3)**	**10 (62.5)**	
N° of passages				
0 N (%)	**1 (1.7)**	**1 (2.3)**	**0**	**0.884**
1 N (%)	**29 (49.2)**	**20.0 (46.5)**	**9 (56.3)**	
2 N (%)	**18 (30.5)**	**14.0 (32.6)**	**4 (25.0)**	
3 N (%)	**6 (10.2)**	**5.0 (11.6)**	**1 (6.3)**	
4 N (%)	**3 (5.1)**	**2.0 (4.7)**	**1 (6.3)**	
5 N (%)	**2 (3.4)**	**1.0 (2.3)**	**1 (6.3)**	
Technique				
Thrombus aspiration N (%)	**29 (49.2)**	**22.0 (51.2)**	**7 (43.8)**	**0.756**
Stent retrieving N (%)	**5 (8.5)**	**3.0 (7.0)**	**2 (12.5)**	
combined technique N (%)	**25 (42.4)**	**18.0 (41.9)**	**7 (43.8)**	
Hemorragic Transformation N (%)	**27 (46.6)**	**17.0 (39.5)**	**10 (66.7)**	**0.070**
Infarct volume (ml) mean ± SD (median)	**17.2 ± 32.9 (7.0)**	**8.8 ± 8.6 (6.3)**	**39.9, ± 57.2 (18.1)**	**0.002**
ECASS				
HI1 N (%)	**13 (48.1)**	**9.0 (52.9)**	**4 (40.0)**	**0.570**
HI2 N (%)	**7 (25.09**	**3.0 (17.6)**	**4 (30.0)**	
PH1 N (%)	**2 (7.4)**	**1.0 (5.9)**	**1 (10.0)**	
PH2 N (%)	**5 (18.5)**	**4.0 (23.5)**	**1 (10.0)**	

sICH N (%)	2 (3.6)	1.0 (2.4)	1 (6.7)	0.450

Table 9. Univariate analysis of ET-1 and NO levels among patients with and without no-reflow phenomenon

	All (N 61)	Good microvascular reperfusion (mCIS>3) (N 44)	No-reflow phenomenon (mCIS≤3) (N 17)	P value
T0 NO (μM) mean ± SD	43.75 ± 18.46	44.71 ± 19.65	41,20 ± 15.40	0.558
T0 ET-1 (pg/ml) mean ± SD	14.37 ± 6.81	13.92 ± 7.20	15,55 ± 5.77	0.464
T0 SBP (mmHg) mean ± SD	143.71 ± 23.27	145.39 ± 25.02	138,78 ± 16.93	0.277
T0 DBP (mmHg) mean ± SD	79.00 ± 11.82	79.73 ± 11.28	76,85 ± 13.47	0.482
T0 MBP (mmHg) mean ± SD	100.63 ± 13.87	101,62 ± 14,16	97,71 ± 13.02	0.353
T1 NO (μM) mean ± SD	19.39 ± 11.15	19.77 ± 11.30	17,71 ± 10.99	0.644
T1 ET-1 (pg/ml) mean ± SD	18.45 ± 7.93	17.55 ± 7.13	22,26 ± 10.37	0.255
T1 SBP (mmHg) mean ± SD	139.27 ± 22.71	143.12 ± 22.82	125.55 ± 17.00	0.022
T1 DBP (mmHg) mean ± SD	73.56 ± 15.74	74.84 ± 15.13	69.00 ± 17.92	0.390
T1 MBP (mmHg) mean ± SD	95.46 ± 15.83	97.60 ± 15.68	87.85 ± 14.66	0.105
ET1 Intra Pre (pg/ml) mean ± SD	17.69 ± 4.48	16.40 ± 3.01	19,75 ± 5,98	0.294
NO Intra Pre (μM) mean ± SD	15.13 ± 6.62	18.58 ± 5.92	9,60 ± 2.80	0.004
ET1 Intra post (pg/ml) mean ± SD	22.54 ± 7.67	22.79 ± 7.62	22,01 ± 8.92	0.886
NO Intra Post (μM) mean ± SD	20.74 ± 8.01	19.52 ± 7.85	23,17 ± 8.91	0.516
T2 NO (μM) mean ± SD	15.22 ± 8.19	14.00 ± 8.06	20,46 ± 7.08	0.084
T2 ET-1 (pg/ml) mean ± SD	16.65 ± 6.48	17.03 ± 6.94	14,95 ± 3.90	0.337
T2 SBP (mmHg) mean ± SD	139.91 ± 19.39	139.46 ± 19.13	141.71 ± 21.83	0.809

