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Improved Bowel Function in Patients with Spina Bifida After Bone Marrow-Derived Mononuclear Cell Transplantation: A Report of 2 Cases

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Case series

Patient: Male, 14 • Female, 11
Final Diagnosis: Improvement in bowel functions after bone marrow mononuclear cells transplantation
Symptoms: Impaired bowel functions • spina bifida
Medication: —
Clinical Procedure: Bone Marrow-Derived Mononuclear Cell Transplantation
Specialty: Pediatrics and Neonatology

Objective: Congenital defects/diseases
Background: Bowel dysfunction is observed in 42.2–71.2% of patients with spina bifida. Traditional treatments yield limited results. The objective of this paper is to report on improvement in bowel function in 2 children with spina bifida following bone marrow-derived mononuclear cells transplantation.
Case reports: Two patients – 14 years old and 11 years old – with bowel dysfunction after myelomeningocele repair underwent 2 BMMNC transplantations without complications. Those patients had normal defecation, assessed through follow-ups of 21 months and 16 months, respectively.
Conclusions: BMMNC transplantation can improve bowel function, as demonstrated in 2 patients with spina bifida.

MeSH Keywords: Adult Stem Cells • Bone Marrow Transplantation • Spina Bifida Occulta

Full-text PDF: <https://www.amjcaserep.com/abstract/index/idArt/909801>

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Background

Spina bifida (SB) is the most common malformation due to neural tube defects and has a prevalence ranging from 3.5 to 42.8 per 10 000 births, with geographic variation. There are 2 types of SB – SB aperta and SB occulta – with different subtypes (myeloschisis, myelomeningocele, meningocele, lipomeningocele, and spinal dorsal dermal sinus tract) [1,2].

Although children with SB have been shown to have a low fatality rate, most of them suffer from long-term functional disorders such as urinary dysfunction, bowel dysfunction, paraplegia, distress, and other problems. These dysfunctions are sequelae of primary anatomical defects of the spinal cord and its injury during pregnancy and after delivery. During pregnancy, neural tissue destruction happened as a result of the spinal cord being exposed to amniotic fluid. Spinal nerves may be further damaged while awaiting surgery or during postnatal repair of SB [3–6].

Among many associated disabilities, bowel dysfunction (constipation, fecal incontinence, or both) is observed in 42.2–71.2% of patients with SB [7,8]. Quality of life of these patients is severely affected and they often experience embarrassment, anxiety, depression, and social isolation [9].

Multiple approaches have been conducted in an attempt to improve bowel function in patients with SB, including adjustment of diet and fluid intake, oral laxatives, antegrade continence enema, transanal irrigation, digital stimulation or extraction of stool, or nerve-stimulation implants, but these treatments sometimes fail to achieve fecal continence [10–12].

Recently, stem cell transplantation (SCT) has emerged as an alternative treatment option for patients with neurologic damage, and it has resulted in promising outcomes [13–19]. Especially in patients with spinal cord injury, improvements of bowel and bladder function were observed at different levels after SCT transplantation [20].

SCT was also tried for SB in animal models and humans. In animals, injection of mesenchymal stem cells into amniotic fluids can induce partial or complete coverage of experimental SB [21–24]. In humans, Gupta et al. published the first paper on BMMNC transplantation for patients with meningocele in 2007; the procedure was safe, without perioperative complications [25]. However, follow-up results have not been reported, and since then, to the best of our knowledge, no other studies using stem cells for SB have been published.

The aim of this report is to present results on the improvement of bowel function after BMMNC transplantation in 2 patients with SB.

