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Endothelial Progenitor Cells influence acute and subacute stroke hemodynamics

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ABSTRACT

Background: Endothelial Progenitor Cells (EPCs) are a circulating stem cell population with *in vivo* capacity of promoting angiogenesis after ischemic events. Despite the promising preclinical data, their potential integration with reperfusion therapies and hemodynamic evolution of stroke patients is still unknown. Our aim was to determine the association of EPCs with acute, subacute and chronic hemodynamic features.

Methods: In this prospective study, we included consecutive patients with ages between 18 and 80 years and non-lacunar ischemic stroke within the territory of a middle cerebral artery. All patients were subject to hemodynamic evaluation by ultrasound at baseline, seven days and three months. We quantified cerebral blood flow (CBF) and assessed early recanalization and collateral flow. Hemorrhagic transformation was graded in Magnetic Resonance imaging performed at seven days. EPCs were isolated from peripheral venous blood collected in the first 24 h and seven days, counted and submitted to functional *in vitro* tests.

Results: We included 45 patients with a median age of 70 ± 10 years. The angiogenic and migratory capacities of EPCs were associated with increased collateral flow in the acute stage and day seven CBF, without statistically significant associations with recanalization nor haemorrhagic transformation. The number of EPCs was not associated with any hemodynamic variable.

Conclusions: The functional properties of EPCs are associated with acute and subacute stroke hemodynamics, with no effect on haemorrhagic transformation.

1. Introduction

The treatment algorithm for acute ischemic stroke has had significant recent updates due to new effective strategies to promote recanalization. However, after the first few hours no therapy has demonstrated meaningful impact on clinical recovery. Endothelial Progenitor Cells (EPCs) are circulating cells that have emerged as a promising treatment strategy, with demonstrated *in vivo* capacity of promoting neovascularization and neurological improvement after stroke [1,2]. Nonetheless, their timing of action and integration within stroke hemodynamics are still unknown, which can be critical for optimization of their clinical effect.

Acute stroke hemodynamics have been recognized as one of the main determinants of clinical evolution [3,4]. Several pathophysiological mechanisms take place in the regulation of vascular responses with distinct implications and at different timepoints. In the hyperacute stage the main determinant of evolution is early recanalization, with a marked clinical impact [5,6]. Collateral pial circulation and cerebral blood flow (CBF) are also essential early hemodynamic mechanisms, installed in the attempt to extend the preservation of cerebral tissue after the vascular insult [7]. After the acute stage, and depending on timing of recanalization and resistance to ischemia, a loss of

Abbreviations: ACA, Anterior Cerebral Artery; CAC, Circulating Angiogenic Cells; CBF, Cerebral Blood Flow; CFU-EC, Collony Forminf Unit-Endothelial Cells; EGM-2, Endothelial Growth Medium-2; EGM-2 MV, Endothelial Growth Medium-2 Microvascular; EPC, Endothelial Progenitor Cell; FBS, Fetal Bovine Serum; HT, Hemorrhagic Transformation; ICA, Internal Carotid Artery; MCA, Middle Cerebral Artery; MNC, Mononuclear Cells; MRI, Magnetic Resonance Imaging; mRS, Modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale; oEPC, Outgrowth Endothelial Progenitor Cell; PCA, Posterior Cerebral Artery; PH, Parenchymal Hemorrhage; TCCD, Transcranial Colour Coded Doppler; VA, Vertebral Artery