T2 DBP (mmHg) mean ± SD	77.74 ± 13.01	76.75 ± 13.17	81.71 ± 12.44	0.374
T2 MBP (mmHg) mean ± SD	98.47 ± 10.86	97.65 ± 10.66	101.71 ± 11.83	0.431

Table 10. Multivariate analysis of predictors of no-reflow phenomenon including pre-interventional intracranial NO levels in the model

	Adjusted OR	p	95% C.I.
NO Intra Pre	0.561	0.075	0.297 – 1.061.
Age	0.851	0.489	0.539 – 1.344
Arterial hypertension	0.344	0.805	0-1614.

Table 11. Univariate analysis of NO and ET-1 levels according to clinical outcome at 24 hours and at 7 days

	Failure of Early Neurological Improvement at 24h	Early neurological improvement at 24h	p	Early neurological improvement at 7days	Failure of Early Neurological Improvement at 7days	p
T0 NO (μM) mean ± SD	37.47 ± 16.89	45.56 ± 18.74	0.237	44.82 ± 18.91	37.62 ± 15.57	0.343
T0 ET-1 (pg/ml) mean ± SD	10.90 ± 5.54	15.37 ± 6.88	0.061	14.64 ± 7.04	12.80± 5.48	0.488
Baseline SBP (mmHg) mean ± SD	139.07 ± 18.95	145.14 ± 24.47	0.358	144.08 ± 23.99	141.14 ± 18.77	0.718

Baseline DBP (mmHg) mean ± SD	**76.15 ± 11.71**	**79.88 ± 11.85**	**0.329**	**79.10 ± 11.70**	**78.28 ± 13.49**	**0.883**
Baseline MBP (mmHg) mean ± SD	**97.13 ± 3.45**	**101.71 ± 14.25**	**0.274**	**100.83 ± 13.95**	**99.24 ± 14.28**	**0.789**
T1 NO (μM) mean ± SD	**12.82 ± 8.91**	**21.71 ± 11.03**	**0.014**	**20.40 ± 10.70**	**11.80 ± 12.638**	**0.208**
T1 ET-1 (pg/ml) mean ± SD	**15.67 ± 6.08**	**19.43 ± 8.35**	**0.125**	**19.14 ± 8.02**	**13.31 ± 5.25**	**0.068**
T2 NO (μM) mean ± SD	**14.63 ± 7.70**	**15.48± 8.56**	**0.784**	**15.16 ± 8.14**	**15.57 ± 9.80**	**0.941**
T2 ET-1 (pg/ml) mean ± SD	**16.65 ± 5.18**	**16.64 ± 7.10**	**0.995**	**16.50 ± 6.79**	**17.64 ± 4.13**	**0.657**

Table 12. Multivariate analysis of predictors of fENI at 24 hours

	Adjusted OR	**p**	**95% C.I.for**
T1 NO	**1,109**	**,023**	**1,014** **1,213**
Baseline NIHSS	**1,041**	**,559**	**,909** **1,192**
Age	**,962**	**,422**	**,874** **1,058**
Sex	**,916**	**,936**	**,108** **7,788**
T1 MBP	**,966**	**,275**	**,907** **1,028**

Table 13. Univariate analysis of NO and ET-1 levels according to 90-day functional outcome

	90-day mRS 3-6	**90-day mRS 0-2**	**p**	**No Hemorrhagic transformation**	**Hemorrhagic transformation**	**p**

