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Endothelial and circulating progenitor cells as prognostic biomarkers of stroke: A systematic review and meta-analysis

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ABSTRACT

Purpose: Endothelial progenitor cells (EPCs) are biomarkers of neurovascular repair in cerebral vascular disease (CVD). Low quantification of EPCs and/or their dysfunction has been associated with stroke severity and post-stroke functionality. This systematic review (SR) and meta-analysis aimed to analyze whether EPC quantification contributes to stroke severity and functional prognosis.

Methods: Articles were selected from the PubMed, ScienceDirect, and Ovid MEDLINE databases, according to the guidelines of the PRISMA 2020 [1] statement. Detailed observational studies of samples from subjects with a clinical diagnosis of CVD (ischemic stroke-IS, hemorrhagic stroke-HS, or transient ischemic attack-TIA) aged >45 years during 2003–2023 were included. Evaluation of study quality was based on the Critical Appraisal Skills Programme checklist(Santamaría, 2017 [2]).

Results: We included 22 articles in our SR. Patients with IS and good functional outcomes had higher EPC levels during the first week of admission than those with worse functional outcomes. Higher EPC levels were associated with reduced infarct growth, improved NIHSS scores at 48 h (OR 0.8; 95 % CI: 0.72–0.90; $p < 0.0002$) 7 ($r = -0.607$; $p < 0.0001$), and 90 days ($r = -0.570$; $p < 0.0001$), with a negative correlation between EPC levels and NIHSS score (overall pooled r = −0,32, 95 % CI: −0.39-0.24), and good functional outcomes with better mRS scores at 24 h, 3, 6, and 12 months (overall pooled SMD 4.51, CI 95 %: 0.70–0.83). Lower EPC quantification and worse functional outcomes during admission were predictors of IS recurrence. Higher EPC levels were associated with better functional outcomes and lower bleeding volumes in patients with HS and were protective markers for the progression high-risk TIA.

Conclusion: EPCs seems to be predictive biomarkers of better clinical outcomes in patients with CVD, exhibiting lower severity (NIHSS) and better functional prognosis (mRS).

1. Introduction

The structural and functional loss of neurons in cerebrovascular disease (CVD) relies on the processes of neuroreparation, neuroplasticity, ischemic tolerance, and preconditioning of the brain parenchyma, as well as on the vascular compensation mechanisms responsible for promoting reperfusion or tissue revascularization, which are essential processes for the survival of growing tissue and repair of injured tissue [3]. The processes of angiogenesis and vasculogenesis, which are key to the regeneration process of ischemic tissue, contribute to its development [4].

Endothelial progenitor cells (EPCs) are a group of circulating cells that exhibit characteristics similar to mature endothelial cells. They are found in hypoxic or ischemic sites within the endothelium, where they differentiate into mature endothelial cells and secrete proangiogenic factors to promote vascularization [5–7]. Current evidence suggests that these cells, which express CD34+, play a crucial role in regulating tissue repair processes following ischemic damage. Notably, EPCs can also differentiate *ex vivo* into endothelial-like cells. While the migration and proliferation of adjacent mature endothelial cells contribute to endothelial repair, EPCs are key players in the early stages of vascular regeneration [8]. This suggests that EPCs may serve as markers of

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endothelial function and cardiovascular risk.

Current evidence on the relationship between EPC quantification and functional prognosis in patients with CVD has several limitations due to the heterogeneity in the collection of clinical-analytical variables, the inclusion of patients with different subtypes of CVD, the small sample size present in the different publications, the short observation period of the studies, and the variability in the methodology used to measure angiogenic properties and quantify these cells.

The aim of this systematic review (SR) is to establish validation of the EPC count as a prognostic biomarker in the recovery of patients with stroke.

2. Methods

In this study, a systematic review (SR) was conducted following the updated guidelines outlined in the PRISMA 2020 statement [1] (Page et al., 2020) for reporting systematic reviews and meta-analyses. The studies included in this SR were selected from publications between 2003 and 2023. Evaluation of study quality was based on the Critical Appraisal Skills Programme checklist [2]. The eligibility criteria for this SR are as follows:

Inclusion criteria:

- Study population
  - o Patients of either sex with an age range of 45 years or older.
  - o Patients with clinical diagnostic criteria for CVD, either ischemic stroke (IS), hemorrhagic stroke (HS), or transient ischemic attack (TIA), according to the *American Heart Association* (AHA) classification.
- Study features
  - o Analytical observational prospective and retrospective cohort, case-control, and cross-sectional research.
- Outcome measures

We collected studies that established a relationship between the quantification and angiogenic properties of EPCs and the following outcome measures:

- o Chronology of CVD: acute stroke (AS), subacute stroke (SS), and chronic stroke (CS).
- o Study of the subtypes of CVD: IS according to the *Oxfordshire Community Stroke Project* (OCSP) and *Trial Org-10,172 Acute Stroke Treatment* (TOAST) classification, HS, and TIA.
- o Clinical outcomes of severity (*National Institutes of Healt Stroke Scale*-NIHSS, *European Stroke Scale*-EES, and *ABCD2*) and functional prognosis (*Modified Rankin Scale*-mRS and *Barthel Index*) of CVD, considering other accessory clinical outcome variables such as infarct volume (IV), residual bleeding volume (RBV), or cognition (CDR or *Clinical Dementia Rating*).
- o Studies attempting to establish associations between angiogenic properties and/or quantification of EPCs and the risk of stroke recurrence, measured as the presence of recurrent symptoms or objective findings of progression to cerebral infarction.

