Developing Clinical Guidelines to Treat Stroke with Stem Cells Part I: Acute and Sub Acute Ischemic Stroke

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Abstract

The evolution of stem cell-based therapies has been exponential in recent years, going from animal models to human clinical trials in a relatively short period of time. The field has also changed the rationales about the main mechanisms of action. While stem cell-based therapies were previously thought to be "just" the replacement of damaged cells, more complex actions have been discovered in the meantime, including paracrine effects, activation of local stem cells towards trans differentiating, neuro protection of local tissues and a systemic and local neuro-immuno modulation. Furthermore, additional methods to repair genetic defects or improve autologous stem cells with relatively and easy techniques are revolutionizing the field. This means that we are currently experiencing how stem cell-based therapies are increasingly employed to develop curative treatments.

A detailed analysis of the American Heart Association's guidelines for the treatment of ischemic stroke, which are the currently most widely accepted current clinical guidelines, will also be provided to address and describe the scientific evidence on which these guidelines are based.

We review the most relevant literature concerning the use of stem cells to treat stroke patients. In this paper, constituting part I out of III, we will specifically focus on acute and sub acute ischemic stroke. This literature review will provide key information on how stem cells' interventions where performed with the analysis of the level of evidence they are providing according to the Oxford Centre of Evidence Guidelines and according to the American Heart Association's adaptation of levels of evidence and classes of recommendations.

Drawing on the gathered information we will then compare the scientific evidence that underlies the current treatment guidelines with the scientific evidence on currently available stem cells publications.

We found that in general, the clinical guidelines for the treatment of ischemic stroke are not based on solid scientific evidence. As a matter of fact, just 2.2% of all the recommendations for ischemic stroke are Level A. Furthermore, we found that evidence of the use of stem cells have the same and sometimes higher level of evidence as the ones used for the guidelines, and in several cases stem cells treatment are associated with lower risks. As a result of this survey and analysis we conclude with a series of recommendations for the use of stem cells in clinical settings for acute and sub acute stroke that could be applied right now. We consider this publication as the basis to develop the first stem cells clinical guideline for a neurological disease, specifically for ischemic stroke likely to be followed by guidelines regarding other kinds of strokes and other neurological diseases. We encourage the consideration of these recommendations in future guidelines for treatment of ischemic stroke.

Keywords: Stroke; Ischemic stroke; Stem cells; Clinical guidelines

Introduction

Importance of evidence-based medicine

Certainly, what has enabled medicine to offer safer and better options for treating patients is the understanding and application of evidence-based medicine [1]. Over the centuries, and owing to great efforts, the field of medicine has changed from the Hippocratic-Galenic empiricism into a more evidence-based medicine that can systematically improve patients' health.

However, a field such as medicine should be always guided by common sense and the treatment of human beings should be based on ethical principles, even if this means that the highest standards of evidence-based medicine cannot be reached. In general, when investigating a new treatment, three main aspects have to be considered to avoid losing the focus:

A. If the release of new treatment is intended, risks and benefits have to be assessed.

B. If there are already treatments available, the new treatment has to be compared to the established ones. (Note: this applies to treatments available for the same objective,

i.e. we cannot compare the use of anticoagulation intended to limit the damage to a stem cell treatment that is intended not only to reduce the damage but also to re-establish functionality).

C.   The treatment should also offer a substantially higher efficacy compared to the currently accepted therapy.

When using stem cells to treat neurological disorders, a sui generis environment emerges, i.e. we are confronted with a very special configuration of factors and actors involved (e.g. scientists, legal regulations, patients, etc.). The main reason for this is that there is a lack of an efficacious therapy not just for ischemic stroke, but also for several other neurovascular and neurological diseases. The use of different treatments that could potentially risk the patient's integrity, as has been argued with SCs therapies in investigational stages, should both offer new treatment options and at the same time improve the development of new therapies. Considering that a treatment that palliates symptoms, as the majority of treatments of neurological diseases, is not even remotely comparable to a treatment intended to cure the disease, such as SCs, a higher risk should be allowed. Furthermore, the totally different goals of currently accepted palliative treatments and healing intentions of SCs make a proper comparison difficult, as normally the new treatments are compared to the gold standard of methods with the same treating goal. A further highlight is that several palliative pharmacological treatments available do not even have safer profiles than SCs, as they are associated with even higher side effects than is reported for SCs therapies. In brief, we are using several currently accepted treatments not intended to fully cure a patient, and hence, the risk-benefit principle is set aside.

We are aware that several inconsistencies exist in the traditional, evidence-based development of treatments including stem cell therapies, such as unpublished knowledge acquired through medical practice, unpublished negative effects of pharmaceutical compounds, large conflict of interests surrounding the development and use of new treatments, little empowerment of the patients and their clinicians regarding the decision for the best therapy, regulatory agencies designed to protect populations and not individuals, low education of clinicians and patients, etc. This topic is not within the scope of this publication. Nonetheless, having this problem in other fields of medicine and other fields of non-biological sciences is enough to understand that this should not be taken as an excuse to limit the development of stem cell therapies to medical practice, conversely the field of SCs could be used to identify new paths for research and development [2]. A recent evaluation of the Cochrane Database of Systematic Reviews found that the existing evidence was unable to support or refute 49% of medical interventions in [3].

We do not encourage that low levels of evidence support the medical practice, since this is often used as an argument against the acceptance of SCs treatments. If half of our practices are not based on substantial evidence and are inefficient and risky, it makes sense to bring new treatments into the field in a more effective way.

In a context where SCs therapies are considered such a controversial field, we decided to carefully present all data in strict accordance with the evidence-based medicine statutes. We even think that a possible 'evolution' of medical research should be adapted to the era of cellular treatments. Our analysis and the resulting recommendations are based on the available scientific evidence and an adapted risk-benefit analysis based on the assumption that a major risk may be taken if the potential benefits of using SCs therapies are significantly greater than the prospect of just palliating the patient's condition with traditional methods. As several reluctant colleagues argue that the use of SCs has a very high risk, it is important to understand that cellular products are not pharmaceutical compounds. This does not mean they do not have side effects, but that they have to be treated differently. Furthermore, in addition to the fact that SCs have lower risks than several other pharmaceutical compounds, all the treatments mentioned as recommendations have passed the preclinical and clinical phases regarding safety.

We therefore consider it necessary to adapt the clinical guidelines for treatments to the recent developments in evidence-based medicine in all fields of medicine including stem cells and possibly even in other, non-biological scientific fields. This of course first requires the adaptation of legal regulations allowing for better medical care.

We encourage the study and testing of any medical intervention, including SCs therapies, to be rooted in evidence-based medicine and to adhere to ethical codes. However, we also encourage the development of path ways to use this evidence to redefine our conceptualization of a gold-standard treatment.

Relevance of the analysis

After reviewing several current clinical guidelines, we found that less than 1% of all the recommendations for neurological diseases in the clinical guidelines are sustained by a level 1A of evidence (Systematic review of RCT with homogeneity, according to the Oxford Centre of Evidence) [4]. When reviewing the scientific evidence of stem cells treatment we found that several stem cell therapies are based on the same or even stronger scientific evidence than the current medical treatments supporting their application in the clinical setting. This means accepted treatments and stem cell treatments nearly have the same level of evidence. Although this evidence is far from level A, it is apparently enough for several clinicians and regulatory agencies to maintain a reluctant posture towards stem cell therapies but not towards other kinds of treatment.

