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Bone marrow nucleated cell concentrate autograft in temporomandibular joint degenerative disorders: 1-year results of a randomized clinical trial

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ABSTRACT

Objective: to assess the reliability of bone marrow nucleated cell (BMNc) intra-articular injection in patients with degenerative temporomandibular joint disorders (TMDs), and to compare its efficacy with that of hyaluronic acid (HA).
Materials and methods: this study was designed as a randomized, controlled trial of parallel groups. Patients affected by degenerative joint mandibular disorders were enrolled in this prospective clinical trial and randomly divided into two groups. The HA group underwent temporomandibular joint (TMJ) arthrocentesis and HA injection, whilst patients in the BMNc group were inoculated with BMNc inside the joint after lavage. Outcome measures were: assessing pain at rest and during motion, joint noises, chewing efficiency, and maximum interincisal opening. A postoperative MRI scan was performed and compared with the preoperative one, while examining for cartilage regeneration. Clinical and radiological data were collected from baseline to 12 months follow-up.
Results: Thirty patients, 15 for each group, complaining of different degrees of unilateral TMD with internal derangement, were enrolled and treated. In both groups, significant clinical improvements were detected after the procedure up to 1 year postoperatively. The BMNc group presented significantly better pain relief than the HA group after 6 months ($p = 0.028$) and 12 months ($p = 0.000$). No significant differences were observed in terms of joint noises. In terms of chewing efficiency, the BMNc group showed positive significant differences after 12 months ($p = 0.000$). Maximum interincisal opening presented significantly better values in the BMNc group after 6 months ($p = 0.001$) and 12 months ($p = 0.000$). No MRI evidence of cartilage regeneration was reported.
Conclusion: intra-articular TMJ BMNc injection improved clinical outcomes in TMD treatment. The Results of this first human-model study are promising but further studies are needed to determine whether BMNc can represent the best treatment for TMDs.

1. Introduction

Temporomandibular disorders (TMDs) represent a wide range of functional modifications and pathological conditions affecting the temporomandibular joint (TMJ), masticatory muscles, and other components of the oro-maxillofacial region (De Riu et al., 2013). TMDs are a major cause of non-dental orofacial pain. The prevalence in the general population is high, with an estimated 40–75% having at least one sign and 33% reporting at least one symptom (Zhang et al., 2015).

One of the most common forms of TMD is internal derangement (ID). It has been reported that 80% of patients with signs and symptoms of TMD have some form of ID of the TMJ (Gorrela et al., 2017). In these patients, cartilage joint lesions are often present, representing a debilitating disease that Results in fibrillation and

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subsequent degradation of the surrounding articular surfaces, possibly involving the subchondral bone, and thus favoring the development of arthritis (Zaki et al., 2017).

TMDs can be treated conservatively or surgically. Conservative treatments include bite wafers, rehabilitation exercises, isometric exercises, masticatory muscle massage, medical treatments (NSAIDs, diazepam, etc.) thermal treatment, and laser therapy. Surgical treatments can be invasive (open approaches) or minimally invasive, including arthrocentesis and arthroscopy. The latter are associated with minimal complications, and for this reason now exceed open surgeries in patients who have failed to respond to conservative treatments (Nitzan (2006); Vaira et al., 2017; Vaira et al., 2018). The efficacy of arthrocentesis in reestablishing normal mouth opening and reducing pain and dysfunction levels has been reported by several authors (Alpaslan et al., 2003; Tan and Krishnaswamy, 2012; De Riu et al., 2013), and is similar to that of the arthroscopy. At the end of the procedure, corticosteroids (Laskin et al., 2006), morphine (List et al., 2001), bupivacaine (Furst et al., 2001), mepivacaine (Zuniga et al., 2007), protein-rich plasma (Al-Delayme et al., 2017), or sodium hyaluronate (Alpaslan et al., 2003) have been injected inside the joint as a long-acting analgesic.

With the development of tissue engineering, intra-articular grafting of autologous bone marrow-derived stem cells (MSCs) has been proposed as biological therapy for patients with knee osteoarthritis, because the procedure is safe, reduces pain, and improves function (Emededin et al., 2012; Orozco et al., 2014; Vega et al., 2015; Gupta et al., 2016; Lamo-Espinosa et al., 2016). Besides their self-renewal and differentiation potential, MSCs produce a wide spectrum of trophic factors in their secretome, with regenerative and immunomodulatory properties, providing the basis for their utilization in patients with osteoarthritic joint disorders (Zhang et al., 2015). However, culture expansion of MSCs is still a complex and uncertain process, which involves technical expertise and sophisticated laboratory logistics, so its widespread application is limited. Recently, some studies have attempted to use uncultured bone marrow-derived nucleated cells (BMNc), mostly CD34 + cells, to repair articular cartilage defects (Chang et al., 2008; Giannini et al., 2009; Zhang et al., 2012), which proved to be effective. However, there are still no human-model reports that have investigated the use of BMNc in the treatment of TMDs.