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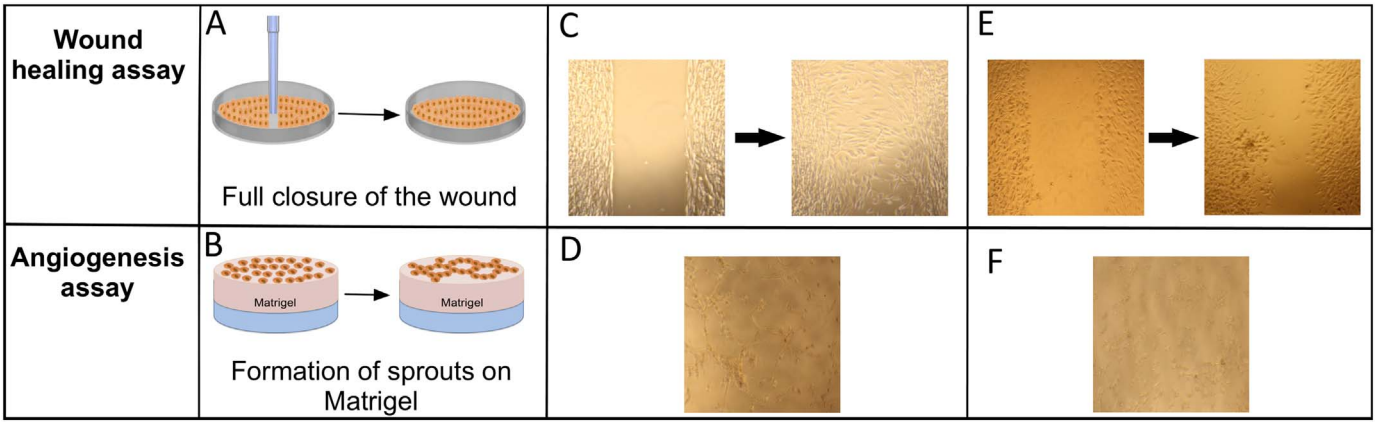

Fig. 1. Representation of *in vitro* wound healing and angiogenesis assays (A and B respectively). Examples with enhanced (C and D) and poor properties (E and F) in each test are shown.

autoregulation may precipitate hyperperfusion injury which may ultimately lead to hemorrhagic transformation [8]. Altogether, these are potentially modifiable hemodynamic responses, crucial to the clinical evolution after stroke.

The potential integration of EPC transplantation within the treating algorithm of stroke will have to take the hemodynamic response into account. As such, in this study we used a multidimensional approach to assess the interplay between EPCs and the several aspects of the patients' hemodynamic state.

2. Methods

2.1. Study population

We included consecutive acute ischemic stroke patients admitted in our department during a period of 27 months (June/2012 to August/2013 and June/2014 to July/2016) in a prospective observational cohort study. All patients with ages between 18 and 80 years and clinically defined non-lacunar strokes within the territory supplied by the Middle Cerebral Artery (MCA) that could have full clinical, neuroimaging and cellular evaluation within 24 h after the onset of symptoms were included (Supplementary Fig. 1 presents the flow chart for study participation, including exclusion criteria). All patients or legal representatives signed written informed consent for study participation. The study design was approved by the local ethics committee (Ref. 130-CE-2011).

At hospital presentation we collected demographic variables, vascular risk factors and quantified stroke severity using the National Institute of Health Stroke Scale (NIHSS) [9]. Functional outcome was graded in person at three months according to the modified Rankin scale (mRS) by vascular neurologist blinded to CBF and *in vitro* data.

2.2. Study design

At day zero (first 24 h after symptom onset) we collected patients' blood for cellular isolation and performed clinical and hemodynamic evaluation. At day 7 ± 2 days patients underwent MRI, repeated hemodynamic evaluation and blood collection for cellular studies. At 3 months ± one week participants had repeated clinical and hemodynamic evaluation. The primary objectives were to determine the associations between the number and functional properties of EPCs with CBF, hemorrhagic transformation, recanalization and flow diversion after ischemic stroke. We also aimed to understand the role of EPCs and CBF within clinical and demographic features as secondary objectives.

2.3. Isolation of EPC sub-populations

We isolated EPCs from 18 mL of peripheral venous blood collected in the first day and at day seven after stroke onset in accordance to previously validated protocols [1,2]. Mononuclear cells (MNC) were isolated from peripheral blood by density gradient centrifugation using Lymphoprep™ density gradient medium.

Three different types of EPCs were analyzed: circulating angiogenic cells (CACs), outgrowth Endothelial Progenitor Cells (oEPCs) and colony forming unit-endothelial cells (CFU-ECs). For CACs, MNCs were plated into 2 μg/cm^2 fibronectin-coated plates (24-well plates; 1.9 × 10^6 cells/well) and cultured in Endothelial Growth Medium-2 Microvascular (EGM-2 MV) containing 5% fetal bovine serum (FBS) during five days. Adherent cells were detached using trypsin, counted and used for functional assays.

For oEPCs and CFU-ECs, 10 × 10^6 MNCs in Endothelial Growth Medium-2 (EGM-2) with 10% FBS were seeded into one well of 2 μg/cm^2 fibronectin-coated 24-well plate. After 48 h, nonadherent cells were collected and 3 × 10^6 cells were replated into three fibronectin-coated 24-well plates. At day 5, colony-forming units were counted manually in four random fields (20 × magnification). The CFU-ECs were detached using trypsin and used for functional studies [10]. The adherent cells at 48 h continued cell culture for 14–21 days [11] to obtain oEPCs. Medium for oEPCs and CFU-ECs was changed every 48 h. All incubation periods were performed at 37 °C and 5%CO_2.