We propose that not just level 1A of evidence should be accepted as treatment, especially in cases where there are no options for patients besides experimental treatments. In this debate, the necessity of RCT or blinded trials arises, especially because SCs therapies are a procedure rather than medication

and conducting clinical studies with "surgical placebos" is ethically controversial [5,6]. There are analyses that describe how series of observational studies found results similar to those obtained from for RCTs [7], which means that certain number of cohort or case-controls studies would yield enough

valuable information to bring treatments to the clinical practice, although we plan to wait for better-design RCT to confirm the results, if possible. In addition to this, a functional pathway of de-implementation of practices is necessary once there is more reliable evidence.
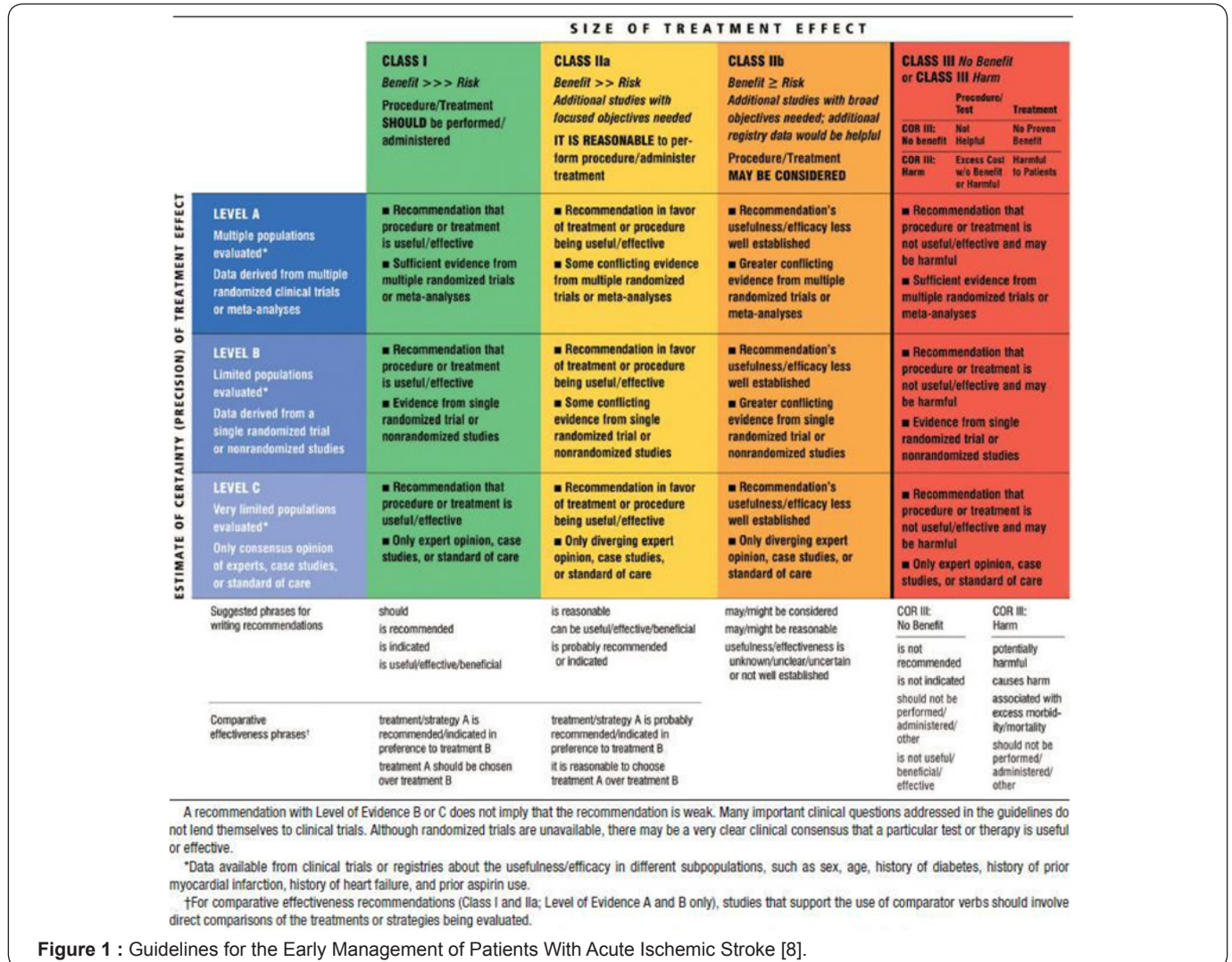
American Heart Association Clinical guidelines

Figure 1 : Guidelines for the Early Management of Patients With Acute Ischemic Stroke [8].

Ischemic stroke: The current management guidelines for acute ischemic stroke by the American heart association (AHA) [8] are widely accepted worldwide and are the ones considered for this survey. They contain certain sections such as public education, designation of stroke centres, stroke care quality improvements, pre-hospital management, emergency evaluation and diagnosis that are not within the scope of our analysis. Only those sections were analyzed that include supportive care and specific treatments which are intended to modify the course of the disease or limit the damage (intravenous fibrinolysis, endovascular interventions, anticoagulants, antiplatelet, volume management, neuro protective agents, surgical interventions and stroke care after hospitalization and treatment of complications). The panel of the AHA adapt the levels of evidence and the formulation and strength of recommendations (Figure

1) that would be used for comparison. The following classes are specified and levels are distinguished: Class I-should be performed, Class IIa-is reasonable to use, IIb– may be considered and class III- is harmful or has no benefit.

Level A-Multiple populations evaluated. Data derived from multiple randomized clinical trials or meta-analyses.

Level B-Limited populations evaluated with data derived from a single randomized trial or nonrandomized studies.

Level C-very limited populations evaluated. Only consensus opinion of experts, case studies, or standard of care.

The pre-hospital stroke management is intended to reduce the time between the initiation of symptoms and the treatment. Currently, SCs therapies are far from available in

pre-hospital care; nonetheless the implementation of SCs therapies as regular practice in the early hospital interventions will certainly contribute to widening the windows of treatment. A general overview of this section describes the current situation of evidence-based medicine in the guidelines. Out of six recommendations, just one is classified as level A and the other five are level B of evidence; however, all of them are recommended as Class I.

In terms of general supportive care (airway support, cardiac monitoring, blood pressure, etc.) the handling is similar to other acute events; therefore it is not considered in this review. Generally, in the section on supportive care, none of the 14 recommendations can be categorized as level A. Four of them are level B (recommending two as Class I, one as Class IIa and one as Class III) and the rest are level C, of which seven are Class I recommendations, two are Class IIa and one is Class IIb.

Intravenous fibrinolysis with rTPA should be performed (Class I-Level A) within 3 hours from the onset of symptoms and within 60 minutes door-to-needle time. Patients who are between 3 and 4.5 hours from the onset of symptoms should also be treated with IV rTPA (Class I - Level B). In both cases, a careful review of inclusion and exclusion criteria is necessary, with additional exclusion criteria in the latter case. Patients within 3 to 4.5 hours with one or more exclusion criteria may be considered (Class IIb-Level C). Specific conditions such as mild stroke deficits, rapidly improving stroke symptoms, recent surgery or recent myocardial infarction may also be considered as candidates (Class IIb-Level C). The use of sonothrombolysis is not well-established (Class IIb - Level B). The use of IV tenecteplase, reteplase, desmoteplase, urokinase and other fibrinolytic agents and IV of ancord or other defibrinogenating agents should only be used in clinical trial situations (Class IIb-Level B). The use of IV streptokinase for the treatment of stroke is not recommended (Class III-Level A). The use of IV rTPA in patients taking direct thrombin inhibitors or direct Xa inhibitors may be harmful and is not recommended (Class III-Level C).

Concerning endovascular therapies, the guidelines recommend that intra-arterial rTPA should be performed in carefully selected patients with major ischemic strokes within 6 hours from the onset of symptoms caused by occlusions of the MCA who are not otherwise candidates for IV rTPA (Class I-Level B). Mechanical Thrombectomy alone or in combination with fibrinolysis for recanalization can be useful in carefully selected patients (Class IIa-Level B), although their ability to improve patient outcomes has not yet been established. Intra-arterial fibrinolysis or mechanical thrombectomy are reasonable in patients who have contraindications to the use of IV fibrinolysis (Class IIa-Level C) or who suffer from large-artery occlusions that have not responded to the IV fibrinolysis as a rescue therapy (Class IIa-Level B). The usefulness of emergent intracranial or extracranial angioplasty and/or stenting is not well-established (Class IIb-Level C).

For anticoagulation treatments, the use of thrombin inhibitors such argatroban and urgent anticoagulation in severe stenos is of an internal carotid artery ipsilateral to an ischemic stroke are not well-established (Class IIb-Level B). The use of urgent anticoagulation to prevent recurrent stroke, halt neurological worsening or improve outcomes after acute ischemic stroke or management of non-cerebrovascular conditions in patients with moderate-to-severe strokes as well as anticoagulation therapy within 24 hours of treatment with IV rtPA are not recommended (Class III-Level A).

Regarding antiplatelet treatment, the use of Aspirin is recommended within 24 to 48 hours after the onset (Class I-Level A); however, this therapy is not recommended as an adjunctive treatment within 24 hours of IV fibrinolysis (Class III-Level C), as a substitute for other acute interventions for treatment of stroke, including rtPA; the use of other agents that inhibit the glycoprteoin IIb/IIIa receptor (Class III-Level B) is not recommended, either. The use of clopidogrel, IV tirofiban and eptifibatide is not well-established (Class IIb-Level C).