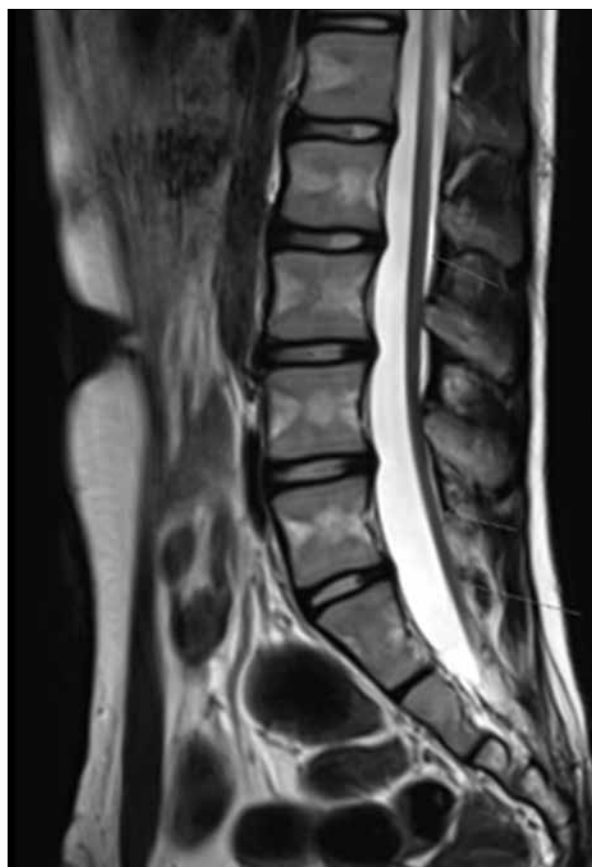


Figure 1. MRI shows a low termination of the spinal cord.

Case Report

Patient 1

A boy was born in October 2001. No abnormality was detected during prenatal ultrasound. However, a myelomeningocele located in the lumbosacral area was detected after birth, which was repaired at 10 days of age, and ventriculoperitoneal shunting was carried out at 3 months of age due to hydrocephalus. After myelomeningocele repair, the patient could not defecate or void voluntarily. Daily enema was required to evacuate stool, and clean intermittent catheterization (CIC) was applied to empty the bladder. Leakage of urine occurred frequently. The patient had never had any urge sensation of defecation or urination.

On examination at Vinmec International Hospital in July 2015:

- Uremia: 5.3 mmol/l, Creatinemia: 96 μ mol/l.
- Urinary tract sonography revealed a thickened bladder wall many diverticula.
- Lumbar spine MRI: The spinal cord terminated at S2 level (Figure 1).
- Cystography showed the bladder with an abnormal shape, mildly deviated to the right side with many diverticula

