Published in final edited form as:

Stroke. 2016 June ; 47(6): 1632–1639. doi:10.1161/STROKEAHA.116.012701.

A Systematic Review and Meta-Analysis of Bone Marrow Derived Mononuclear Cells in Animal Models of Ischemic Stroke

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Abstract

Background and Purpose—Bone marrow derived mononuclear cells (BMMNCs) offer the promise of augmenting post-stroke recovery. There is mounting evidence of safety and efficacy of BMMNCs from pre-clinical studies of ischemic stroke (IS), however their pooled effects have not been described.

Methods—Using PRIMSA guidelines, we conducted a systematic review of pre-clinical literature for intravenous use of BMMNCs followed by meta-analyses of histological and behavioral outcomes. Studies were selected based on pre-defined criteria. Data were abstracted by two independent investigators. Following quality assessment, the pooled effects were generated using mixed effect models. Impact of possible biases on estimated effect size was evaluated.

Results—Standardized mean difference (SMD), 95% confidence interval (CI) for reduction in lesion volume was significantly beneficial for BMMNC treatment (SMD −3.3, 95% CI: −4.3, −2.3), n = 113 each for BMMNC and controls. BMMNC treated animals (n = 161) also had improved function measured by cylinder test (SMD −2.4, 95% CI: −3.1, −1.6), as compared to controls (n = 205). A trend for benefit was observed for adhesive removal test and neurological deficit score. Study quality score (median: 6, Q1-Q3: 5-7) was correlated with year of publication. There was funnel plot asymmetry, however the pooled effects were robust to the correction of this bias and remained significant in favor of BMMNC treatment.

Conclusions—BMMNCs demonstrate beneficial effects across histological and behavioral outcomes in animal IS models. Though study quality has improved over time, considerable degree of heterogeneity calls for standardization in the conduct and reporting of experimentation.

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Disclosures: SIS is a site investigator for industry clinical trials by SanBio, Let's Cure CP, Mission Connect, Athersys, and Aldagen. SIS is consultant for Neural Stem, Mesoblast, ReNeuron, and San Bio (all funding goes to UT Health).

Keywords

Stroke; Bone Marrow Cells; Animal Experimentation

Introduction

Stroke imposes tremendous mortality and morbidity burden.[1] Despite the established benefit of intravenous tissue plasminogen activator (IV rtPA), it is estimated that only about 7% of ischemic stroke (IS) patients receive IV rtPA in the US,[2] and intra-arterial therapy is beneficial in only a selected subset of IS patients.[3] Cellular therapy is another investigative modality that offers considerable hope and promise to promote post stroke recovery.[4]

A number of cell types have been investigated in pre-clinical studies and in clinical trials. Bone marrow derived mono-nuclear cells (BMMNCs) are a heterogeneous group of cells consisting of varying proportions of differentially matured B-cells, T-cells, monocytes, as well as a smaller proportion of progenitor cells such as hematopoietic stem cells, mesenchymal stem cells (MSCs), endothelial progenitor cells, and very small embryonic-like cells. The relative ease of processing, potential for intravenous (IV) or intra-arterial (IA) administration, and opportunity of an autologous harvest make them an attractive option for pre-clinical testing and clinical applications.

The evidence of beneficial effect of BMMNCs in animal models of IS has been mounting over the past decade. It has been demonstrated that they lead to a reduction in ischemic lesion volume and improvement in behavioral outcomes.[5-9] There is evidence that BMMNCs cross the blood brain barrier,[10] exert neuro-protective effects,[11, 12] and lead to post-ischemic angiogenesis and neurogenesis.[13-15] It has also been demonstrated that IS may lead to activation of BMMNCs resulting in paracrine mediated modulation of post-stroke inflammatory responses.[16]

The growing evidence of safety and benefit of BMMNCs in pre-clinical models of IS has led to initial clinical testing of these cells by different investigators.[17-28] Despite testing in pre-clinical models and application in the clinical milieu, there are a number of unanswered questions regarding the use of BMMNCs in IS patients pertaining to dose, timing, route of administration and autologous vs. allogeneic approach. It is therefore important to study the pooled treatment effects of BMMNCs in relevant pre-clinical models of IS and explore sources of heterogeneity. We therefore aimed to conduct a systematic review and meta-analysis of BMMNCs in animal models of IS.