The aim of this study was to test the hypothesis that office-based TMJ arthrocentesis with intra-articular injection of BMNc has a better clinical outcome compared with arthrocentesis with intra-articular injection of hyaluronic acid (HA) in patients with degenerative TMDs. The trial has been reported according to the CONSORT statement (http://www.consort-statement.org) for improving the quality of reporting of parallel-group, randomized, controlled trials.

2. Materials and methods

2.1. Study design

This randomized, controlled trial was conducted at the Maxillofacial Surgery Unit, University Hospital of Sassari, Italy, between 2016 and 2018. Patients were treated by the same maxillofacial surgeon (GDR). The investigation was conducted according to the principles embodied in the Helsinki Declaration of 1975 for biomedical research involving human subjects, as revised in 2013, and the study protocol was approved by the University of Sassari Ethical Committee (No. 297/2016). All patients were treated and followed up at the same Maxillofacial Surgery Unit.

Patients affected by severe unilateral temporomandibular joint disorders with internal derangement were recruited by offering an arthrocentesis treatment. The study protocol and the nature of the research were thoroughly explained, and all participants provided written informed consent prior to enrollment in the trial. Patients were enrolled in accordance with the following inclusion criteria:

- Age between 18 and 70 years.
- Chronic temporomandibular arthritic disorder with history of pain and joint noises for at least 1 year.
- Spontaneous and evoked TMJ pain (with mandibular movements and direct TMJ compression).
- Wilkes stages III or IV (internal derangement).
- Joint noises, such as crepitation and clicking.
- Limited mouth opening.
- Magnetic resonance imaging evidence of cartilage surface defects.
- Already treated with conservative methods (occlusal splint, pharmacological and/or physio-kinesio therapy) for at least 3 months without satisfactory benefit.

The exclusion criteria were as follows:

- Previous TMJ traumas and fractures.
- Previous TMJ surgeries.
- Only click and/or mouth opening limitations.
- Bilateral severe TMJ derangements.
- TMJ ankylosis.

2.2. Clinical procedures

All patients were clinically evaluated and their medical histories recorded. Preliminary screening was performed to evaluate potential patients' eligibility. Patients who met the selection criteria underwent dynamic MRI (Samsung Electronics Co., Seoul, South Korea) to evaluate articular damage of the TMJ, and afterwards were definitively enrolled.

The 30 patients were randomly divided into two groups of 15, using a computer-generated random number table:

- HA group: patients underwent arthrocentesis using Ringer's lactate solution plus intra-articular injection of hyaluronic acid (HA).
- BMNc group: patients underwent arthrocentesis with Ringer's lactate solution plus intra-articular injection of bone marrow nucleated cell concentrate (BMNc).

Immediately before surgery an opaque envelope containing a randomization code to assign one of the two different procedures was opened.

In the HA group the procedure was performed in total aseptic conditions, under local anesthesia to block the auriculotemporal nerve. The points for needle insertion were marked on the skin according to the method suggested by Nitzan (2006). A line was drawn from the middle of the tragus to the outer canthus of the eye (Holmlund-Hellsing line). The posterior entry point was located along the cantho-tragal line, 10 mm from the middle of the tragus line and 2 mm below. The anterior entry point was placed 10 mm further forward along the line and 10 mm below. An 18-gauge needle was inserted into the superior joint space through the posterior entry point. Approximately 2 ml of Ringer's lactate solution were then injected to distend the joint. A second 18-gauge needle was inserted into the distended compartment through the anterior entry point to establish a free flow of the solution through the superior joint space. A syringe filled with Ringer's lactate solution was then connected to one of the needles, and fluid was injected into the superior joint space. The second needle provided

an outflow for the solution, which was collected in a kidney dish. A total of 200–250 ml of solution was used to wash the superior joint space. During the lavage, the mandible was moved using opening, excursive, and protrusive movements to facilitate lysis of adhesions. After the lavage, 2 ml of sodium hyaluronate (Hyalgan® 20 mg/2 mL, Fidia Farmaceutici S.p.A, Abano Terme, Italy) was injected inside the upper joint space.

In the BMNc group, bone marrow nucleated cells were drawn from the iliac crest under local anesthesia using a specific collection and concentration kit (Marrowstim™, Biomet Biologics Inc., Warsaw, IN, USA) [Video 1]. The entry point was marked on the skin above the iliac crest, at least 2 cm behind the anterior superior iliac spine [Fig. 1A]. After infiltration with local anesthetic, a sharp trocar was pushed inside the iliac crest marrow cavity. At least 30 ml of medullary blood was withdrawn [Fig. 1B] and then transferred to a concentration tube. This latter was centrifuged at 3200 revolutions per minute for 15 min. During the medullary blood processing, TMJ arthrocentesis and lavage was performed as described for the HA group [Fig. 1C]. After centrifugation the cell-poor plasma was eliminated while the BMNc concentrate was drawn into a sterile syringe. Finally, 2 ml of BMNc was injected inside the upper joint space after the lavage [Fig. 1D]. For each patient, a 0.5 ml sample of BMNc concentrate was sent to the laboratory for cell count analysis.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.jcms.2018.11.025.