Exclusion criteria:

Research examining the association between the angiogenic properties and/or quantification of EPCs and the following primary endpoints:

- o Modifiable risk factors for CVD.
- o Secondary preventive medical-surgical treatments for CVD.
- o Other associated medical vascular diseases other than CVD.
- o Specific biomarkers outside the angiogenic/cytometric properties of EPCs.

- Study features:
  - o Experimental studies.

The initial search for studies and the review of their references were conducted on August 1, 2023, and on August 3, 2023, respectively, in the PubMed, ScienceDirect, and Ovid MEDLINE databases.

For the search strategy, performed on August 1, 2023, free text terms and MeSH (*Medical Subject Headings*) terms were used, applying the following Boolean operators "AND" and "NOT": <<(((Endothelial progenitor cells) AND (Stroke)) AND (Prognosis)) and < <"Endothelial Progenitor Cells"[MeSH Terms] AND "Stroke"[MeSH Terms]>>, respectively. The terms were entered in English and filters were used for temporality (2003−2023), age (Middle Aged + Aged: 45+ years), species (humans), and type of study (observational studies).

The search with these filters generated approximately 32, 871 and 100 results on the PubMed, ScienceDirect and Ovid MEDLINE platforms respectively. Once the search was performed in the corresponding databases, the bibliographic managers Zotero and Mendeley were used to eliminate duplicate articles. The process of study selection is shown in the attached flow diagram (Fig. 1). The quality of the studies was quantified using the *Critical Appraisal Skills Programme* (CASP) checklist of the *Oxford Centre for Triple Value Healthcare*.

2.1. Statistical analysis

Statistical analyses were performed using *Revman Web*. Graph generation was performed using the *Meta-Mar* tool. A random-effects model was used to calculate the pooled effect size because heterogeneity was expected because of differences in the study design, study population, methodology in the quantification of EPCs, analysis of severity or functional prognosis of CVD at different time periods, and outcome measures. A meta-analysis of the Correlation and the Standardized Mean Difference were calculated for the analysis of stroke severity (NIHSS) and functional prognosis (mRS) respectively.

Statistical heterogeneity was assessed using Higgin's I2 statistic. Study-level estimates were considered heterogeneous if the I2 value was >50 %. An I2 value of 50 %–75 % was considered substantial heterogeneity, and an I2 value >75 % was considered considerable heterogeneity. Funnel plots were used to examine potential publication biases.

3. Results

The current SR includes a sample of 2205 patients, with a mean age of cases ranging from 58.7 ± 10.3 years (Marek Kukumberg et al., 2020) to 76.9 ± 7.8 years (Kamini Rakkar et al., 2023). The study by Antoine Dunac et al. (2006) covered a wider age range, between 25 years in the control subjects and over 80 years in the cases. In the case of Navarro-Sobrino et al. (2010), the average age of the participants was not specified. Most of the studies have been conducted in male patients, constituting around 68.9 % of the total sample. Only in the work of Yushuang Deng et al. (2017) was a higher percentage of women than males recruited (57 % *vs.* 43 %). In 3 studies (Rokhsareh Meamar et al., 2016; Navarro-Sobrino et al., 2010; Antoine Dunac et al., 2006), the sex of the participants was not specified, and in two studies (Tomás Sobrino et al., 2007; Alexander Woywodt et al., 2012), the percentage of male and female patients was the same.

Regarding the methodology of the studies, the most studied CVD was IS. The only studies that evaluated patients with HS were Juan Pías-Peleteiro et al. (2016) and Tomás Sobrino et al. (2011). The only work that evaluated both IS and HS was that of Kon Chu et al. 2008. TIA was evaluated by Rokhsareh Meamar et al., 2016; Wang Zhao et al., 2022; Pol Camps-Renom et al., 2021; and Marek Kukumberg et al., 2020 (the latter two synchronically with IS). The most commonly used method for measuring and analyzing EPCs was quantitative assessment by flow cytometry, except in the work of Tomás Sobrino et al. (2007) and Kon Chu et al. (2008), where qualitative evaluation by cell culture was used. Akihiko Taguchi et al. (2008) did not specify the cell assessment

