

ORIGINAL ARTICLE

Autologous bone marrow cell transplantation in acute spinal cord injury—an Indian pilot study

HS Chhabra, K Sarda, M Arora¹, R Sharawat¹, V Singh, A Nanda, GM Sangodimath and V Tandon**Study design:** Phase- I/II, prospective, randomized, single-blind, controlled pilot study.**Primary Objective:** To determine the safety and feasibility of autologous bone marrow transplantation in patients with acute spinal cord injury (SCI) via two routes of transplantation as compared with controls.**Setting:** Indian Spinal Injuries Center, New Delhi.**Methods:** Twenty-one subjects with acute, American Spinal Injury Association Impairment Scale (AIS) A (complete), traumatic SCI with neurological level T1–T12, were recruited and randomized into three groups of seven subjects each. Two groups underwent cell transplantation through the intrathecal or intralaminar route, whereas the third served as control. Participants were assessed at baseline and followed up at 6 months and 12-months post enrollment. Safety and tolerability were evaluated by monitoring for any adverse events. Efficacy was assessed through neurological, functional and psychological evaluation, as well as through electrophysiological studies and urodynamics.**Results:** Surgery was tolerated well by all participants. There were no significant adverse events attributable to the procedure. There was no significant improvement in the neurological, electrophysiological or urodynamic efficacy variables. A statistically significant improvement in functional scores as evaluated by the Spinal Cord Independence Measure and International Spinal Cord Injury Scale was observed in all groups.**Conclusions:** The procedure is safe and feasible in AIS A participants with thoracic-level injuries at 12-months follow-up. No efficacy could be demonstrated that could be attributed to the procedure.*Spinal Cord* (2016) **54**, 57–64; doi:10.1038/sc.2015.134; published online 18 August 2015

INTRODUCTION

There is a huge base of preclinical evidence that suggests the safety and clinical efficacy of cellular therapies after spinal cord injury (SCI).¹ Most of the human trials published so far have had major limitations due to flaws in design and documentation, thus severely compromising the evidence base in favor of cellular transplantation for human SCI.² The authors had previously undertaken a prospective pilot study involving autologous olfactory mucosal transplantation in five chronic, motor, complete SCI subjects with neurological level C5–T12.³ The procedure was found to be relatively safe and feasible; however, no efficacy could be demonstrated. The failure of this and a similar study⁴ to replicate the efficacy previously reported by Lima *et al.*⁵ highlights the need to analyze the factors responsible for difference in outcomes of various clinical trials on human SCI. It also emphasizes the need to understand the factors that underlie the gap between the preclinical 'bench' and clinical 'bedside' findings. We hence planned to undertake another study to address the issues of 'cell population' selected, 'time of transplant or transplantation window' and the 'route of transplantation'.

Bone marrow transplantation for SCI has been the focus of attention in the past few years. There have been extensive preclinical studies demonstrating their potential role.¹ Moreover, several hypotheses have been proposed to explain the potential mechanism

of action of bone marrow stem cells in SCI models. First, bone marrow cells (BMCs) improve neurologic deficit by generating either neural cells or myelin-producing cells.^{6,7} Second, transplanted BMCs do not differentiate into neurons; rather, they work by guiding axonal regeneration by producing extracellular matrix.⁸ Third, transplanted BMCs promote compensatory mechanisms to reorganize the neural network and activate endogenous stem cells.⁹

BMCs have been transplanted by direct injection into the injured spinal cord,¹⁰ through intravenous injection,^{10,11} intrathecal injection¹² or through injection into the spinal artery.¹⁰ Of these, the majority of recent studies have undertaken transplantation via direct injection into the lesion or via intrathecal injection, as both strategies directly target the site of injury.^{12–15}

Hence, we undertook the study to document the safety and feasibility of administering enriched BMCs in complete acute SCI using these two modes of cell delivery as a pilot/ prelude to a larger clinical trial using this form of therapy.

MATERIALS AND METHODS

Ethics statement

This trial was approved by the Ethics Committee of Indian Spinal Injuries Centre as well as by the Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). Additional approval was also obtained from the Indian

Council of Medical Research (ICMR). The study was registered in the ICMR database (<http://ctri.nic.in/Clinicaltrials/login.php>) under the number CTRI/2014/01/004316. Ethical guideline provisions from the Helsinki Declaration were followed. All good manufacturing practice (GMP) and good clinical practice (GCP) standards required for undertaking studies with autologous minimally manipulated cells (as used in this study) were followed to ensure quality of the cell population isolated and transplanted. Written informed consent was obtained from each subject.

Patients and selection criteria

The study was designed as a Phase- I/II (Pilot), prospective, randomized, single-blind, controlled trial over a period of 5 years. The eligibility criteria are given in Table 1. Twenty-one subjects with acute traumatic SCI (10–14 days post injury) between neurological levels T1 and T12 and complete motor and sensory loss were enrolled in this study.