The use of vasopressors is recommended in cases where a systemic hypotension is producing neurological sequels (Class I - level C). The use of albumin for acute ischemic stroke is not well-established (Class IIb - Level B). The use of devices to augment cerebral blood flow and drug-induced hypertension is not common (Level B-Class IIb) and recommended exclusively for the use in clinical trials. Hemodilution and vasodilatory agents such as pentoxifylline are not recommended (Class III-Level A).

In the case of neuroprotective strategies, the continuation of statins is recommended as reasonable treatment (Class IIa-Level B). The use of hypothermia or trans cranial near-infrared laser therapy is not common (Class IIb

– Level B). No pharmacological agents with putative neuroprotective actions have shown to improve outcomes after stroke and are therefore not recommended (Class III-Level A). The use of hyperbaric oxygen may be harmful and is not recommended except for stroke secondary to air embolization (Class III-Evidence B).

The efficacy of surgical interventions such as urgent or emergent carotid endarterectomy is not acknowledged (Class IIb-Level B).

In terms of intra hospital care after the first intervention, admission to the stroke unit and immediate rehabilitation are recommended (Class I-Level A). Antibiotics for suspected pneumonia and anticoagulants should be prescribed (Class I-Level A). The use of standardized stroke care order sets, the assessment of swallowing and, if necessary, the use of feeding devices are also recommended (Class I-Level B), and an NG tube is preferred in the first 2 to 3 weeks after the event (Class IIa-Level B). The early mobilization of less severely affected patients, treatment of concomitant medical diseases and early preventive

measures against recurrent strokes should be performed (Class I-Level C). Other general care measurements such as Aspirin for patients that cannot receive anticoagulants to treat DVT (Class IIa - Level A) and intermittent compression devices (Class IIa - Level B) are reasonable to use. Nutrition supplements (Level B), prophylactic antibiotics (Level B) and bladder catheters (Level C) have no benefits (Class III). Routine placement of bladder catheters is not recommended (Class III-Level C).

Acute complications occur and are consequently treated in 25% of the patients and a close measurement of intracranial pressure and neurological monitoring during the first days are recommended (Class I-level A). De compressive surgical evacuation for space-occupying cerebellar infraction and malignant oedema of the cerebral hemisphere should be carried out (Class I-Level B). Acute seizures should be treated with standard procedures as applied in other acute situations (Class I -Level B). Ventricular drainage is useful for acute hydrocephalus (Class I-Level C). Corticosteroids (Class III - Level A) and prophylactic anticonvulsants (Class III-Level C) are not recommended.

Stem cells scientific evidence

In this section we analyze published SCs literature for the treatment of ischemic stroke using the same criteria of the AHA guidelines to determine the level of evidence and class of recommendation. By conducting this classification, we obtained recommendations that are comparable to the current recommendations in the management of ischemic stroke regarding levels of evidence.

A recent meta-analysis including 7 clinical trials [9] found some improvements in the European stroke scale and motor subscale (ESS and ESMs) in patients treated with different kinds of SCs; they also found that other clinical indicators did not display significant differences between treated and control groups, concluding that the effects of SCs treatments on the recovery of ischemic stroke are not ideal.

Another meta-analysis [10] of 5 clinical trials found that bone marrow-derived stem cells (BM-SCs) might generate some benefits by lowering the grade of impairment caused by ischemic stroke. However, they concluded that larger RCTs are required to further confirm the effectiveness. A recent non-systematic review [11] analyzed the clinical trials available for ischemic stroke and concluded that more evidence from RCT is needed. Another comprehensive review [12] reported that the majority of the clinical studies concerning stroke and stem cells are small, nonrandomized and uncontrolled; however, they report that cell therapies seem to be safe, feasible and potentially efficacious. Another publication of a systematic review and a single arm meta-analysis [13] concluded that SCs are effective according to single arm clinical studies; however, it is stated in this publication that clinical benefits of SCs therapies for patients with stroke need further investigation and revaluation to test their clinical efficacy.

As described in the AHA guidelines, data derived from a meta-analysis is considered one of the highest levels of evidence possible (Level A). Still, we identified several methodological aspects in the above mentioned meta-analysis that limit the correct interpretation of the evidence towards clinical applications: this study considers all types of stroke (including both ischemic and hemorrhagic stroke), all kinds of SCs sources (autologous, allogenic, embryonic, etc.), all routes (IC, IV, IA, IT) and different times of application (acute, sub acute and chronic) in a single analysis. In our view, SCT should not be considered as a single treatment; each SCs source, route of administration and time of administration represents a single and different intervention that should be analyzed and standardized separately. Furthermore, different types of stroke regarding the cause and time of intervention should also be considered as separate diseases in order to generate more adequate analyses that have a real impact in the clinical setting.

We definitely agreed upon and recognized the need to develop future larger RCT and better metanalyses. However, today the only modifying treatment available in the AHA recommendations with level A (RCT or meta-analyses) is the IV rTPA, the use of which is known to be limited due to the small window of time during which treatment is possible and the long list of contraindications. Besides this, the alternative options of treatment when IV rTPA is contraindicated are recommendations with Level B (single randomized trial or nonrandomized studies) or C (only consensus opinion of experts, case studies or standard care). Therefore, it is fair to propose SCs therapies recommendations based on the information available from studies with evidence below level A. As the AHA guidelines highlight, "a level of Evidence B or C in the recommendation does not imply that the recommendation is weak because many important clinical questions addressed in the guidelines do not lend themselves to clinical trials. Although randomized trials are unavailable, there may be a very clear clinical consensus that a particular test or therapy is useful or effective".

Since the potential sources of evidence level A mentioned above [9-13] imply such important limitations for our analysis towards a clinical guideline, we decided to review the individual publications cited in these sources by assigning a level of evidence and a class of recommendation in the same way as the AHA did for their own evidence and recommendations. We assigned the trials analyzed to their respective disease group (ischemic, hemorrhagic or chronic) and categorized them according to time of intervention (acute <7 days after onset of symptoms, sub acute >7 days and <3 months, and chronic treatments >3 months). We evaluated further relevant trials discussed in other publications, including only English publications in our study and excluding those only dealing with the mobilization of SCs with G-CSF and not including a reinfusion of SCs. Acute Ischemic stroke (<7 days onset of symptoms) (Table 1.1-1.3).

Table 1.1: Stem cells scientific evidence and recommendations for ischemic stroke.

Specific Disease	Sub groups	Source (author, year)	1 Frame Type of cell	Harvesting site	Manipulation	Dose	# of applications
Ischemic stroke	Acute	Savitz et al. [14]	MNC	Autologous - BM	Separation	10×10	1
		Furtado de Mendonça et al. [15]	MNC	Autologous-BM	Separation	$30 \times 10 / 10 \times 10^7$	2(10min interval)
		Friedrich et al. [16]	NP MNC	Autologous-BM	Separation	22×10^7	1
		Moniche et al. [17]	NP/NR MNC	Autologous-BM	Separation	1.5×10^8	1
	Sub acute	Bang et al. [18]	NP/NR MSCs	Autologous-BM	Expansion/cultivation	5×10^7	2
		Lee et al. [19]	NP/NR MSCs	Autologous-BM	Expansion/cultivation	5×10^7	2
		Prasad et al. [20]	NP/NR MNCs	Autologous-BM	Separation	8×10^7	1
		Prasad et al. [21]	NP/NR MNC	Autologous-BM	Separation	28×10^7	1
		Honmou et al. [22]	NP/NR MSCs	Autologous-BM	Expansion/cryo	$0.6\text{-}1.6 \times 10^8$	1
		Correa et al. [23]	NP/NRMNC	Autologous-BM	Separation + label Tc99	3×10^7	1
		Battistella et al. [24]	NP/NR MNC	Autologous-BM	Separation + label Tc99	$1\text{-}5 \times 10^8$	1
		Rosado-de-Castro et al. [25]	NP/NRMNC	Autologous-BM	Separation	$1\text{-}5 \times 10^8$	1
		Han et al. [26]	NP/NR MSCs	Allogenic-hUCB	Separation and cultivation	1.2×10^7	3
		Qiao et al. [27]	NP/NR MSCs / NSPCs	Allogenic-hEmbrio	Cultivation	0.5×10^6/kg	4

1. Frame - Frame of action (neuroprotection - NP, neuroregeneration - NR). 2. Improvement - According to the specific goal (Grades: T – Total / M – Moderate / L – Low / NI - No improvement / NA – Not available). 3. Risk/Benefit (Acceptable or not acceptable). 4. Level of evidence - Individual Level of evidence according to the Oxford Centre for Evidence-based Medicine. 5. Grade of recommendation - Individual Grade of recommendation according to the Oxford Centre for Evidence-based Medicine. 6. Patients per recommendation - Total of number of patients treated in all publications that are considered for each recommendation. 7. MRI- decreased ischemic area. PET – increased metabolism and increased metabolism by PET. 8. L-limiting CNS damage / Rf - Recovery of function / M – Mortality / Rt – Regeneration of tissue / S - Survival / Rec – Recanalization. 9. without a clear relationship to the procedure. 10. MRI - Reduction of atrophy and ventricle dilatation. 11. Importance of having less involvement of the SVZ for better outcomes. 12. Newcastle-Ottawa Scale (NOS).

