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Radiologic Cerebral Reperfusion at 24 h Predicts Good Clinical Outcome

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Abstract

Cerebral reperfusion and arterial recanalization are radiological features of the effectiveness of thrombolysis in acute ischemic stroke (AIS) patients. Here, an investigation of the prognostic role of early recanalization/reperfusion on clinical outcome was performed. In AIS patients ($n = 55$), baseline computerized tomography (CT) was performed ≤ 8 h from symptom onset, whereas CT determination of reperfusion/recanalization was assessed at 24 h. Multiple linear and logistic regression models were used to correlate reperfusion/recanalization with radiological (i.e., hemorrhagic transformation, ischemic core, and penumbra volumes) and clinical outcomes (assessed as National Institutes of Health Stroke Scale [NIHSS] reduction ≥ 8 points or a NIHSS ≤ 1 at 24 h and as modified Rankin Scale [mRS] < 2 at 90 days). At 24 h, patients achieving radiological reperfusion were $n = 24$, while the non-reperfused were $n = 31$. Among non-reperfused, $n = 15$ patients were recanalized. Radiological reperfusion vs. recanalization was also confirmed by early increased levels of circulating inflammatory biomarkers (i.e., serum osteopontin). In multivariate analysis, ischemic lesion volume reduction was associated with both recanalization ($\beta = 0.265$; $p = 0.014$) and reperfusion ($\beta = 0.461$; $p < 0.001$), but only reperfusion was independently associated with final infarct volume ($\beta = -0.333$; $p = 0.007$). Only radiological reperfusion at 24 h predicted good clinical response at day 1 (adjusted OR 16.054 [1.423–181.158]; $p = 0.025$) and 90-day good functional outcome (adjusted OR 25.801 [1.483–448.840]; $p = 0.026$). At ROC curve analysis the AUC of reperfusion was 0.777 ($p < 0.001$) for the good clinical response at 24 h and 0.792 ($p < 0.001$) for 90-day clinical outcome. Twenty-four-hour radiological reperfusion assessed by CT is associated with good clinical response on day 1 and good functional outcome on day 90 in patients with ischemic stroke.

Keywords Ischemic stroke · Reperfusion · Recanalization · Ischemic core · Penumbra · Inflammation

Introduction

The early pharmacological or mechanical restoration of the blood flow in the ischemic brain is recommended to treat acute ischemic stroke (AIS) [1, 2]. The effectiveness of thrombolysis is mainly driven by the establishment of early recanalization and/or reperfusion [3]. Although they are closely related, these pathophysiological conditions can be radiologically identified. For instance, thrombolysis may fail to induce recanalization [4, 5], but reperfusion may be

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maintained by collateral circulation. In turn, successful recanalization does not consistently lead to reperfusion in case of distal thrombus embolization [6] or no-reflow phenomenon [7]. Despite these pathophysiological aspects, the term "recanalization" and "reperfusion" have been long-time used interchangeably [8]. Nowadays, advances in imaging methods have been applied to discriminate these entities. In addition, it is now possible to routinely visualize the ischemic core, the penumbral tissue, and the state of collateral blood supply. Recently, the clinical benefit of recanalization achieved by endovascular treatment was demonstrated in seven clinical trials, whereas the prognostic relevance of reperfusion in predicting improved clinical and radiological outcome was reported in some of these and other clinical trials [9–12]. This study, enrolling patients with AIS and undergoing serial examination with computerized tomography (CT), has been designed to compare the prognostic value of reperfusion vs. recanalization after an AIS. Whereas long-term (day 90) good functional outcome was set as primary endpoint, we have also planned to investigate potential correlations between reperfusion and serum inflammatory biomarkers.

Methods

Patients Selection and Clinical Assessment

We retrospectively analyzed clinical and radiological data from $n = 55$ consecutively AIS patients admitted to the Neuroscience Department of Ferrara University Hospital during the period from April 2016 to December 2017. All patients were treated according to the current guidelines [13, 14]. Inclusion criteria were: (i) presentation at the hospital within 8 h from symptom onset; (ii) baseline and follow-up confirmation of a large vessel anterior AIS based on CT findings represented by the absence of intracranial hemorrhage on non-contrast CT (NCCT) at admission, the presence of a territorial hypoperfused area on CT perfusion (CTP) at onset and the presence of a territorial hypoattenuated area on NCCT at 24 h; (iii) baseline and follow-up CT imaging carried out at established time points after symptom onset (NCCT, CT angiography [CTA], and CTP at admission, NCCT, CTA, and CTP at day 1 and NCCT at day 90). Exclusion criteria included the detection of brain stem infarct or intracerebral hemorrhage at admission NCCT, the inability to complete multimodal CT protocol at baseline and follow-up, history of strokes with residual deficit; contraindications to iodinated contrast agent, pregnancy, age < 18 years, clinical instability and/or poor quality of CT acquisition due to motion artifacts. Lacunar AIS were also excluded, due to the low sensitivity of CTP in detecting them [15, 16]. To select a more homogenous population, we further excluded minor strokes (defined by a NIHSS < 4) as well as patients receiving intra-arterial thrombolysis and mechanical thrombectomy. As previously reported [17],

we further excluded patients with lacunar/undetermined or posterior circulation stroke, NIHSS < 4 at enrollment and those receiving intra-arterial thrombolytic therapy as well. Type of reperfusion therapy (if any) was recorded. The Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria were used to categorize AIS subtypes. [18], whereas the National Institutes of Health Stroke Scale (NIHSS) was used to score the disease severity at onset, day 1, and day 90 after AIS [19]. The Local Ethics Committee approved this study, in accordance with the guidelines of the Declaration of Helsinki. All patients gave informed consent before entering in the study.