All subjects underwent standardized treatment including surgical stabilization and rehabilitation. In all subjects, the procedure of surgical stabilization (\pm decompression) was not dependent on the cell implantation procedure. Before BMC transplantation, they underwent magnetic resonance imaging and motor as well as somatosensory evoked potential (MEP and SSEP) examination.

Neurological assessment was performed by blinded assessors as per International Standards for Neurological Classification of SCI (ISNCSCI). Baseline hematological and biochemical tests were also undertaken. Table 2 summarizes the table of activity schedule of the subjects at the prescreening visit.

The enrolled subjects ($n=21$) were randomized and divided into three groups. Randomization was carried out using a randomization box that was pre-set before the start of the study. The first group ($n=7$) received BMCs through injection into the injured spinal cord site via durotomy (Group A). The procedure of exposure of the spinal cord, including the laminectomy, for transplantation of cells was discussed with the subjects. The second group ($n=7$) received BMCs through the intrathecal route (Group B). The third group ($n=7$) was the noninterventional (control) group (Group C). All assessments were conducted by a blinded assessor. The researchers administering the intervention were not blinded to the randomization schedule.

Bone marrow harvesting and stem cell enrichment

We used a point-of-care device with a closed bag system to procure enriched autologous BMCs (SmartPreP2 Bone Marrow Aspirate Concentrate System, Harvest, Plymouth, MA, USA). Before initiating the pilot study on transplantation of autologous BMCs in persons with acute SCI, a validation of

Table 1 Eligibility criteria

Inclusion criteria	Male and female subjects with traumatic spinal cord injury aged between 18 and 50 years. Acute spinal cord injury between 10 days and 14 days post injury. Traumatic spinal cord injury with neurological level of injury between T1 and T12. Complete injury (AIS A).
Exclusion criteria	Subject whose medical condition requires mechanical ventilation. Subjects with pathological fracture. Spinal Injuries in subjects with Ankylosing Spondylitis. Subjects with pre-existing severe medical disease that would affect the outcome, like severe diabetes, rheumatoid arthritis. Subjects with psychological disorders. Pregnant subjects.

Table 2 Schedule of evaluation, assessments, subjects' visit and procedure during the study

Particulars	Pre-screening	Screening	Baseline	Day of procedure	Post-op period	Month 6	Month 12
Demography/medical history	X	X	X			X	X
Inclusion–exclusion criteria	X	X	X	X			
Informed consent	X						
Physical examination	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
Screening MRI/CT/myelogram of spinal cord	X					X	X
EMG of four limbs		X				X	X
Psychological assessment		X				X	X
Urodynamics study			X			X	X
SSEP			X			X	X
MEP			X			X	X
Pre-operative laboratory tests			X				
PRO questionnaire	X		X			X	X
ISNCSCI examination	X	X	X			X	X
Voluntary movement in additional muscle groups not included in ISNCSCI	X	X	X			X	X
Modified Ashworth Scale	X	X	X			X	X
WISCI	X	X	X			X	X
SCIM	X		X			X	X
Investigational procedure				X			

Abbreviations: CT, computed tomography; EMG, electromyography; MEP, motor-evoked potential; MRI, magnetic resonance imaging; ISNCSCI, International Standards for Neurological Classification of Spinal Cord Injury; PRO, patient reported outcome; SCIM, spinal cord independence measure; SSEP, somatosensory evoked potential; WISCI, Walking Index of Spinal Cord Injury. Note: vital signs include oral body temperature, systolic and diastolic blood pressure in sitting and supine positions and pulse rate.

the device and its stated functions was undertaken using bone marrow aspirate (BMA) from two subjects after obtaining their informed consent. A volume of 60 ml of BMA was harvested by means of the aspiration technique from the posterior iliac crest using a 6 lumen Jamshidi-type trocar needle and 20-ml syringes, preflushed with heparin (concentration of 1000 units per ml) and processed in accordance with the manufacturer's instructions. The ready-to-inject stem cell fraction had an approximate volume of 7–10 ml. Once the total and viable cell count of an aliquot of the cells was obtained, a further centrifugation of the concentrate at 1500 r.p.m. for 5 min at room temperature (Model No. 5804 R, Eppendorf AG, Hamburg, Germany) was undertaken to obtain a concentration of 1×10^8 cells ml^{-1} .

Transplantation of BMCs through injection into the injured spinal cord site via durotomy

Although the BMA was harvested and enriched, laminectomy was performed from one vertebra above the injury site to one below using the standard procedure in order to provide sufficient access to the transplantation site. The dura mater was incised, sparing the arachnoid, which was subsequently opened separately with micro-scissors. The injury site was located using high-power microscopic magnification from normal tissue above the normal tissue below the lesion. Aliquots (300 μl) of cell suspension (total volume of 1.8 ml) were injected into six separate positions surrounding the lesion site (two above, two below and two at the lesion site) with the injection depth of 5 mm from the dorsal surface and 5 mm lateral from the midline. A volume of 2×10^8 cells were injected at a rate of 300 $\mu\text{l min}^{-1}$ using a 21-gauge needle attached to a 1 ml syringe. To prevent cell leakage through the injection track, the injection needle was left in position for 5 min after completing the injection. The dura mater and arachnoid were then closed. The muscle and skin were closed layer by layer.