Clinical and radiological data were collected at baseline and at 7 days, 1 month, 6 months, and 12 months after the procedure.

2.3. Outcome measures

A number of clinical parameters were assessed preoperatively and at 1 week, 1 month, 6 months, and 12 months after the procedure (Al-Delayme et al., 2017):

1. TMJ pain assessment, at rest and during motion, using a 10 cm visual analog scale (VAS) ranging from 'no pain' to 'worst pain ever experienced'. Absence of pain was scored as 0. If pain was present the patient was asked to select a field from 1 cm to 10 cm.
2. Chewing efficiency, using a 10 cm VAS with 0 being 'worst efficiency ever' and 10 being 'best efficiency ever'.
3. Joint noises, using a 10 cm VAS scale, with 0 for 'no sound' and 10 for 'joint noise that is easily perceptible from outside'.
4. Maximum interincisal opening (MIO).

For each parameter, the appropriate score was recorded on a form by one uninvolved, blinded operator.

2.4. Dynamic MRI

All patients underwent a dynamic MRI scan of the TMJ at baseline and 12 months after treatment. The same experienced radiologist evaluated the MRI images in a blinded way, by assessing cartilage lesions, disk displacement, intra-articular effusion, and subchondral bone alterations. MRI findings were classified as proposed by Wurm et al. (2018), with the scoring system comprising three groups to identified the three parameters assessed: disk (group D), luxation (group L), and osseous components (group J) [Table 1]. Using this system numbers can be combined to describe complex conditions (e.g. L14 is a ventromedial luxation while D14 is a disk displacement with reduction and perforation).

2.5. Randomization

Randomization codes were created using dedicated computer software (Excel, Microsoft, Redmond, WA, USA), combining a sequence of randomized, non-consecutive numbers that matched the two different procedures (HA group, BMNc group). An

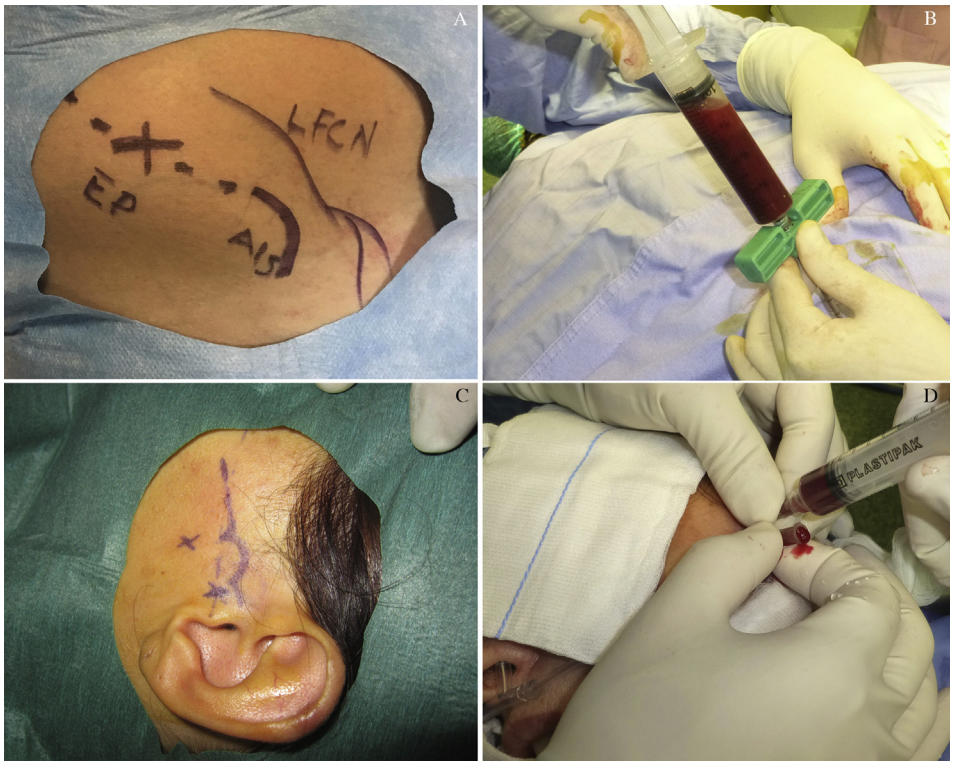

Fig. 1. BMNc transcutaneous harvesting from the iliac crest and injection of cell concentrate into the upper temporomandibular joint space. The process is described in the materials and methods section.

Table 1
MRI score system.