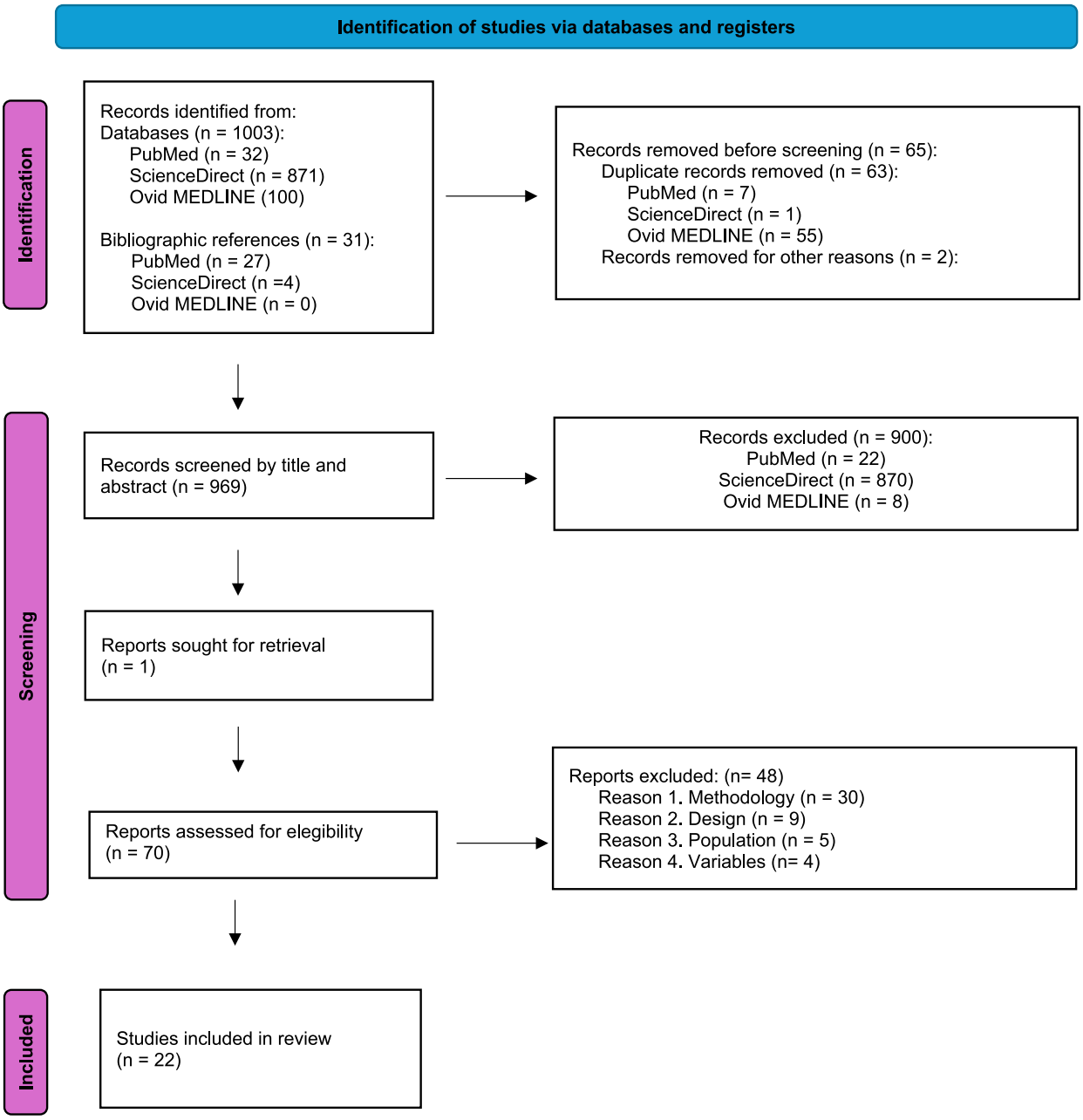

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 flow diagram.

methodology. B. Machalinski et al. (2006) and Navarro-Sobrino et al. (2010) employed a mixed method to analyze the percentage of EPCs by flow cytometry and the levels of granulocyte and macrophage colony-forming units (CFU-GM), erythrocyte-forming units (BFU-E), and functional and vasculogenesis capacity by *ex vivo* cell culture.

Some studies also assessed alternative aspects to the severity and functional prognosis of CVD, such as cognition (Akihiko Taguchi et al., 2008) using the *Clinical Dementia Rating* (CDR) scale, the risk of stroke recurrence (Rokhsareh Meamar et al., 2016; Wang Zhao et al., 2022; Joan Martí-Fàbregas et al., 2015), IV (Tomás Sobrino et al., 2007; Nai-Wen Tsai et al., 2013; Kon Chu et al., 2008; Yushuang Deng et al., 2017), or RBV (Juan Pías-Peleteiro et al. 2016). The severity of CVD was analyzed in all studies using the NIHSS, except in the study by Alexander Woywodt et al. (2012), which additionally employs the ESS, and in the study by Wang Zhao et al. 2022, which uses the ABCD2 scale for studying patients with TIA.

A summary of the main results of each study included in the SR can be found in Table 1: Main characteristics of the included studies.

A. Ischemic stroke

Eighteen different studies were included in the SR on the relationship between EPCs and IS. An association was found between EPC quantification and the functional prognosis of IS. Patients with IS and good functional outcomes had higher levels of EPCs during the first week of admission than those with worse functional outcomes [21], and the increase in these cells during the first week was associated with reduced infarct growth, improvement in NIHSS at 7 and 90 days, and good functional outcomes at 24 h, 3 [9], 6 [14] months and 1 year of follow-up [30], considering EPCs as a prognostic biomarker for IS [22]. Furthermore, patients with a history of IS for >1 year and decreased levels of EPCs showed a significant worsening in functional and neurological prognosis [10].

EPC levels are a separate predictor of severe neurological

Table 1
Main characteristics of the included studies.