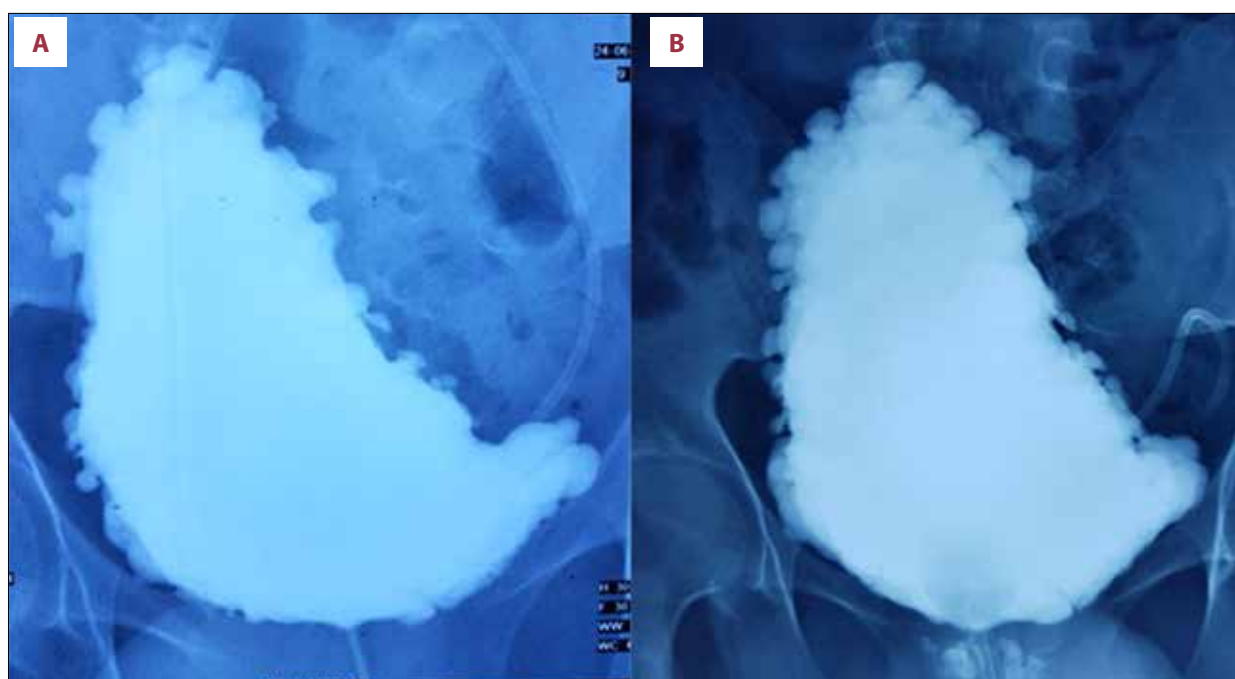


Figure 2. (A) Pretransplantation cystography of the first patient demonstrates many diverticula. (B) Post-transplantation cystography of the first patient still shows many diverticula.

Table 1A. Rectal manometry before and after BMMNCs transplantations of the first patient.

Indicator	Pre-transplantation	Post-transplantation	Normal value [26]
Rectal pressure: Resting/squeeze/strain	9.8/9.6/24	10.5/14.8/23.7	No information
Anal resting pressure	16	44.4	96 mmHg
Anal squeeze pressure	18	76.8	229 mmHg
First sensation	None	20 ml	22.1 ml
Urge	No defecation urge sensation	60 ml	55 ml
Rectoanal inhibitory reflex (RAIR)	40 ml	20 ml	18.6 ml

(Figure 2A). Rectomanometry revealed no rectal sensation (Table 1A).

- The urodynamic investigation revealed very poor detrusor function during filling phase (Table 1B, Figure 3A).

Diagnosis of bowel and bladder dysfunction was confirmed, and BMMNC transplantation was indicated after receiving approval of the hospital board and written informed consent from the parent. BMMNC transplantation performed twice with an interval of 6 months.

The first BMMNC transplantation was performed on August 10th, 2015

Bone marrow aspiration was performed through the bilateral anterior iliac crests under general anaesthesia in the operating

room. We obtained 250 ml of bone marrow. The density gradient centrifugation method using Ficoll was conducted to separate and collect BMMNCs.

The total blood components and the number of BMMNCs were analyzed and calculated before and after the Ficoll separating process using a Beckman Coulter LH780. To identify the number of hematopoietic progenitor cells (CD34+) from the patient's bone marrow after the Ficoll process, the samples were freshly prepared and incubated with Stem Kit™ Reagent (Beckman Coulter) according to the manufacturer's protocol, followed by running through a flow cytometer (Navios Beckman Coulter, Navios software V2.0). Before transfusion, the BMMNC solutions were examined for Mycoplasma and Endotoxin using the MycoAlert™ PLUS Mycoplasma detection kit (Lonza) and Endosafe-PTS kit (Charles River), respectively.

Table 1B. Urodynamic investigation before and after transplantations of the first patient.

Indicator	Pre-transplantation	Post-transplantation	Normal value [27,28]
Vesical capacity	273 ml	333 ml	480–510 ml
First sensation of voiding	200ml	185 ml	211 ml (200–330 ml)
Strong desire to void			>350 ml
P det max	5 cm H ₂ O	15 cm H ₂ O	6–15 cm H ₂ O
Detrusor function during filling phase	Poor detrusor contraction	Improved	Stable
Leakage	The leak was not noticed during filling phase		