Group D		Group L		Group J	
D0	No abnormal findings	L0	None	J0	No abnormal findings
D1	Disk displacement with reduction	L1	Ventral	J1	Subchondral sclerosis, slight signal alterations
D2	Disk displacement without reduction	L2	Lateral	J2	Osteophytes, subchondral cysts, erosion, edema
D3	Disk degeneration	L3	Dorsal	J3	Joint gap narrowed
D4	Disk perforation	L4	Medial	J4	Joint shape changed
D5	Disk missing	–	–	J5	Ankylosis

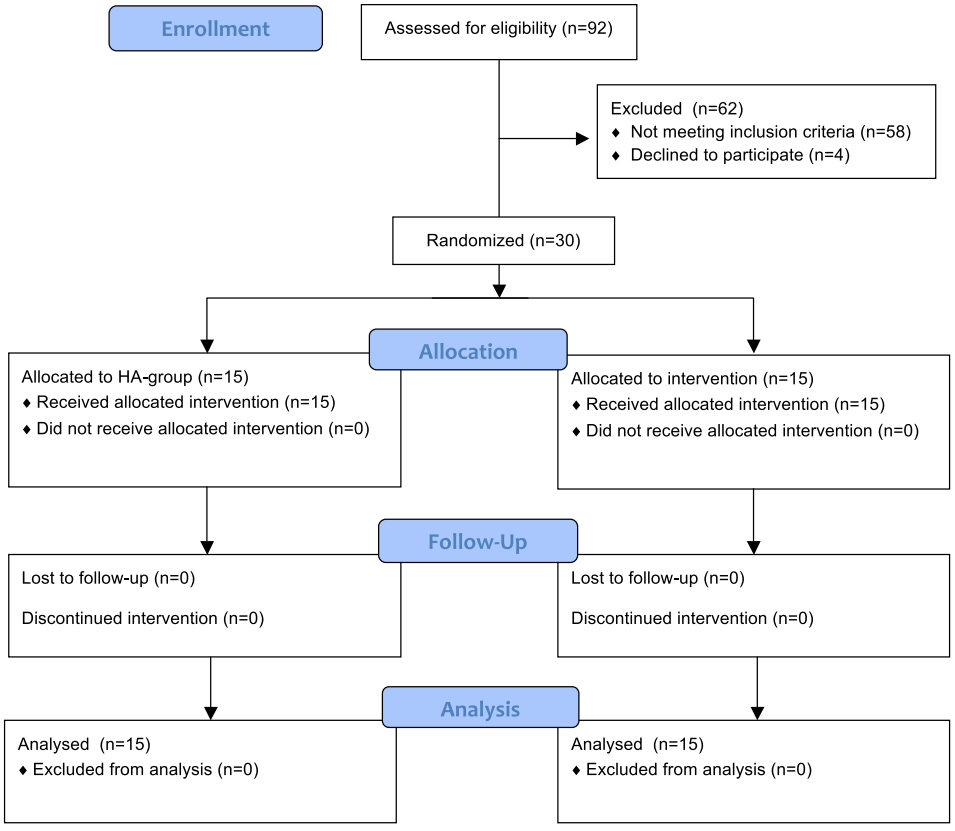

Fig. 2. CONSORT flow diagram.

Table 2
General characteristics of the patients included in the two groups.

	HA group	BMNc group	*p*-value[a]
Gender			
Male	1 (6.7%)	0 (0%)	
Female	14 (93.3%)	15 (100%)	
Age	44.5 ± 12.6 years (range 33–61 years)	48.2 ± 10.2 years (range 35–67 years)	0.371
Duration of symptoms	33.2 ± 11.6 months (range 13–55 months)	30.1 ± 18.6 months (range 13–60 months)	0.553
Preoperative pain at rest VAS	7.8 ± 1.15 cm	8.2 ± 1.01 cm	0.898
Preoperative pain during motion VAS	8.53 ± 0.99 cm	8.73 ± 0.88 cm	0.998
Preoperative joint noises VAS	7 ± 0.76 cm	7.07 ± 0.96 cm	1.000
Preoperative chewing efficacy VAS	4.6 ± 1.12 cm	4.53 ± 0.99 cm	0.815
Preoperative MIO	23.2 ± 2.45 mm	22 ± 2.67 mm	0.369

[a] Kolmogorov–Smirnov test for independent groups of data. Confidence interval: 95%. Significance level set at $p \leq 0.05$.

independent operator (EC), not previously involved in the trial, was aware of the random sequence and had access to the randomization lists stored in a password-protected computer. The random codes were enclosed in sequentially numbered, identical, opaque, sealed envelopes. Envelopes were opened sequentially immediately before surgery. Data were collected onto spreadsheets (Excel) by an independent medical doctor (LAV).

2.6. Statistical analysis

The collected data were analyzed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics for quantitative variables were presented as mean ± standard deviation (SD). Baseline and postoperative data were compared using a Wilcoxon signed-rank test, while the Kolmogorov–Smirnov Z test

Table 3
Pain at rest and during movement VAS. Results and statistical analysis.