Methods

The protocol was developed based on Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines.[29] It was approved by all authors and an external member. For detailed protocol, methods, and PRISMA checklist please see http://stroke.ahajournals.org.

Study selection

Studies were included if they described experiments exclusively on IV administration of autologous, allogeneic, or xenogeneic BMMNCs for pre-clinical models of focal cerebral ischemia in mice and rats.

Search Strategy

We conducted search for literature in MEDLINE, PUBMED, EMBASE, SCOPUS, Cumulative Index of Nursing and Allied Health Literature (CINAHL), and Animal Welfare Information Center (AWIC) databases. Elements of the research question were divided into search components (SC), and searched separately followed by combination of SCs. The search results were documented at each step to ensure repeatability. The abstracts were reviewed by hand for relevance, and studies were excluded based on pre-defined criteria.

Data Extraction and Quality Assessment

Data were extracted by two independent abstractors and entered electronically. One abstractor was blinded to the journal, title, and the authors. For articles reporting data only as figures, quantitative methods were used as described in the protocol (https://stroke.ahajournal.org). Each selected study was assessed for quality based on published standards.[30]

Statistical Analysis

Study characteristics are provided using descriptive analyses. Effects sizes i.e. improvement in outcome for BM MNC treated animals relative to the control group, and were calculated using Hedges' G.[31] Heterogeneity was quantified using the I^2 statistic, and weights were assigned using mixed effect models. Sources of heterogeneity were explored by meta-regression.[32] Publication and / or selection bias was evaluated using funnel plots,[33] and symmetry was formally tested using the Egger test.[34] Trim and fill approach was used to correct for funnel plot asymmetry.[35] Robustness of estimates to the effect of potentially missed or negative studies was evaluated using Fail-Safe N approach.[36, 37] Alpha of 0.05 was used for statistical testing, and analyses were performed using STATA 13 and Comprehensive Meta-Analysis.

Results

Study Characteristics

Initial search generated a total of 399 records. Figure 1 illustrates the review process leading to finally selected 22 manuscripts; all published in peer-reviewed journals.[5-7, 9, 13, 38-54] An experiment within a study was considered independent if data for a separate control group were available. More than 90% of experiments were done on various species of rats, with 66.3% using allogeneic BMMNCs. The most commonly employed doses were 10 and 30 million cells/ kg in about 63% of the studies. In approximately 75% of experiments BMMNCs were injected within 24 hours of stroke onset. Table 1 summarizes characteristics of the included studies.

Outcome Measures

A total of 15 outcomes were identified from included studies and relevant data were abstracted. Five outcomes were measured in 77% of experiments. These 'Major Outcomes', and number of animals in control / experimental groups for pooled analyses are: stroke lesion size absolute reduction (n = 113/113) and relative reduction (n = 83/66), cylinder test (n = 161/205), adhesive removal by use of paralyzed limb (n = 69/62) and by time to removal (n = 67/49), neurological deficit score (NDS) (n = 74/74), and modified neurological deficit score (mNDS) (n = 48/48). For details on major and other outcomes please see https://stroke.ahajournals.org.

Pooled estimates

The BMMNC treated animals had significantly reduced stroke lesion volume and enhanced recovery of sensorimotor modalities as measured by cylinder test, adhesive removal test, and NDS. Standardized mean difference (SMD) and 95% confidence interval along with number of animals in the control and intervention group for each of the five major outcomes are summarized in table 2. The corresponding forest plots for lesion size and cylinder test are show in Figures 2a/2b, and 3. Forest plots for other major outcomes are included in the online supplement, please see https://stroke.ahajournals.org.