2.4. Functional tests of EPCs

The wound healing capacity of oEPCs and CFU-ECs was evaluated using the *in vitro* wound-healing (scratch) assay. In brief, cells were plated in 2 μg/cm^2 fibronectin-coated 96-well plates and cultured until they form a monolayer. Then, the wounds were created by scratching the cell layer with a 200 μL pipette tip. Migratory capacity was quantified as the percentage of wound closure after 24 h (Fig. 1).

The angiogenic capacity of oEPCs and CFU-ECs were determined by the sprout formation on Matrigel (using an IBIDI μ-slide angiogenesis kit). The total tube length and number of branching points (*i.e.* points featuring more than two connections) were manually measured in four random fields (20 × magnification) 24 h after plating (Fig. 1).

The migratory capacity of CACs was determined by transwell migration. In short, 2 × 10^4 CACs were placed in the upper chamber of a modified Boyden chamber (2 μg/cm^2 fibronectin-coated). The chamber was placed in a 24-well culture dish containing EBM-2 and human recombinant VEGF (50 ng/mL) and incubated for 24 h. The lower side of the filter was then washed with PBS and fixed with 4% paraformaldehyde. For quantification, cell nuclei were stained with 4′,6-diamidino-2-phenylindole. Cells migrating into the lower chamber were counted manually in 3 random microscopic fields (20 × magnification).

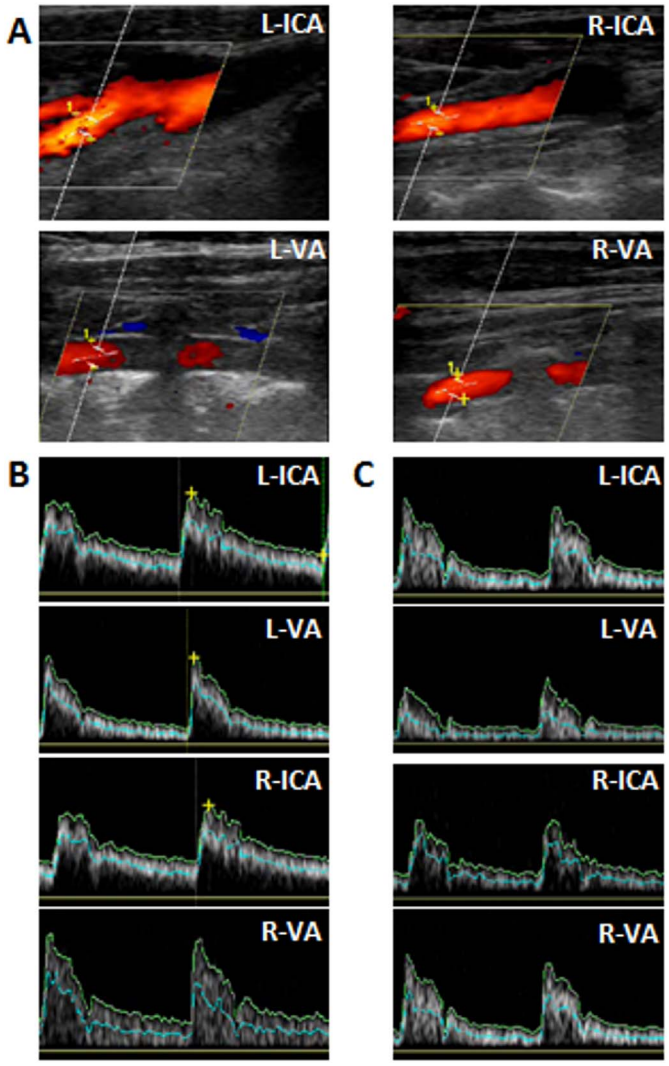

Fig. 2. Quantification of Cerebral Blood Flow (CBF). In (A) the identification of both Internal Carotid Arteries (ICA) and Vertebral Arteries (VA) is shown in continuous colour Doppler images. The parallelogram shown in yellow indicates the colour box steer in the right-to-left/caudal-cephalic orientation of flow. The vessel diameter used for the quantification of CBF is measured for each artery and indicated as the number "1". (B) and (C) represent the blood flow velocity analysis (in cm/seg) during two cardiac cycles of two patients: patient represented in (B) has milder NIHSS at admission, flow diversion and higher CBF than the patient in (C). The blue line in each flow analysis represents the mean flow velocity (used for CBF quantification) and the yellow line indicates the maximum flow velocity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.5. Hemodynamic evaluation