Study Endpoint and Power Estimation

The primary endpoint was to determine whether the occurrence of radiological reperfusion might predict long-term (day 90) good functional outcome, defined as a modified Rankin scale (mRS) at day 90 < 2 [17, 20, 21]. The sample size was computed based on an expected prevalence of complete reperfusion of 45%, taking into account a prevalence of good functional outcome of 65% and a minimum of six-fold increased incidence in reperfused patients [17, 21]. According to our power calculation for two proportion comparison, the minimum sample size requested to detect a six-fold increase in the incidence of good functional outcome with a power of 80% and with a two-sided alpha error of 5% was of 12 patients. As secondary endpoint, we investigated the ability of reperfusion to predict early favorable clinical response, defined as a reduction of ≥ 8 points on the NIHSS [17] during the first day after AIS, and the occurrence of haemorrhagic transformation during the first 7 days after AIS. Finally, potential correlations between reperfusion and serum biomarkers of inflammation has been explored. Two blinded independent investigators adjudicated the study endpoints.

Imaging Acquisition Protocol

All images were conducted on 64-slice scanners and included (i) NCCT carried out from the skull base to the vertex; (ii) CTA performed from the carotid bifurcation to vertex; (iii) CTP that covered a total of 4 cm from the basal ganglia to the lateral ventricles. CTP studies were obtained with a dynamic first-pass bolus-tracking methodology according to a one-phase imaging protocol consisting of an acquisition of 50-s continuous (cine) scans, which started 5 s after the automatic injection of 40 ml of non-ionic contrast agent at the rate of 4 ml/s.

Imaging Processing and Analysis

According to EXTEND-IA trial [22], the severity of arterial occlusion was judged on CTA at onset using a modified version of the Thrombolysis in Myocardial Infarction (TIMI)

grading system: complete occlusion (TIMI score = 0–1); partial occlusion (TIMI score = 2); no occlusion (TIMI score = 3). Patients with TIMI score ranging between 0 and 1 were categorized as occluded, whereas a TIMI score of 2 and 3 defined the not occluded ones. The site of occlusion was assessed as previously indicated [23]. All CTP scans were assessed using a commercially available delay-sensitive deconvolution software (CT Perfusion 3, GE Healthcare, Waukesha, WI). For each CTP scan, time-density curves for the arterial input function and venous output functions were obtained from the anterior cerebral artery and superior sagittal sinus, respectively. The AIF was corrected for partial volume averaging using the VOF-TDC. Cerebral blood flow (CBF), cerebral blood volume (CBV), and mean-transit-time (MTT) CTP maps were generated for each patient by deconvolution of tissue TDCs and the AIF. CBF, CBV, and MTT values were expressed in ml/min/100 g, ml/100 g, and seconds, respectively. Large blood vessels were automatically excluded from calculation by the software. Color coded functional CTP map scales were set at 0–100 ml/min/100 g for CBF, 0–8 ml/100 g for CBV, and 0–20 s for MTT. After identification by visual inspection on MTT and CBV maps, three different regions of interest (ROIs) were drawn freehand by two neuroradiologists (A.B. and E.F., with 5- and 20-year experience, respectively) on every section in which they were visible according to the classical CTP mismatch model [24]: (i) MTT lesion indicating total hypoperfusion; (ii) CBV lesion referring to infarct core; (iii) MTT-CBV lesion representing ischemic penumbra (Fig. 1). This last ROI was outlined on MMT maps where the ROI corresponding to CBV defect were automatically superimposed. Discrepancies between readers were resolved by consensus adjudication. The sum of these lesion areas was then multiplied by slice thickness to obtain core and penumbra (CTP MTT-CBV mismatch) volumes, respectively. Final infarct volume was measured on follow-up NCCT at 3 months after symptom onset with a multi-slice planimetric method by summation of the hypodense areas, manually traced on each

slice in which they were detectable, multiplied by slice thickness [25]. The type of HT was recorded by NCCT at 24 h and at 7 days post ictus. In agreement with EXTEND-IA trial [22], recanalization was scored on CTA at 24 h with an adaptation of the TIMI grading system: persistent occlusion (TIMI score = 0–1); partial recanalization (TIMI score = 2); full recanalization (TIMI score = 3). Patients with TIMI score ranging from 2 to 3 were considered as recanalized, whereas patients with TIMI score of 0 and 1 were classified as not recanalized. All patients not occluded on CTA at admission had a TIMI score of 3 at 24 h-CTA and therefore, were considered as recanalized. Radiological reperfusion was evaluated by reperfusion index that measures the percentage reduction of baseline MTT lesion at 24 h. For this purpose, visually identified MTT defect volume was obtained by a manual multi-slice planimetric method at admission and at 24 h [26]. Patients with a reperfusion index > 75% were considered as reperfused. Reduction of the ischemic volume was expressed as the difference between total hypoperfusion volumes at admission (baseline) and at 24 h.