T0 NO (μM) mean ± SD	**46.18 ± 20.60**	**39.67 ± 13.88**	**0.241**	**49.69 ± 18.23**	**38.76 ± 17.69**	**,075**
T0 ET-1 (pg/ml) mean ± SD	**13.37 ± 6.36**	**16.03 ± 7.40**	**0.257**	**13.87 ± 5.75**	**13.93 ± 7.80**	**,977**
Baseline SBP (mmHg) mean ± SD	**142.30 ± 26.49**	**145.40 ± 19.08**	**0.617**	**140.35 ± 25.99**	**144.36 ± 17.84**	**,513**
Baseline DBP (mmHg) mean ± SD	**77.23 ± 12.01**	**81.12 ± 11.46**	**0.226**	**78.82 ± 12.85**	**78.72 ± 11.10**	**,976**
Baseline MBP (mmHg) mean ± SD	**98.92 ± 14.94**	**102.67 ± 12.46**	**0.315**	**99.33 ± 16.22**	**100.72 ± 10.47**	**,710**
T1 NO (μM) mean ± SD	**18.41 ± 12.81**	**20.81 ± 8.27**	**0.466**	**19.70 ± 12.62**	**19.48 ± 10.14**	**,953**
T1 ET-1 (pg/ml) mean ± SD	**18.68 ± 8.94**	**18.11 ± 6.39**	**0.812**	**16.48 ± 6.68**	**18.46 ± 6.76**	**,374**
ET1IntraPre (pg/ml) mean ± SD	**21.22 ± 5.63**	**16.11 ± 3.03**	**0.053**	**18.85 ± 5.38**	**16.40 ± 3.86**	**,458**
NOIntraPre (μM) mean ± SD	**12.46 ± 8.20**	**16.31 ± 5.93**	**0.441**	**13.27 ± 4.35**	**17.23 ± 6.86**	**,256**
ET1Intrapost (pg/ml) mean ± SD	**25.98 ± 5.76**	**21.38 ± 8.16**	**0.334**	**21.80 ± 6.28**	**22.00 ± 8.95**	**,967**
NOIntraPost (μM) mean ± SD	**24.09 ± 8.97**	**19.62 ± 7.90**	**0.496**	**25.61 ± 7.12**	**19.34 ± 7.63**	**,215**
T2 NO (μM) mean ± SD	**14.10 ± 7.58**	**17.66 ± 9.32**	**0.306**	**13.60 ± 6.77**	**17.89 ± 9.85**	**,200**
T2 ET-1 (pg/ml) mean ± SD	**15.87 ± 6.21**	**18.36 ± 7.05**	**0.352**	**16.82 ± 6.38**	**16.36 ± 6.91**	**,853**

Table 14. Correlations between ET-1 and NO at different time points and stroke severity, baseline blood pressure and infarct volume

		T0 NO	T0 ET-1	T1 NO	T1 ET-1	ET1 IntraPre	NO IntraPre	ET1 Intrapost	NO IntraPost	T2 NO	T2 ET-1
Baseline NIHSS	**r**	**0.074**	**0.126**	**0.040**	**0.374**	**0.360**	**-0.733**	**-0.070**	**0.208**	**0.145**	**0.088**
	p	**0.651**	**0.438**	**0.801**	**0.015**	**0.227**	**0.004**	**0.830**	**0.516**	**0.429**	**0.632**
Post interventional NIHSS	**r**	**0.009**	**-0.075**	**-0.068**	**0.044**	**0.306**	**-0.436**	**-0.501**	**-0.038**	**0.092**	**-0.005**
	p	**0.955**	**0.644**	**0.667**	**0.782**	**0.309**	**0.137**	**0.097**	**0.905**	**0.618**	**0.980**
24H NIHSS	**r**	**-0.026**	**-0.123**	**-0.161**	**0.035**	**0.113**	**-0.377**	**-0.590**	**-0.142**	**0.204**	**0.090**
	p	**0.876**	**0.455**	**0.308**	**0.827**	**0.713**	**0.204**	**0.043**	**0.659**	**0.264**	**0.626**
7 days/Discharge NIHSS	**r**	**-0.161**	**-0.120**	**-0.211**	**-0.022**	**0.008**	**-0.380**	**-0.266**	**0.007**	**-0.015**	**0.091**
	p	**0.328**	**0.468**	**0.181**	**0.890**	**0.980**	**0.201**	**0.404**	**0.984**	**0.937**	**0.620**
Baseline SBP	**r**	**0.142**	**0.104**	**0.008**	**0.188**	**0.136**	**-0.364**	**0.081**	**-0.454**	**-0.042**	**0.050**
	p	**0.408**	**0.548**	**0.962**	**0.252**	**0.689**	**0.272**	**0.824**	**0.827**	**0.826**	**0.793**