Transplantation by intrathecal route

Subjects received BMCs ($\sim 2 \times 10^8$ cells per 1.8 ml) through a lumbar puncture injection performed in the L3–4 interspace taking all aseptic precautions.

Follow-up schedule

The list of activities performed at the time of discharge, at 6-month and 12 month intervals post enrollment is given in Table 2.

Safety outcome

Safety was assessed by documenting the ascending level of SCI, neuropathic pain, aseptic meningitis, rejection of transplant, deleterious changes in MRI, changes in hematology, serum chemistry and urinalysis tests, vital signs and systemic examination.

Primary efficacy end point

Blinded assessors examined subjects for improvement of at least one American Spinal Injury Association Impairment Scale (AIS) grade by 12 months or change from baseline of ≥ 10 points in total motor score.

Secondary efficacy end points

Blinded Assessors examined for any voluntary movement by additional muscle groups not included in ISNCSCI, improvements by at least 1 point in the Walking Index of Spinal Cord Injury (WISCI) scale, decrease in spasticity by one grade or more as assessed by the Modified Ashworth Spasticity scale, improvement in the American Spinal Injury Association sensory examination, improvement in SCIM score, improvements in electromyogram, SSEP and MEP, significant change in any of the variables included in urodynamic assessment, improvements in any of the variables included in Patient Reported Outcome (PRO) using ISCIIS as well as changes in psychological evaluation by Beck Depression Inventory.

Assessments

The same reviewer was used throughout the study to obviate inter-rater variability. Neurological and functional evaluations were made by a trained physiotherapist and the findings reviewed by another senior.

Electrophysiological and urodynamic studies were carried out by the same neurologist and urologist, respectively, whereas psychological assessment was carried out by the same psychologist. Magnetic resonance imaging was reported by the same radiologist but the findings were validated by another radiologist.

Statistical analyses

Descriptive data are presented as mean \pm s.e.m. All statistical analyses were performed using SPSS software (SPSS Inc. Released 2007 SPSS for Windows, version 16.0., SPSS Inc., Chicago, IL, USA). Difference in mean scores between the groups was compared by means of the paired *t*-test. Missing values were excluded from analysis. $P < 0.05$ was considered significant.

RESULTS

Study subjects

Twenty-one subjects met the eligibility requirements and were enrolled into this study (demographic details in Table 3 and Supplementary Table 3). The first subject was enrolled into the study on 09 February 2011 and the last on 17 November 2012.

Follow-up data at 6 months and 12 months are available for all subjects, except for three (#006, #011 and #021) who did not come for the follow-up at 6 months and for two (#006 and #021) at 12 months after the procedure.

Safety variables

The enriched cell concentrate was reported to be sterile and had no aerobic, anaerobic or fungal elements. The concentrate was also reported to be mycoplasma and endotoxin free in all subjects. Safety data revealed that surgery was tolerated well by all subjects. There were no surgery-specific complications, except for one case, in which the sutures dehiscid day 2 postoperatively. The hematological and biochemical laboratory findings were clinically insignificant postoperatively in all but one subject. The subject #011 developed a liver abscess post transplantation. The subject opted to get transferred to another center for further treatment and was lost to follow-up at 6 months and came for the 1-year follow-up. No adverse events were reported in any other subject. Magnetic resonance imaging evaluation did not reveal any infection, hemorrhage, tumor cells or increase in cord diameter in any of the subjects.

Feasibility end points. It is an easy and simple technique. Table 4 depicts the number and characterization of the cell population transplanted in all subjects. The bone marrow harvesting and stem cell enrichment procedure consistently provided the required number of cells for transplantation.

Efficacy end points. The total American Spinal Injury Association motor scores of subjects are depicted in Figure 1. None of the subjects had significant improvement in motor score. There was no improvement in motor score of any additional muscle group not included in ISNCSCI. One participant (intervention group) improved by six points (from 0 to 6) and three subjects (one in the control group and two in the intervention group) improved by five points each (from 0 to 5) on the WISCI scale. Spasticity, as assessed by the Modified Ashworth Spasticity Scale, decreased by two grades in three subjects and by one grade in two subjects. On the contrary, it increased by one grade in two subjects. There was no statistically significant difference between the groups. The details of sensory scores for light touch and pin-prick are provided in Figure 1.