Blood Collection and Quantification

Blood samples were collected at different time points using a butterfly to reduce membrane shear stress and then drawn in tubes to obtain serum. Samples were collected at baseline and at day 1 after AIS. Hematology parameters and blood chemistry, including plasma glucose, triglycerides, total cholesterol, high-density lipoprotein, and low-density lipoprotein cholesterol (to assess dyslipidemia) were measured by routine auto-analyzer.

Measurement of a Serum Biomarker Associated with Reperfusion

Serum osteopontin (OPN), a biomarker associated with cerebral reperfusion [27, 28], were measured by colorimetric enzyme-linked immunosorbent assay (ELISA), following
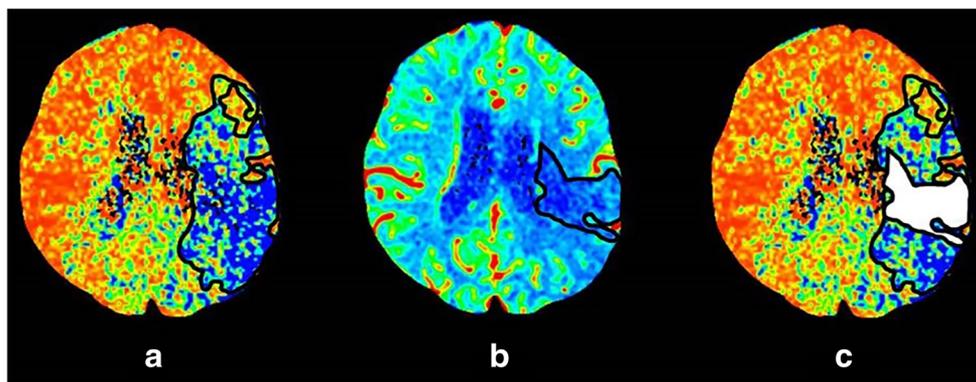

Fig. 1 Color coded functional CT perfusion map. After identification by visual inspection on mean-transit-time (MTT) and cerebral blood volume (CBV) maps, 3 different regions of interest were drawn freehand on every section according to the classical CTP mismatch model: **a** MTT lesion indicating total hypoperfusion; **b** CBV lesion referring to infarct core; **c** MTT-CBV lesion representing ischemic penumbra

manufacturer's instructions (R&D Systems, Minneapolis, MN). The limits of detection for OPN was 62.5 pg/ml. Mean intra- and inter-assay coefficients of variation (CV) were below 8% for all markers [29].

Statistical Analysis

Analyses were performed with IBM SPSS Statistics for Windows, Version 23.0 (IBM CO., Armonk, NY). Categorical data are presented as relative and absolute frequencies. Continuous variables were expressed as median and interquartile range (IQR) as the normality assumption was not demonstrated. Difference between two time points was presented as delta (Δ). Intergroup comparisons were drawn by Fisher's exact test and Mann-Whitney U test, as appropriate. Conversely, the dichotomized classification of reperfusion and recanalization was compared by McNemar test instead comparison between paired sample was drawn by Wilcoxon rank sum test ranked. Multiple linear regressions were performed to model the lesion growth and final infarct volumes with the recanalization and reperfusion. Multivariate logistic regression was used to evaluate the predictive role of recanalization and reperfusion toward good clinical response and good functional outcome. Finally, the prognostic ability was assessed in a post-hoc manner based upon receiver operator characteristic (ROC) curve (MedCalc 12.5, MedCalc Software, Ostend, Belgium). The area under the curve (AUC) was given with 95% confidence interval (CI). For all statistical analyses a two-sided p value < 0.05 was considered as statistically significant.

Results

Patients' Characteristics

Baseline clinical characteristics of the whole cohort ($n = 55$) are listed in Table 1. Patients' median age was 69 years (55–76), with a slight prevalence of hypertension (58.2%). In most cases the time window between symptom onset and CT study performed was less than 3 h (81.8%), thus accounting for a high rate of reperfusion therapy with r-tPA (70.9%). More precisely, in our study population, 49/55 (89.1%) patients were treated ($n = 39$ with intravenous thrombolysis with r-tPA and $n = 10$ with mechanical thrombectomy), whereas 6/55 (10.9%) did not receive any therapy due to the presence of hypodensity > 1/3 cerebral hemisphere, as indicated by ASPECTS ≤ 7, on admission NCCT ($n = 3$), and the current use of anticoagulant with INR > 1.7 ($n = 3$). At day 1 after AIS, reperfusion was present in 24 patients (43.6%) (Table 2). In 15 patients (27.3%), there was a pattern characterized by recanalization without reperfusion (Table 2). Conversely, no patients had reperfusion without

recanalization, whereas in 24 patients (43.6%) both reperfusion and recanalization were observed. A McNemar test on the dichotomized classification of reperfusion and recanalization confirmed a significant discrepancy between these two parameters ($p < 0.001$) (Table 2).