Table 1.2 : Stem cells scientific evidence and recommendations for ischemic stroke.

Disease Specific	Sub groups	Source (author, year)	Time since onset	Route of application	Concomitant treatment	Follow up	Specific goal	8 Clinical effects
Ischemic stroke	Acute	Savitz et al. [14]	24 to 72 hours	IV	NO	6 months	L	ImprovementmRS, NIHSS,BI
		Furtado de Mendonça et al. [15]	72 hours	IA	NO	60 days	L	ImprovementmRS, NIHSS,BI
		Friedrich et al. [16]	2 to 7 days	IA	NO	6 months	L, R, M	ImprovementmRS, NIHSS
		Moniche et al. [17]	6 days	IA	NO	1, 3 and 6months	L, Rf, Rt	NO
		Bang et al. [18]	4 to 5 and 7 to 9weeks	IV	NO	1, 3, 6, 12months	L, Rf, Rt	ImprovementmRS, BI
		Lee et al. [19]	5 and 7 weeks	IV	NO	up to 5 years	L, Rf, Rt, S	ImprovementmRS, Survival

	Sub acute	Prasad et al. [20]	7 to 30 days	IV	NO	Up to 52 weeks	L / Rf	ImprovementmRS, NIHSS,BI
		Prasad et al. [21]	18 days	IV	NO	up to 1 year	Rf, Rt, S	NO
		Honmou et al. [22]	<6 months	IV	Physicaltherapy	Up to 1 year	Rf	Improved NIHSS
		Correa et al. [23]	9 days	IA	NO	4 months	L	Improved NIHSS
		Battistella et al. [24]	<90 days	IA	NO	Up to 180 days	Rf, Rt, S	ImprovementmRS,NIHSS,BI
		Rosado-de-Castro et al. [25]	<90 days	IA / IV	NO	Up to 180 days	Rf, Rt, S	ImprovementmRS, NIHSS,BI
		Han et al. [26]	up to 66 days	IT	NO	5, 15, 27, 41, 60	Rf, Reca, Rt	NIHSS
		Qiao et al. [27]	Up to 1 year	IV, IT	NO	Up to 24 months	L, Rf, Rt	mRs, NIHSS, BI

1. Frame - Frame of action (neuroprotection - NP, neuroregeneration - NR). 2. Improvement According to the specific goal (Grades: T – Total / M – Moderate / L – Low / NI - No improvement / NA – Not available). 3. Risk/Benefit (Acceptable or not acceptable). 4. Level of evidence - Individual Level of evidence according to the Oxford Centre for Evidence-based Medicine. 5. Grade of recommendation - Individual Grade of recommendation according to the Oxford Centre for Evidence-based Medicine. 6. Patients per recommendation -Total of number of patients treated in all publications that are considered for each recommendation. 7. MRI- decreased ischemic area. PET – increased metabolism and increased metabolism by PET. 8. L- limiting CNS damage / Rf - Recovery of function / M – Mortality / Rt – Regeneration of tissue / S – Survival / Rec – Recanalization. 9. without a clear relationship to the procedure. 10. MRI - Reduction of atrophy and ventricle dilatation. 11. Importance of having less involvement of the SVZ for better outcomes. 12. Newcastle-Ottawa Scale (NOS).

Table 1.3 : Stem cells scientific evidence and recommendations for ischemic stroke.

Disease Specific	Sub groups	Source (author, year)	Other Tests	Imaging Tests	Improvement 2	benefit 3	Patients (Control)
Ischemic stroke	Acute	Savitz et al. [14]	NO	NO further infarct expansion	No L	A	10
		Furtado de Mendonça et al. [15]	NO	7 MRI,PET	No M	A	1
		Friedrich et al. [16]	NO	NO	No M/M/ M	A	20
		Moniche et al. [17]	β nerve growth-factor	NO	9 Seizures NI	NA	10(10)
	Sub acute	Bang et al. [18]	NO	10 MRI	No L/L/ NI	A	5(25)
		Lee et al. [19]	SDF-1α	11 MRI	No L/M/NI/ M	A	16(36)
		Prasad et al. [20]	NO	No homing	No M/M	A	11
		Prasad et al. [21]	NO	NO	No NI	NA	58(60)
		Honmou et al. [22]	NO	12 MRI	No L	A	12
		Correa et al. [23]	NO	Tc-99m homming	No M	A	1
		Battistella et al. [24]	NO	2of6 homming	Seizures NA	NA	6
		Rosado-de-Castro et al. [25]	NO	Low brain uptake	Seizures NA	NA	6
		Han et al. [26]	NO	Arteryrecanalization	No M	A	1
		Qiao et al. [27]	NO	NO	No M	A	6

1. Frame - Frame of action (neuroprotection - NP, neuroregeneration - NR). 2. Improvement-According to the specific goal (Grades: T – Total / M – Moderate / L – Low / NI - No improvement / NA – Not available). 3. Risk/Benefit (Acceptable or not acceptable). 4. Level of evidence - Individual Level of evidence according to the Oxford Centre for Evidence-based Medicine. 5. Grade of recommendation - Individual Grade of recommendation according to the Oxford Centre for Evidence-based Medicine. 6. Patients per recommendation-Total of number of patients treated in all publications that are considered for each recommendation. 7. MRI- decreased ischemic area. PET – increased metabolism and increased metabolism by PET. 8. L- limiting CNS damage / Rf - Recovery of function / M – Mortality / Rt – Regeneration of tissue / S – Survival / Rec – Recanalization. 9. without a clear relationship to the procedure. 10. MRI - Reduction of atrophy and ventricle dilatation. 11.Importance of having less involvement of the SVZ for better outcomes. 12. Newcastle-Ottawa Scale (NOS).

Intravenous trials

Savitz et al. [14] conducted an open-label, historically controlled trial in 10 patients with acute ischemic stroke in the MCA territory within 24 to 72 hours of the onset. The clinical condition of the patients was a NIHSS between 6 and 15 in the right hemisphere and 6 to 18 in the left hemisphere without hemorrhagic transformation. They received treatment according to the AHA guidelines including IV rTPA for the patients arrived before 4.5 hours. Following the initial procedures, they were treated with 7-10 x106 IV autologous BM-MNC per kg (viability>94% / $1x10^7$ CD34+and $1x10^5$ CD133+ per kg) with normal saline after density gradient separation. The patients were compared with clinically paired control groups selected from a database of the same facility scored prospectively by the stroke service. No study-related severe adverse event up to 6 months appeared. One patient died for a non-related cell-therapy cause without completing the 6 months assessment. All patients improved the NIHSS from day 1 to 7 and the condition of the surviving patients constantly improved during the following 6 months. Their mRS had also improved by one point after 6 months (5 patients achieved a mRS of 0-2 and 7 of them achieved a BI >90). Two patients suffered infarct expansion assessed by MRI 30 days after the onset. The comparison to historical controls suggests that the patients treated showed a better outcome at 90 days in mRS, even when compared with just the subset that received IV rtPA. (Level of evidence 4/Grade of recommendation C-Oxford). (Level C / Recommendation Class IIb - AHA).

Intra-arterial trials

Furtado de Mendonça et al. [15] reported a case of a 54-year old woman with acute ischemic stroke in the MCA territory that received intra-arterial $30x10^7$ and $10x10^7$ autologous BM-MNC (>90% cell viability) in 3ml with a 10 minutes interval 3 days after the onset of symptoms. Seven days after the intervention, a slight decrease in the ischemic area was shown by MRI diffusion sequence and a hypo perfusion by SPECT, with an increased metabolism revealed by means of a FDG-PET in the left parietal cortex correlated with a partial improvement in speech and right hemiparesis. The NIHSS changed from 17 to 14, the mRS from 4 to 3 and the BI from 5 to 60 at 29 days, and continued to improve until day 60 (Level of evidence 5 / Grade of recommendation D-Oxford). (Level C / Recommendation Class IIb - AHA).