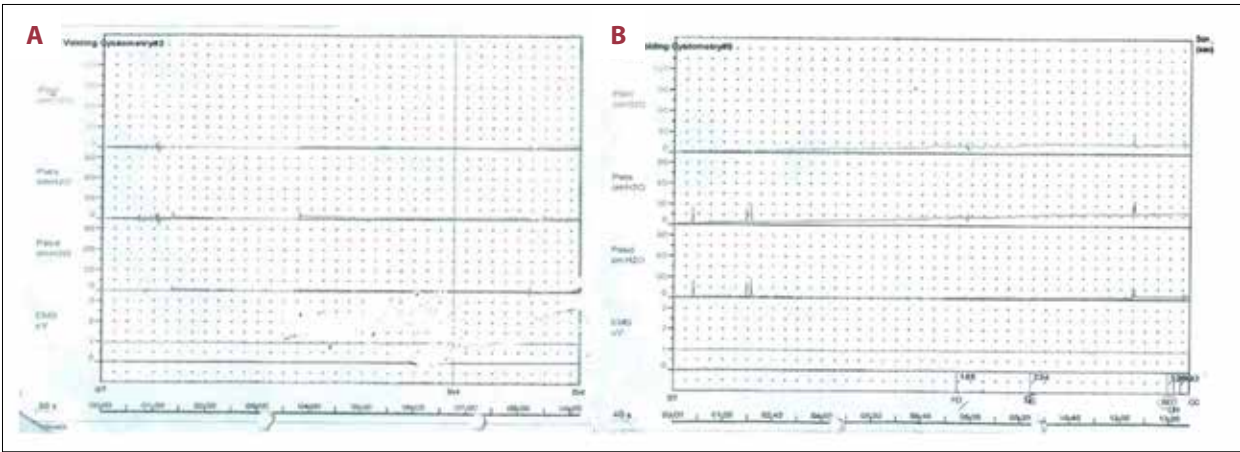


Figure 3. (A) Pre-transplantation urodynamic investigation of the first patient reveals a very underactive detrusor function. (B) Post-transplantation urodynamic investigation of the first patient shows increased detrusor function.

A transplant solution with a volume of 10 ml was obtained after processing, containing a total of 2.22×10^8 MNCs and 2.89×10^7 CD34 cells (Figure 4A), equal to 4.93×10^6 MNC/kg and 6.43×10^5 CD34 cells/kg, respectively, and were infused intrathecally between the 4th and 5th lumbar space. There were no adverse events during or after the procedure. The patient was discharged 2 days after the transplantation.

Evolution after the first transplantation:

- Bowel function: At 5 months after the transplantation, the patient could feel the urge to defecate and was able to defecate once every 2–3 days.
- Bladder function: He could feel the urge to urinate and was able to urinate about 10 ml at a time.

The second stem cell transplantation was carried out on July 6th, 2017.

We collected and processed 250 ml of bone marrow, as above. After processing, 10 ml of solution containing a total of 3×10^8 MNC and 1.69×10^7 CD34 cells (Figure 4B), which was approximately 6.67×10^6 MNC/kg and 3.76×10^5 CD34 cells/kg,

respectively, were infused intrathecally through the 4th and 5th lumbar space without perioperative adverse events. The patient was discharged 2 days after the transplantation.

Follow-up at 21 months after the first transplantation.

- Bowel function: The patient was able to defecate once every 1D2 days without soiling or fecal incontinence. Rectal sensation was observed on rectomanometry (Table 1A).
- Bladder function: Increased detrusor contractility was observed on urodynamic investigation (Table 1B, Figure 3B).

He could feel the urge to urination and was able to urinate voluntarily about 40 ml at a time; however, his bladder did not entirely empty, so CIC was still required. Many diverticula still appeared on cystography (Figure 2B).

Patient 2

A girl was born in January 2005. A small mass located in the lumbar coccygeal area was detected after birth. However, no investigation was carried out. At 9 months old, she could not defecate spontaneously. Urinary infections occurred repeatedly.