The hemodynamic evolution was assessed through serial cervical and transcranial neurosonological exams at admission (day zero), six hours, seven days and three months after stroke (3 MHz sector-probe and 11 MHz linear-probe respectively; General Electric Logiq7). All exams were performed with patients lying in a supine position after at least 10 min rest, collecting data on flow diversion, recanalization and CBF. Flow velocities were assessed bilaterally using transcranial colour coded Doppler (TCCD) with angle correction in anterior, middle and posterior cerebral arteries (ACA, MCA and PCA respectively). Flow diversion was defined as a high-velocity, low-resistance flow signal in the ACA-A1 or PCA (P1–P2 segments) ipsilateral to the occluded MCA; the ACA or PCA flow velocity had to be equal to or higher than the non-affected MCA [12]. Recanalization was defined as grades four or five by the Thrombolysis in Brain Ischemia scale in the TCCD performed six hours after symptom onset. Cerebral blood flow (CBF) was quantified as the sum of flow volumes in both internal carotid arteries (ICA) and vertebral arteries (VA) (Fig. 2A). Intravascular flow volumes were determined by the ultrasound equipment's software using the angle-corrected mean flow velocity and the vessel's luminal diameter perpendicular to its longitudinal axis at the same location as the flow measurement [13]. ICAs were assessed at least 2 cm after bifurcation and VAs in their V2 segment, at the C4-C5 intertransverse area. All arteries were measured in a straight segment for three times and the calculated mean taken for CBF analysis.

Table 1
Univariate association of baseline variables with Cerebral Blood Flow (CBF) at day zero. In the first rows the mean CBF of patients with and without dichotomous variables is presented. In the bottom rows the correlation coefficients between CBF and continuous variables are presented. All *p*-values presented are corrected for multiple comparisons.

	CBF at day 0		p
	With risk factor	Without risk factor	
Male gender	481.3 (119.6)	438.0 (167.9)	0.717
Hypertension	448.7 (129.3)	489.1 (178.4)	0.739
Diabetes mellitus	516.1 (118.0)	446.4 (148.8)	0.550
Atrial fibrillation	457.3 (171.0)	464.2 (113.0)	0.964
Dyslipidemia	443.2 (126.4)	507.0 (182.9)	0.616
Hyperuricemia	433.8 (115.6)	470.7 (154.5)	0.670
Heart failure	493.3 (208.7)	457.4 (139.9)	0.881
Ischemic heart disease	505.8 (161.0)	453.5 (142.9)	0.704
Previous stroke	385.0 (131.5)	464.2 (145.6)	0.717
Smoking	478.5 (158.7)	458.8 (145.2)	0.925
Peripheral artery disease	523.5 (151.5)	454.3 (144.4)	0.736
Obesity	418.2 (113.7)	487.3 (157.2)	0.546
Previous statin	414.9 (101.4)	482.0 (157.8)	0.565
Previous antiplatelet	445.7 (119.0)	464.5 (134.0)	0.908
Intravenous thrombolysis	499.8 (144.7)	404.0 (127.8)	0.213
Hemorrhagic transformation	451.6 (135.4)	461.8 (147.4)	0.884
Recanalization	509.9 (134.9)	426.5 (143.6)	0.325
Flow diversion	519.3 (136.6)	375.9 (112.5)	0.022*
	Correlation coefficient		
NIHSS at admission	− 0.414		0.055
Glucose at admission	0.057		0.928
Age	− 0.024		0.919
Weight	− 0.191		0.523

* $p < 0.05$.

2.6. Neuroimaging

Hemorrhagic transformation was classified in the gradient echo sequences of the MRI performed on day seven. Only type 2 parenchymal hemorrhages (PH2) were considered as hemorrhagic transformation [14].