Friedrich et al. [16] treated 20 patients with ischemic stroke under the MCA territory between 2 to 7 days after the onset of symptoms, NIHSS >8, spontaneous recanalization and persistent disabling deficits in an open-label, non-controlled trial. The patients received 22 x 10^7 (mean) intra-arterial autologous BM-MNC (>90% viability after isolation of density gradient on Ficoll over a 30 minutes period) through a micro-catheter positioned within the MCA origin of the affected hemisphere. The clinical assessment was made at 1, 3, 7, 30, 60, 90 and 180 days after the procedure, together with imaging (CT and MRI) and EEG on day 7, 30, 60 and 90. No serious adverse events related to the procedure were observed (clinical, laboratory, EEG and imaging). Two patients died: one of a myocardial infarct and the other developed hemorrhagic transformation before the SCs therapy. The mortality rate in this study was thus below the level reported for similar populations. There was a significant reduction in the median scores of NIHSS between pre-treatment and the 180-day follow-up period (p<0.001). Six of the patients displayed satisfactory improvement (mRS of 0–NIHSS<8; mRS of 0-1–NIHSS (8–14); mRS 0-2-NIHSS>14) and another eight achieved a good clinical outcome (mRS ≤2) at 90 days. No statistically significant changes were found in the infarct, densities or volumes between groups of improvers and non-improvers. (Level of evidence 4 / Grade of recommendation C-Oxford). (Level C / Recommendation Class IIb - AHA).

Moniche et al. [17] published a nonrandomized controlled trial with a sample of 10 patients (10 controls) with MCA territory ischemic stroke within 6 days from the onset of symptoms. They had a NIHSS≥8. The treated group received $1.5x10^8$ autologous BM-MNC (92% viability / $3.3x10^6$ CD34+) in the M1 segment of the infarct-related MCA at low pressure (0.5 to 1 ml/min). Clinical, blood and adverse effects were assessed at 1, 3 and 6 months together with MRI in the last evaluation. Both groups showed similar results in the baseline assessment. Two patients in the treated group presented isolated partial seizures after 3 months (no clear relation with procedure). No patient experienced serious adverse events (hemodynamic, neurological, new ischemic lesions, tumour or death) during the procedure, hospitalization or follow-up visits. No statistically significant difference in neurological function was found between the groups at a follow-up after 6 months. A correlation between the level of CD34+ infused and the clinical outcome was found and the patients treated with BM-MC had a peripheral β-nerve growth factor. (Level of evidence 2b / Grade of recommendation B-Oxford). (Level B / Recommendation Class III - AHA).

Sub acute Ischemic stroke (between 7 days and 3 months from the onset of symptoms) (Table 1.3-1.5).

Table 1.4: Stem cells scientific evidence and recommendations for ischemic stroke.

Disease Specific	Sub groups	Source (author, year)	Clinical Condition	Type of Study	No's	Level of Evidence	Grade of Recommendation	Number of patients treated	Patients Per Recommendation
		Savitz et al. [14]	6 - 15 NIHSS	Case Series historically Controlled	5	4	C	10	10

Ischemic stroke	Acute	Furtado de Mendonça et al. [15]	14 NIHSS	Case report		5	D	1	31
		Friedrich et al. [16]	>8 NIHSS	Case Series	3	4	C	20	
		Moniche et al. [17]	>8 NIHSS	Open label non-randomized controlledtrial	6	2b	B	10	
	Sub acute	Bang et al. [18]	>7 NIHSS	single blinded, non-randomized	2	2b	B	5	102
		Lee et al. [19]	>7 mNIHSS	single blinded,RCT	7	1b	A	16	
		Prasad et al. [20]	>7 mNIHSS	case series	4	4	C	11	
		Prasad et al. [21]	>7 NIHSS	Open label, multicentreRCT		1b	A	58	
		Honmou et al. [22]	≤3 mRS	Case series	3	4	C	12	
		Correa et al. [23]	5 NIHSS	Case Report		5	D	1	13
		Battistella et al. [24]	4 - 13 NIHSS	Case series	4	4	C	6	
		Rosado-de-Castro et al. [25]	4 - 13 NIHSS	Case series		4	C	6	
		Han et al. [26]	35 NIHSS	Case report		5	D	1	1
		Qiao et al. [27]	3 - 16 NIHSS	Case series		4	C	6	1

1. Frame - Frame of action (neuroprotection - NP, neuroregeneration - NR). 2. Improvement According to the specific goal (Grades: T – Total / M – Moderate / L – Low / NI - No improvement / NA – Not available). 3. Risk/Benefit (Acceptable or not acceptable). 4. Level of evidence - Individual Level of evidence according to the Oxford Centre for Evidence-based Medicine. 5. Grade of recommendation - Individual Grade of recommendation according to the Oxford Centre for Evidence-based Medicine. 6. Patients per recommendation.Total of number of patients treated in all publications that are considered for each recommendation. 7. MRI- decreased ischemic area. PET – increased metabolism and increased metabolism by PET. 8. L- limiting CNS damage / Rf - Recovery of function / M – Mortality / Rt – Regeneration of tissue / S – Survival / Rec – Recanalization. 9. without a clear relationship to the procedure. 10. MRI - Reduction of atrophy and ventricle dilatation. 11. Importance of having less involvement of the SVZ for better outcomes. 12. Newcastle-Ottawa Scale (NOS).

Table 1.5: Stem cells scientific evidence and recommendations for ischemic stroke.

Disease Specific	Sub groups	Source (author, year)	Level of Evidence	Grade of Recommendation for each individual study (According to the criteria used for the AHA for their evidence)
Ischemic stroke	Acute	Savitz et al. [14]	C	Class II b (May be considered)
		Furtado de Mendonça et al. [15]	C	Class II b (May be considered)
		Friedrich et al. [16]	C	Class II b (May be considered)
		Moniche et al. [17]	B	Class III (no benefit)
	Sub acute	Bang et al. [18]	B	Class II a (reasonable)
		Lee et al. [19]	B	Class II a (reasonable)
		Prasad et al. [20]	C	Class II a (reasonable)
		Prasad et al. [21]	B	Class III (no benefit)
		Honmou et al. [22]	C	Class II b (May be considered)
		Correa et al. [23]	C	Class II b (May be considered)
		Battistella et al. [24]	C	Class III (no benefit / or harm)
		Rosado-de-Castro et al. [25]	C	Class III (no benefit / or harm)

		Han et al. [26]	C	Class III
		Qiao et al. [27]	C	Class III

1. Frame - Frame of action (neuroprotection - NP, neuroregeneration - NR). 2. Improvement. According to the specific goal (Grades: T – Total / M – Moderate / L – Low / NI - No improvement / NA – Not available). 3. Risk/Benefit (Acceptable or not acceptable). 4. Level of evidence - Individual Level of evidence according to the Oxford Centre for Evidence-based Medicine. 5. Grade of recommendation - Individual Grade of recommendation according to the Oxford Centre for Evidence-based Medicine. 6. Patients per recommendation. Total of number of patients treated in all publications that are considered for each recommendation. 7. MRI- decreased ischemic area. PET – increased metabolism and increased metabolism by PET. 8. L- limiting CNS damage / Rf - Recovery of function / M – Mortality / Rt – Regeneration of tissue / S – Survival / Rec – Recanalization. 9. without a clear relationship to the procedure. 10. MRI - Reduction of atrophy and ventricle dilatation. 11. Importance of having less involvement of the SVZ for better outcomes. 12. Newcastle-Ottawa Scale (NOS).

Intravenous trials

Bang et al. [18] conducted an RCT with 30 patients (5 treated and 25 controls) diagnosed with sub acute MCA territory infarct and persisting severe neurological deficits (>7 NIHSS) after 7 days of admission in spite of standard management. The patients received IV 5×10^7 autologous BM-MSCs after 30 days of cultivation. The first boosting took place 4 to 5 weeks and the second one 7 to 9 weeks after the onset of symptoms. The patients did not present serious or cell-related side effects. Despite similar baseline values, the BI and mRS of treated patients after infusion was better than that of the controls at 3, 6, and 12 months. The NIHSS improvement from the day of the first boosting until 1 year after the symptoms' onset was not substantial. No patients showed any structural changes (including tumour formation) in the brain after the infusion relative to the baseline. The volumetric analysis indicated that there was no difference between the groups regarding the magnitude of apparent changes in infarct volume between the initial DWI and the follow-up MRI (p=0.661). However, atrophy within peri-infarct areas and secondary dilations of the adjacent ventricle were less prominent in treated patients than in controls. (Level of evidence 2b / Grade of recommendation B-Oxford). (Level B / Recommendation Class IIa - AHA).