Time interval	Group	Pain at rest					Pain during motion				
		VAS score mean ± SD (cm)	Wilcoxon test (observation time vs preop)		Kolmogorov–Smirnov test (HA group vs BMNc group)		VAS score mean ± SD (cm)	Wilcoxon test (observation time vs preop)		Kolmogorov–Smirnov test (HA group vs BMNc group)	
			Z	p-value[a]	Z	p-value[b]		Z	p-value[a]	Z	p-value[b]
Preop	HA group	7.8 ± 1.15			0.365	0.999	8.53 ± 0.99			0.365	0.999
	BMNc group	8.2 ± 1.01					8.73 ± 0.88				
1 week	HA group	4.07 ± 0.96	−3.431	0.001	0.183	1.000	4.4 ± 0.99	−3.453	0.001	0.183	1.000
	BMNc group	4.07 ± 1.03	−3.427	0.001			4.6 ± 0.99	−3.437	0.001		
1 month	HA group	1.47 ± 0.99	−3.462	0.001	0.548	0.925	2.13 ± 1.36	−3.425	0.001	0.548	0.925
	BMNc group	1.13 ± 0.92	−3.455	0.001			1.17 ± 0.96	−3.441	0.001		
6 months	HA group	2.67 ± 1.17	−3.430	0.001	1.461	0.028	3.47 ± 0.99	−3.436	0.001	1.826	0.003
	BMNc group	1.27 ± 0.7	−3.428	0.001			1.73 ± 0.79	−3.442	0.001		
1 year	HA group	4.8 ± 1.37	−3.310	0.001	2.739	0.000	5.47 ± 1.19	−3.425	0.001	2.556	0.000
	BMNc group	1.87 ± 0.35	−3.441	0.001			2.33 ± 0.72	−3.436	0.001		

[a] Wilcoxon signed-rank test for dependent paired data. Confidence interval: 95%. Significance level set at $p \leq 0.05$.
[b] Kolmogorov–Smirnov test for independent groups of data. Confidence interval: 95%. Significance level set at $p \leq 0.05$.

was used to analyze differences between the groups. The level of statistical significance was set at $p < 0.05$ with a 95% confidence interval.

3. Results

Of the 92 patients screened for eligibility 62 did not meet the selection criteria — 58 did not meet the inclusion criteria, while four declined to participate [Fig. 2].

Thirty patients (1 male and 29 female), with an average age of 47.2 ± 14.3 years (range 33–67 years) complaining of different degrees of unilateral (one side with prevalent signs and symptoms) TMD disorders with internal derangement, were enrolled and treated.

TMD symptoms dated, on average, 32.5 ± 17.8 months (range 13–60 months). Before the surgery, the preoperative differences between the two groups, for all the parameters analyzed, were not significant. General characteristics of the two groups are summarized in Table 2.

No major complications relating to the surgical procedure were detected in any patients. In the BMNc group, cell counts using the concentrate samples confirmed the presence of nucleated cells in all the patients, with a median value of 276,520 cells/μL (range: 192320–355910).

Results for the investigated parameters are outlined below.

3.1. Pain

In both groups, pain at rest and during motion was significantly reduced after the procedure in all the follow-up controls. VAS scores presented the best values after 1 month (HA group 1.47 ± 0.99 at rest and 2.13 ± 1.36 during movement; BMNc group 1.13 ± 0.92 at rest and 1.17 ± 0.96 during movement), without significant differences between the two groups ($p = 0.925$ at rest and during movement). In both groups, the scores had worsened after 6 months (HA group 2.67 ± 1.17 at rest and 3.47 ± 0.99 during movement; BMNc group 1.27 ± 0.7 at rest and 1.73 ± 0.79 during movement) and again at 1 year (HA group 4.8 ± 1.37 at rest and 5.47 ± 1.19 during movement; BMNc group 1.87 ± 0.35 at rest and 2.33 ± 0.72 during movement). However, these scores remained significantly better than the preoperative data over the whole observation period. At 6 months and 1 year the two groups presented significantly different scores, with better Results for the BMNc group (6 months $p = 0.028$; 1-year $p = 0.000$). Table 3 and Chart 1 summarize the results obtained.

3.2. Joint noises

Compared with the preoperative Results, in both groups joint noises were significantly reduced over the whole observation period, with the best results at 6 months (HA group 2.4 ± 0.51; BMNc group 1.73 ± 0.59). No significant differences were found between the two groups [Table 4 and Chart 2].

3.3. Chewing efficiency

Chewing efficiency significantly improved after the procedure in both groups with better Results at 6-month control (HA-Group 8.13 ± 0.74; BMNc-group 8.8 ± 0.94). At 1-year control, significantly better results were found in the BMNc-group (HA-Group 5.87 ± 0.83; BMNc-group 8.07 ± 0.88; p = .000) [Table 5] [Chart 3]

3.4. Maximum interincisal opening

MIO improved after the procedure, with significantly better scores at all the postoperative follow-up stages. Values for the two groups were significantly different at 6 months (HA group 34.93 ± 0.8; BMNc group 37.53 ± 1.81; $p = 0.001$) and at 1 year (HA group 28.07 ± 2.25; BMNc group 33.8 ± 1.74; $p = 0.000$), with better Results in the BMNc group [Table 6 and Chart 4].