Reperfusion and Recanalization Weakly Influences Inflammatory Biomarkers and Radiological Features of Cerebral Injury

Ischemic core and penumbra volumes did not differ in patients categorized as recanalized vs. non-recanalized and reperfused

Table 1 Clinical characteristics of study population ($n = 55$) at admission

Demographic	
Age, years (IQR)	69 (55–76)
Male, no. (%)	29 (52.7)
Hypertension, no. (%)	32 (58.2)
Atrial fibrillation, no. (%)	23 (43.4)
Active smokers, no. (%)	18 (33.3)
Previous smokers, no. (%)	5 (9.1)
Diabetes, no. (%)	4 (7.3)
Dyslipidemia, no. (%)	13 (23.6)
Biochemical	
Total WBC[#], no. $\times 10^9$ (IQR)	7.91 (6.70–9.54)
Neutrophil count, no. $\times 10^9$ (IQR)	4.83 (3.71–6.74)
Lymphocyte count, no. $\times 10^9$ (IQR)	2.1 (1.53–2.58)
Serum glycaemia, mg/dL (IQR)	112 (97–143)
INR[*], no. (IQR)	1.09 (1.02–1.19)
Clinical/ radiological	
Time window to CT[†]	
0–3 h, no. (%)	45 (81.8)
3–6 h, no. (%)	9 (16.4)
6–8 h, no. (%)	1 (1.8)
TOAST[‡] classification	
Atherothrombotic, no. (%)	32 (58.2)
Cardio-embolic, no. (%)	23 (43.4)
Intravenous r-tPA[§], no. (%)	39 (70.9)
NIHSS[‖]	10 (7–14)
Total hypoperfused volume, ml (IQR)	88.03 (36.48–129.18)
Ischemic core volume, ml (IQR)	5.74 (0.51–17.18)
Ischemic penumbra, ml (IQR)	59.63 (27.04–102.32)

Data are expressed as median (interquartile range [IQR]) or number [no.] (percentages [%])

[#] WBC: white blood cells

[*] INR: international normalized ratio

[†] CT: computerized tomography

[‡] TOAST: Trial of Org 10172 in Acute Stroke Treatment

[§] r-tPA: recombinant tissue plasminogen activation

[‖] NIHSS: National Institutes of Health Stroke Scale

Table 2 Radiological characteristic at day 1

		p value
Reperfusion, *n* (%)	24 (43.6)	
Recanalization, *n* (%)	39 (70.9)	
Reperfusion vs. recanalization		< 0.001
No-reperfusion, no-recanalization	16 (29.09)	
Recanalization without reperfusion ("no-reflow"), *n* (%)	15 (27.3)	
Reperfusion without recanalization, *n* (%)	0 (0.0)	
Reperfusion and recanalization, *n* (%)	24 (43.6)	

Data are expressed as median (interquartile range [IQR]) or number [no.] (percentages [%]). Comparison was drawn by Fisher's exact test

vs. non-reperfused (Fig. 2a–c). Conversely, reperfusion determined a great reduction of the ischemic volume during the first day after AIS (Δ baseline-day 1, 78.59 vs. 14.59 ml; *p* < 0.001). A lesser but significant benefit was also observed in

the group of recanalized patients (Δ baseline-day 1, 37.95 vs. 13.30 ml; *p* = 0.025). OPN was tested as a known circulating mediators increased after cerebral reperfusion [27, 28]. Noteworthy, when non-reperfused and reperfused patients were analyzed separately, no difference was shown in demographic, biochemical and clinical/radiological parameters (Table 3). Whereas no difference was observed at baseline, a significant increase was observed the day after AIS (*p* = 0.002), thus determining a significant difference among the two study groups (*p* = 0.004) (Fig. 3). Multilinear regression analysis further confirmed an independent association of volume lesion reduction at day 1 with both recanalization (29.425 [6.271–52.580]; *β* = 0.265; *p* = 0.014) and reperfusion (*B* 46.803 [95% CI 29.138–64.469]; *β* = 0.461; *p* < 0.001) (Table 4). However, only the reperfusion was independently associated with final infarct volume at day 90 after AIS (*B* − 54.236 [95% CI 93.203- -15.270]; *β* = −0.333; *p* = 0.007) (Table 4).