Lee et al. [19] conducted an observer-blinded RCT in 52 patients (16 treated and 36 controls) diagnosed with sub acute ischemic stroke in the MCA territory with severe persistent neurological deficit (>7 points mNIHSS). The treated group received IV 5×10^7 autologous BM-MSCs (4 weeks of cultivation and 95% viability) at 5 and 7 weeks after the onset of symptoms. After an average of 2.5 years, the treated patients had a lower mortality rate than the controls. No serious or cell-related side effects were described. 3.5 years after the onset of symptoms, 21 out of 36 controls, as compared to 4 out of 16 treated patients, displayed deterioration according to the mRS. The patients in the treated group increased in proportion by 0-3 points in the mRS compared to controls that did not improve the mRS. The biomarker follow-up demonstrated a correlation between serum levels of SDF-1α and clinical parameters (BI and mRS) that support evidence of MSCs homing in the CNS. A correlation between less infarcted SVZ and functional improvement was also seen in the DWI-treated group and the infarct volume did not change in both groups. This means that improvements in mRS appear when the SVZ is less involved and the effects of MSCs are unpredictable when the SVZ is severely damaged. (Level of

evidence 1b / Grade of recommendation A-Oxford). (Level B / Recommendation Class IIa - AHA).

Prasad et al. [20] carried out an open label, non-controlled trial in 11 patients with sub acute ischemic stroke (between 7 to 30 days) in the MCA and/or ACA territory. They had a GCS >8, BI of 50, NIHSS of >7 and no evident hematoma with clinical stability. The patients were treated with a mean of 8×10^7 IV autologous BM-MNCs (viability mean 95.8%) after 4 hours of separation process. The patients were subjected to a follow-up examination after 24-48 hours, 4-6, 24 and 52 weeks, where no serious adverse event was recorded. Seven patients had favourable outcomes in BI, 10 in NIHSS and 6 in mRS. (Level of evidence 4 / Grade of recommendation C-Oxford). (Level C / Recommendation Class IIa - AHA).

Two years later, the same group, Prasad et al. [21], conducted a multicentre RCT in 58 patients (60 controls) with sub acute (>7 until 30 days) MCA or ACA territory ischemic stroke without thrombolysis or endovascular therapy; with a GCS >8, a BI≤50 and a NIHSS≥7; who were unable to walk without aid or to raise their upper limbs, and who did not display any changes in clinical status for ≥48 hours. The patients received conventional treatment and IV 28×10^7 autologous BM-MC (>90%viability, 2×10^7 CD34+) without expansion or cultivation 18 days after the onset of symptoms. Monitoring of side effects after infusion was performed until 7 days after infusion and blinded assessment with clinical details (NIHSS, mRS and BI); also, MRI, PET and EEG were conducted (day 7, 90, 180 and 365). Patients in both groups had similar characteristics except for infarct volume, which was higher in the control arm. At day 90 and 180, no statistically significant difference between the groups was observed regarding BI, mRS, NIHSS (adjusted by infarct size) infarct volume, EEG or PET (Level of evidence 1a / Grade of recommendation A-Oxford) (Level B / Recommendation Class III - AHA).

Honmou et al. [22] treated 12 patients in an open-label non-controlled trial with 0.6-1.6 $\times 10^8$ IV autologous MSCs (>95% viability) after 20 days of expansion in human serum and cryopreservation together with standard treatment and formal rehabilitation. The patients presented supratentorial ischemic stroke within 6 months after onset (11 of them before 90 days), mRS ≤3 as well as non-severe consciousness impairments (Japan Coma Scale 0 to 100) prior to and after infusion. No significant side effects were observed after 1, 2, 4, 7 and 14 days; 1, 3, 6

months and 1 year. The patients increased the rate of change in NIHSS before and after MSCs infusion (from 0.04 to 0.36 per day). A reduction of NIHSS of 4 or more points in 4/12 patients and 5 or more 3/12 patients was observed within the first 7 days after infusion and maintained for one year. At least a 15% reduction in lesion volume was observed in 7 patients with a tendency to be correlated with the clinical status. (Level of evidence 4 / Grade of recommendation C-Oxford) (Level C / Recommendation Class IIb-AHA).

Intra-arterial trials

Correa et al. [23] treated a patient with sub acute ischemic stroke in the MCA territory 9 days after the onset of symptoms. He received Tc-99m labeled $3x10^7$ autologous BM-MNCs delivered in the MCA; no adverse events occurred. The perfusion brain images revealed intense accumulation of BM-MNCs in the ipsilateral hemisphere and enhanced uptake in brain, liver and spleen. The patient displayed improved NIHSS after 4 months of symptom onset (Level of evidence 5 / Grade of recommendation D-Oxford) (Level C / Recommendation Class IIb - AHA).

Battistella et al. [24] performed a non-control and non-blinded clinical trial in 6 patients with sub acute ischemic stroke in MCA territory (<90 days of onset of symptoms and recanalization with thrombolysis) with 4 to 13 NIHSS. The patients received intra-arterial labelled (99mTc) $1-5 \times 10^8$ autologous BM-MNCs (>93% viability) obtained after Ficoll-density centrifugation and directly injected into the M1 portion of the MCA via femoral artery access within 10 minutes. Assessment was made at admission and after 1, 3, 7, 30, 60, 120 and 180 days. Two patients presented seizures, which however were not related to cell therapy; besides, no other major side effects were observed. At day 180, the clinical scores (NIHSS, BI and mRS) of the patients had improved. Two patients presented brain homing of the cells after 24 hours of the transplantation. (Level of evidence 4 / Grade of recommendation C-Oxford) (Level C / Recommendation Class III - AHA).

Rosado-de-Castro et al. [25] reported the continuation of the study by Batistella et al. as an open-label, nonrandomised controlled trial in patients with sub acute ischemic stroke in the MCA territory. In this second report, another 6 patients were treated (5 IV and 1 IA). Whole-body images SPECT indicated that after 2 hours, the IA route led to greater uptake in the liver and spleen and lower uptake in the lungs as compared to the IV route. Both groups had a similarly low uptake in the brain. Seizures occurred in 7 out of 12 patients and it cannot be ruled out that this was secondary to the transplantation. No clinical changes were properly reported. (Level of evidence 4 / Grade of recommendation C-Oxford) (Level C / Recommendation Class III - AHA).

Intrathecal trials with non-autologous cells

Han et al. [26] reported a case of a 17-year old man with sub acute bilateral infarction of the Pons, midbrain and right superior cerebellum due to basilar artery dissection. The patient was treated with 3 IT units of $1.2x10^7$ human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) from HLA allele match donors after 35, 50 and 76 days of onset. The patient showed improvements of gag reflex, relaxation of muscular tone, and voluntary eyeball movement. The patient also displayed improvements of the NIHSS from 35 to 25, and down to 20 after 40 and 60 days. The patient also presented an improvement in the recanalization of the basilar artery after transplantation assessed by MRI and aMRI. (Level of evidence 5 / Grade of recommendation D-Oxford). (Level C / Recommendation Class IIa - AHA).

Qiao et al. [27] treated 6 patients with ischemic stroke in the territories of the MCA and/or ACA after 1 week to 2 years after the onset (acute, sub acute and chronic). The patients were given full standard stroke care. The first group received four IV infusions of $0.5×10^6$/kg MSCs-derived from umbilical cord (MSCs-UC). The second group received one time $0.5×10^6$/kg UC-MSCs and 3 more infusions of $0.5×10^6$/kg UC-MSCs together with $5×10^6$/kg IT neuron stem/progenitor cells (NSPCs) from the subependymal zone of aborted fetuses' brains. All treatments had a 1-week interval between them. The patients were followed up after 2 years. One patient died 10 months after the transplantation due to a recurrent massive cerebral infarction. No other major adverse effects were reported in the clinical, laboratory and imaging long-term follow-up. Five patients showed NIHSS improvements by more than two points after 3 months. All patients improved in the BI and mRS. The improvements could be observed after a mean of 19.8 days. The improvements were more significant in the patients receiving NSPCs. The patients treated in the acute or sub acute phases also better recovered than that the ones treated in chronic phases. (Level of evidence 4 / Grade of recommendation C-Oxford). (Level C / Recommendation Class IIb - AHA).

