Neurourology and Urodynamics

A Novel Cell Therapy for Stress Urinary Incontinence, Short-Term Outcome

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Aims: The aim of this study was the safety assessment of urethra injections of autologous total nucleated cells (TNCs) along with platelets, which focused on the outcome over a 6 month period. **Methods:** An open, prospective study was conducted on 9 patients with severe stress urinary incontinence (SUI). At the baseline, 1, 3, and 6 months after external urethral sphincteric and submucosal injections of autologous TNCs along with platelets, the patients were assessed according to cough tests, Q-Tip tests, urodynamics, 1 hr pad tests, upper tract ultrasonography (UTU), post voiding residue (PVR), International Consultation on Incontinence Questionnaire-Urinary incontinence (ICIQ-UI), and International Consultation on Incontinence Modular Questionnaire-Quality of Life (ICIQ-QOL). On the 3rd month post-injection, the maximum urethral closure pressure (MUCP) and abdominal leak point pressure (ALPP) were measured in one patient with intrinsic sphincteric deficiency (ISD; the baseline: ALPP < 60 and MUCP < 30 cmH_2O). **Results:** No complications were observed after injection. At 6-months' follow up (F/U), all the patients considered themselves clinically cured with eight women completely continent and one marked improvement. Mean age was 48.9 ± 13.8 years. Before the injection, urodynamics, UTU, and PVR were normal and cough tests, 1 hr pad tests were positive in patients. At 1, 3, and 6 months post-injection, there was a significant improvement in ICIQ-UI, ICIQ-QOL ($P < 0.05$). UTU and PVR were normal, cough tests, and 1 hr pad tests were negative, except for ISD patient with severe coughs (at month 3: ALPP $= 92$ and MUCP > 30 cmH_2O). **Conclusion:** Cell therapy consisting of intrasphincteric and submucosal injections of autologous TNCs along with platelets in SUI patients is a feasible and safe procedure. The results point out those subjects cured or with marked improvement after 6 months F/U. *Neurourol. Urodynam.*

Key words: peripheral blood; platelets; stem cells; stress urinary incontinence

INTRODUCTION

Stress urinary incontinence (SUI) is defined as the involuntary leakage of urine on effort, exertion, sneezing or coughing. The well-established risk factors for the development of SUI are pregnancy and vaginal childbirth, vaginal or pelvic surgery, aging, obesity, white race, and smoking.[1] Treatment for SUI includes surgical and non-surgical options. The gold standard treatment option available is an invasive surgical procedure, which uses transvaginal tape (TVT). Ward and Hilton[2] reported only 63% of the TVT group was objectively cured at 2 years. The subjective cure rate was even less impressive at 43%. Also, adverse events have been reported due to TVT, perforation or laceration of the vessels, nerves, bladder, urethra or bowel during placement, erosion of the vaginal or urethral mucosa or the bladder wall, and fistula development.[3]

Also, disappointing results were obtained in non-surgical options. Duloxetine is used in pharmacotherapy for treatment of SUI, and is available in Europe, however, it was not approved for use by the FDA.[4] The use of injectable bulking agents such as polytetrafluoroethylene (PTFE, Teflon), bovine collagen, silicone particles, and carbon beads has yielded short-term success rates in the treatment of SUI. However, such bulking agents cause chronic inflammation and initiate the foreign body giant cell response and other complications associated with the encapsulation of biomedical materials.[5]

"The use of one body part for another or the exchange of parts from one person to another was mentioned in the medical literature even in antiquity and captured the imagination of many over time." (Campbell's Urology 2012, chapter 74 page: 2168).

Abbreviations: SUI, stress urinary incontinence; TNCs, total nucleated cells; F/U, follow up; U/A, urine analysis; U/C, urine culture; ICIQ-UI, International Consultation on Incontinence Modular Questionnaire-Urinary Incontinence; ICIQ-QOL, International Consultation on Incontinence Modular Questionnaire-Quality of Life; ALPP, abdominal leak point pressure; MUCP, maximum urethral closure pressure; UPP, urethral pressure profilometry; ISD, intrinsic sphincteric deficiency; PVR, post voiding residue; UTU, upper tract ultrasonography; ICS, International Continence Society; UFL, uroflowmetry; HES, hydroxyethyl starch; BMSCs, bone marrow-derived mesenchymal stem cells; MDPSCs, muscle-derived progenitor stem cells; HUCB, human umbilical cord blood stem cell; TVT, transvaginal tape; EPC, endothelial progenitor cells; CEC, circulating endothelial cells; SCP, synergetic cell population.