There was improvement in SCIM score from baseline visit to follow-up in all subjects (Figure 2, Supplementary Table 1). There was significant improvement as compared with baseline in all groups with regard to bladder function, bowel function,

Table 3 Details of injury and intervention administered

ID (BMC/ISIC)	Grp	Level of Injury	Mode of Injury	Injury Mechanism	Procedure done	Date of injury	Date of decompression Surgery	Stem cell transplantation date	Laminectomy for primary surgery	Laminectomy for cell implantation
001	B	T6	FFH	Fracture dislocation	Posterior reduction and stabilization with pedicle screw T4-T6 and T8-T9 with bone grafting	28 Jan 11	31 Jan 11	11 Feb 11	None	None
002	A	T12	FFH	Fracture dislocation	Posterior reduction and stabilization with pedicle screw T10-L1 with bone grafting.	05 Feb 11	09 Feb 11	18 Feb 11	None	Yes
003	C	T12	RTA	Compression	Posterior reduction and stabilization with pedicle screw T11-L2	11 Feb 11	14 Feb 11	Control	Inferior lamina T12	
004	B	T3	RTA	Fracture dislocation	Posterior reduction and stabilization with pedicle screw T2-T5 with bone grafting	14 Mar 11	21 Mar 11	25 Mar 11	None	None
005	B	T12	FFH	Burst fracture	Posterior stabilization with pedicle screw T10-T11, L1-L2 with decompression transpedicular with bone grafting	22 Mar 11	25 Mar 11	06 Apr 11	None	None
006	C	T11	FFH	Fracture dislocation	Posterior stabilization with pedicle screw T8-L1	31 Jul 11	08 Aug 11	Control	None	None
007	B	T11	RTA	Fracture dislocation	Posterior stabilization with pedicle screw T10-L1	05 Aug 11	10 Aug 11	19 Aug 11	None	None
008	A	T10	RTA	Mechanism ^a unknown	Posterior decompression	15 Aug 11	29 Aug 11	29 Aug 11	T9-T11 laminectomy	Yes
009	A	T11	RTA	Fracture dislocation	Posterior decompression and stabilization with pedicle screw T10-L1	07 Sep 11	10 Sep 11	16 Sep 11	T12 laminectomy	Undertaken during primary surgery
010	A	T6	RTA	Burst fracture	Posterior stabilization with pedicle screw T5-T8	18 Sep 11	28 Sep 11	28 Sep 11	None	Yes
011	A	T7	FFH	Fracture dislocation	Posterior Stabilization with pedicle screw T6-T8 and Decompression	05 Jan 12	13 Jan 12	18 Jan 12	T6 laminectomy	Yes
012	C	T12	RTA	Fracture dislocation	Posterior stabilization with pedicle screws T9- T12	08 Jan 12	11 Jan 12	Control	T11-T12 laminectomy	
013	C	T10	RTA	Fracture dislocation	Posterior stabilization with pedicle screw T9-T12	25 Jan 12	08 Feb 12	Control	T11 laminectomy	Yes
014	A	T11	RTA	Burst Fracture	Posterior decompression and stabilization with pedicle screws T9-L1	30 Jan 12	02 Feb 12	08 Feb 12	None	Yes
015	B	T5	RTA	Fracture dislocation	Posterior decompression and stabilization with pedicle screw T6-T7	08 Feb 12	10 Feb 12	18 Feb 12	None	None
016	B	T5	RTA	Compression	Anterior column reconstruction with cage with pedicle screw T4-T5, T7-T8, and T6 corpectomy	01 May 12	02 May 12	13 May 12	T4-T5 laminectomy	
017	A	T8	RTA	Flexion distraction	Posterior decompression with pedicle screw fixation T7-T10	04 Jul 12	06 Jul 12	16 Jul 12	None	Yes
018	B	T12	FFH	Burst fracture	Posterior decompression and pedicle screw fixation T11-L1	20 Jul 12	23 Jul 12	30 Jul 12	None	None
019	C	T10	FFH	Fracture dislocation	Posterior stabilization with pedicle screw T9-T12	26 Sep 12	28 Sep 12	Control	None	None
020	C	T11	FFH	Fracture dislocation	Posterior stabilization with pedicle screw fixation at T11-L1 with interbody bone grafting T11-T12	24 Oct 12	27 Oct 12	Control	None	None
021	C	T11	FFH	Flexion distraction	Posterior stabilization with pedicle screw T9-T12	04 Nov 12	05 Nov 12	Control	None	None

Abbreviation: FFH, Fall from height; RTA, Road traffic accident; SCIMORA-Spinal Cord Injury Without Radiological Abnormality.