Exploration of heterogeneity and meta-regression

The pooled estimated for included experiments in all meta-analyses exhibited considerable degree of heterogeneity (I^2 values > 70% for all analyses). Univariate meta-regression was conducted to study the effect of dose, timing, and study quality on observed heterogeneity for lesion volume and cylinder test. No significant effects were observed.

Study Quality

The median (Q1,Q3) quality score for was 6 (5-7) and the range was 4 – 10. The experimental quality criteria that were least adhered to were reporting of power and sample size calculations, use of animal models with relevant comorbidities, and reporting of allocation concealment procedures. The coefficient of meta-regression for study quality with effect size for lesion volume was 1.44 (p = 0.06), and there was a statistically significant correlation between study quality and year of publication (p = 0.03). Only six (27.2%) published articles directly or in-directly reported details on immunophenotyping of BMMNCs.

Assessment of bias and sensitivity analysis

Funnel plots for effect size of BMMNCs as measured by lesion size and cylinder test were asymmetric (p < 0.001 for both). However, the pooled effect size under the random effects model remained statistically significant in favor of BMMNCs for lesion volume (SMD: −2.03, 95% CI: −3.48, −1.06) and cylinder test (SMD: −1.24, 95% CI: −2.09, −0.39) after the trim and fill procedure (Figure 4a/4b). The classic Fail-Safe N analysis yielded the lesion volume and cylinder test effect size of BMMNCs to be robust against 748 and 846 potentially missed null studies, respectively. Furthermore, the Orwin Fail-Safe N analysis

indicated combined effect sizes to rise above −0.5 if 19 and 12 studies are added respectively to lesion volume and cylinder test analyses with SMD of 1.

Discussion

In the rapidly evolving field of cellular therapy for IS, there are a number of un-answered questions with respect to the choice of cell type, timing, route of administration, safe and effective dose, and the purported mechanism of action. As the evidence generated from pre-clinical studies forms the basis for designing clinical trials, it is important to explore the pooled effects of animal studies, and investigate the various sources of heterogeneity. Prior reviews have either pooled results for a number of neurological disorders,[55] or have included multiple different cell types for IS.[56] Other reviews have focused solely on MSCs manufactured from various tissues.[57] Some of these studies did not generate an effect size or analyze study quality,[58] whereas others pooled results by including various routes of delivery.[57] To our knowledge, this is the first systematic review and meta-analysis of BMMNCs in experimental stroke models. The aim was to focus on bone marrow MNCs, administered solely via IV delivery, in a clearly defined disease model of small animal focal cerebral ischemia – while examining study quality, and pooling estimates of most commonly and homogenously measured outcomes.

We employed a comprehensive search and robust data assimilation procedure. For the 22 studies that were finally selected, 15 different outcomes were analyzed. A number of behavioral tests in pre-clinical models of stroke have been reviewed in the literature.[59] Meta-analyses were only performed for outcomes that were consistent in measurement and reporting. The pooling procedures employed and outcome reported were similar to other meta-analyses.[57]

Based on arbitrarily defined quantification of effect size,[60] our observed effect seizes for beneficial effect of BMMNCs on histological and behavioral outcomes were very large (between −3.3 and −1.04). All estimates other than modified neurological severity score and time to adhesive removal were statistically significant. The number of animals included for these two outcomes in the pooled analyses were small; it is therefore possible that lack of statistical significance for these end-points is a function of small sample size. Though methodological differences do not permit a direct comparison with previously conducted meta-analyses, a prior meta-analysis has reported similar favorable effect sizes for MSCs in IS models for modified neurological severity scale and adhesive removal test respectively.[57] Also, another meta-analysis that included multiple cell types, reported a comparable SMD for reduction in infarct lesion size in stem cell treated animals.[56] We therefore believe that observing large beneficial effect sizes in pre-clinical pooled data is not unique to our analysis.