Fig. 2 Box-whisker representative plots of the impact of recanalization/reperfusion of radiological findings. The values of ischemic core volume (**a**), ischemic penumbra (**b**), and difference (Δ) in hypoperfused volume between onset at 24 h (**c**) are represented in non-recanalized/recanalized and non-reperfused/reperfused patients
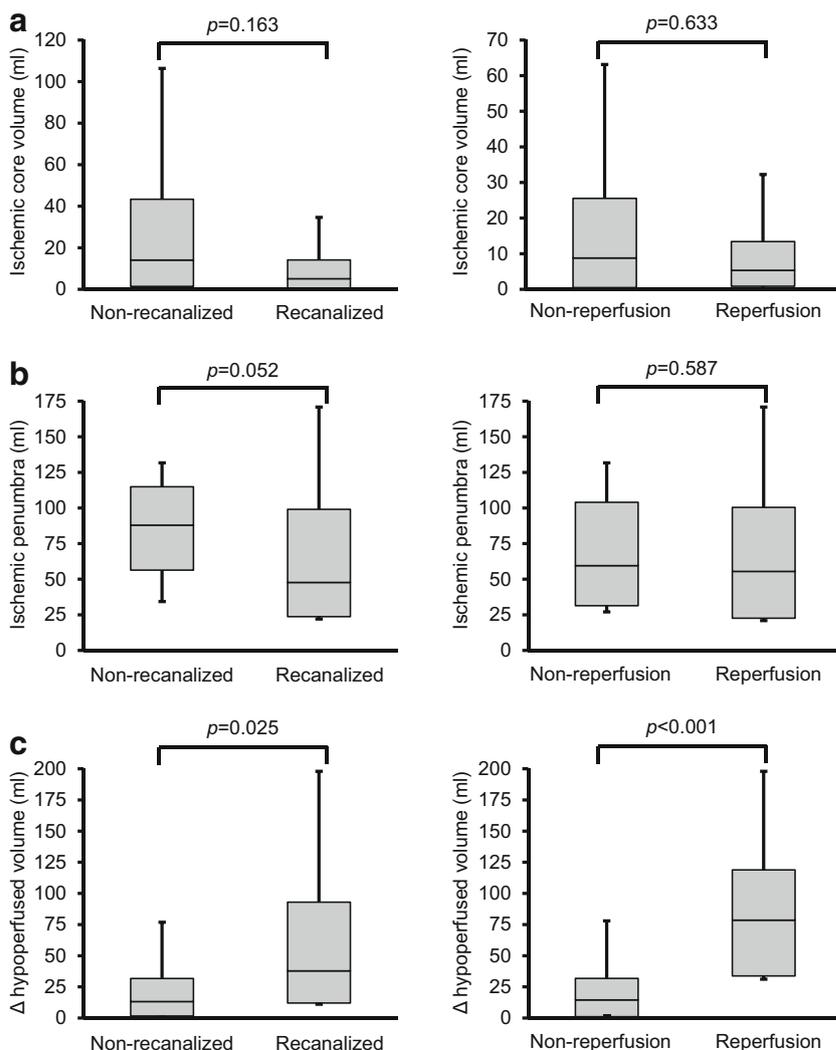
Table 3 Clinical differences among non-reperfused and reperfused patients at baseline

	Non-reperfused ($n = 31$)	Reperfused ($n = 24$)	p value
Demographic			
Age, years (IQR)	67 (58–76)	71 (46–78)	0.939
Male, no (%)	17 (54.8)	12 (50.0)	0.789
Hypertension, no. (%)	19 (61.3)	13 (54.2)	0.783
Atrial fibrillation, no. (%)	12 (38.7)	11 (45.8)	0.783
Active smokers, no. (%)	13 (41.9)	5 (21.7)	0.151
Previous smokers, no. (%)	3 (9.7)	2 (8.3)	1.000
Diabetes, no. (%)	3 (9.7)	1 (4.2)	0.624
Dyslipidemia, no (%)	10 (32.3)	3 (12.59	0.116
Biochemical			
Total WBC[#], no. $\times 10^9$ (IQR)	7.91 (6.99–10.01)	7.8 (6.26–9.01)	0.396
Neutrophil count, no. $\times 10^9$ (IQR)	5.11 (3.69–7.36)	4.45 (3.74–6.42)	0.665
Lymphocyte count, no. $\times 10^9$ (IQR)	2.1 (1.65–2.74)	2.13 (1.18–2.58)	0.773
Serum glycaemia, mg/dL (IQR)	116 (103–166)	107 (91–130)	0.096
INR*, no. (IQR)	1.08 (1.01–1.13)	1.13 (1.02–1.28)	0.098
Clinical/ radiological			
Time window to CT[†]			0.214
0–3 h, no. (%)	24 (77.4)	21 (87.5)	
3–6 h, no. (%)	7 (16.422.6)	2 (8.3)	
6–8 h, no. (%)	0 (0.0)	1 (4.2)	
TOAST[‡] classification			
Atherothrombotic, no. (%)	19 (61.3)	13 (54.2)	
Cardio-embolic, no. (%)	12 (38.7)	11 (45.8)	0.783
Intravenous r-tPA[§], no (%)	21 (67.7)	18 (75.0)	0.765
NIHSS[‖]	11 (7–20)	10 (7–14)	0.208

Comparison were drawn by Mann-Whitney U test, Fisher's exact test or Kruskal-Wallis test, as appropriate. Data are expressed as median (interquartile range [IQR]) or number [no.] (percentages [%])

[#] WBC: white blood cells

*INR: international normalized ratio

[†] CT: computerized tomography

[‡] TOAST: Trial of Org 10172 in Acute Stroke Treatment

[§] r-tPA: recombinant tissue plasminogen activation

[‖] NIHSS: National Institutes of Health Stroke Scale

Reperfusion at 24 h Is a Strong Predictor of Good Outcome After an AIS

Reperfusion, but not recanalization, was able to predict good clinical response at 24 h also after adjustments for age, gender, glycaemia, time window to CT, intravenous thrombolysis, NIHSS, ischemic core and penumbra volumes at onset (OR 16.054 [1.423–181.158]; $p = 0.025$) (Table 5). Though non-significant, reperfusion shown a trend toward the prediction of hemorrhagic transformation during the first 7 day after AIS (adjusted OR 0.153 [0.022–1.047]; $p = 0.056$) (Table 5). However, the most relevant result was the predictive ability of reperfusion toward 90-day good functional outcome, assessed by mRS (adjusted OR 25.801 [1.483–448.840];

$p = 0.026$) (Table 5). ROC curve analysis, further characterized the predictive ability of reperfusion by showing an AUC of 0.777 (sensitivity 88.89%, specificity 64.86%; $p < 0.001$) and 0.792 (sensitivity 88.89%, specificity 75.68%; $p < 0.001$) for the good clinical response and good long-term clinical outcome, respectively (Fig. 4).