Author, year	Sampling size (n)	Age range	Type and period of the study	CVD studied	Analysis of endpoints	Summary of the results and conclusions
Juan Pías-Peleteiro et al. 2016	46	72,7± 10,8	OPC (2012–2014)	HS	12-month mRS and RBV at 6 months	Good functional outcomes were associated with increased EPC levels at 72 h and day 7. Separate association between EPC levels on day 7 and mRS $\leq$ 2 at 12 months. Negative correlation between EPCs on day 7 and RBV.
Tomás Sobrino et al. 2007	48	70,7 ± 10	OPC (2005–2006)	Non-lacunar IS	mRS, IV, and NIHSS	Good functional results associated with increased CFU-EC on the 7th day and at 3 months. An increase in CFU-EC during the first week was associated with a reduction in IV. An increase in CFU-EC was associated with an improvement in NIHSS at 7 and 90 days.
Kamini Rakkar et al. 2023	121	Cases: 76,9 ± 7,8 Controls: 73,3 ± 7,2	OPC (2017–2019)	Cortical and lacunar IS	mRS, BI, and IS subtypes	Increase in EPCs in patients with IS vs. healthy subjects at admission and at 30 days. No significant differences were observed between the quantification and functionality of EPCs and the IS subtype. No correlation was observed between mRS at 90 days after stroke and the functionality and quantification of EPCs. EPCs as a diagnostic marker but not as a prognostic marker.
Rokhsareh Meamar et al. 2016	60	Cases: 63 ± 10,5 Controls: 62,2 ± 12	OPCC (2013–2014)	TIA	CVD recurrence	High levels of EPCs in patients with the first TIA event compared with controls. EPCs do not prove to be predictive markers for CVD recurrence in patients with TIA.
Akihiko Taguchi et al. 2008	40	73,1 ± 1,1	OPC (UTP)	IS	BI, NIHSS, and CDR	IS and low EPC levels were associated with worse BI and CDR. No significant differences between EPCs quantification and NIHSS and mRS.
Wang Zhao et al. 2022	159	HR-AIT: 61,48 ± 4,03 NHR-AIT: 56,74 ± 8,35	OCS (2019–2021)	TIA	ABCD2 and CVD recurrence	Higher levels of EPCs in "NHR-AIT" group vs. "HR-TIA" group. Lower proliferation, migration, and adhesion capacity of EPCs in HR-TIA than in NHR-TIA. EPCs were significantly demonstrated to be protective markers in HR-TIA and predictive of progression to HR-TIA.
Nai-Wen Tsai et al. 2013	130	Cases: 61,8 ± 12,4 Controls: 57,7 ± 11,6	OPC (2011−2012)	IS (SVIS, LVIS)	mRS, BI, NIHSS, and IV	Lower levels of EPCs in patients vs. controls in the first 24 h and 7 days after stroke. Lower levels of EPCs in patients with LVIS vs. SVIS within 24 h of stroke. Non-significant correlation between EPC levels, NIHSS, mRS, and IV. Lower levels of EPCs in the "poor outcome group" vs the "good outcome group" in 24 h after stroke. EPC levels at admission are separate factors in clinical and functional outcomes at 6 months.
Kon Chu et al. 2008	160	Cases: -CS 61,5 ± 9,6 -AS 63,1 ± 11,6 Controls: 58,4 ± 11	OPCC (UTP)	HS and IS (LAA, CE y SVIS)	NIHSS, IV, and CVD chronology	EPC levels increased progressively after stroke. Lower levels of EPCs in AS vs. CS and healthy individuals. "CE group" presented significantly higher levels and better functional activity of EPCs vs. "LAA group", "SVIS group" and "HS group". IV and NIHSS higher in "outgrowth cells-positive group" versus "outgrowth cells-negative group" at admission. NIHSS at entry is a significant separate predictor of cell growth.
Joan Martí-Fàbregas et al. 2013	146	70,8 ± 12,2	OPC (UTP)	IS	NIHSS, CVD chronology, and mRS	Higher EPC levels on the 7th day vs. at admission and 3 months after stroke. Stroke etiology is a significant separate predictor of EPC levels at admission. Better functional outcomes in patients with high levels of EPCs, LAA, and SVIS at 3 months.
B. Machalinski et al. 2006	37	Cases: 67 ± 29 Controls: 58 ± 26	OCS (UTP)	IS	Mobilization	Higher levels of EPCs in patients with IS vs. controls. IS as a trigger for increased mobilization of EPCs.
Navarro-Sobrino et al. 2010	46	Not specified.	OPCC (2007–2008)	IS	Mobilization, CVD chronology and NIHSS	Higher levels of EPCs in AS. vs. controls. Higher in vitro cell growth of EPCs in subacute IS vs. controls. Positive correlation between stroke severity by NIHSS and the degree of cell growth. Greater capacity for vasculogenesis and secretion of pro-angiogenic factors in SS.

(*continued on next page*)

Table 1 (*continued*)