Table 4 Characteristics of the cell population transplanted in all subjects

S.No.	Case No. (ISIC/BMC)	Age	Sex	Body wt (kg)	Volume of bone marrow aspirated	Volume of bone marrow after concentration	Total cell yield	Total no. of mononuclear cells	% Viable	% CD34 ⁺ of total nucleated cells	Total no. of CD34 ⁺ cells obtained	No. of CD 34 ⁺ cells transplanted
1	001	26	M	85	60	9	9.9 × 10 ⁸	4.2 × 10 ⁸	98.10	0.95	9.4 × 10 ⁶	1.8 × 10 ⁴
2	002	30	M	100	60	10	7.2 × 10 ⁸	5.4 × 10 ⁸	99.00	0.98	7.0 × 10 ⁶	1.9 × 10 ⁴
3	004	20	M	55	60	10	8.4 × 10 ⁸	3.5 × 10 ⁸	98.40	0.96	8.0 × 10 ⁶	1.9 × 10 ⁴
4	005	20	F	45	60	10	9.8 × 10 ⁸	4.6 × 10 ⁸	98.80	1.10	10.7 × 10 ⁶	2.1 × 10 ⁴
5	007	18	M	44	60	9.5	9.7 × 10 ⁸	4.5 × 10 ⁸	98.25	0.94	9.1 × 10 ⁶	1.8 × 10 ⁴
6	008	24	M	50	60	10	8.5 × 10 ⁸	5.2 × 10 ⁸	98.88	0.97	8.2 × 10 ⁶	1.9 × 10 ⁴
7	009	24	M	45	60	10	10.1 × 10 ⁸	4.7 × 10 ⁸	98.56	0.98	9.8 × 10 ⁶	1.9 × 10 ⁴
8	010	22	M	56	60	10	9.8 × 10 ⁸	5.0 × 10 ⁸	99.20	1.12	10.9 × 10 ⁶	2.0 × 10 ⁴
9	011	37	F	58	60	10	9.8 × 10 ⁸	5.0 × 10 ⁸	99.20	0.95	9.3 × 10 ⁶	1.8 × 10 ⁴
10	014	30	M	84	60	9.8	8.9 × 10 ⁸	4.7 × 10 ⁸	99.50	0.92	8.3 × 10 ⁶	1.8 × 10 ⁴
11	015	18	M	64	58	9.5	9.9 × 10 ⁸	5.0 × 10 ⁸	99.70	0.94	9.3 × 10 ⁶	1.9 × 10 ⁴
12	016	26	M	65.5	60	10	9.7 × 10 ⁸	4.5 × 10 ⁸	98.90	0.88	8.6 × 10 ⁶	1.9 × 10 ⁴
13	017	33	F	65	60	9.8	9.8 × 10 ⁸	4.8 × 10 ⁸	99.40	0.98	9.6 × 10 ⁶	2.1 × 10 ⁴
14	018	20	M	68	60	9.7	9.8 × 10 ⁸	5.1 × 10 ⁸	98.70	0.96	9.4 × 10 ⁶	2.0 × 10 ⁴

ASIA MOTOR & SENSORY SCORES

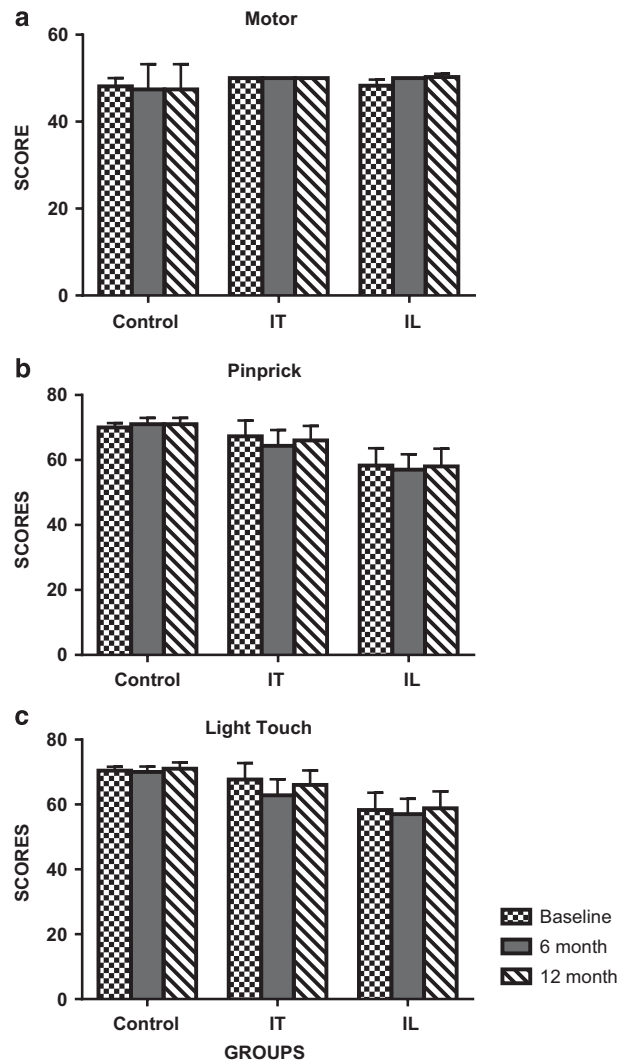


Figure 1 (a) American Spinal Injury Assessment Motor scores of all participants at baseline and at 6-monthly follow-ups. (b) International Standards for Neurological Classification of Spinal Cord Injury Sensory Examination (pin-prick) scores of all participants at baseline and at 6-monthly follow-ups. (c) International Standards for Neurological Classification of Spinal Cord Injury Sensory Examination (light touch) scores of all participants at baseline and at 6-monthly follow-ups.

injury-related pain, physical function and life impact at 6 and 12 months' follow-up on ISICIS assessment (Table 5, Supplementary Table 2). The majority of subjects chose not to answer the sexual function component of the ISICIS questionnaire; hence, data were not analyzed for this variable.