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Considering the above mentioned treatment options, there is an urgent unmet clinical need in the development of a new minimally invasive approach and effective treatment modality for SUI. Cell therapy for the regeneration of injured tissues has recently been extensively investigated at the experimental level both in vitro and in vivo, and its clinical application in a variety of fields has recently been studied.[6]

There are few reports[6–12] that use stem cells from different sources used in the treatment of SUI, especially with various success rates in the short-term and long-term outcomes. We report here on the first nine SUI patients included in a larger clinical trial aiming to evaluate the treatment of SUI patients using autologous stem cells and platelets derived from peripheral blood. To the best of our knowledge, this is the first time that this combination of peripheral blood components has been applied in the treatment of SUI.

MATERIAL AND METHODS

Patients

Nine patients with SUI that did not respond to conventional therapy were included in the present study at Imam Reza Academic Hospital from June 2011 until March 2012. The patient's characteristics are presented in Table I.

The study protocol and informed-consent forms were reviewed and approved by the Human Research Ethics Committee of Mashhad University of Medical Sciences. All patients signed the informed consent essential for this study.

Inclusion criteria were: female outpatients, predominant clinical diagnosis of severe SUI, have discrete episodes of incontinence (that are dry between episodes and not continuously leaking urine, synchronous with increased intra-abdominal pressure from coughing, sneezing, and exercising, with mild cystocele (grade 1 and 2 according to POP-Q system) or without cystocele, normal uroflowmetry (UFL), normal pressure/flow cystometry [Normal cystometry considered as normal intravesical pressure ($<$5–20 cmH_2O) during filling and normal capacity (300–500 cm^3) without any uninhibited contracture], normal electromyography (bladder and striated sphincter coordination), which was performed during urodynamic studies and positive cough tests was performed at the beginning of the urodynamic study with a full bladder. For patient with positive cough tests, abdominal leak point pressure (ALPP) tests were performed in order to signify the amount of intra abdominal pressure during leakage. ALPP $<$ 60 cmH_2O and more than 90 cmH_2O has been considered as intrinsic sphincteric deficiency (ISD) and urethral hypermobility without l ISD or with little ISD, respectively. ALPP between 60 and 90 cmH_2O has been considered as equivocal. Urethral pressure profilometry (UPP) was performed in all of the patients. Exclusion criteria were: known vesicoureteral reflux, vaginal prolapse beyond the introitus, or other significant pelvic floor abnormalities, neuromuscular disorder (e.g., muscular dystrophy, multiple sclerosis), uncontrolled diabetes, pregnancy, lactating, or plans to become pregnant during course of the study, morbid obesity (defined as 100 pounds over their ideal body weight, or BMI $\geq$ 40) and not expected to benefit from treatment, current or acute conditions involving cystitis or urethritis with a history of urogenital cancer scheduled to receive radiation treatment to the vicinity, or history of radiation treatment to the urethra or adjacent structures, current use of any medications for the treatment of urinary incontinence or any mental or physical disability.

At the baseline, the patients were evaluated with cough and Boney tests, Q-Tip test, urodynamic studies, 1 hr pad tests, upper tract ultrasonography (UTU), post voiding residue (PVR), routine laboratory tests, urine analysis (U/A), and culture, the subjective symptoms and quality of life (using a validated disease-specific questionnaire—the International Consultation on Incontinence Questionnaire-Urinary incontinence (ICIQ-UI) and -quality of life (ICIQ-QOL). In the ICIQ-UI was calculated at baseline and repeated 1, 3, and 6 months after treatment. A higher score in the ICIQ-UI and the ICIQ-QOL indicated an unfavorable and a favorable condition, respectively.

The blood was taken 1 day before the injection and 1 g cephazoline was given intravenously 1 hr before the injection. After P-TNCs-M injection, catheter was placed and then was removed the next day to allow maturation, and the patients were discharged with oral antibiotics till 5 days (tablet: ciprofloxacin 500 mg—BID).

Primary out comes 1, 3, and 6 months after the injection, include cough tests, UTU, UFL, PVR, 1 hr pad tests, ICIQ-UI and ICIQ-QOL were also measured. In one patient with an abdominal leak point less than 60 (demonstrating of intrinsic sphincter deficiency), maximal urethral closure pressure (MUCP) was performed before and 3 months after the operation. In this patient, ALPP was done at 3 months follow up (F/U). The total period of F/U was 6 months.