Study quality was assessed using Stroke Therapy Academic Industry Roundtable (STAIR) recommended objective scoring ciretira.[30] The importance of assessing study quality has been repeatedly emphasized, and a prior review of MSCs reported a positive correlation between effect size and study quality.[57] Meta-regression yielded a similar trend in our analysis, showing a 44% increase in effect size for one point score increase in study quality

(p = 0.06). All included studies were published within the last 10 years, (95% and 77% during the last 7 and 4 years, respectively). We also noted a statistically significant correlation between study quality and year of publication. This result may be indicative of better implementation of and adherence to quality standards over time. The quality criteria that were not addressed in most studies were sample size / power calculations, concealment of allocation, and testing of animals with relevant comorbidities. Lack of sample size justification in pre-clinical experimentation in neuroscience is prevalent, and attention has been drawn to its detrimental influence on overestimation of effect size.[61] Standardization in experimentation and measurement, along with development of data repositories for pre-clinical disease models may provide these estimates for investigators. Allocation concealment is necessary to minimize selection bias, and lack thereof is another factor potentially leading to exaggeration of treatment effects.[62] The importance of using disease specific animal models was emphasized in various STAIR publications, and is regarded by some as necessary for any successful translation of a purported new therapy for IS.[63]

We recognize that our results are not immune to publication and small study effect biases. We used funnel plots to examine the possibility of these biases, and observed considerable asymmetry resulting from lack of null or negative studies. This asymmetry was also quantified using Egger's test which was found to be statistically significant. We made corrections for apparent asymmetry of the funnel plots, using trim and fill approach, and found that our corrected estimates, though reduced in magnitude of effect, remained statistically significant in favor of BMMNC therapy. We further explored the sensitivity of our estimates to the effect of addition of non-significant studies, and found that a considerably large number of null or negative studies would need to be added to make our estimates statistically not significant. We are also limited by a relatively small number of studies compared with other meta-analyses that fit the specific inclusion criteria. We chose to be specific in our search criteria in order to describe the effects of a specific type of cell therapy in a relevant pre-clinical model using an intravenous delivery. Despite these restrictive selection criteria, a considerable degree of heterogeneity in estimates was observed. We performed univariate meta-regression to study the possible effects of measured variables on effect sizes but did not find any significance. A possible reason could have been a small number of experiments per each outcome. Having fewer studies has also resulted in a relatively small number of animals in experimental and control groups for our pooled analyses. We acknowledge the impact of small sample size on pooled estimates, as has been discussed in literature.[64]

Our results indicate the IV BMMNCs have significantly beneficial pooled effects on IS lesion size, the cylinder test, the adhesive removal test (as measured by proportional use of the paralytic limb), and neurological deficit score in experimental models of IS. These behavioral tests indicate that BMMNCs carry the potential to improve both modality-specific limb function and overall neurological outcome on a composite score. Estimated effects seem large but are overall robust to potential biases. Compared to other cell therapies, BMMNCs have similar effect sizes and carry the advantage that they can be prepared from patients and re-administered intravenously in more acute time windows after stroke. However, there is a considerable degree of unexplained heterogeneity within experiments despite using restrictive inclusion criteria for study selection. Although the overall study

quality has significantly improved over time, standardization of conduct and measurement of pre-clinical experimentation for various structural and behavioral outcomes of cerebral ischemia may be an important focus area for experts in the field.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding: SIS is supported by NIH R01 NS071127.