Discussion

In this study, reperfusion at 24 h after AIS consistently overcame recanalization in predicting radiological evolution of ischemic lesion and clinical outcomes. Indeed, patients with MTT reperfusion index > 75% experienced a greater

Fig. 3 Box-whisker representative plots of the modification in circulation osteopontin (OPN). Circulating OPN levels were quantified at baseline and after 1 day from stroke onset. Intergroup comparisons and overtime change were assayed
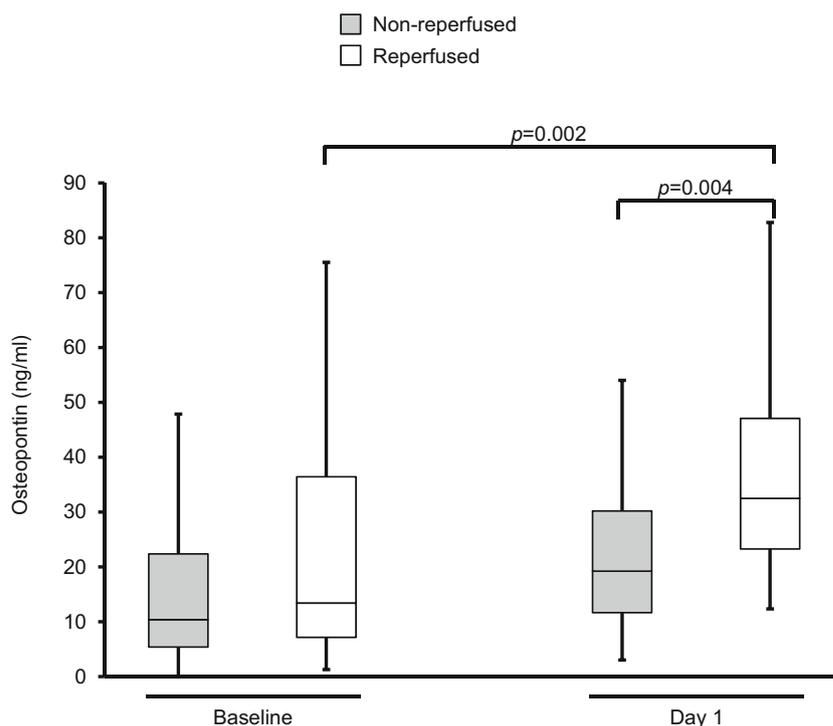

Table 4 Multiple linear regression for predicting the reduction of ischemic lesion volume at day 1 and the final infarct at day 90

	B (95% CI)	β	p value
Volume lesion reduction			
Age	−0.069 (−0.640 to 0.502)	−0.019	0.809
Gender, male	7.361 (−8.481 to 23.202)	0.073	0.354
Glycemia	−0.105 (−0.324 to 0.115)	0.109	0.341
Time window to CT[#]	−4.948 (−23.525 to 13.628)	−0.044	0.594
Intravenous r-tPA*	−2.095 (−21.594 to 17.404)	−0.019	0.830
NIHSS[†] at onset	−0.139 (−1.753 to 1.475)	−0.016	0.863
Ischemic core volume	0.554 (0.290 to 0.819)	0.334	<0.001
Ischemic penumbra	0.663 (0.496 to 0.830)	0.587	<0.001
Reperfusion at day 1	46.803 (29.138 to 64.469)	0.461	<0.001
Recanalization at day 1	29.425 (6.271 to 52.580)	0.265	0.014
Final infarct volume			
Age	−0.851 (−41.309 to 191.681)	−0.142	0.180
Gender, male	−22.466 (−57.409 to 12.477)	−0.139	0.202
Glycemia	−0.058 (−0.542 to 0.426)	−0.027	0.809
Time window to CT	42.518 (1.540 to 893.945)	0.233	0.042
Intravenous r-tPA	22.079 (−20.931 to 65.090)	0.124	0.307
NIHSS at onset	2.289 (−1.271 to 5.849)	0.163	0.202
Ischemic core volume	1.270 (0.687 to 1.854)	0.476	<0.001
Ischemic penumbra	−0.060 (0.428 to 0.308)	0.033	0.745
Reperfusion at day 1	−54.236 (−93.203 to −15.270)	−0.333	0.007
Recanalization at day 1	−1.606 (−52.681–49.469)	−0.009	0.950