Platelets and Total Nucleated Cells Separation

One hundred twenty milliliters peripheral blood was taken and total nucleated cells (TNCs) and platelets were prepared

TABLE I. Patient's Characteristics, Past Medical History and Number of Children and Mode of Delivery

No.	Age	Weight	Gravid	Number of children	Mode of delivery	Past medical history
1	61	58	5	5	NVD	—
2	30	90	2	2	NVD	—
3	65	74	13	10	NVD	—
4	55	70	4	3	C/S	—
5	31	68	3	3	NVD	Curettage
6	34	72	4	4	NVD	—
7	61	68	4	4	NVD	—
8	55	64	3	2	NVD	—
9	48	74	5	5	NVD	—
Mean $\pm$ SD	48.88 $\pm$ 13.8	70.88 $\pm$ 8.78	4	4		

NVD, normal spontaneous vaginal delivery, C/S: caesarian.

according to standard procedures. The platelets were prepared by first centrifugation at 2,000*g* for 2 min and then second centrifugation at 4,000*g* for 8 min and the supernatant plasma was removed to a final volume of 4 ml.[13] TNCs were separated and concentrated to 96% purity and to a final volume of about 6 ml using hydroxyethyl starch (HES).[14] HES (HAES-steril 10%; Fresenius Kabi, Germany) was added to the blood to obtain a final concentration of 2%. The blood was allowed to stand for 45 min to allow red blood cells (RBC) sedimentation. The supernatant was slowly separated and centrifuged at 400*g* for 12 min. After completion of centrifugation, the supernatant plasma was removed to a final volume 6 ml and mixed with 4 ml platelets. The total volume of platelets and TNCs mixture (P-TNCs-M) was 10 ml.

Periurethral Injection of Platelets and Total Nucleated Cells Mixture

The transurethral endoscopic injection of P-TNCs-M was carried out under general anesthesia. A 21-Fr rigid cystoscope was used for injection of P-TNCs-M. The cystoscope was inserted into the urethra. Under endoscopic vision, a puncture needle was passed through the cystoscope into the urethra at the region of the external urethral sphincter. After puncturing the urethra (by a needle with an 18 gauge thickness) at the region of the external urethral sphincter under endoscopic vision, the P-TNCs-M was injected. Initially, 8 ml was injected at a depth of 5 mm into the rhabdosphincter at 1.5, 3, 4.5, 6, 7.5, 9, 10.5, and 12 O'clock positions (1 ml in each position). Subsequently, 2 ml of P-TNCs-M was equally injected into the submucosal spaces at 3 and 9 O'clock positions to facilitate coaptation of the urethral mucosa by the bulking effect.

Statistical Analysis

For the statistical analysis, the GraphPad Instant statistical package was used (GraphPad Software, Inc.). All parameters were given as mean ± SD. The paired *t* tests and descriptive analysis were employed. The level of statistical significance was set to $P < 0.05$.

RESULTS

There was no morbidity during and after the injection. Mean age of the patients was 48.9 ± 13.8 years. Before the injection, all patients had SUI with positive cough tests and 1 hr pad tests, normal upper urinary tract ultrasonography and normal PVR. Urodynamic findings were normal except for one patient (No. 3) ALPP < 60 cmH_2O and demonstrating of ISD. ALPP were 60–90 (77.66 ± 9.44 cmH_2O) in rest of the patients. The resting UPP was normal in all patients except for patient No. 3. The data on the identification of various cell types before and after process were presented at Table II. The injection took about 10–15 min under general anesthesia.

Eight patients had cured (completely dry) and one patient (patient No. 3 with 13 gravid) had marked improvement according to their ICIQ-UI and ICIQ-QOL. Ultrasonography of the upper urinary tract, PVR and UFL were normal in all patients at 1, 3, and 6 months after, without any evidence of bladder outlet obstruction. Cough test was done at 1, 3, and 6 months in all patients which was negative except for 1 patient (patient 3 with 13 gravid) which was positive in severe cough and ALPP measurement was 92 at 3 month F/U (Table III).

None of the patients had voiding dysfunction, urinary retention or urinary tract infection after P-TNCs-M injection.