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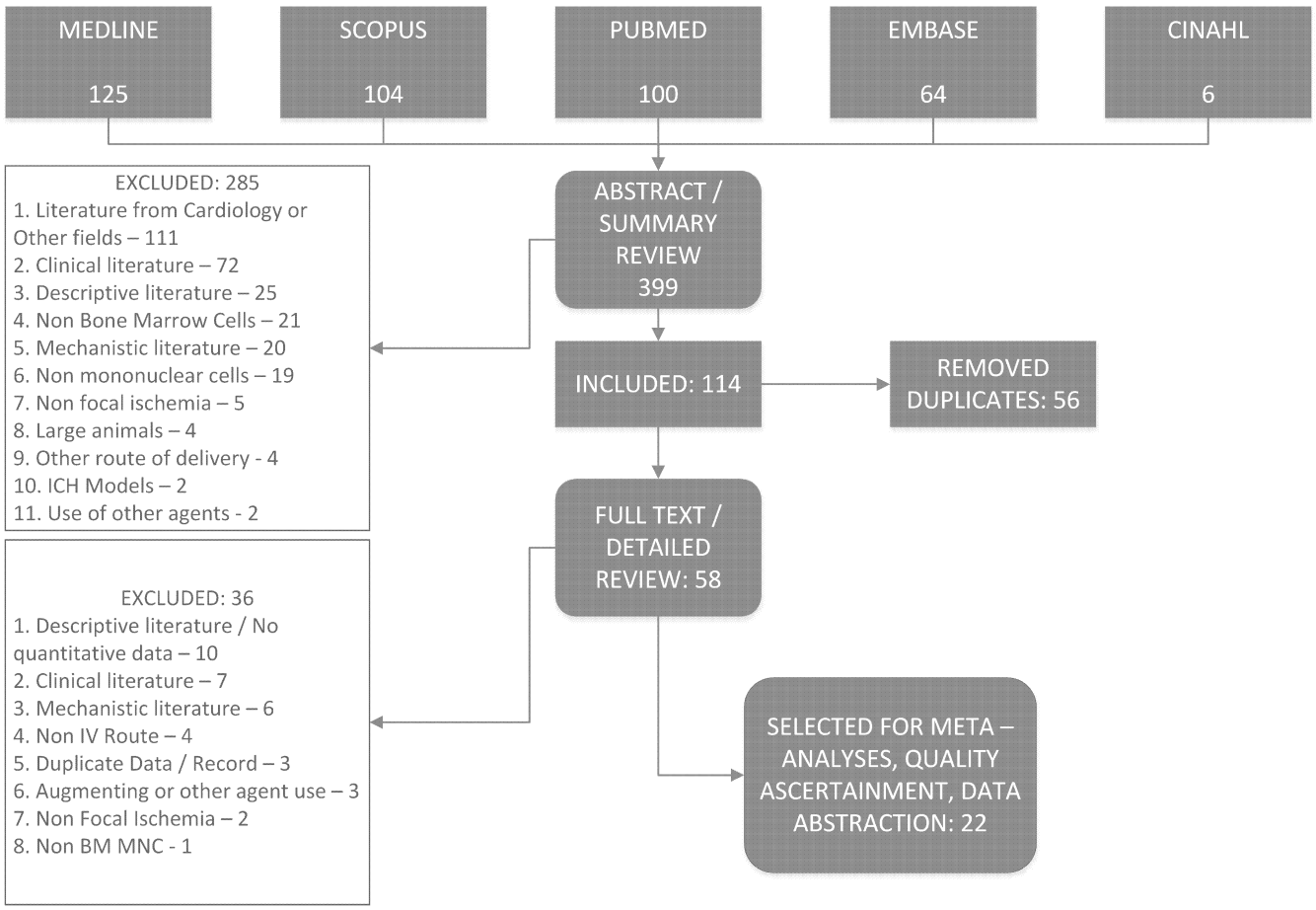

Figure 1.

PRISMA flow diagram for review and selection process of studies included in systematic review and meta-analyses of BM MNCs in animal models of cerebral ischemia. The number of search results at each stage of selection along with reasons for exclusion are documented.