2.7. Statistical analysis

Normality of all study variables was assessed by the Shapiro-Wilk test. The association of patients' demographics and vascular risk factors with CBF at admission, seven days and three months was evaluated using independent samples *t*-test, Pearson or Spearman correlation as indicated by the variables' characteristics. We determined the independent association of EPCs cell number and functional properties with CBF at different timepoints using multivariable linear regression. To evaluate the association of EPC cell number and functional properties with hemorrhagic transformation, recanalization and flow diversion we used binary logistic multivariate regressions. We then assessed the association of CBF, hemorrhagic transformation, recanalization and flow diversion with mRS at three months through uni- and multivariable ordinal regression. All univariate analysis were

Table 2
Association of cellular variables at days zero and seven with CBF at day zero, day seven and 3 months. All results were obtained by multivariable regression adjusted for baseline NIHSS, intravenous thrombolysis and flow diversion. SE: Standard Error

Variable		CBF at day 0		CBF at day 7		CBF at 3 months	
		B (SE)	p	B (SE)	p	B (SE)	p
Day 0	CAC number	0.189 (0.124)	0.134	0.273 (0.156)	0.09	0.189 (0.143)	0.197
	oEPC number	0.101 (0.267)	0.707	0.481 (0.329)	0.154	0.161 (0.287)	0.579
	CFU-EC number	0.079 (0.292)	0.787	− 0.333 (0.382)	0.39	− 0.2 (0.313)	0.528
	CAC migration	− 4.076 (1.496)	0.01*	− 0.011 (2.097)	0.996	0.953 (2.088)	0.651
	oEPC migration	0.381 (1.084)	0.729	1.35 (1.431)	0.359	0.59 (1.182)	0.625
	CFU-EC migration	1.954 (0.957)	0.052	0.37 (1.419)	0.797	− 0.341 (1.229)	0.784
	oEPC sprout number	− 0.852 (1.662)	0.612	3.125 (1.968)	0.127	3.518 (1.922)	0.081
	CFU-EC sprout number	− 1.778 (1.755)	0.32	4.913 (1.836)	0.014*	1.699 (2.022)	0.411
	oEPC sprout length	− 0.04 (0.014)	0.789	0.016 (0.017)	0.34	0.028 (0.016)	0.096
	CFU-EC sprout length	− 0.01 (0.017)	0.554	− 0.024 (0.02)	0.235	0.013 (0.018)	0.476
Day 7	CAC number			0.061 (0.257)	0.816	0.144 (0.219)	0.516
	oEPC number			0.886 (0.414)	0.041*	0.423 (0.316)	0.191
	CFU-EC number			0.396 (0.35)	0.267	0.636 (0.32)	0.057
	CAC migration			1.244 (2.433)	0.612	1.506 (1.903)	0.435
	oEPC migration			0.641 (1.492)	0.674	2.294 (2.148)	0.311
	CFU-EC migration			1.6 (1.014)	0.133	1.68 (1.248)	0.2
	oEPC sprout number			3.433 (1.486)	0.03*	1.12 (1.471)	0.455
	CFU-EC sprout number			2.404 (1.881)	0.214	− 1.056 (1.806)	0.565
	oEPC sprout length			0.021 (0.007)	0.009*	0.009 (0.007)	0.259
	CFU-EC sprout length			0.033 (0.02)	0.108	0.024 (0.018)	0.211

* $p < 0.05$.

Table 3
Association of EPC cell number and functional properties with recanalization, flow diversion and hemorrhagic transformation. Results were obtained by multivariable regression adjusted for baseline NIHSS, intravenous thrombolysis and flow diversion.