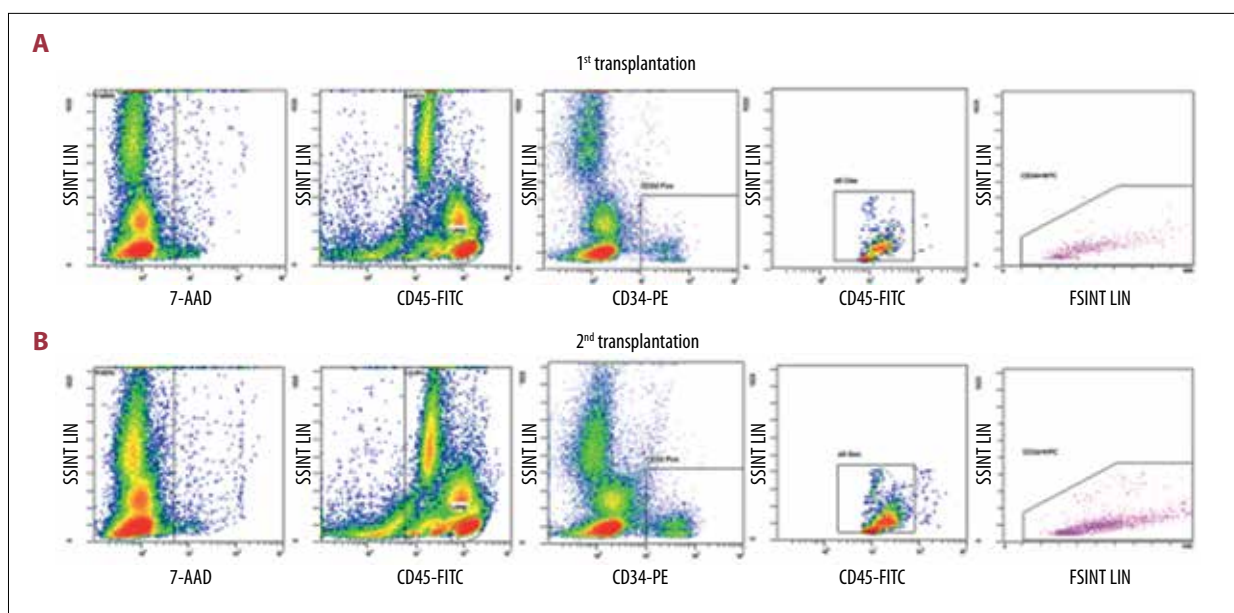


Figure 4. Flow cytometry of CD34+ analysis of the first patient. Calculation of CD34+ population from the BMMNCs at the 1st transplantation (A) and 2nd transplantation (B).



Figure 5. Spinal MRI of the second patient demonstrates a low termination of the spinal cord.

MRI revealed SB with a small myelomeningocele located at L5 and S1. Daily colon enemas were required to evacuate stool. The operation to repair the myelomeningocele was performed at 28 months old. After the operation, she was still unable to

defecate spontaneously and dysuria occurred. A daily colon enema and CIC were required. The child never experienced an urge to defecate or to urinate.

On examination at Vinmec International Hospital in May 2016:

- Uremia: 3.07 mmol/l, Creatininemia: 60 μ mol/L.
- Abdominal ultrasound showed a left dilated ureter and irregular bladder wall with many diverticula.
- Vertebral MRI: The spinal cord terminated at S3–S4 level (Figure 5).
- Cystography showed an irregular thickness of the bladder wall, with multiple pseudodiverticula, especially in the bladder fundus region (Figure 6A).
- Rectomanometry: The first rectal sensation was perceived at 40 ml and constant sensation at 100 ml (Table 2A).
- The urodynamic investigation showed a high vesical pressure and P det (Table 2B, Figure 7A).

The diagnosis of bowel and bladder dysfunction was confirmed. The first BMMNC transplantation was performed on July 29th, 2016 with the approval of the Hospital's Board of Directors and written informed consent from her parent.

Similar BMMNC volume, preparation, and quality control were applied as described in the first patient. A 10-ml solution containing 5.2×10^8 MNCs and 3.65×10^7 CD34 cells in total (1.68×10^7 MNC/kg and 1.18×10^5 CD34 cells/kg, respectively) were infused intrathecally through the vertebral space between the 4th and 5th space (Figure 8A).