TABLE II. The Data on the Identification of Various Cell Types Before Process of Separation and After Process for Final Product

Cell type	TNCs* ($\times 10^8$)	MNCs* ($\times 10^8$)	Platelets($\times 10^{10}$)	RBC depletion %*
Pre-process	7.02 ± 1.18	2.00 ± 0.35	3.46 ± 0.45	NA
Post-process	6.76 ± 1.22	1.88 ± 0.32	3.01 ± 0.11	97.8 ± 1.2
% Yield	96.19 ± 1.92	94.37 ± 1.08	87.95 ± 8.74	

MNCs, mononuclear cells; NA, not applicable.
*Mean ± SD.

For the patient with ISD (No. 3), baseline MUCP measurement was less than 30 cmH_2O and it was repeated at 3 months F/U, which was more than 30 cmH_2O.

ICIQ-UI base line for patients was 18.33 ± 0.6 and it was reduced to 1.11 ± 0.5 after 1 month. There was significant difference between their scores by paired sample *t* test ($P < 0.001$). At the 3rd and 6th months of F/U ICIQ-UI scores were under one (0.44 ± 0.4). ICIQ-QOL base line for patients was 28.8 ± 3.7 and it was increased to 94.11 ± 12.8 after 1 month. The levels of the ICIQ-QOL was test with repeated measure general linear models and it showed that significant difference among ICIQ-QOL in 1st, 3rd, and 6th months after injection ($P < 0.001$). ICIQ-UI and ICIQ-QOL were presented in Tables III and IV at base line, 1, 3, and 6 months after the operation. There was no recurrence of the symptoms after 6 months.

DISCUSSION

This pilot study shows that the treatment of SUI with mixture of peripheral blood TNCs and platelets is safe and effective method with 8 of patients completely cured and 1 marked with significant improvements 6 months after a single injection.

SUI pathophysiology is caused by weakening of the pelvic floor muscles that support the bladder and urethra (e.g., urethral hypermobility) and/or by weakness of the urethral sphincter (i.e., intrinsic sphincter deficiency [ISD]). Hypermobility occurs when the normal pelvic floor muscles can no longer provide the necessary support to the urethra and bladder neck. As a result, the bladder neck is free to drop when any downward pressure is applied and thus, involuntary leakage occurs. ISD is related to the weakening of the urethral sphincter muscles or the closing mechanism related to contraction. As a result of this weakening, the sphincter does not function normally regardless of the position of the bladder neck or urethra. Cellular aging, cell death (apoptosis and necrosis), and renewal continue throughout life and through all tissues. During aging, apoptosis supersedes renewing capacity which imbalances between cell death and renewal. Therefore, the higher prevalence of SUI among elderly persons is a symptom of increasingly poor and eventually failing tissue regeneration in the vesico-urethral apparatus.[15]

Considering the pathophysiology of SUI, the success and efficacy of SUI treatment depends on the reconditioning of phenotypically altered cells, which is augmenting sphincter regeneration. Multipotent stem cells can be differentiated to the myogenic lineage (skeletal and smooth muscle cells), neural and endothelial cell lineages and stem-cell therapy offers a potential cure of the deficient sphincter by regenerating the damaged muscle and connective tissues. Cells transplanted

TABLE III. Outcome of the Patients in 1, 3, and 6 Month Follow-Up

Mode of evaluation		ICIQ-UI			ICIQ-QOL			UTU		Pad test		UFL		PVR		Cough test	
Time of evaluation	Baseline	1st	2nd	3rd	Baseline	1st	2nd	3rd	Baseline	1st, 3rd, 6th	Baseline	1st, 3rd, 6th	Baseline	1st, 3rd, 6th	Baseline	1st, 3rd, 6th	
Patients number																	
1	16	3	0	0	30	88	98	98	N	N	+	N	N	N	N	+	–
2	21	0	0	0	28	98	100	102	N	N	+	N	N	N	N	+	–
3	21	4	4	4	24	65	67	65	N	N	+	N	N	N	N	+	P (ALPP = 92 cmH_2O at 3rd)
4	17	0	0	0	31	108	108	108	N	N	+	N	N	N	N	+	–
5	16	0	0	0	35	101	101	101	N	N	+	N	N	N	N	+	–
6	17	0	0	0	24	95	98	98	N	N	+	N	N	N	N	+	–
7	18	3	0	0	33	99	99	99	N	N	+	N	N	N	N	+	–
8	20	0	0	0	28	88	95	98	N	N	+	N	N	N	N	+	–
9	19	0	0	0	27	105	105	110	N	N	+	N	N	N	N	+	–
Mean ± SD	18.33 ± 0.6	1.11 ± 0.5	0.44 ± 0.4	0.44 ± 0.4	28.8 ± 3.7	94.11 ± 12.8	96.7 ± 11.8	97.6 ± 13									