Variable		Recanalization		Flow diversion		Hemorrhagic transformation	
		OR(95%CI)	p	OR(95%CI)	p	OR(95%CI)	p
Day 0	CAC number	1.004 (0.998–1.01)	0.163	1.001 (0.997–1.006)	0.544	0.992 (0.979–1.005)	0.213
	oEPC number	1.006 (0.996–1.016)	0.225	0.989 (0.976–1.002)	0.11	1.001 (0.984–1.018)	0.893
	CFU-EC number	1.004 (0.993–1.016)	0.454	1.003 (0.992–1.013)	0.616	0.997 (0.978–1.016)	0.723
	CAC migration	0.992 (0.934–1.052)	0.779	1.066 (0.994–1.143)	0.073	0.88 (0.746–1.038)	0.13
	oEPC migration	0.986 (0.945–1.029)	0.531	1.04 (1.0–1.081)	0.053	1.036 (0.975–1.101)	0.249
	CFU-EC migration	0.983 (0.945–1.023)	0.404	1.049 (1.003–1.096)	0.036*	0.969 (0.908–1.033)	0.333
	oEPC sprout number	0.965 (0.901–1.034)	0.312	1.101 (1.022–1.187)	0.012*	1.021 (0.93–1.121)	0.663
	CFU-EC sprout number	0.966 (0.904–1.033)	0.313	1.289 (0.991–1.672)	0.059	0.995 (0.906–1.092)	0.909
	oEPC sprout length	1.0 (1.0–1.001)	0.549	1.0 (1.0–1.001)	0.46	1.001 (1.0–1.002)	0.11
	CFU-EC sprout length	1.0 (0.999–1.001)	0.417	1.004 (1.0–1.008)	0.037*	0.998 (0.994–1.002)	0.369
Day 7	CAC number					0.995 (0.982–1.007)	0.404
	oEPC number					0.984 (0.956–1.013)	0.269
	CFU-EC number					1.0 (0.983–1.017)	0.996
	CAC migration					0.966 (0.864–1.081)	0.551
	oEPC migration					1.002 (0.936–1.072)	0.955
	CFU-EC migration					0.992 (0.94–1.048)	0.782
	oEPC sprout number					0.95 (0.837–1.079)	0.432
	CFU-EC sprout number					0.797 (0.511–1.245)	0.319
	oEPC sprout length					1.0 (0.999–1.001)	0.662
	CFU-EC sprout length					0.998 (0.994–1.003)	0.998

* $p < 0.05$.

corrected for multiple comparisons by false discovery rate, and the corrected *p*-values are presented. All multivariable models included the variables with $p < 0.1$ in univariate association with CBF at any timepoint (NIHSS and flow diversion) as well as intravenous thrombolysis, due to its recognized clinical and hemodynamic effect. Statistical significance was set for $p < 0.05$.

The sample size calculation was based on estimating large treatment effects ($d = 0.8$) due to stringent inclusion criteria, a between-group comparison with a 0.05 one-sided alpha significance level and 80% power, that would require a total sample population of 42 patients. We included 45 patients to account for potential dropouts and technical failures in MRI.

2.8. Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

3. Results

We included a total of 45 patients in our study population, median age 70 years, (interquartile range: 10) and 24 (53.3%) male. At three months 5 (11.1%) were dead and 22 patients (48.9%) showed good clinical outcome (mRS between 0 and 2). Univariate associations of baseline characteristics with CBF at days zero are presented in Table 1 (in Supplementary Table 1 we present the univariate associations of