There was no change in electromyogram, SSEP or MEP in any of the subjects. There was no significant change in any variable of the urodynamic assessment in all subjects, except for reduced compliance observed in five subjects.

DISCUSSION

Despite the huge base of preclinical evidence supporting the restoration of neuronal function through cellular interventions, the clinical

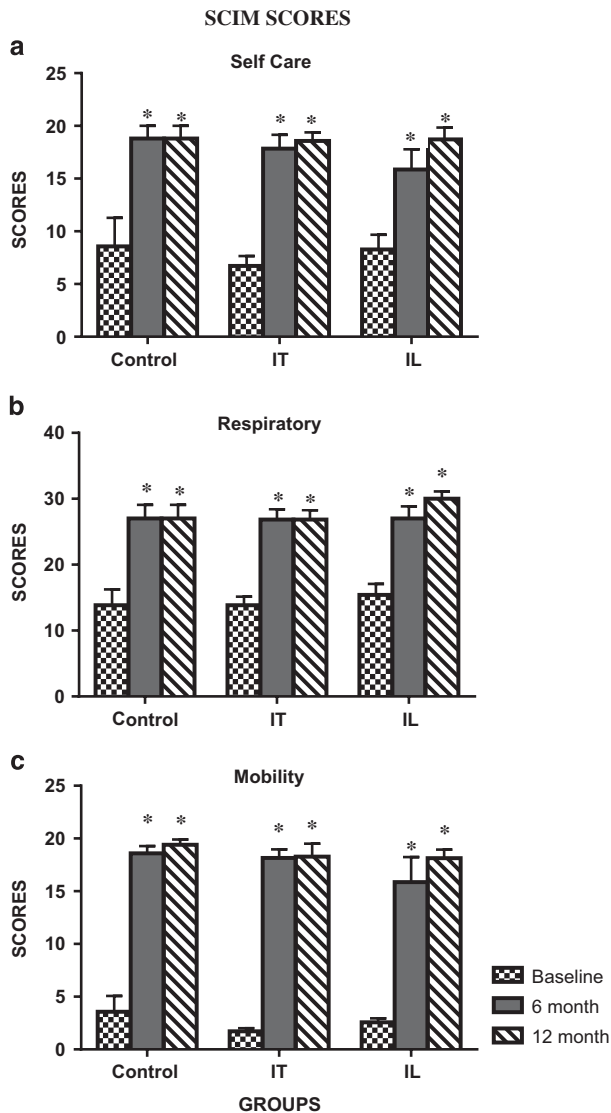


Figure 2 SCIM scores of all participants at baseline and at 6-monthly follow-ups: (a) self-care score; (b) respiratory score; and (c) mobility score.

evidence has not been that encouraging. A huge gap still remains between the 'bench' and the 'bedside' and needs to be bridged.¹⁶ This may be due to the inherent limitations of the preclinical studies as their conclusions are dependent upon the mechanism of injury and the animal model used.² Also, factors such as cell population used, timing of intervention, patient selection criteria, spontaneous recovery confounding the interpretation of results, poor trial design and lack of standardized outcome measures underlie the low level of clinical evidence base supporting the efficacy of cellular transplantation in case of SCI.^{2,17} The trial was thus designed to address these factors with an aim to provide robust, standardized and validated data regarding the potential of stem cell interventions in case of SCI.

As compared with incomplete SCI subjects (AIS B, AIS C and AIS D), motor complete SCI subjects (AIS A) have fairly limited and predictable neurological recovery. Most of the spontaneous neurological recovery in AIS A subjects is likely to occur within the Zone of Partial Preservation.¹⁸ Hence, only AIS A subjects were included in the study.

The potential of regeneration and recovery of the spinal cord may reduce with time.^{1,17} Hence, even though it is preferable to recruit chronic SCI subjects in such a trial (as any potential for spontaneous recovery would have plateaued by that time), we chose to do this study in acute SCI patients.