Normal, N; negative, –; positive, +.

into the urethra can serve as a natural bulking agent and become innervated into the host muscle, potentially improving urethral function.[16]

Stem cells has been used from various sources such as bone marrow-derived mesenchymal stem cells (BMSCs), chondrocytes, muscle-derived progenitor stem cells (MDPSCs), adipose derived mesenchymal stem cell, human umbilical cord blood stem cell (HUCB) in preclinical, and clinical studies in order to replenish the lost cells during the tissue regeneration of damaged rhabdosphincter.[17]

Corcos et al.[18] showed that the rats BMSCs have the ability to differentiate towards smooth and striated muscle phenotypes, and the periurethral injection of BMSCs significantly improved the injured rhabdosphincter. However, for clinical use, BMSCs is limited due to highly invasive and painful procedures of bone marrow aspiration, a decline in differentiation potential and BMSC number with increased age, and a low yield of BMSCs upon processing and senescence of such cells during culture.[19]

The autologous chondrocytes were the first cells type, which used in 32 patients. This treatment was safe, effective, and durable, with 50% of patients remaining completely dry 12 months after a single injection.[12]

In a clinical trial,[10] autologous MDPSCs were injected under transurethral ultrasound guidance into the rhabdosphincter of the mid-urethra. Separate injections of a suspension of autologous fibroblasts, and collagen, which functioned as carrier material, were additionally performed into the submucosa cranial and caudal to the injection side of the MDPSCs with improvements in quality of life and thickness and contractility of the urethral sphincter, with a success rate reported of over 90% for women and over 50% for men.

For eight women, Carr et al.[9] reported more modest and realistic improvements and changes, with one patient achieving complete continence and four indicating improvement from the baseline 1 year after the initial injection of MDPSCs. Also, a multicentre clinical study showed that intrasphincteric injection of autologous MDPSCs at various doses reduced the amount of leakage during a 24-hr pad tests and lowered the incidence of diary-reported stress leaks over 3 days. These improvements started as early as 1 month after injection and lasted through the study's end point of 6 months, with some evidence suggesting that the higher doses of MDPSCs are associated with greater and faster improvement of incontinence symptoms.[11]

Adipose-derived stem cells (ADSCs) can express specific striated muscle markers form multinucleated cells characteristic of myotubules, and have been shown to regenerate the functional capacity of damaged skeletal muscle.[20] In a two cases study, ADSCs were injected periurethrally in two male patients after radical prostatectomy. SUI improved after 2 weeks of injection and persisted up to 12 weeks after treatment.[6]

In a clinical study, 39 women with incontinence stemming from intrinsic sphincter deficiency, mixed incontinence, or urethral hypermobility; underwent transurethral allogenic HUCBs cell injections. At 12 months after treatment, 72% of patients had more than 50% improvement of their incontinence symptoms.[7]

The ideal cells for tissue engineering should be autologous cells, easily procured from minimally invasive procedures, provide sufficient quantities of cells, exhibit potency of differentiation to regenerate multiple tissues, and proliferate quickly in a well-controlled manner.

In this study, we decided to use the peripheral blood TNCs along with platelets as the first approach of cell therapy for

TABLE IV. The Difference of ICIQ-UI and ICIQ-QOL at Baseline and 1, 3, and 6 Months After Treatment

		P-value			*P*-value
ICIQ-UI-Base	ICIQ-UI-1mon	0.000	ICIQ-QOL-Base	ICIQ-QOL-1mon	0.000
	ICIQ-UI-3mon	0.000		ICIQ-QOL-3mon	0.000
	ICIQ-UI-6mon	0.000		ICIQ-QOL-6mon	0.000
ICIQ-UI-1mon	ICIQ-UI-Base	0.000	ICIQ-QOL-1mon	ICIQ-QOL-Base	0.000
	ICIQ-UI-3mon	1.000		ICIQ-QOL-3mon	0.332
	ICIQ-UI-6mon	1.000		ICIQ-QOL-6mon	0.194
ICIQ-UI-3mon	ICIQ-UI-Base	0.000	ICIQ-QOL-3mon	ICIQ-QOL-Base	0.000
	ICIQ-UI-1mon	1.000		ICIQ-QOL-1mon	0.332
	ICIQ-UI-6mon	1.000		ICIQ-QOL-6mon	1.000
ICIQ-UI-6mon	ICIQ-UI-Base	0.000	ICIQ-QOL-6mon	ICIQ-QOL-Base	0.000
	ICIQ-UI-1mon	1.000		ICIQ-QOL-1mon	0.194
	ICIQ-UI-3mon	1.000		ICIQ-QOL-3mon	1.000

P-value <0.05 is considered significant difference.