3.5. MRI findings

Disk displacement, with reduction and cartilage lesions, was presented in all 30 patients included in the study. Lesions detected included thinning of the disk, with some fissures and/or perforations, and unevenness of the condylar cartilage. Initial signs of osteoarthritis were present in 12 patients (40%), while a narrowed joint gap was detected in 16 patients (53.3%).

In all cases in both groups, cartilage or bony lesion healing was detected by the MRI imaging analysis carried out 12 months after the procedure [Table 7] [Fig. 3].

4. Discussion

As with other articular cartilages, TMJ cartilage lacks an intrinsic regenerative capacity, so many approaches have been proposed to

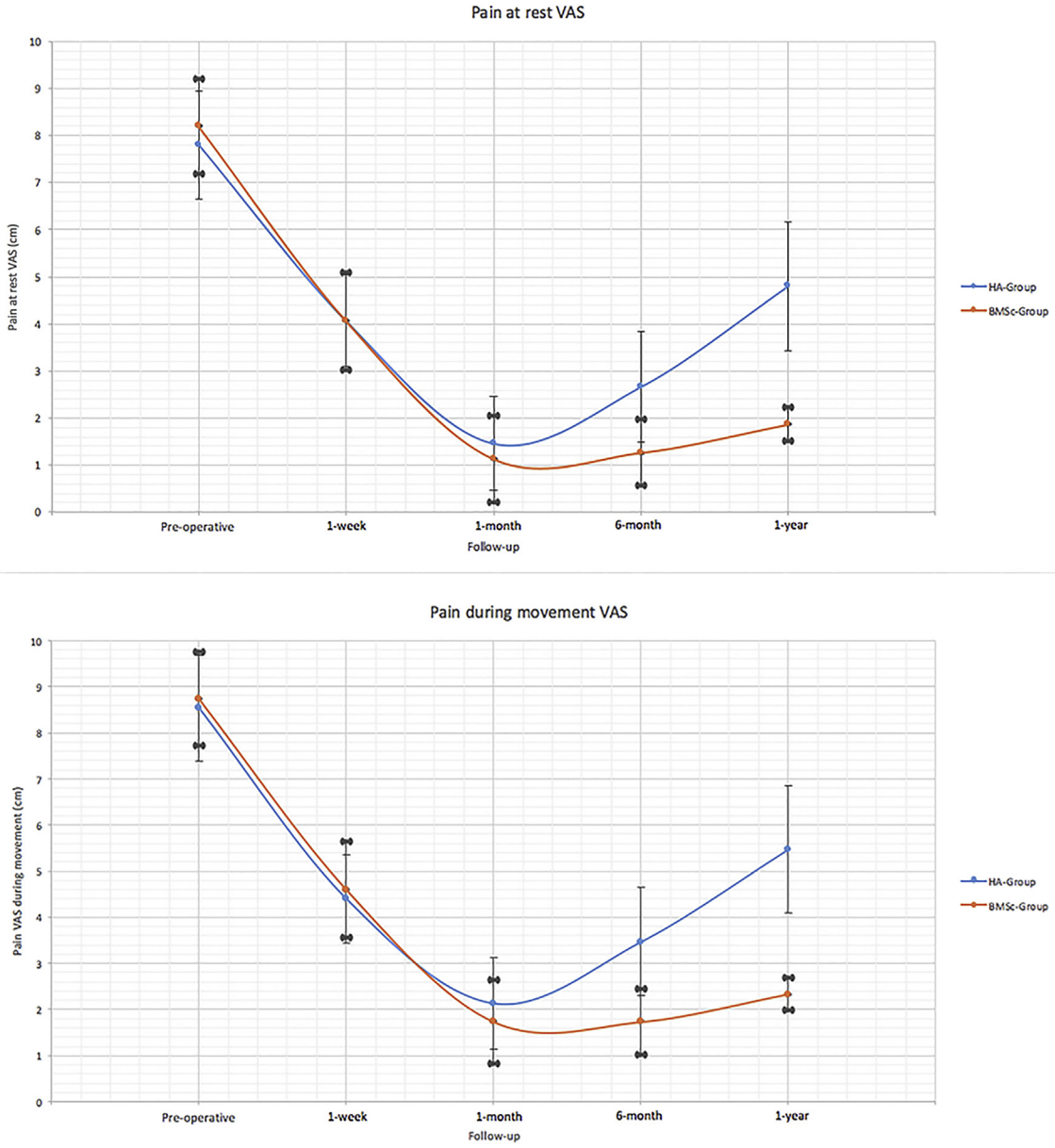

Chart 1. Pain at rest and during movement VAS trend for different follow-up times.

Table 4
Joint noises VAS. Results and statistical analysis.