Baseline DBP	**r**	**0.022**	**0.199**	**0.085**	**0.348**	**-0.164**	**0.048**	**0.126**	**0.071**	**-0.231**	**0.229**
	p	**0.898**	**0.243**	**0.606**	**0.030**	**0.631**	**0.889**	**0.729**	**0.846**	**0.218**	**0.225**
Baseline MBP	**r**	**0.094**	**0.169**	**0.051**	**0.299**	**-0.051**	**-0.191**	**0.057**	**-0.300**	**-0.152**	**0.155**
	p	**0.587**	**0.323**	**0.756**	**0.065**	**0.882**	**0.573**	**0.875**	**0.399**	**0.423**	**0.414**
Infarct volume	**r**	**-0.111**	**-0.122**	**-0.072**	**0.049**	**0.136**	**-0.449**	**-0.051**	**0.229**	**-0.042**	**-0.017**
	p	**0.538**	**0.499**	**0.681**	**0.779**	**0.674**	**0.143**	**0.882**	**0.499**	**0.827**	**0.930**

Figures

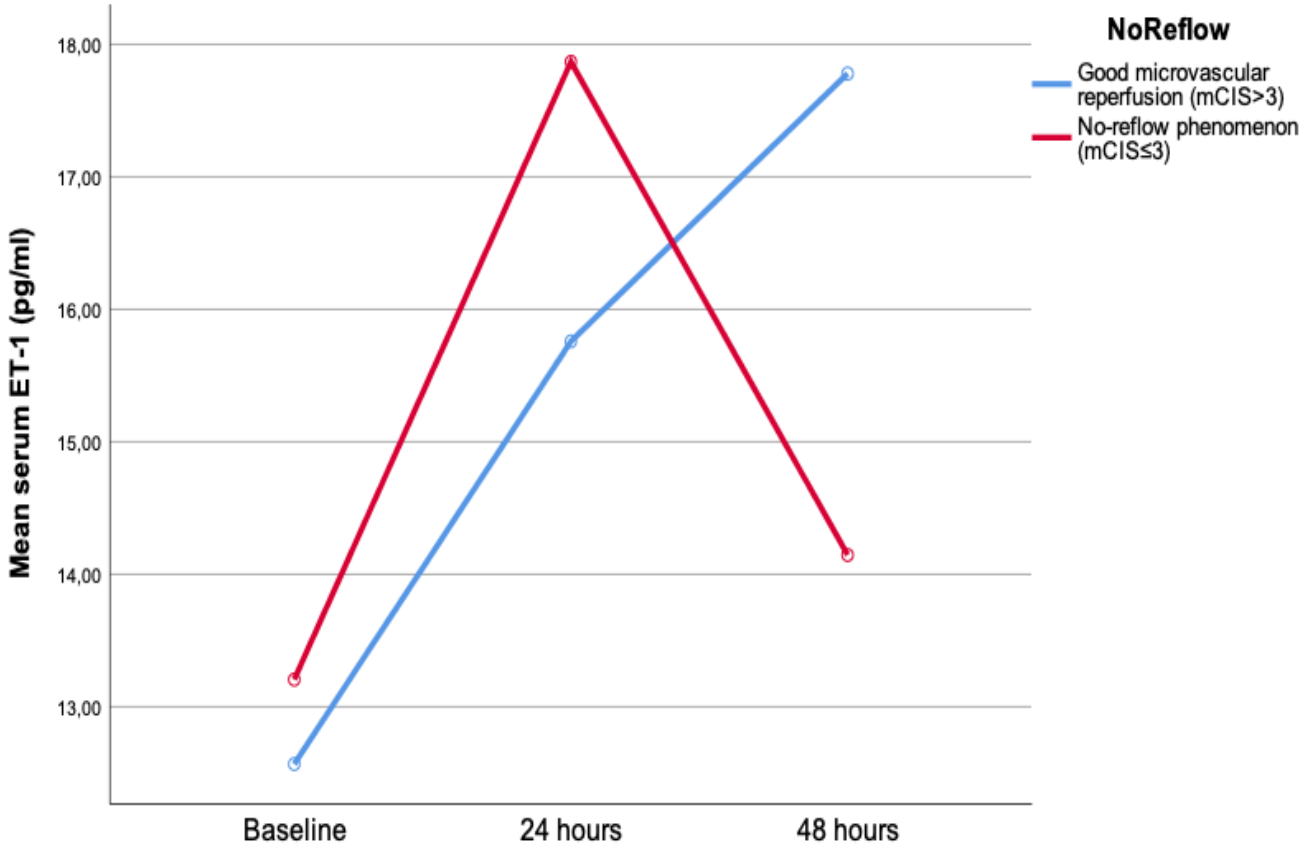


Figure 5. Serum ET-1 at three time points in no-reflow phenomenon groups. (ET-1= endothelin-1)