Author, year	Sampling size (n)	Age range	Type and period of the study	CVD studied	Analysis of endpoints	Summary of the results and conclusions
Dominik Sepp et al. 2014	96	68,4	OPCC (UTP)	IS	Mobilization	Increased mobilization and significant EPC levels during the first 24 h to 5 days after IS. The baseline level of EPCs in IS was lower than that in controls.
Yushuang Deng et al. 2017	113	Cases: 65,1 ± 10,2 Controls: 64,4 ± 9,6	OPCC (2015–2016)	IS	Mobilization and IV	EPC levels increased on the 7th day after IS, peaked at 14 days, and decreased after 21 days. No significant correlation between EPCs and IV levels on days 1 and 14 after IS. Significant positive correlation between IV and an increase in EPCs.
Tomás Sobrino et al. 2011	32	67,1 ± 10,8	OPC (2009)	HS	RBV, and mRS	EPC levels at 7 days were associated with good functionality at 3 months. Negative correlation between EPC levels at 7 days and RBV at 3 months.
Tomohiro Kawano et al. 2016	89	70,5 ± 11,6	OPC (2012−2013)	PATI	EPCs, mRS, and NIHSS	The "SW group" had lower levels of EPCs compared to the "Non-SW group". Negative correlation between EPC levels and NIHSS at admission. Low EPC levels at admission are considered as separate predictive biomarkers of worse clinical-functional outcomes in patients with PATI.
Pol Camps-Renom et al. 2021	80	74,3 ± 10,4	OPC (2016–2019)	IICA and TIA	EPCs and mRS at 3 months and 1 year	Patients with IICA and CTA with increased levels of EPCs associated with better mRS at 3 months and after 1 year of follow-up. "High mobilizers" presented lower NIHSS one month after admission compared to "Poor mobilizers".
Antoine Dunac et al. 2006	50	Cases: > 80 años Controls: 25–45	OPCC (UTP)	IS (LAA)	Mobilization, NIHSS, and mRS	Negative correlation between NIHSS and cell mobilization at 3 months. Association between higher cell mobilization and better mRS at 3 months. EPCs are predictive biomarkers that favor the neurological and functional recovery process at 1 and 3 months.
Hon-Kan Yip et al. 2007	198	Cases: -Group 1: 65,8 ± 11 -Group 2: 66,8 ± 9,9	OPCC (2005–2006)	IS	NIHSS	Higher levels of EPCs in IS vs. control subjects at risk. Lower levels of EPCs in group 1 vs. group 2 at 48 h after symptoms. Correlation between EPC levels and improvement in NIHSS score ≥ 4 points at 21 days after IS. Low levels of EPCs are separate predictors of severe neurological deterioration (NIHSS ≥ 12) at 48 h and of adverse clinical outcomes (recurrent IS, NIHSS ≥ 12 at 90 days or death).
Marek Kukumberg et al. 2020	237	Cases: 58,7 ± 10,3 Controls: 51 ± 17	OPCC (2011–2015)	IS and TIA	Quantification and functionality of EPCs and their proangiogenic factors	Increased levels of EPCs in patients with IS/TIA aged 45 years vs. age-matched controls. EPC quantification was upregulated in patients with IS/TIA 12 months after stroke. EPCs of patients with IS/TIA showed impaired tubular formation compared with controls. Age and previous IS/TIA influence the angiogenic capacity of EPCs.
Joan Martí-Fàbregas et al. 2015	121	70,1 ± 12,6	OPC (UTP)	IS	CVD recurrence and EVs	Lower quantification of EPCs and worse mRS increased the risk of VE. The absence of EPCs was predictive of new VE in patients with IS.
Francesca Cesari et al. 2009	67	73	OPC (UTP)	IS	NIHSS	Lower levels of CPCs in IS TACI < POCI < PACI/LACI. Negative correlation between NIHSS scores at admission and CPCs. Higher CPC levels in NIHSS < 12. Patients discharged home had higher levels of CPCs and EPCs and lower NIHSS than patients requiring inpatient rehabilitation. CPC levels are predictive biomarkers for better clinical and functional outcomes.
Alexander Woywodt et al. 2012	129	Cases: 71 Controls: 51	OPCC (UTP)	IS (LAA, CE y SVIS)	ESS, NIHSS, and mRS	Lower levels of EPCs in healthy controls. Higher levels of EPCs in SVIS > CE > LAA without statistical significance. No correlation between EPCs, ESS/NIHSS and mRS. EPCs are biomarkers of endothelial damage and neurorepair in IS

Abreviatures: OPC (Observational prospective cohort). OPCC (Observational prospective case-control). (OCS) Observational cross-sectional. EPCs (Endothelial progenitor cells). CVD (Cerebral vascular disease). IS (Ischemic stroke). HS (Hemorrhagic stroke). IV (Infarct volume). CFU-EC (Colony-forming units-endothelial cells). TIA (Transient ischemic attack). mRS (Modified Rankin Scale). BI (Barthel Index). NIHSS (National Institutes of Healt Stroke Scale). RBV (Residual bleeding volume). CDR (Clinical Dementia Rating). SVIS (Small-vessel ischemic stroke). LVIS (Large-vessel ischemic stroke). Penetrating artery territory infarction (PATI). CS (Chronic stroke). AS (Acute stroke). LAA (Large artery aterosclerosis or atherothrombotic stroke). CE (Cardioembolism or cardioembolic stroke). SUA (Stroke of undetermined etiology). ACIS (Anterior circulation ischemic stroke). TACI (Complete anterior circulation stroke). PACI (Partial anterior circulation stroke). POCI (Posterior

circulation stroke). LACI (Lacunar stroke). CTA (Carotid atherosclerosis). SW (Symptomatic worsening). RF (Risk factors). VE (Vascular events). OCSP (Oxfordshire Community Stroke Project). ESS (European Stroke Scale). UTP (Unspecified time period).

deterioration at 48 h and adverse clinical outcomes (recurrent IS, NIHSS ≥12 at 90 days after IS or death); therefore, EPC levels were significantly lower in patients with severe neurological impairment and poor outcome at 24–48 h after symptom onset [11,14,24] and were significantly higher in patients with IS than in at-risk controls [11,12,15]. In addition, age and previous stroke are two factors that influence the angiogenic capacity of these cells, and an increase in the number of EPCs is triggered during recovery from IS [13–20,22].

Focusing on stroke chronology, there has been a process of continuous cell mobilization since the vascular event (VE), demonstrating that IS acts as a trigger for the increased mobilization of EPCs and that their levels and capacity for the induction of vasculogenesis in patients with SS and CS were higher than those with AS [15,17,18] and healthy controls [25]. Specifically, there are contradictory results on the exact timing of higher increased cell mobilization; therefore, some researchers found that EPC levels reached peak levels at the first 24 h to 5 days after the onset of IS symptoms [19], on the 7th day [16] and the 14th day [20]. In fact, some researchers have attempted to analyze the mobilization process according to the subtype of IS, such that EPC levels in patients with LAA stroke reached their peak at 7 days, decreased after 21 days, and returned to normal at 90 days, in contrast to those with CE or lacunar stroke, whose levels progressively decreased until day 90 [25].