Preclinical studies suggest that transplants are more likely to survive if the procedure is undertaken during the subacute phase (10–14 days post injury) as the secondary cascade events in the acute phase of injury may affect the survival of the graft.^{19,20} In a clinical trial Yoon *et al.*²¹ have reported a better outcome following cell transplant in their acute and subacute injury group as compared with the chronic injury group. Also, as per guidelines for the conduct of clinical trials for SCI as developed by the ICCP Panel, any therapy initiated within the acute phase of injury (up to ~72 h post injury) would need a very large number of subjects to identify significant differences due to the intervention as a result of high probability (20%) of spontaneous recovery in AIS A subjects at this stage.²² In the subacute phase of injury, the probability of spontaneous recovery in case of AIS A subjects reduces to 10%, leading to a reduction in the number of subjects required to detect a potentially significant benefit. Hence, we chose to undertake the intervention in the subacute (10–14 days post injury) phase of injury. If during manipulation of cervical spinal cord regions of critical healthy spinal cord tissue rostral to the injury are compromised, it may lead to severe adverse events.²² Thus, for this study subjects with cervical neurological level lesions were not included.

The study design was in conformance with the 'Guidelines for the conduct of clinical trials for spinal cord injury' as developed by the International Campaign for Cures of SCI Paralysis panel.²²

Bone marrow transplantation for SCI has been the focus of attention in the past few years. There have been extensive preclinical studies that have demonstrated their potential role. Transplanted BMCs were found to improve neurological deficit in CNS injury models by generating neural cells or myelin-producing cells.⁶ The use of BMCs for stem cell therapy in SCI subjects has more advantages compared with embryonic stem cells; therefore, the cell population has been used more widely.¹ Extensive scientific data on BMCs are also available from wide-ranging experiences in bone marrow transplantation for hematological diseases.²³ Bone marrow stem cell-based therapy is not associated with carcinogenesis, which is an adverse event documented in case of embryonic stem cell therapy.²⁴ It is also possible to use these cells for allogenic transplantation as there is little risk of immunological rejection or graft-versus-host reactions.²⁵

The safety of transplanted unmanipulated autologous bone marrow in subjects with SCI has been reported by Sykova *et al.*²⁶ However, the authors could not confirm that the observed beneficial effects were due to cellular intervention.²⁶ Other groups have transplanted enriched or expanded bone marrow mesenchymal stem cells in SCI subjects either alone or in combination with CD34+ cells or GM-CSF.^{21,25} However, because of various limitations of these studies, the outcomes reported are inconclusive.²

This study was initially planned to transplant the Ficoll-purified mononuclear cell population as used in other studies. However, because of regulatory constraints in India that did not allow the use of a Ficoll-purified population, an enriched BMC population was transplanted. Before undertaking the transplantation, a validation study was undertaken by us where the enriched bone marrow population was analyzed for CD34+ cell and mononuclear cell percentage. These were found to be comparable to that reported for Ficoll-purified cells.²⁷ The sterility of processed cells was also established by us.

Table 5 ISCIS^a scores at baseline, 6-month and 12-month follow-up

Group	Control			Intrathecal			Intralesional		
	B	6	12	B	6	12	B	6	12
Bladder	23.3±1.6	16.2±2.0	15.4±1.8	21.4±3.7	16.2±2.4	16.7±2.6	31.4±1.3	17.8±2.3	19.0±2.8
Bowel	26.3±0.7	18.4±1.9	16.8±1.9	24.8±2.1	16.1±3.4	18.1±2.5	29.7±2.2	19.3±2.6	19.6±2.3
Pain	20.1±0.7	11.0±1.9	11.4±1.9	19.4±2.1	13.8±2.3	14.1±1.8	19.3±2.1	15.0±2.4	13.8±2.2
Physical function	60.1±2.8	48.2±2.6	44.4±3.3	65.7±6.0	55.2±5.2	50.0±5.2	57.4±3.3	49.4±4.8	50.3±5.3
Life impact	32.4±3.4	20.0±2.4	18.0±1.7	31.3±0.6	21.8±3.6	24.0±3.1	34.4±1.6	27.3±2.7	27.0±3.0
Sexual function ^b	—	—	—	—	—	—	—	—	—
Total	162.2±2.7	119.8±2.9	118±2.6	162.6±3.1	123.1±3.3	134.9±2.5	172.2±2.3	134.8±2.4	141.7±2.5

Abbreviation: ISCIS, International Spinal Cord Injury Scale.

^aScores ± s.e.m.^bNot analyzed because of insufficient data

The procedure was found to be safe, as revealed by our 12-month follow-up findings. There was no incidence of infection/meningitis. There was one incidence of liver abscess in a subject who received the cells via intrathecal transplantation. The exact cause of the liver abscess could not be determined. The prescreening and screening assessments of the subject had been normal and the transplanted material was found to be negative for aerobic and anaerobic bacteria, mycoplasma and fungus. The subject had opted to get transferred to another center for further treatment and the abscess had completely resolved. The subject was lost to follow-up at 6 months. At the 12-month follow-up visit, the liver abscess was documented by the other center as a fungal infection. As the transplanted material was reported to be sterile, the cause of the infection may have been factors other than the transplanted material. The subject had been referred to us from another hospital where the subject had received primary treatment for the SCI. This could be a case of hospital-acquired or coincidental infection.