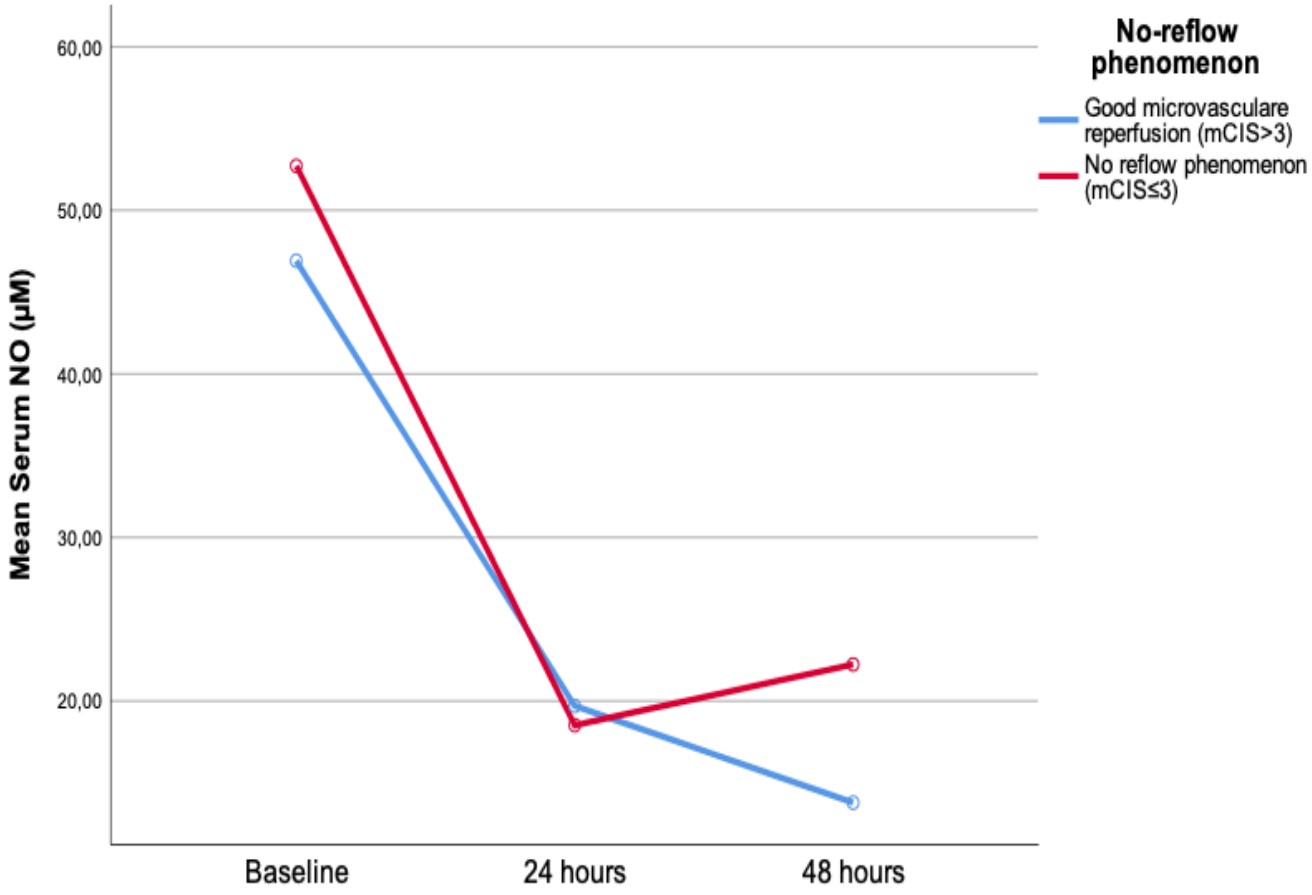


Figure 6. Serum NO at three time points in no-reflow phenomenon groups. (NO=nitric oxide)