Conflicting results have been found regarding the quantification and functionality of EPCs, IS subtype (TOAST and OCSP classification), and functional prognosis. It has been reported that EPC levels in patients with LVIS were lower than those in patients with SVIS 24 h after stroke [14]. Patients with CE stroke had significantly higher levels and better functional activity of EPCs than the group of patients with LAA stroke, small vessel occlusion stroke, and acute HS, with cell growth being higher in the group of patients with CE and LAA stroke than in the other two groups [15]. However, some researchers found that patients with IS on admission of lacunar etiology had higher levels of EPCs (68 cells/mL) than patients with CE (46 cells/mL) and LAA (32 cells/mL) stroke, in sequential order [25]. Better functional outcomes were observed in patients with high levels of EPCs and LAA and small-vessel strokes at 3 months [16]. In addition, patients with a clinical diagnosis of PACI and LACI had higher levels of EPCs than those with a clinical diagnosis of POCI and TACI [24].

Finally, in terms of VE recurrence, lower quantification of EPCs and worse functional outcome during admission were predictors of recurrent VE in patients with AS during patient follow-up, but they were not associated with an increased risk of stroke recurrence (IS, HS or TIA) [23].

In summary, EPC levels are related to clinical and functional outcomes after the VE. Patients with good functional outcomes were found to have higher levels of EPCs during the first week of admission than those with worse outcomes. In addition, lower levels of EPCs were associated with worsening functional and neurological prognoses in patients with a history of IS. Although differences in EPC levels were observed between IS patients and healthy controls, the predictive ability for long-term functional outcomes appears to be limited. Mobilization of EPCs also appears to be influenced by IS etiology and the severity of neurological damage, but there is no clear distinction between IS subtypes. In addition, age and previous history of IS may also influence the angiogenic capacity of EPCs.

B. Hemorrhagic stroke

Two different studies were included in the SR on the relationship between EPCs and HS. An association was found between EPC quantification and the functional prognosis of HS. Patients with HS and good functional outcome showed higher levels of EPCs at 72 h and on day 7, and their levels on day 7 were negatively correlated with RBV [26]. In the same vein, EPC levels at 7 days were independently associated with a good functional outcome and lower RBV at 3 months [27].

C. Transient ischemic attack

Two different studies were included in the SR on the relationship between EPCs and TIA. An association was found between EPC quantification and the functional prognosis of TIA. EPC levels were found to be increased in the group of patients with the first episode of TIA compared with control subjects, demonstrating a higher mobilization of EPCs in the first group of patients [28]. In terms of the relationship between EPC levels and the severity of neurological damage in patients with TIA and the risk of CVD recurrence, some researchers found that the non-high-risk TIA group of patients had higher levels of EPCs than the high-risk group (ABCD2 ≥ 4), with EPCs showing less proliferation, migration, and adhesion capacity and a higher rate of stroke recurrence in the latter group, concluding that EPCs are protective markers for high-risk TIA and that their values are predictive factors in the progression of TIA to high-risk TIA [29].

3.1. Meta-analysis of severity and functional outcomes of CVD

Due to the expected considerable heterogeneity between the different studies, only the statistical analysis of severity (NIHSS) and functional outcomes (mRS) of patients with CVD (IS, HS and TIA) and their relationship with the quantification of EPCs was performed, with only a qualitative review of the other outcome variables mentioned above.

3.2. Meta-analysis of CVD severity (NIHSS)

A meta-analysis of the correlation between two variables was performed, focusing on the relationship between endothelial progenitor cell (EPC) quantification and stroke severity, measured by the National Institutes of Health Stroke Scale (NIHSS). The sample sizes and correlation values from each study were collected. Out of the 22 studies included in this systematic review (SR), 10 provided a detailed analysis of NIHSS stroke severity. However, two studies (Antoine Dunac et al., 2007, and Alexander Woywodt et al., 2012) were excluded from the statistical analysis due to differences in cellular analysis methodologies, which assessed cell mobilization or growth *in vitro*, rather than absolute EPC quantification.

A higher EPC count was generally associated with lower NIHSS stroke severity, indicating a negative correlation. The overall pooled correlation for lower stroke severity (NIHSS) with higher EPC quantification was - 0.20 (−0.57; 0.23) under the random-effects model (Fig. 2). The analysis of the 8 included studies showed considerable heterogeneity ($I^2 = 93$ %) (Fig. 3).

3.3. Meta-analysis of CVD functional outcomes (mRS)

The sample size of the good and poor prognosis groups (N1 and N2) was collected, as well as the mean number of EPCs and their standard deviations in each group, and the Standardized Mean Difference was calculated. Of the 22 studies included in this SR, 12 performed a detailed analysis of the mRS functional outcomes of the CVD studied. However, 4 studies (Alexander Woywodt et al. 2012; Kamini Rakkar et al. 2023; Joan Martí-Fàbregas et al. 2013 and Joan Martí-Fàbregas et al. 2015) did not define the absolute values of EPCs in the prognostic groups, so they were not included in the statistical analysis. An inverse relationship was defined between the levels of EPCs and functional outcomes when the Std. Mean Difference > 0 (higher levels of EPCs are associated with