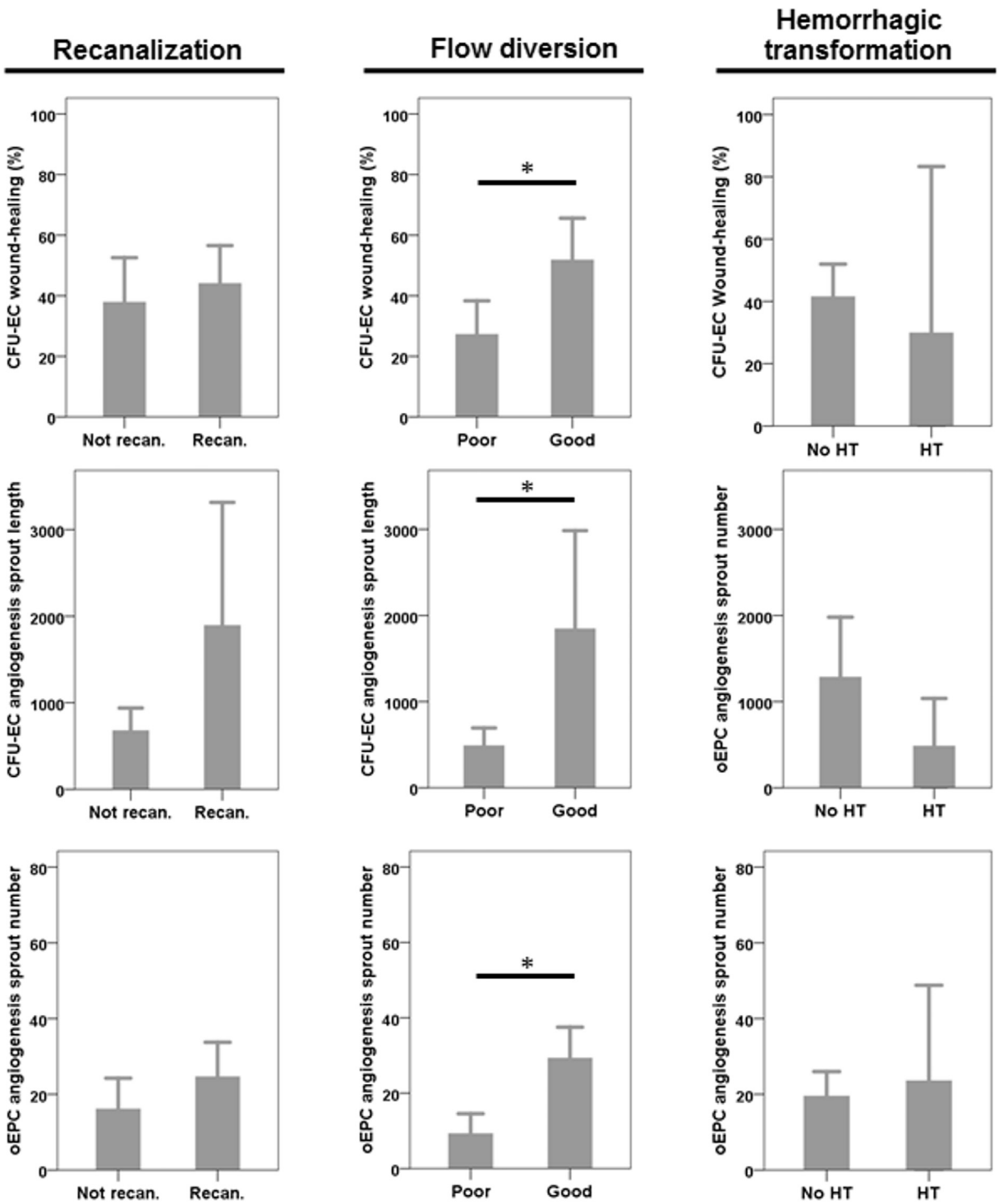

Fig. 3. Association of the functional properties of EPCs at day zero with recanalization (Recan.), flow diversion and hemorrhagic transformation (HT). Values are presented as mean and 95% confidence interval. *p < 0.05. Flow diversion is presented as "good" or "poor".

baseline variables with CBF at day seven and three months). At day zero, CBF was associated with the presence of flow diversion, with no statistically significant associations at day seven nor day 90. Fig. 2 (B and C) represents the flow velocities in two patients with distinct CBF and clinical outcome.

The multivariable associations of CBF at different timepoints with number and functional properties of EPCs are presented in Table 2. At day zero, CBF was associated with the migratory properties of CACs. CBF at day seven was associated with the angiogenic properties of day zero CFU-ECs and day seven oEPCs. At three months no statistically significant associations were identified between CBF and EPC properties. No cellular variable was associated with recanalization nor hemorrhagic transformation on multivariable analyses (Table 3). Flow diversion showed an independent association with oEPC sprout number, CFU-EC migration and sprout length at day zero (Fig. 3).

We also evaluated the association of each hemodynamic variable with functional outcome at three months (Table 4). Recanalization and flow diversion were independently associated with a better clinical outcome.

Table 4
Associations of hemodynamic variables with functional outcome at three months (multivariate ordinal regressions are adjusted for NIHSS, intravenous thrombolysis and flow diversion). The p-values presented in univariate analysis are corrected for multiple comparisons. mRS: modified Rankin scale; CBF: Cerebral Blood Flow; OR: Odds Ratio; CI: Confidence Interval.

Variable	mRS at 3 months Univariate OR (95%CI)	p	mRS at 3 months Multivariable OR (95%CI)	p
CBF at day 0	− 0.005 (− 0.009 to − 0.001)	0.020*	0.001 (− 0.004 to 0.006)	0.607
CBF at day 7	− 0.004 (− 0.008 to 0.000)	0.084	− 0.003 (− 0.008 to 0.001)	0.159
CBF at 3 months	− 0.004 (− 0.009 to 0.001)	0.109	− 0.005 (− 0.011 to 0.001)	0.093
Recanalization	− 2.670 (− 3.994 to − 1.347)	< 0.001*	− 1.718 (− 3.150 to − 0.286)	0.019*
Hemorrhagic transformation	1.207 (− 0.466 to 2.881)	0.157	1.546 (− 0.336 to 3.428)	0.107
Flow diversion	− 3.751 (− 5.319 to − 2.184)	< 0.001*	− 3.416 (− 5.005 to − 1.827)	< 0.001*

* $p < 0.05$.