Zariffa *et al.*²⁸ suggested that deterioration of three or more thoracic sensory levels or loss of upper-extremity motor function are rare events and may be used to track the safety of therapeutic intervention in early-phase acute SCI clinical trials.²⁸ The absence of this in our subjects also suggests the safety of the intervention. A longer follow-up is being undertaken to establish long-term safety.

The stringent inclusion–exclusion criteria resulted in a slow recruitment of subjects. This problem had also been faced by another similar study with similar inclusion–exclusion criteria.⁴ Another factor that slowed down the recruitment in our study was that subjects, who initially showed interest in our study, later opted for one of the numerous other centers in the country offering some form of cellular transplantation ‘cure’.

Single blinding of assessors may have led to decreased compliance to trial follow-up visits by the control subjects. This may have resulted in two subjects being lost to follow-up (at both 6- and 12-month visits) in the control group. Only one subject was lost to follow-up from the interventional groups at the 6-month visit but was recovered at the 12-month visit.

Our study established the feasibility of the procedure. It was possible to aspirate bone marrow from the patient and transplant it within 2 h of aspiration. The cell population studied in our study was an enriched BMC population and not a Ficoll-purified mononuclear cell population as reported by others. The data provided by our study regarding the composition of the cell population transplanted are of importance as the data provide leads to correlating the number of cells and transplanted cell population with clinical findings in the follow-up visits. This would also be useful for standardizing the dose of transplanted cells in future studies.

There was no significant improvement in the primary efficacy variable, that is, the American Spinal Injury Association motor score of the subjects. Such an outcome corresponds to the natural history of complete SCI.¹⁷ The study was just a pilot study and a larger study with a longer follow-up is required to determine the efficacy of the procedure.

There was also no significant improvement in the secondary efficacy variables other than SCIM or ISCIS. The statistically significant improvement in functional examination (SCIM) scores without any significant changes in neurological (American Spinal Injury Association motor or sensory score) or electrophysiological (electromyogram, SSEP or MEP) outcomes suggests that the improvements in SCIM may have been because of compensation and exploitation of neuronal plasticity by functional training and not because of regeneration. Curt *et al.*¹⁷ have postulated this mechanism for improvement in activities of daily living of subjects that occurs without any change in neurological condition. The functional improvement could be responsible for the statistically significant improvement in scores of physical function and life impact (as demonstrated through the ISCIS questionnaire). The improvement in scores of bladder and bowel function could be due to the bladder and bowel training.

The findings of our study differ from those of other human trials undertaking transplantation of autologous BMCs in SCI subjects published so far^{13,15,21,25,26,29} in that no efficacy could be demonstrated in our study that could be attributed to the procedure. This disparity may be attributed to our stringent trial design with only AIS A subjects, type of cell population transplanted and validated outcome measures used by us to analyze the effect of BMC transplantation in persons with acute SCI. Some of the previous studies had also enrolled incomplete SCI subjects and thus any improvement could have been due to the natural history of the disease. Further, assessors were not blinded in most of these studies and many also lacked appropriate control groups.

Recent publications on the mechanisms involved in repair and regeneration post SCI provide valuable insights regarding potential barriers to regeneration after SCI.²⁴ These need to be addressed by scientists and clinicians to define new strategies for achieving repair. Basic scientific research should be directed toward providing a rational basis for tailoring specific combinations of clinical therapies to different types of SCI. Functional regeneration should be the primary goal of any approach being tested and it is important that this be tested by scientifically validated and universally accepted outcome measures and tools.

Because of the involvement of multiple cell types and the complexity of SCI, it is becoming increasingly clear that a single approach as

used in our study may not be successful in achieving SCI repair.² A multifactorial approach involving inhibition of secondary cascade events, molecular therapy, cell transplantation, bioactive scaffolding matrix, growth factor supplementation and scar removal is required to address this situation. Multicentric studies involving a standardized and validated approach, a stringent trial design with appropriate outcome measures and rehabilitation protocol are a must to understand and achieve the potential of such combinatorial strategies (including cellular therapy) in case of SCI.

The following could be the limitations of this study:

- The small sample size may not be enough to conclude on the efficacy of the study.
- The outcomes may have been affected by an inappropriate number of transplanted cells.

CONCLUSIONS

The procedure of BMC transplantation is relatively safe and feasible in AIS A subjects with thoracic-level injuries at 12-month follow-up. A full follow-up of 5 years in all 21 subjects will further reinforce the safety of the procedure. No efficacy could be demonstrated that could be attributed to the procedure. However, it may not be possible to conclude on the efficacy of the procedure because of the limitations of the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA ARCHIVING

There were no data to deposit.

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Supplementary Information accompanies this paper on the Spinal Cord website (<http://www.nature.com/sc>)