SUI, because of the least invasive method of obtaining the autologous cells such as multipotent cells and endothelial progenitor cells (EPC), which take part in tissue regeneration. Porat et al. found a novel human cell population derived from the peripheral blood, termed synergetic cell population (SCP), which considered as a potential source of autologous treatment for a variety of diseases. The SCP was capable of differentiating into a variety of cell lineages such as angiogenic, neural or myocardial lineages.[21] Asahara demonstrated the presence of both circulating endothelial cells (CEC) and EPC in peripheral blood.[22]

In regeneration medicine, tissue repair is strongly dependant on the formation of new blood vessels and capillaries, which two different processes with cooperation of CECs and EPCs through proliferation, migration and remodeling take place such as vasculogenesis, the in situ assembly of capillaries from undifferentiated endothelial cells (ECs), and angiogenesis, the sprouting of capillaries from pre-existing blood vessels. Angiogenesis is a morphogenic process, which describes the formation of new blood capillaries from EC of pre-existing vessels. When converting to the angiogenic stage, ECs will capture new properties that enable them to neovascularize the tissue. When the new vessels are in place and the vascular network matures, the neovascular ECs resume their quiescent phenotype.[3] Angiogenesis has been known for a long time to be essential for muscle repair.[7] Indeed, endothelial cells (ECs) stimulate myogenic cell growth and, inversely, differentiating myogenic cells to promote angiogenesis.[23]

The rational beyond using the platelets in this study is due to the release of bioactive factors and an extensive documentation of in vitro and in vivo studies demonstrating the safety and efficacy of these factors in the muscle regeneration.[24]

In platelets, bioactive factors are in the α-granules and the dense granules. In tissue regeneration, the muscle heals through the 3 phases: inflammation, proliferation, and remodeling. These bioactive factors are active during each of these phases. The α-granules contain growth factors such as transforming growth factor-β, platelet-derived growth factor, insulin-like growth factor, fibroblast growth factor, epidermal growth factor, vascular endothelial growth factor, and endothelial cell growth factor. These growth factors play important roles in cell proliferation, chemotaxis, cell differentiation, and angiogenesis. The dense granules contain serotonin, histamine, dopamine, calcium, and adenosine. These non-growth factors have fundamental effects on the biologic aspects of tissue repair.[25] In general, the bioactive factors play a central role in the healing processes by modulating the recruitment, duplication, activation, and differentiation of different cell types in muscle repair.

Timothy reported that Cugat (unpublished data) showed an autologous PRP injection directly into the tear in a group of athletes included 8 soccer players and 6 basketball players (accounting for a total of 16 muscular injuries). The return-to-play interval was diminished in each group according to severity, and in the less severe injuries (grades I and II), a greater than 50% reduction in return to play was reported. At regular intervals, F/U included clinical assessment as well as ultrasonic imaging, which confirmed progressive healing of the injured muscle. They concluded that PRP can be helpful in returning athletes to sport, with a shorter time of restoration and rehabilitation.[25]

LIMITATIONS OF OUR STUDY

In this study, since the mixture of TNCs and PRP were injected, it is not possible to define which one has marked effects on improvement. Future research is needed to distinguish the effects of each of them in detail by choosing different groups of patients. PRP and TNC should be injected separately in two other groups of patients.

Any further conclusions regarding the clinical effects of injections of autologous TNCs along with platelets have to be drawn cautiously due to the limited number of patients. The sample size for the study was calculated to provide us with enough power to demonstrate the differences found in clinical events. Despite this, we believe that the outcome can provide the basis for a larger trial, specifically sized to assess differences in long-term (more than 1 year) clinical outcomes. Another limitation of the study includes the fact we looked this treatment just for patients and as ethical issues we could have a placebo group (injecting normal saline).

In conclusion, the injection of peripheral blood TNCs and platelets is safe and effective in treatment of SIU is a feasible and safe procedure with 6 months F/U. The results point out those subjects cured or marked improvement in 6 months F/U. This study suggests that based on these positive preliminary findings, a larger adequately powered trial is needed to investigate and confirm the efficacy of procedure in long-term outcome.

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