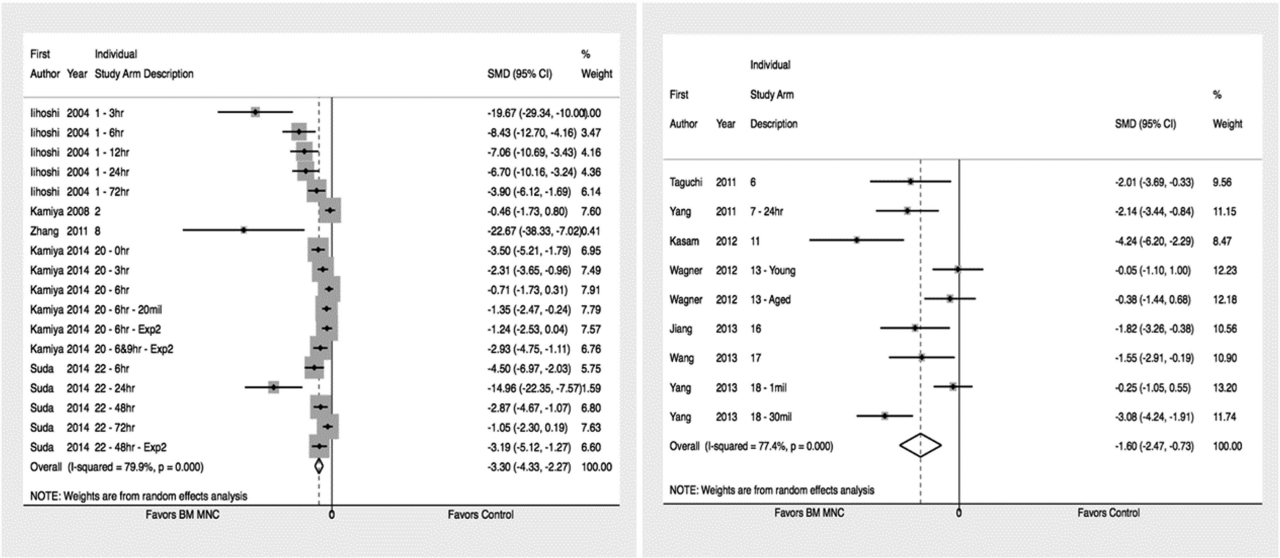

Figure 2a and 2b.

Forrest plot for effect size for IV BM MNCs on absolute reduction (figure 2a) and relative change to the non-infarct side (figure 2b). Weights have been calculated using random effects model. Degree of heterogeneity in the pooled estimates is represented at I^2 statistic. The studies included in meta-analysis of absolute[5,6,41,54] and relative[39,40,44,46,49,50] decrease in infarct size are cited.