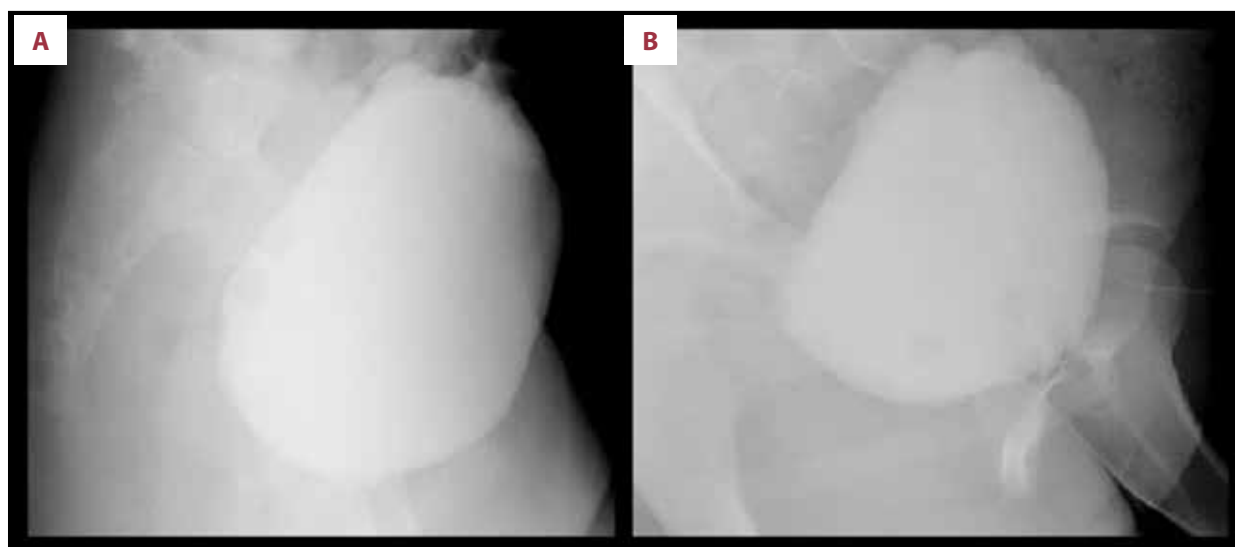


Figure 6. (A) Pre-transplantation cystography of the second patient shows multiple diverticula. (B) Post-transplantation cystography of the second patients still shows multiple diverticula.

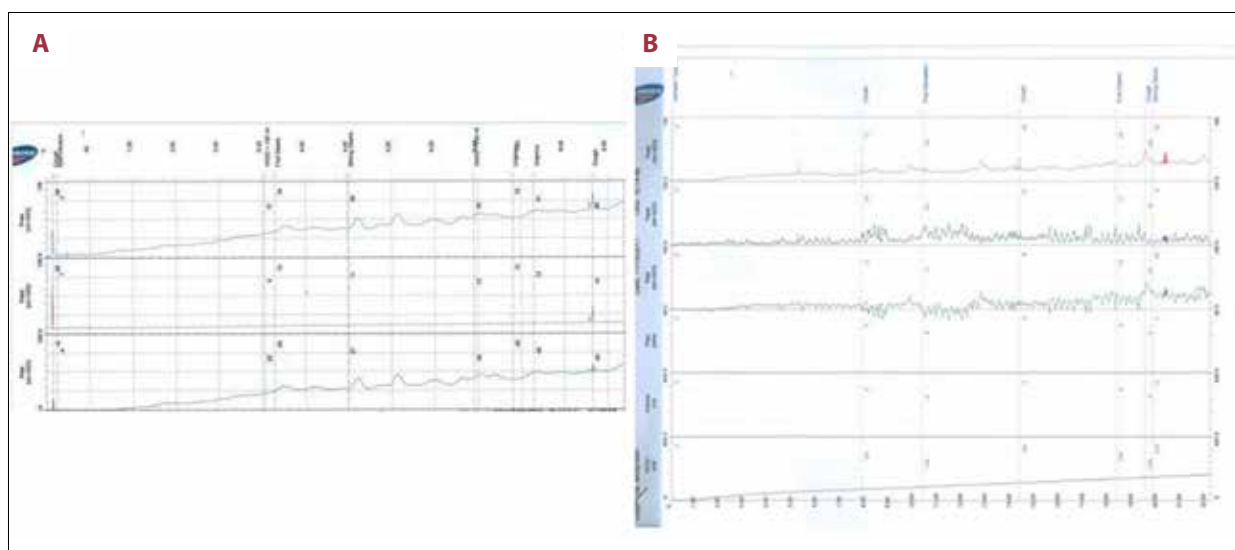


Figure 7. (A) Pre-transplantation urodynamic investigation of the second patient shows hyperactive detrusor functions. (B) Post-transplantation urodynamic investigation of the second patient reveals reduced hyperactive detrusor function.