Volume of lesion growth is defined as the Δ baseline-day 1

WBC white blood cells

[#] CT: computerized tomography

*r-tPA: recombinant tissue plasminogen activator

[†] NIHSS: National Institutes of Health Stroke Scale

Table 5   Multiple logistic regression for predicting good clinical response at day 1 (reduction of ≥ 8 points on the NIHSS or a NIHSS score ≤ 1), occurrence of hemorrhagic transformation and good functional outcome (modified Rankin Scale, 0–1) at day 90

	OR (95% CI)	p value
Good clinical response (day 1)		
Age	1.090 (1.002–1.185)	0.044
Gender, male	3.316 (0.475–23.150)	0.227
Glycemia	0.928 (0.870–0.990)	0.024
Time window to CT[#]	1.751 (0.108–28.333)	0.693
Intravenous r-tPA*	0.746 (0.056–9.940)	0.825
NIHSS[†] at onset	1.235 (0.978–1.559)	0.076
Ischemic core volume	0.946- (0.898–0.996)	0.034
Ischemic penumbra	1.002 (0.982–1.023)	0.811
Recanalization at day 1	7.721 (0.296–201.188)	0.219
Reperfusion at day 1 ≥ 75%	16.054 (1.423–181.158)	0.025
Hemorrhagic transformation (onset-day 7)		
Age	1.014 (0.941–1.092)	0.720
Gender, male	0.476 (0.076–2.976)	0.427
Glycemia	1.020 (0.998–1.042)	0.076
Time window to CT	0.156 (0.007–3.573)	0.245
Intravenous r-tPA	1.197 (0.119–12.010)	0.879
NIHSS at onset	1.052 (0.862–1.284)	0.616
Ischemic core volume	1.074 (1.007–1.147)	0.030
Ischemic penumbra	1.000 (0.981–1.019)	0.984
Recanalization at day 1	0.744 (0.065–8.575)	0.812
Reperfusion at day 1 ≥ 75%	0.153 (0.022–1.047)	0.056
Good functional outcome (day 90)		
Age	0.950 (0.854–1.058)	0.0.351
Gender, male	0.348 (0.035–3.471)	0.370
Glycemia	0.968 (0.939–0.998)	0.034
Time window to CT	0.071 (0.004–1.347)	0.078
Intravenous r-tPA	0.050 (0.002–1.054)	0.054
NIHSS at onset	0.734 (0.569–0.949)	0.018
Ischemic core volume	0.964 (0.922–1.007)	0.095
Ischemic penumbra	1.008 (0.985–1.032)	0.507
Recanalization at day 1	1.618 (0.178–14.700)	0.669
Reperfusion at day 1 ≥ 75%	25.801 (1.483–448.840)	0.026

WBC white blood cells

[#] CT: computerized tomography

*r-tPA: recombinant tissue plasminogen activator

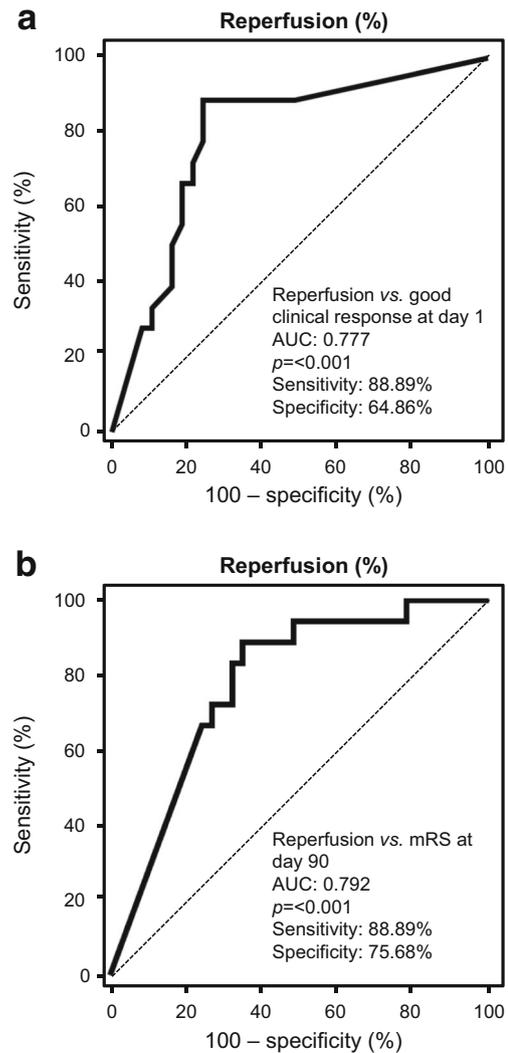[†] NIHSS: National Institutes of Health Stroke Scale