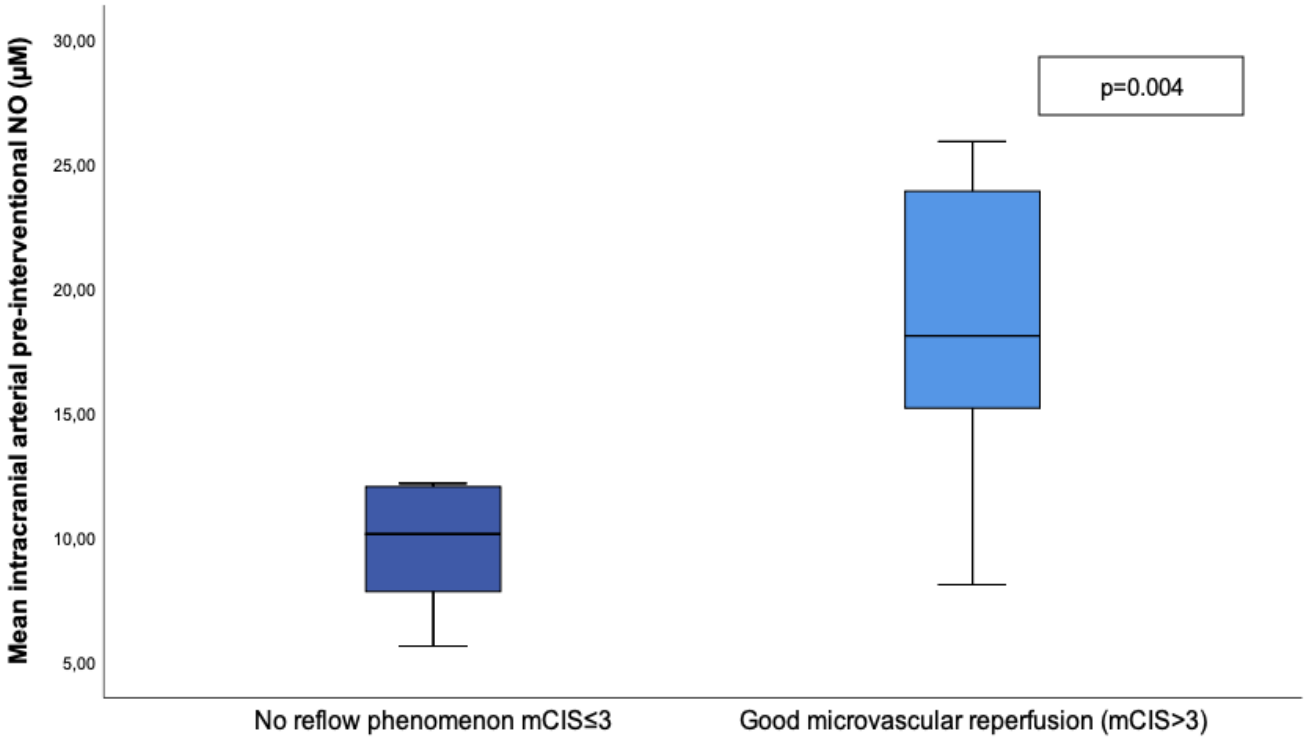


Figure 7. Pre-interventional intracranial arterial NO in patients with and without no-reflow phenomenon. (NO=nitric oxide)