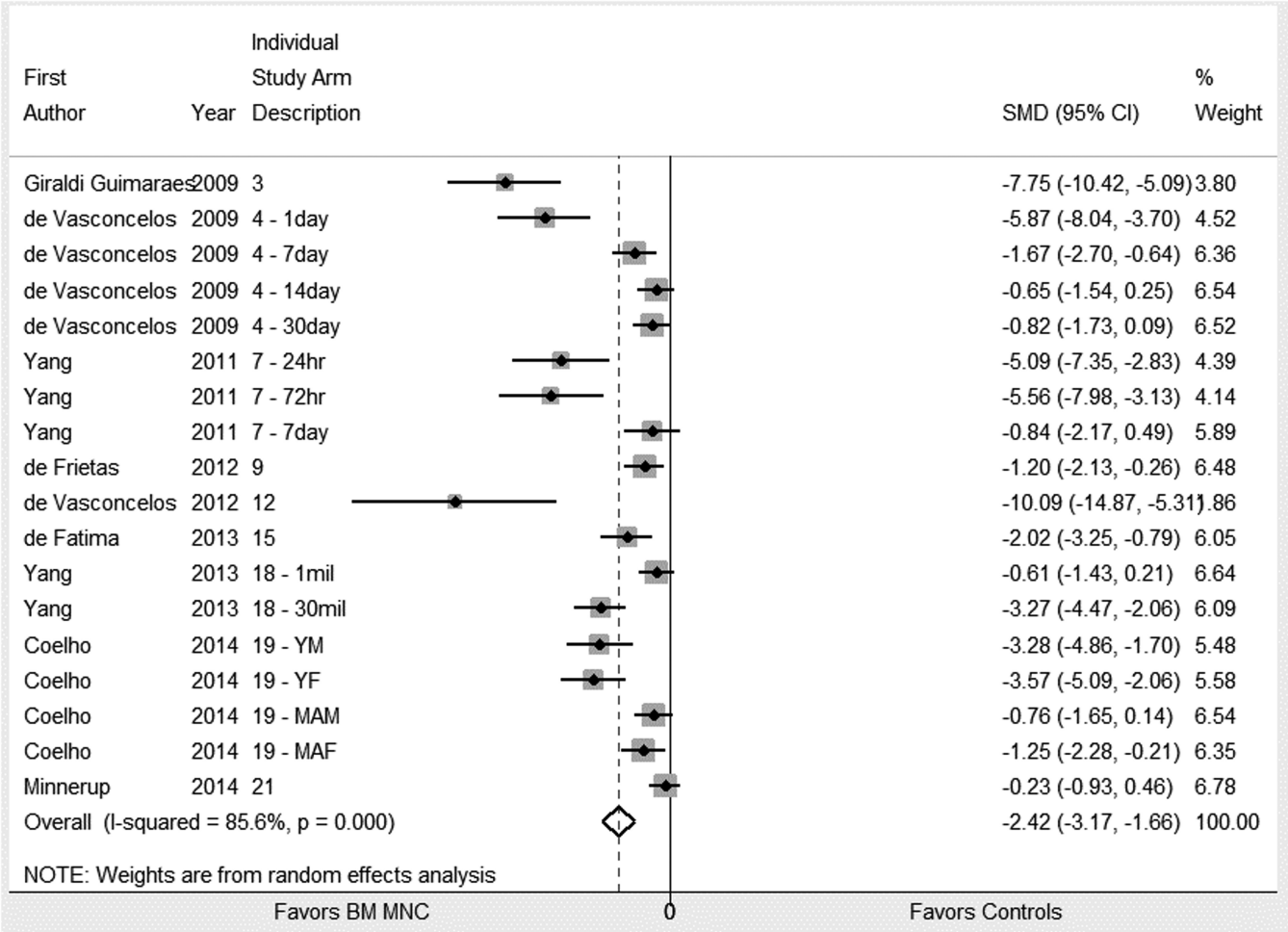

Figure 3.

Forrest plot for effect size for IV BM MNCs on cylinder test. Weights have been calculated using random effects model. Degree of heterogeneity in the pooled estimates is represented at I^2 statistic. The studies included in the meta-analysis for effect of IV BMMNC on cylinder test are cited.[7,38,40,42,45,48,51-53]

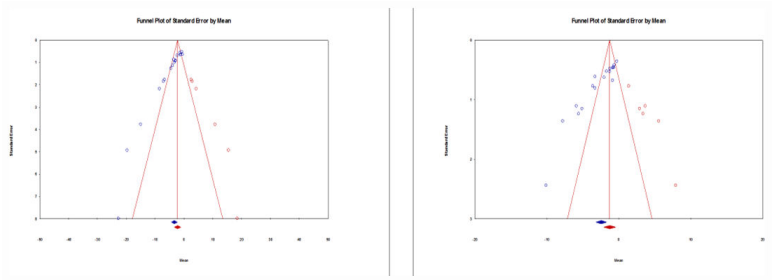

Figure 4a / 4b.

Funnel plot with standardized mean (X Axis) and standard error (Y Axis) for studies included in meta-analysis for absolute reduction in lesion size (4a) and cylinder test (4b). The bubbles in blue are estimates from actual studies, whereas the bubbles in red are hypothetical studies included during the trim and fill approach to correct for asymmetry of the funnel plot. The diamonds below the X Axis represent actual estimates of effect (blue) and correct estimates (red) after trim and fill. Null value is represented by Zero on the X Axis.