Time interval	Group	Joint noises				
		VAS score mean ± SD (cm)	Wilcoxon test (observation time vs preop)		Kolmogorov–Smirnov test (HA group vs BMNc group)	
			Z	*p*-value[a]	Z	*p*-value[b]
Preop	HA group	7.0 ± 0.76			0.183	1.000
	BMNc group	7.07 ± 0.96				
1 week	HA group	4.47 ± 0.83	−3.432	0.001	0.548	0.925
	BMNc group	4.0 ± 0.85	−3.451	0.001		
1 month	HA group	3.4 ± 0.51	−3.458	0.001	1.095	0.181
	BMNc group	2.67 ± 0.62	−3.431	0.001		
6 months	HA group	2.4 ± 0.51	−3.457	0.001	1.095	0.181
	BMNc group	1.73 ± 0.59	−3.436	0.001		
1 year	HA group	4.13 ± 0.64	−3.443	0.001	0.913	0.375
	BMNc group	3.67 ± 1.17	−3.423	0.001		

[a] Wilcoxon signed-rank test for dependent paired data. Confidence interval: 95%. Significance level set at $p \leq 0.05$.
[b] Kolmogorov–Smirnov test for independent groups of data. Confidence interval: 95%. Significance level set at $p \leq 0.05$.

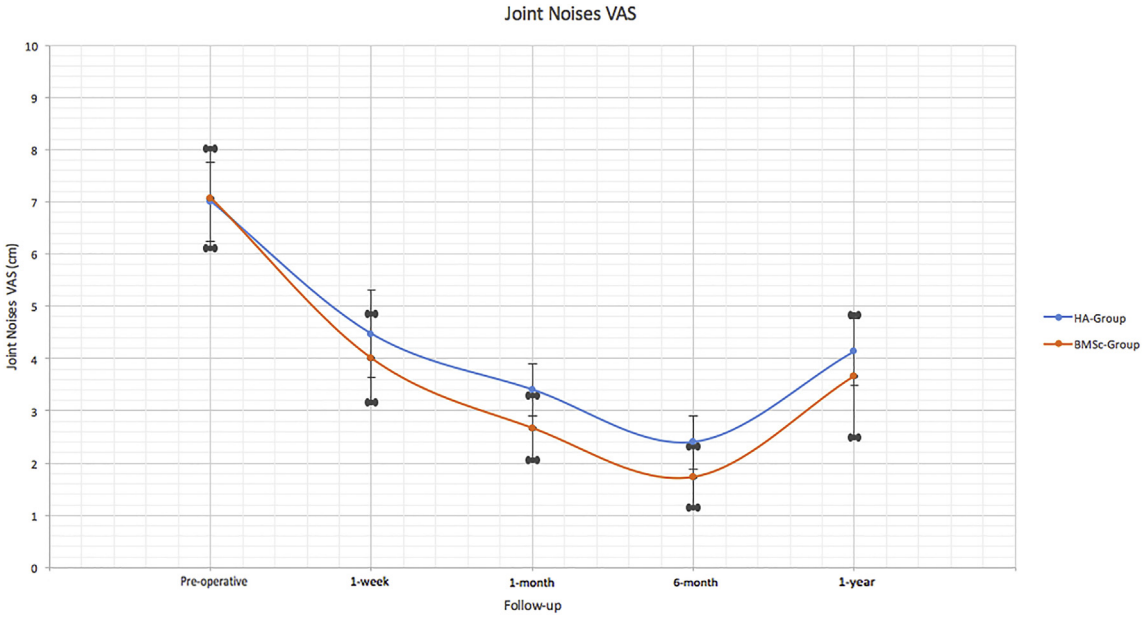

Chart 2. Joint noises VAS trend during the observation period.

Table 5
Chewing efficiency VAS. Results and statistical analysis.

Time interval	Group	Chewing efficiency				
		VAS score mean ± SD (cm)	Wilcoxon test (observation time vs preop)		Kolmogorov–Smirnov test (HA group vs BMNc group)	
			Z	*p*-value[a]	Z	*p*-value[b]
Preop	HA group	4.6 ± 1.12			0.365	0.999
	BMNc group	4.53 ± 0.99				
1 week	HA group	6.0 ± 0.84	−3.111	0.002	0.183	1.000
	BMNc group	6.0 ± 0.76	−3.205	0.001		
1 month	HA group	7.27 ± 1.03	−3.345	0.001	0.548	0.925
	BMNc group	7.67 ± 0.82	−3.352	0.001		
6 months	HA group	8.13 ± 0.74	−3.432	0.001	1.095	0.181
	BMNc group	8.8 ± 0.94	−3.451	0.001		
1 year	HA group	5.87 ± 0.83	−2.709	0.007	2.191	0.000
	BMNc group	8.07 ± 0.88	−3.347	0.001		

[a] Wilcoxon signed-rank test for dependent paired data. Confidence interval: 95%. Significance level set at $p \leq 0.05$.
[b] Kolmogorov–Smirnov test for independent groups of data. Confidence interval: 95%. Significance level set at $p \leq 0.05$.

increase its healing capacity. Viscosupplementation therapy and hyaluronic acid injections following arthrocentesis has gained popularity as a treatment option for larger joints (Al-Delayme et al., 2017). Many publications on TMJ disk displacement and degeneration have reported the use of low-molecular-weight hyaluronic acid intra-articular injection, which improves symptom control and leads to a chondroprotective effect (Guarda-Nardini et al., 2007; Manfredini et al., 2009).