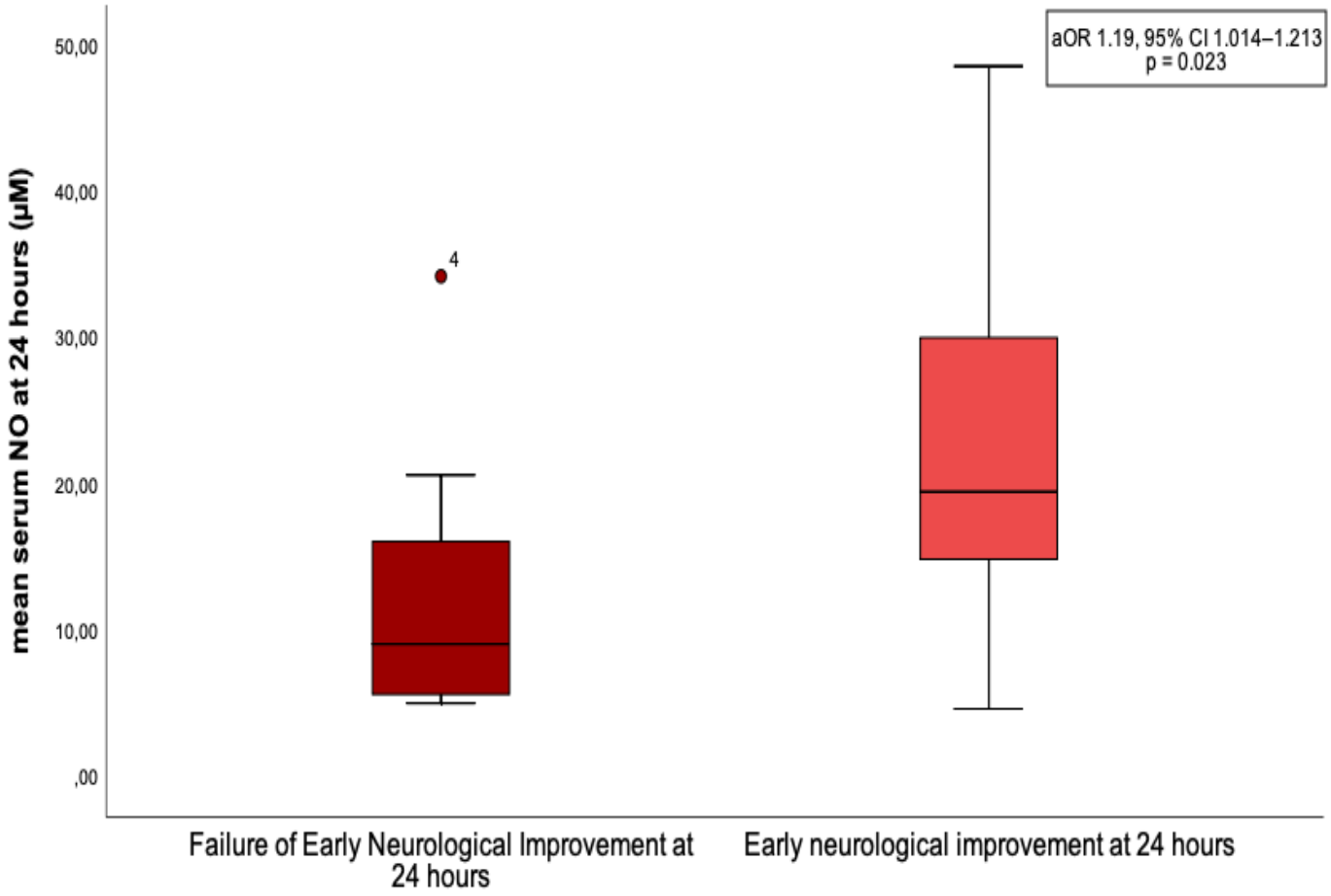


Figure 8. Serum NO at 24 hours in patients with and without fENI at 24 hours. (NO=nitric oxide; fENI= failure of early neurological improvement)

GENERAL CONCLUSION

Technical success of revascularization therapies in AIS does not guarantee the expected clinical results for all patients. Namely, reperfusion of cerebral parenchyma does not depend just on main arteries patency, rather on small vessels status.

No-reflow phenomenon is a potential target for future therapies to be associated to MT and IVT. Evidence on no-reflow phenomenon in human brain is scarce, hence a widely reproducible marker of this condition is needed.

In the first part of our research, we identified a radiological marker of no-reflow phenomenon. We used post-interventional DSA, which has two main advantages: first, this method is suitable for all AIS patients with LVO, since after every endovascular procedure, a final DSA is performed to establish cerebral circulation status; second, the dynamic nature and the high spatial resolution of DSA give more detailed information on microvascular reperfusion, thus allowing to identify absence or delay of parenchymal phase.

For our scope, we created an angiographic score and then set a cut-off for no-reflow phenomenon identification, e.g. microvascular impairment involving all MCA territory. Our angiographic evaluation of no-reflow phenomenon predicted early clinical worsening at 24 hours and at 7 days, but was not associated with 90-day functional outcome. Previous studies reported that high microvascular resistance predicted three months functional status[106] after MT. Nonetheless, differently from the abovementioned study, our research was specifically focused on patients who reached a successful recanalization, hence our data are scarcely comparable. As compared to MRI studies[36], our method showed higher incidence of no-reflow phenomenon, which would be in line with data on failure of early neurological improvement[13].