Evolution after the first transplantation

- Bowel function: At 4 months after the transplantation, the patient experienced urge sensation of defecation and was able to defecate voluntarily 1–2 times per day without laxatives.
- Bladder function: The patient developed urinary urge and was able to urinate spontaneously at a small volume; however, her bladder could not be emptied completely, so CIC was still needed.

The 2nd stem cell transplantation was carried out on March 24th, 2017

Similar BMMNCs volume, preparation and quality control were applied as described previously. A 10-ml solution containing 6.4×10^8 MNCs and 1.12×10^7 CD34 cells (approximately 1.78×10^7 MNC/kg and 3.1×10^5 CD34 cells/kg, respectively) were infused intrathecally through the vertebral space between the 4th and 5th space without adverse events (Figure 8B). The patient was discharged 2 days after the transplantation.

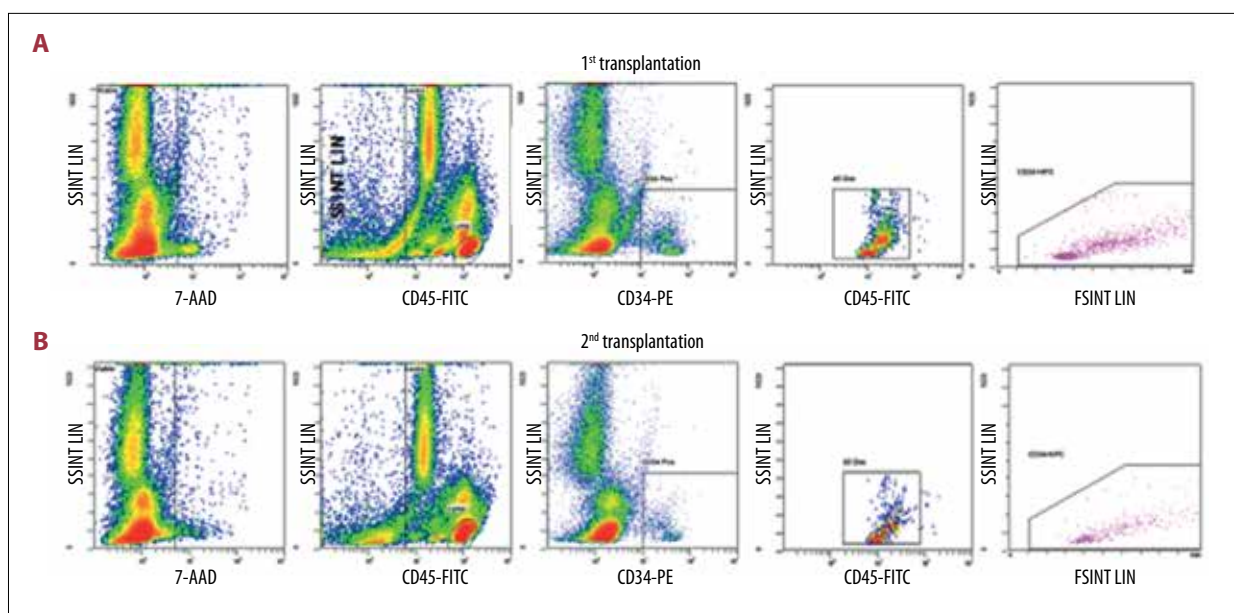


Figure 8. Flow cytometry of CD34+ analysis of the second patient. Calculation of the CD34+ population from the BMMNCs at the 1st transplantation (A) and 2nd transplantation (B).

Table 2A. Rectal manometry before and after BMMNCs transplantations of the second patient.