Fig. 4 Receiver operator characteristic (ROC) curve analysis. The predictive value of reperfusion toward good clinical response at 24 h (a) and long-term functional outcome assessed by modified Rankin scale (mRS) at day 90 (b)

reduction of ischemic lesion volume during the first day after AIS and a smaller infarct volume at 90 days. According to radiological findings, reperfusion was also associated to more favorable prognosis in terms of good clinical response at 24 h and good functional outcome assessed 90 days after AIS. Noteworthy, we observed some discrepancies between recanalization and reperfusion rates. As compared to previous studies [17, 26], we did not observe reperfusion without recanalization, whereas the recanalization occurred without reperfusion in 15 patients. This implies that, in some cases, reopening of the occluded artery is not associated with the restoration of microcirculatory blood flow [4]. On the other hand, it is important to emphasize that reperfusion can be non-nutritional inducing a luxury perfusion, exceeding metabolic demand, in non-viable tissue evolving into infarct [30] or a deterioration of ischemic but salvageable brain tissue due to an impairment of neurovascular unit mediated by molecular and cellular mechanisms [31]. Nevertheless, the present study further emphasizes a conceptual difference between reperfusion and recanalization. The discrepancy between angiographically successful recanalization and non-favorable clinical outcome might be explained by a non-visible alteration in microcirculation, as occurs in case of distal thrombus embolization [6] or no-reflow phenomenon. In the latter case, swollen

astrocyte, pericyte and endothelial cells determine the narrowing of microvascular lumen and failure of microcirculatory reperfusion despite clot removal [32, 33]. The capillary narrowing usually starts 1 h after AIS and contributes to brain injury by promoting oxidative stress [32]. Not surprisingly, more recent experimental studies [34] and clinical trials [35, 36] pointed out the role of early (or even ultra-early) reperfusion as key determinant of stroke outcome [5, 37, 38]. Our results are then consistent with previous studies based on both CT [26] and magnetic resonance [39–41], in supporting a role of reperfusion as surrogate marker of clinical outcome independently of recanalization. More specifically, we emphasized the role of earlier repeated follow-up imaging in improving the prognostic value of reperfusion [42]. Another critical matter of discussion is the role of inflammation in ischemic/reperfusion (I/R) injury [43]. Various studies in the last decade emphasized the detrimental role of inflammatory response in AIS. We previously observed that delayed rise in serum OPN was associated with poor long-term clinical outcome after an AIS [44]. Similarly, time-dependent changes in serum OPN were also correlated with left-ventricular volume and function in patients with myocardial infarction undergoing successful reperfusion [45]. Yet, numerous clinical studies failed to demonstrate any positive outcomes of anti-inflammatory strategies in stroke patients [46]. Rather, the timing of inflammatory response is increasingly emerging as main determinant of I/R injury outcome. Whereas persistent inflammation exerts negative effects on tissue repair, early inflammatory response seems to drive tissue healing [47]. With this aim, we focused on the early change in circulating OPN, reporting a significant rise in reperfused patients. Nevertheless, long-term kinetic of OPN after AIS and potential relationship with radiological findings still remains unknown and any potential explanation is highly speculative.

Study Limitations

Firstly, the study cohort may not be considered representative of a general stroke population as whole and future population-based studies are warranted to validate our results. Whereas we were able to demonstrate the prognostic role of reperfusion independently of intravenous thrombolysis, our sample size was not powerful enough to discriminate the effect of other therapeutic approaches (i.e., intra-arterial thrombolysis and mechanical thrombectomy). Similarly, larger studies are required to investigate the setting of minor stroke, where the expected differences may be smaller. Secondly, recanalization/reperfusion imaging was obtained relatively late, and recanalization/reperfusion observed at 24 h after AIS may not have the ability to salvage viable ischemic tissue at risk. However, the time from admission to recanalization/reperfusion imaging and to discharge imaging was similar for

all patients, thus not introducing a bias in the analysis. Thirdly, the evaluation of reperfusion by CTP is incomplete because this technique is not able to obtain metabolic information which are crucial, in combination with hemodynamic parameters, to establish whether the restoration of microvascular circulation corresponds to an actual tissue recovery or leads to reperfusion injury [48]. In this way, only positron emission tomography can provide both hemodynamic and metabolic data useful to better understand tissue fate [49]. Fourthly, the estimation of final infarct volume with 24 h NCCT could be affected by a distortion of residual cavity due to retraction effects related to gliosis. However, it is currently accepted that final infarct volume is more accurately delineated in images obtained 30 or 90 days after stroke [50]. Fifthly, it is well-known that threshold-based fully-automated software are superior to manually tracing technique we used in this study for quantitative volumetric analysis of CBV and MTT alterations [51]. Nevertheless, the calculation of CBV and MTT lesion volume by a manual multi-slice planimetric method is still considered reliable in defining core and penumbra [52] and reperfusion on CTP [53]. Finally, different studies have described a bi-modal delayed peak of OPN (a serum biomarker associated with reperfusion) after AIS [54]. Therefore, future prospective cohorts should include earlier and later time points in order to able to correlate radiological evolution (in terms of recanalization/reperfusion) with overtime change in circulating OPN.

Conclusion

In conclusion, we were able to show that reperfusion occurring during the first day after AIS in predicting better radiological evolution of ischemic lesion and clinical outcome. Also, reperfusion, but not recanalization was associated with an increase in serum levels of OPN, an inflammatory molecule potentially related with post-ischemic cerebral pathophysiology. Future studies correlating radiological reperfusion and potential biomarkers are warranted to investigate the fine-tuned mechanisms underlying cerebral ischemia/reperfusion salvage.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Research Involving Human Participants and/or Animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research

committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

This article does not contain any studies with animals performed by any of the authors.

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