England et al. [28] Performed a prospective, single-center, double-blinded, randomized, placebo-controlled trial in 60 (20 controls) patients with sub acute stroke (10 days / hemorrhagic and ischemic). The patients received subcutaneous human recombinant G-CSF (1 x106 units / kg, equivalent to 10 µg/kg, Neupogen) or matching subcutaneous saline once per day for 5 days. Eight patients (6 treated and 2 controls) underwent a collection of peripheral CD34+ for labeling and IV reinjection on the same day. An MRI-tracking on day 10 suggested the presence of migrated-labeled cells in the site of infarction in only 1 participant. No significant difference in the number of patients with serious adverse events was reported. No statistically significant difference manifested itself between treatment and placebo groups with respect to clinical measurements such as mRS, BI, NIHSS, Mini-Mental, etc. However, a trend in NIHSS improvement was observed among the treated subjects at day 90. A trend toward reduced lesion volume at day 90 was shown in G-CSF–treated patients. In comparison with placebo, G-CSF significantly elevated the CD34+ cell count at day 5; at day

10 it had returned to normal (Level of evidence 1b / Grade of recommendation A-Oxford) (Level B / Recommendation Class III - AHA).

Jiang et al. [29] treated 4 young (<60 years old) patients with sub acute ischemic (3) and hemorrhagic (1) stroke using a IA (M1 segment of MCA) single dose of 2x107 (diluted in 20ml saline / rate 1ml per min) allogenic, cultured and cryopreserved UC-MSCs that did not show any significant neurological functional improvement after undergoing standard medical treatment. The UC-MSCs had a negative HLA-DR. No major complications including immune response or recurrence (TIA) occurred within 6 months. Two of the ischemic stroke patients showed muscle strength improvement in their upper extremities and one also in their lower extremities. Two of the ischemic stroke patients presented improvements in the mRS at 90 and 180 days of therapy (Level of evidence 4 / Grade of recommendation C-Oxford) (Level C / Recommendation Class IIb - AHA).

SCs Recommendations for ischemic stroke (Table 1.6)

Table 1.6: Stem cells scientific evidence and recommendations for ischemic stroke.

Sub groups	Source (author, year)	Stem Cells Recommendation	Level of Evidence	Grade of Recommendation
Acute	Savitz et al. [14]	Within 24 to 72 hours from the onset of symptoms in patients with acute ischemic stroke who are not candidates to thrombolysis and/or other endovascular therapies and/or did not respond successfully to those treatments, the intravenous infusion of 10millionaBM-MNCperkgafteron-sitesepararation may be considered in order to promote neuro protection, always as an additional intervention to the current AHA recommendations and without delaying the regular interventions.	C	Class II b(May be considered)
	Furtado de Mendonça et al. [15]	Within 7 days from the onset of symptoms in patients with acute ischemic stroke who are not candidates to thrombolysis and/or other endovascular therapies and/ordidnotrespondsuccessfully to those therapies, the intra-arterial infusion of 220 million a BM-MNC after separation may be considered in order to promote neuroprotection, always as an additional intervention to the current AHA recommendations without delaying the regular interventions.	B	Class II b(May be considered)
Sub acute	Bang et al. [18]	Within 7 to 90 days from onset of symptoms in patients with subacute ischemic stroke who are candidates for disease-modifying treatments or did not respond to them, intravenous infusion regulations is reasonable to promote neuroprotection and early neuroregeneration, always additional treatment to the current AHA recommendations without delaying the regular interventions.	B	Class II b(reasonable)
	Lee et al. [19]			
	Prasad et al. [20]			
	Prasad et al. [21]			
	Correa et al. [23]	Within 7 and 90 days from the onset of symptoms in patients with subacute ischemic stroke who arenotcandidatesfordisease-modifyingb treatments or did not respond to them ,intra-arterial infusionof 50 to 500 millions a BM-MNCsafterseparationmayb econsideredforneuroprotectionandearly neuroregeneration, always as an additional treatment to the current AHA recommendations withoutdelayingtheregularinterventions.	B	Class II b(May be considered)
	Battistella et al. [24]			
	Rosado-de-Castro et al. [25]			
	Han et al. [26]	There is not enough evidence to recommend infusion of either IV or IA non-autologous SCs inthesubacuteperiodafterischemicstroke, exceptunderaclinicaltrial.	C	Class III
	Qiao et al. [27]		C	

1. Frame - Frame of action (neuroprotection - NP, neuroregeneration - NR). 2. Improvement - According to the specific goal (Grades: T – Total / M – Moderate / L – Low / NI - No improvement / NA – Not available). 3. Risk/Benefit (Acceptable or not acceptable). 4. Level of evidence - Individual Level of evidence according to the Oxford Centre for Evidence-based Medicine. 5. Grade of recommendation - Individual Grade of recommendation according to the Oxford Centre for Evidence-based Medicine. 6. Patients per recommendation - Total of number of patients treated in all publications that are considered for each recommendation. 7. MRI- decreased ischemic area. PET – increased metabolism and increased metabolism by PET. 8. L-limiting CNS damage / Rf - Recovery of function / M – Mortality / Rt – Regeneration of tissue / S – Survival / Rec – Recanalization. 9. without a clear relationship to the procedure. 10. MRI - Reduction of atrophy and ventricle dilatation. 11. Importance of having less involvement of the SVZ for better outcomes. 12. Newcastle-Ottawa Scale (NOS).

According to the literature reviewed in this paper we can propose the following recommendations:

A.   Within 24 to 72 hours from the onset of symptoms in patients with acute ischemic stroke who are not candidates to thrombolysis and/or other endovascular therapies and/or did not respond successfully to those treatments, the intravenous infusion of 10 million aBM-MNC per kg after on-site separation may be considered in order to promote neuroprotection, always as an additional intervention to the current AHA recommendations and without delaying the regular interventions. (Class IIb-Level C).

B.   Within 7 days from the onset of symptoms in patients with acute ischemic stroke who are not candidates to thrombolysis and/or other endovascular therapies and/or did not respond successfully to those therapies, the intra-arterial infusion of 220 million aBM-MNC after separation may be considered in order to promote neuroprotection, always as an additional intervention to the current AHA recommendations without delaying the regular interventions. (Class IIb-Level B).

C.   Within 7 to 90 days from onset of symptoms in patients with sub acute ischemic stroke who are not candidates for disease-modifying treatments or did not respond to them, intravenous infusion of 50 to 80 million aBM-MSCs or 280 million aBM-MNCs after separation and/or expansion under GMP regulations is reasonable to promote neuroprotection and early neuroregeneration, always as an additional treatment to the current AHA recommendations without delaying the regular interventions.(Class IIa-Level B).

D.   Within 7 and 90 days from the onset of symptoms in patients with sub acute ischemic stroke who are not candidates for disease-modifying treatments or did not respond to them, intra-arterial infusion of 50 to 500 millions aBM-MNCs after separation may be considered for neuroprotection and early neuroregeneration, always as an additional treatment to the current AHA recommendations without delaying the regular interventions. (Class IIa-Level C).

E.   There is not enough evidence to recommend infusion of either IV or IA non-autologous SCs in the sub acute period after ischemic stroke, except under a clinical trial.

Considerations of the proposed SCs recommendations

A.   It is important to stress that none of the above recommendations are allowed by the regulatory agencies in most parts of the world.

B.   The number of patients in the SCs publications is not as stable as would normally be expected of a guideline recommendation. However, we are talking about a clinical situation in which no other treatment options are available. Furthermore, the fact that any other therapy is not geared towards neuroregeneration and neuroprotection makes the assessment of a significant sample size not entirely possible. It would also be inaccurate to take sample size as a parameter, since other accepted treatments have different treatment objectives.

C.   Additionally, it has been shown that even when taking into account all stroke publications, there are too few clinical trials that report the sample size calculation and even if they do, most of them are unsubstantial [30]. We definitely encourage the validation of results with larger samples; however, the reported lack of significant sample size in other already accepted and recommended treatments in stroke suggests that not all accepted treatments can be supported by scientific evidence, and it therefore still seems reasonable to recommend SCs therapies based on the information available.

D.   The exact dose has not been precisely determined and changes with the route of infusion; however, based on the evidence available we identified a dose that is safe and provides therapeutic effects. It should be stressed that even though higher doses may contribute to better outcomes, it should be carefully considered whether or not to increase doses. For the time being, a good strategy is to base further escalating doses on body weight [14,31].

A direct comparative analysis of current clinical recommendations and potential stem cell recommendations

The recommendations of the AHA regarding public education, creation of stroke centers, stroke care quality improvements, pre-hospital management, emergency evaluation and diagnosis were not evaluated in detail because they are not comparable to SCs therapies, and the introduction of SCs therapies as recommendations would not change this for now, apart from providing wider windows of treatment. As a futuristic idea, we might want to consider the use of allogenic cryopreserved SCs and/or autologous BM-derived cells as pre-hospital treatment in the field.