Indicator	Pre-transplantation	Post-transplantation	Normal value [26]
Rectal pressure: Resting/squeeze/strain	5/6/15 mmHg	3/12/23 mmHg	No information
Anal resting pressure	18	47	94 mmHg
Anal squeeze pressure	26	67	206 mmHg
First sensation	40 ml	20 ml	14.7 ml
Urge	No defecation urge sensation	80 ml	36.3 ml
Rectoanal inhibitory reflex (RAIR)	20 ml	10 ml	14.7 ml

Table 2B. Urodynamic investigation before and after transplantations of the second patient.

Indicator	Pre-transplantation	Post-transplantation	Normal value [27,28]
Vesical capacity	230 ml	246 ml	360–400 ml
First sensation of voiding	105 ml	124 ml	No information
Strong desire to void	140 ml	219 ml	No information
P det max	50 cm H ₂ O	40 cm H ₂ O	<20 cm H ₂ O
Detrusor function during filling phase	Overactive	Improved	Stable

Follow-up 16 months after the first transplantation:

- Bowel function: The patient was able to defecate spontaneously 1–2 times per day without soiling or fecal incontinence. Rectal sensation was improved. The first sensation was perceived at 20 ml and constant sensation at 60 ml (Table 2A).
- Bladder function: The patient had urinary urge and was able to urinate a small amount, but CIC was still required to empty her bladder completely. Urodynamic analysis showed decreased vesical pressure and P det (Table 2B, Figure 7B). Multiple diverticula were still observed on cystography (Figure 6B).

Discussion

To the best of our knowledge, this is the first report showing improvement of bowel function in patients with SB after BMMNC transplantation. Our results reveal that intrathecal stem cell transplantation was safe and effective for 2 patients with SB. There were no intraoperative or postoperative complications. Bowel function was remarkably improved after BMMNC transplantation. These 2 patients could feel the urge to defecation and pass stool voluntarily at 5 months and, 4 months, respectively, after the first transplantation. At follow-up 21 months and 16 months, respectively, after the first transplantation, the patients achieved normal defecation. Oral laxatives or other supportive measures were not required to evacuate stools.

Improvement of bladder function was not significant compared to that of bowel function in our patients after BMMNC transplantations. Although urodynamic investigation showed some improvements in the 2 patients and they could feel the urge to urinate and could spontaneously void a small amount of urine, their bladders could not be emptied completely.

The failure to achieve urinary continence may have been due to performing BMMNC transplantation at the late stage, when the bladder already has suffered from severe fibrosis and has poor function. To determine whether better bladder function could be achieved through earlier STC transplantation, additional studies with larger patient numbers and longer follow-up need to be performed and evaluated.

The mechanism by which stem cells improve bowel and bladder function in SB patients is still poorly understood. However, there are similarities between spinal cord injury in patients with SB and in patients with spinal cord injury due to trauma. The improvement of bowel and bladder function in our 2 patients could be explained based on knowledge of STC transplantation in patients with spinal cord injury.

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In animals with spinal cord injury, transplantation of bone marrow-derived cells or bone marrow mesenchymal stem cells can produce myelin repair, improvement of degeneration of axons, an increase of axonal regrowth, promotion of neurite outgrowth of spinal neurons, and secretion of neurotrophic factors [29]. Evidence of improved bladder function has been reported in 2 studies, which illustrated the potential mechanism of SCT in spinal cord injury via inhibition of unmediated C-fiber sprouting from bladder afferents. These effects contributed to the decreases of C-fiber formation in bladders, directly controlled by bladder-to-bladder spinal micturition reflex [30,31]. In patients with spinal cord injury, Jiang et al. showed improved bowel function at different levels in 9 of 12 cases (60%), and improved urinary bladder function in 8 of 10 patients (80%) after stem cell transplantation [20].

Conclusions

In conclusion, BMMNC transplantation significantly improved defecation function in 2 patients with SB. A study with a more patients and longer follow-up is required to confirm the efficacy of this treatment.

Statement

All laboratory equipment and stem cell preparations were provided by Vinmec Research Institute of Stem Cell and Gene Technology. Clinical standard operations and drugs were performed and provided by medical doctors and staff at Vinmec International Hospital.

Conflict of Interests

The authors have declared that no competing interests exist.

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