4. Discussion

The main finding of our study is the association of EPC properties such as angiogenesis and migration with hemodynamic properties, namely increased flow diversion at day zero and CBF at day seven. Our results further show no statistical association between EPC properties and recanalization or hemorrhagic transformation.

The associations between the angiogenic properties of oEPCs and CFU-ECs with subacute CBF suggests the involvement of these cells in the formation of new blood vessels. Yet, it is important to note that this association was not visible in all functional tests, nor for all EPC lineages. In fact, previous studies in mice have demonstrated a weak but significant association between angiogenesis and CBF, which is more pronounced after seven days [15] and prolongs at least up to 21 days [16]. This subacute association of angiogenesis with CBF is reinforced by recent findings associating blood-brain barrier permeability of stroke patients at seven days with good clinical outcome and more proficient EPCs [2], and are in line with our current results, where no association was found between EPCs and CBF at three months. Moreover, the interpretation of our data within these previous reports, support the notion that the hemodynamic impact of the EPCs can only be partially explained by the effect of angiogenesis on CBF [16], as could be expected considering the anatomically restricted area of neoangiogenesis in comparison to the more widespread evaluation implicated in CBF. An alternative mechanism relates to the possibility that the presence of EPCs with enhanced functional properties might be a marker of a more proficient vascular response to the ischemic insult and not a local consequence of their action, although the concomitant association with flow diversion and the dampening of effect at three months suggests a more direct relation.

Flow diversion in the acute stage was associated with CBF at day zero, functional properties of day zero EPCs and functional outcome at three months. The link between angiogenesis and collateral circulation has been suggested in previous animal model studies [17,18], but had never been studied in humans. The biological mechanisms implied in the development of collateral circulation in the context of an ischemic insult seem to vary over time. In the acute and subacute stages two processes appear to promote collaterals: shear fluid stress precipitated by the pressure gradient caused by the acute thrombus and angiogenesis, as hypoxia triggers endothelial cells to sprout and form capillary networks [19]. In this last critical step, EPCs take a pivotal role through their paracrine (mainly through CAC subpopulation) and direct angiogenic properties (mostly oEPCs and CFU-ECs). As stated earlier, the association of flow diversion in the first hours after stroke with the functional properties of EPCs may also suggest that both mechanisms (EPCs and flow diversion) may coexist as markers of an efficient response to stroke.

A potential offset of promoting a network of new vessels is hemorrhagic transformation. In fact, previous studies on animal models have yielded conflicting results considering the relation between angiogenesis and angiogenic factors (such as VEGF) with hemorrhagic transformation [16,20]. Nonetheless, this seems to be a time-dependent response: in the first hours after stroke VEGF promotes acute BBB disruption with hemorrhagic transformation, whereas in the subacute stage the enhancement of effective neoangiogenesis improves CBF and neurological recovery [16,20]. Our study supports the concept that in stroke patients enhanced functional properties of EPCs promote flow diversion with no effect on hemorrhagic transformation.

No effect was identified by EPCs on early arterial recanalization. Although no previous study had assessed the association of EPCs with recanalization in ischemic stroke, early works on EPCs suggested the rationale for an effect on recanalization, albeit being more likely on venous thrombi, due to the organization of fibrin meshwork and cellular composition of the venous thrombi [21].

Our study has inherent limitations that should be taken under consideration in its interpretation. Firstly it was a single centre study with a relatively reduced sample size, however, the strict inclusion criteria and features of the study population suggest external generalizability of the findings. Moreover, cellular assessment at two timepoints may be limited within the intricate pathology of stroke. Nonetheless, these timepoints have been suggested as pathologically important in preclinical studies and are both potential treatment windows in stroke patients. Moreover, performing comprehensive and serial evaluations of hemodynamic and neurological status allowed a rational and clinically meaningful interpretation of results.

5. Concusions

The functional properties of EPCs are associated with enhanced flow diversion and subacute CBF, with no impact on recanalization nor hemorrhagic transformation. These results promote the need and help the design of interventional trials on the efficacy of EPCs in acute stroke, reinforcing the integration of these cellular therapies within current therapies.

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Conflict of interests

The authors have nothing to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2017.12.028.

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