The second phase of our research focused on potential vasoactive agents involved in no-reflow phenomenon. In contrast to our expectations, ET-1 did not show to be determinant in patients with angiographic no-reflow phenomenon. On the other side, our data showed important differences in NO levels, both before and after MT. We suppose that NO might play different roles before and after flow restoration. In particular, when main artery is still occluded endothelial NO would have a protective effect, preventing microvascular occlusion, and no-reflow phenomenon. Conversely, after reperfusion nNOS and iNS activity would promote oxidative stress and parenchymal damage.

Reperfusion injury is a complex condition, involving release of vasoactive and pro-thrombotic agents which acts mainly on parenchymal capillaries and inflammatory cells and mediators, causing blood brain barrier rupture and neuronal damage. Clinical studies exploring each one of these different factors, and their mutual influence on clinical outcome are needed.

Our score is the first created to identify specifically no-reflow phenomenon in AIS patients and, at the same time, to show an association with clinical outcome. As future direction of our research, we plan to extend it to other stroke centers to get an external validation. Our score might represent a useful tool to identify patients developing no-reflow phenomenon. Albeit limited by small numbers, our results outline a key role of NO in MT outcomes, and we plan to continue our research enlarging our cohort.

Several studies tested the efficacy of NO donors on AIS patients, without showing clinical benefit[101–104]. If our results will be confirmed, they could support new clinical studies, testing NO donor on selected population of AIS patients, who are candidate to endovascular treatment.

MT has given, for the first time, the possibility to study AIS pathophysiology from a closer look, thus we think that acquiring more data from intracranial arterial blood testing is

crucial. Among our future aims, we hope to include coagulation and inflammation markers in our analyses, to get a more complete overview of microvascular pathophysiology in AIS.

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