Treatments such as thrombolysis, endovascular interventions, anticoagulants, antiplatelet, volume management, neuroprotective agents, surgical interventions, stroke care after hospitalization and treatment of complications were analyzed. Out of these, just IV fibrinolysis and endovascular treatments seem to have actual effects on the diseases. The use of IV rTPA is certainly effective; however, the scope of patients that can be treated this way is limited due to the time that has passed since the onset and the narrow criteria of inclusion. Furthermore, the side effects of rTPA cannot be ignored or downplayed (i.e. angioedema, seizure)-even if they are outweighed by the benefits of the treatment, this does not mean it is a harmless procedure. Based on the information currently available, it is clear that the treatment of acute ischemic stroke is insufficient and time-dependent.

The time problem has not yet been entirely overcome by public health policies or faster emergency services and may persist in the future. Especially in developing countries [32], better, different, less expensive and less risky treatments should be sought, not only in the research but also with a view to a prompt implementation in the clinical settings.

In terms of general supportive care (airway support, cardiac monitoring, blood pressure, etc.), out of 14 recommendations, four are level B (2 Class I, 1 Class IIa and 1 class III) and the rest are level C, of which seven are class I, two are class IIa and one is class IIb.

Regarding IV fibrinolysis, three out of 12 recommendations can be categorized as level of evidence A (one of the 12 recommendations with level A advise not to treat because harm risk), five are level B and four are level C. Expectedly, those recommendations categorized as level A of evidence are Class I recommendations. However, in level B category, the class of recommendation varies: three are Class I and two are Class IIb, even though the level of evidence is the same. Among the recommendations with level C of evidence, one is Class IIa, two are IIb and one is III.

For endovascular therapies, two out of 11 recommendations are level A, whereby it is stated that they should just be used if the patient is not eligible for IV rTPA. The other recommendation detailed which retrievers are preferred. Both of the recommendations mentioned above are Class I recommendations. Four of them are level B, of which two are recommended as Class I, one as Class IIa and one as Class IIb. Finally, five are level C, with one of those recommended as Class I concerning the necessity of a specialized centre, 1 as Class IIa and 3 as Class IIb.

The use of anticoagulation agents is summarized in 5 recommendations, three of which are Class III with level A and level B, and the other two are level B and Class IIb.

From 6 antiplatelet recommendations, just one is level A and it is recommended as Class I. From the remaining ones, the three with level C are Class III and Class IIb and the two with level B are recommended as Class III, i.e. it is recommended to avoid their use.

Regarding the use of vasopressors, two out of six recommendations are Class A and advised not to be used as Class III, three are Class B and recommended as Class IIb, and one is Level C and recommended as Class I.

From five neuroprotective recommendations, four are level B, 3 of them are recommended as Class IIb and one as Class III; the other recommendation is level A and Class III, advising not to use any neuroprotective pharmacological compound. In the general measures for acute settings, four out of 15 recommendations are level A recommended as Class I and one as Class IIa; seven are classified as level B, three of which are Class I, two are Class IIa and two Class III. Four of them are level C, of which three are Class I and one is Class III.

Regarding the treatment of acute complications, two out of 8 recommendations are level A, recommended as Class I and III. Three are level B and recommended as Class I, and three are level C and recommended as Class I, IIb and III.

Just 3 out of 136 recommendations of actual interventions have level A of evidence (2.2% of the recommendations are based on what is considered good scientific evidence. The rest categorized as levels B or C or even without purity of evidence, are still repeatedly recommended as reasonable (Class IIa) or even as actual treatment options (Class I).

It seems that in several cases, procedures with the same level of evidence are indistinguishable from the class of recommendation; also different levels of evidence are sometimes assigned to the same class of recommendation.

What is more, a discrepancy between the written recommendation and the description of the class of recommendation was found in several sections. This means for instance that the guidelines state "recommend their use just in clinical trials", when a Class IIa or IIb classification generally means that it is reasonable to consider this treatment in clinical practice (e.g. "The use of IV tenecteplase, reteplase, desmoteplase, urokinase and other fibrinolytic agents and IV of ancord or other defibrinogenating agents should only be used in a setting of clinical trial (Class IIb / may be considered-Level B)").

Some extraordinary cases could also be observed, such as the endovascular approach using IA rTPA, which is actually recommended as Class I (it should be performed) with a level B of evidence, without an established optimal dose and even without the FDA's approval for intra-arterial infusion.

Thus, what the above examples suggest is that even though there is a relatively low level of scientific evidence, the experience of experts is highly important to reach consensus on important issues in the field.

Furthermore, the efficacy of some recommendations even with Level A of evidence and recommended as Class I is somehow low because they do not lead to evident improvement of the patient's condition (e.g. cognitive treatments in patients with stroke). In this regard, we think that the rates of positive outcomes in patients treated with SCs, even if they are not ideal for now, are similar to what has been reported by other trials for the currently recommended treatments (i.e. the mRS of patients treated with SCs and the mRS in the Prolyse in Acute Cerebral Thromboembolism II (PROACT-II) trial have improved very similarly [33]. Sometimes SCs treatments have slightly lower outcomes as compared to other intra-arterial recanalization treatments [34-36], though with a safer profile in the SCs treatment).

As far as side effects are concerned, even the rTPA (the only one supported by level A evidence and fully recommended as treatment) is controversial. Although widely accepted and used as the only accepted treatment, recent publications have shown that there is an absolute excess risk of intracerebral haemorrhage in the three relevant subgroups regarding time to treatment, stroke severity, and age [37].

Another fact that should not be neglected is that 6 out of 15 writers had a research grant provided by a private company, 5 out of 15 received research support from private companies, 2 out of 15 have ownership interest and 14 out of 15 have a consultant/advisory position in a private institution. This is remarkable, but it is not the intention of this publication to further discuss possible conflicts of interests around the decisions about clinical guidelines.

Bearing the above in mind, it is fair to propose other recommendations which, although they are not ideal and should be studied in more detail, could expand the scope of possibilities for patients today who would otherwise have no promising interventions at their disposal.

The recommendations of SCs that we provided for acute and sub acute ischemic stroke, even if only tested with a low number of patients per trial, have consistent results and a fairly safe profile.

Important aspects for SCs interventions include: the time interval between the onset of stroke and the time of cell therapy. Even though not to the same extent as for rTPA, these factors also play an important role for the efficacy of the treatment, which is why earlier interventions should be considered [38]. The level of viability in the cells infused, cell selection between hole BM-MNCs and BM-MSCs may be considered in the future to determine which cell is better at which point of the intervention. It is furthermore crucial for the success of this procedure to have a high number of cells, since a subdosis, like in Prasad et al. [21], often leads to failure. These considerations have to be taken into account to ensure the most efficient treatment. It seems that the dose is more important than the hyper acute application; therefore, a high number of cells in the sub acute period (after cultivation) may represent the ideal situation, with further boosters in the rehabilitation period.

We have seen that in the acute and sub acute phase, several strategies could be used in order to include SCs therapies in the treatment without delaying the regular treatments and with the possibility of contributing to a better outcome. By implementing the SCs recommendations with a relatively high safety profile and mostly with relatively low exigencies of technology and operator experience, more robust information without the high cost and bias of a RCT could be provided in the community setting after allowing these procedures to be performed more widely. This phenomenon has been observed in other treatments such as rTPA, which confirmed its efficacy with larger populations based on community experience before a total certainty of its efficacy was known.

Conclusion

For the treatment of acute and sub acute ischemic stroke, several recommendations are put forward by the AHA. These recommendations define different classes and levels of evidence and in several cases they are not congruent with each other. Furthermore, in some cases the recommendations have low success rates, several side effects and have not even been approved by the FDA. With the four punctual recommendations involving SCs therapies, we could open up new possibilities of limiting the damage and potentiating the recovery. Currently, more information is gathered with the aim of improving such therapies. The reluctance to new approaches to treat these patients will in any case delay the applications of new technologies that could successfully improve the outcome for patients.

The phenomena described for the treatment of acute and sub acute stroke are also applicable for other types of stroke (part II and III of this publication), other neurological and non-neurological diseases. It is time to realize that saying that "there is no scientific evidence for clinical implementation" is no longer valid. We are aware that further research should be conducted on this matter, but we are also convinced that patients should be offered the best options possible, especially when regular treatments are inapplicable or have turned out unsuccessful.

This publication could serve as the basis to work on the clinical guidelines for stem cell therapies to treat acute and sub acute stroke followed by other neurovascular and neurological diseases.

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