Table 1

Summary characteristics of the 22 studies included in meta-analysis

Characteristics	Summary Data
Study Characteristics	
Year of publication – n (%)	
2004 – 2008	2 (9.1)
2009 – 2010	3 (13.6)
2011 – 2012	8 (36.4)
2013 – 2014	9 (40.9)
Journal Impact Factor – median (Q1 – Q3)	2.96 (2.54 – 4.13)
Quality Score – median (Q1 – Q3)	6 (5 – 7)
Presence of additional non IV BM MNC arms	13 (59.1)
Animal Characteristics	
Animal Type – n (%)	
Rats	20 (90.9)
Mice	2 (9.1)
Animal Species / Type – n (%)	
Rats (n = 20)	
Wistar	8 (40.0)
Sprague-Dawley	6 (30.0)
Long Evans	3 (15.0)
SHR[*] / SHR – SP[†]	3 (15.0)
Mice (n = 2)	
SCID[‡]	1 (50.0)
BALB / c[§]	1 (50.0)
Animal Gender – n (%)	
Male	19 (86.4)
Female	1 (4.6)
Both	1 (4.6)
Not specified	1 (4.6)
Animal Weight Categories – n (%)	
18 – 20 gr	1 (4.6)
220 – 450 gr	15 (68.2)
600 – 800 gr	1 (4.6)
Not specified	5 (22.7)
Cell Characteristics	
Cell source – n (%)	
Allogeneic	14 (63.3)
Autologous	7 (31.8)

Characteristics	Summary Data
Study Characteristics	
Human	1 (4.6)
Bone Marrow Harvest (autologous cell source) – n (%)	
After experimental stroke	5 (71.4)
Before experimental stroke	2 (28.5)
Cell Dose (n = 24, more than 1 experiment / study included)	
30 million cells	7 (29.2)
20 million cells	1 (4.2)
10 million cells	8 (33.3)
8 million cells	1 (4.2)
5 million cells	3 (12.5)
3 million cells	1 (4.2)
1 million cells	3 (12.5)
Timing (n = 37, more than 1 experiment / study included)	
≤ 12 hours	11 (29.7)
24 hours	17 (45.9)
48 hours	2 (5.4)
72 hours	3 (8.1)
> 72 hours	4 (10.8)
Site of delivery - n (%)	
Femoral Vein	7 (31.8)
Tail Vein	7 (31.8)
Jugular	5 (22.7)
Not specified	3 (13.6)
Stroke type Characteristics	
Mechanism of ischemia - n (%)	
MCAO// - Intraluminal Occlusion	8 (36.4)
MCAO - Coagulation / Ligation	6 (27.3)
Thermocoagulation	5 (22.7)
Vasoconstrictor Peptide	2 (9.1)
Cortical Ablation	1 (4.6)
Type of ischemia - n (%)	
Permanent	14 (63.6)
Transient	8 (36.4)
Duration of transient ischemia (n = 8)	
180 minutes	2 (25.0)
90 minutes	4 (50.0)
60 minutes	1 (12.5)
45 minutes	1 (12.5)

*SHR: Spontaneously Hypertensive Rats.

[†] SHR - SP: Spontaneously Hypertensive Rats -Stroke Prone.

[‡] SCID: Severe combined immunodeficiency.

[§] BALB/c: Bagg Albino (inbred research mouse strain).

[//] MCAO: Middle Cerebral Artery Occlusion

Table 2

Pooled estimates from meta-analysis of major outcomes

Outcome	Number of animals Control / Intervention	Pooled SMD (95% CI)	P value
Lesion Size			
Absolute Reduction	113 / 113	−3.3 (−4.33, −2.27) *	< 0.001
Percent Reduction	83 / 66	−1.6 (−2.47, −0.73) *	< 0.001
Cylinder Test	161 / 205	−2.42 (−3.17, −1.66) *	< 0.001
Adhesive Removal Test			
Use of paralyzed limb	69 / 62	1.17 (0.51, 1.84) *	0.001
Time to adhesive removal	67 / 49	−1.96 (−4.48, 0.56)	0.13
Neurological Deficit Score	74 / 74	−1.04 (−1.8, −0.27) *	0.008
Modified Neurological Deficit Score	48 / 48	−1.6 (−3.38, 0.18)	0.078

SMD: Standardized Mean Difference

* SMD shows significantly favorable effect of BMMNC treatment