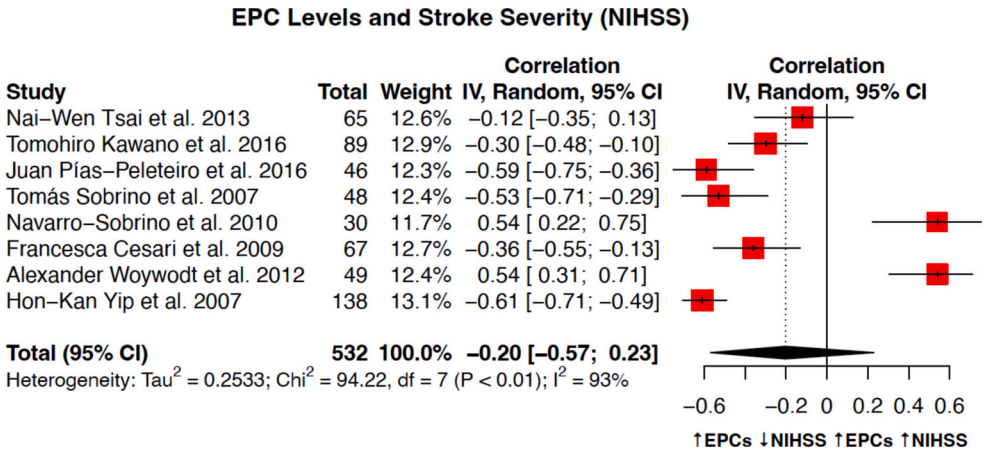

Fig. 2. Random effects model forest plot showing the relationship between EPC levels and stroke severity as measured using the NIHSS score. Higher EPC levels were associated with lower NIHHS stroke severity when a negative correlation was found.

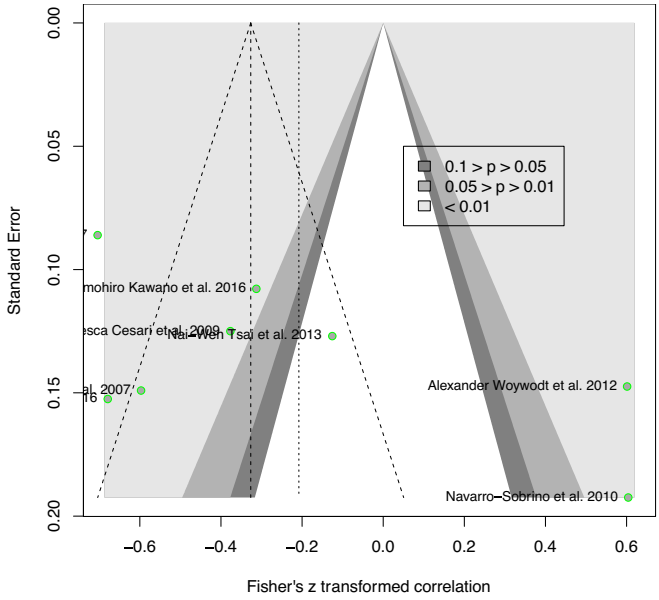

Fig. 3. Funnel plot showing considerable heterogeneity among studies examining the relationship between EPC levels and stroke severity as measured using the NIHSS score.

lower mRS) and a direct relationship when the Std. Mean Difference < 0 (higher levels of EPCs are associated with higher mRS).

The overall pooled Std. Mean Difference for lower functional outcome of CVD (mRS) with higher quantification of EPCs was 4,51 (0,70; 8,33) in the random effects model (Fig. 4).

4. Discussion

EPCs may play a critical role in the pathophysiology of ischemic stroke (IS) by promoting the restoration of endothelial and blood-brain barrier dysfunction, enhancing angiogenesis, and contributing to vasculogenesis through the formation of collateral vessels. These functions suggest that EPCs could serve as biomarkers of favorable prognosis in IS, as described by Dunac et al. (2006), Sobrino et al. (2007), and Yip et al. (2007). However, other studies present conflicting results. Martí-Fàbregas et al. (2013) found no association between EPC quantification and overall patient outcomes, although better functional outcomes were observed in patients with large artery atherosclerosis (LAA) stroke and higher EPC levels. This variability in findings may be influenced by the different stroke subtypes, as some studies suggest higher EPC levels in small vessel strokes (Woywodt et al., 2012; Tsai et al., 2013), while others, like Chu et al. (2008), report higher EPC levels in cardioembolic strokes.

Several studies have examined the relationship between EPC levels and clinical outcomes in IS. Most studies support a positive correlation between higher EPC levels and improved functional recovery, with

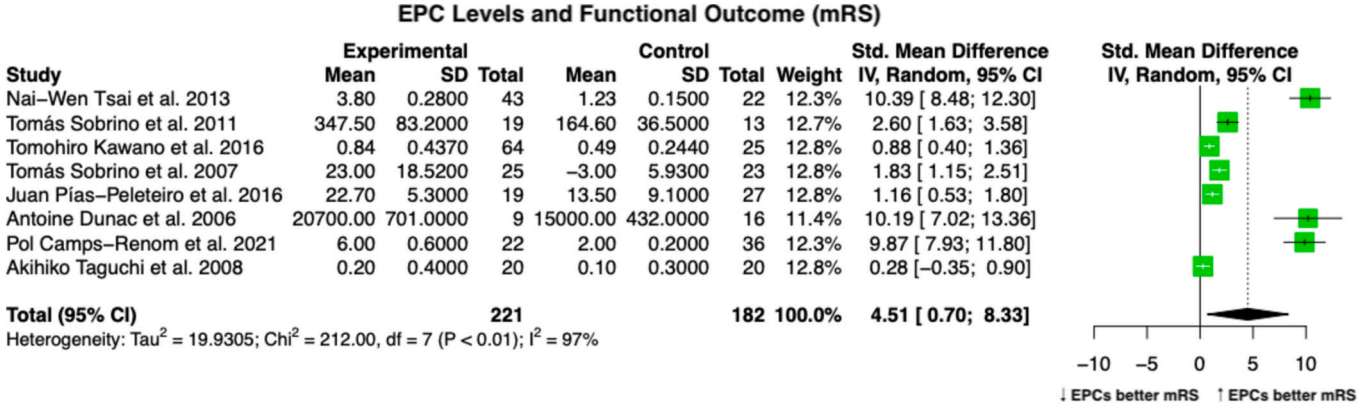

Fig. 4. Forest plot showing the relationship between EPC levels and stroke functional outcome as measured by the mRS. An inverse relationship was defined between EPC levels and functional outcome when the Std. Mean Difference > 0 (higher levels of EPCs are associated with lower mRS) and a direct relationship when the Std. Mean Difference < 0 (higher levels of EPCs are associated with higher mRS).

lower NIHSS and mRS scores at follow-up (Yip et al., 2007; Kawano et al., 2016; Camps-Renom et al., 2021). In contrast, some studies, such as Rakkar et al. (2023) and Martí-Fàbregas et al. (2013), found no significant association between EPC levels and clinical outcomes, emphasizing the need for caution when interpreting these results. The timing of cell mobilization may be a key factor, as studies have shown that the acute phase of stroke triggers a compensatory mechanism of EPC mobilization, potentially influencing recovery (Sepp et al., 2014; Deng et al., 2017).

Meta-analyses on stroke severity (NIHSS) and functional outcomes (mRS) across the included studies reveal mixed findings. For severity, some studies (Chu et al., 2008; Navarro-Sobrino et al., 2010) report a compensatory increase in EPC mobilization in more severe strokes, suggesting a complex relationship between EPC levels and stroke severity. However, most studies indicate a protective role of EPCs, with higher levels associated with better functional outcomes (Sobrino et al., 2011; Dunac et al., 2006). Additionally, the temporal dynamics of EPC mobilization after IS vary, with some studies indicating a peak at seven days (Sobrino et al., 2011; Martí-Fàbregas et al., 2013), while others suggest intermittent release (Dunac et al., 2006).

The current evidence linking EPC quantification with stroke severity and recovery has several limitations. These include heterogeneity in study design, small and variable sample sizes, differences in measurement techniques (*e.g.*, flow cytometry *vs.* cell culture), and inconsistent inclusion of stroke subtypes. Additionally, while many studies focus on IS, few have examined transient ischemic attacks (TIA) or hemorrhagic stroke (HS). These factors limit the generalizability of the findings, and further research with larger, more consistent designs is needed to clarify the role of EPCs in stroke prognosis.

Therefore, the heterogeneity observed across the included studies is a crucial factor to address. It would have been ideal to conduct a meta-regression and a multi-trait meta-analysis to boost the power of the meta-analysis and explore the potential influence of covariates such as study design, methods of EPC quantification, mean age, percentage of male patients, sample size, and other study-specific factors. Meta-regression and multi-trait meta-analysis were not performed for the following reasons:

1. Most original studies did not provide detailed data on these covariates, limiting our ability to analyze their influence systematically.
2. The relatively small number of included studies reduces the statistical power required to perform a robust meta-regression analysis.
3. The inherent heterogeneity of study designs and the variability in outcome measures across studies.
4. Differences in study methodologies, timing of blood sample collection, and methods of measuring EPCs. For example, while most studies used flow cytometry, others employed cell culture techniques, making direct comparisons difficult.
5. A significant variability was found in outcome reporting across the studies, which hindered the possibility of pooling data for multivariate analysis. Therefore, we performed subgroup analyses only when applicable, focusing on more homogeneous outcome measures, such as the NIHSS and mRS scores.

Despite this, we have considered heterogeneity by applying a random-effects model to our meta-analysis, which better accommodates the variability between studies.

5. Summary and conclusions

The following conclusions can be drawn from this SR, which attempts to respond to the aims stated previously.

1. The role of EPCs in vascular repair processes in patients with cerebrovascular disease (CVD) has been suggested in several studies, though the mechanisms are not yet fully understood. While evidence supports their involvement in endothelial repair, further research is needed to clarify their exact contribution to stroke recovery.
2. Increased quantification and proangiogenic properties of these cells are associated with improved outcomes in some studies, suggesting a potential role in the restoration of endothelial and blood-brain barrier dysfunction or damage. However, the exact mechanisms by which EPCs influence angiogenesis and vasculogenesis remain to be fully elucidated, and more research is required to confirm these findings.
3. EPCs and CPCs are predictive biomarkers for better clinical and functional outcomes in patients with IS. Their quantification and angiogenic properties correlate with the severity and functional outcome of patients with CVD, as measured by the NIHSS and mRS scales.

CRediT authorship contribution statement

Juan Francisco García Granado: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Francisco Javier Rodríguez Esparragón:** Investigation, Formal analysis, Conceptualization. **Jesús María González Martín:** Visualization, Supervision, Methodology. **Sara E. Cazorla Rivero:** Visualization, Supervision, Methodology. **Ayoze Nauzet González Hernández:** Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization.

Registration and protocol

This SR has not been registered nor has a specific protocol been drafted.

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Declaration of competing interest

The authors of this SR declare that they have no conflicts of interest.

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