In recent years, stem cells have gained popularity as a possible biological therapy for various diseases (Squillaro et al., 2016), including knee cartilage reparation.

Stem cells are derived from two major sources: mesenchymal stem cells (MSCs) and embryonic stem cells (ESCs) (Thomson et al., 1998; Ahtiainen et al., 2013; Zaki et al., 2017). Among stem cell sources reported for cartilage repair and tissue engineering, MSCs represent the most widely used, and have been isolated from a multitude of tissues (Zhang et al., 2015), including bone marrow (Gupta et al., 2016; Zaki et al., 2017). The use of autologous adult bone marrow-derived stem cells in research can be divided in three areas: nucleated cell concentration; isolated MSCs without culture expansion; and isolated MSCs with culture expansion. The adult stem cell fraction is present in the nucleated cells of the marrow. Most of these cells are CD34 + hematin progenitors (destined to differentiate into blood components), while very few (1/10,000–1/1,000,000 nucleated cells) are actually MSCs capable of differentiating into bone, cartilage, or muscle (Centeno et al., 2008).

In humans, nucleated cells are isolated from an aspirate of bone marrow, which is typically harvested from the superior iliac crest of the pelvis using a trocar. The composition of these nucleated cells is diverse, and includes mesenchymal stem cells (MSCs), hematopoetic stem cells, monocyte precursor cells, macrophages, T cells, B cells, dendritic antigen presenting cells, natural killer cells and neutrophils. The actions of these cells, both in isolation and symbiotically, once introduced into arthritic joints, may help improve pain and function by replenishing damaged joint structures and reducing the catabolic immune response, thus alleviating the symptoms and progression of the disease (Centeno et al., 2015).

Only a few reports have investigated the effect of in vitro chondrogenic differentiated MSCs in animal TMJ models, using surgically made lesions (Ahtiainen et al., 2013; Chen et al., 2013;

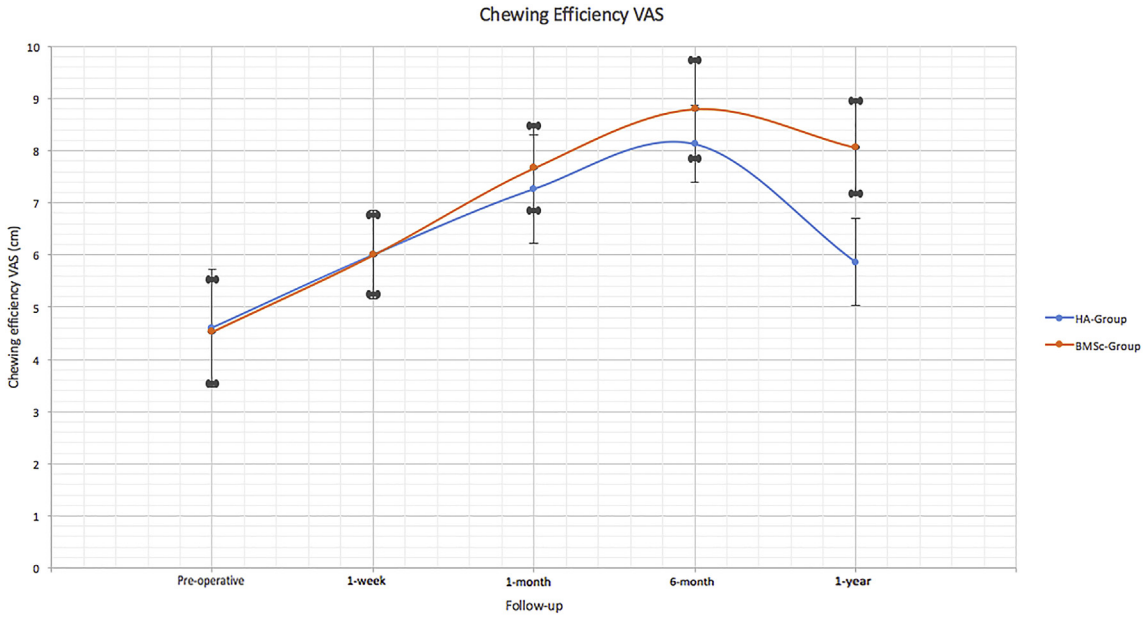

Chart 3. Maximum interincisal opening (MIO) trend during the observation period.

Table 6
Maximum interincisal opening (MIO) VAS. Results and statistical analysis.

Time interval	Group	Maximum interincisal opening (MIO)				
		MIO mean ± SD (mm)	Wilcoxon test (observation time vs preop)		Kolmogorov–Smirnov test (HA group vs BMNc group)	
			Z	*p*-value[a]	Z	*p*-value[b]
Preop	HA group	23.2 ± 2.45			0.913	0.375
	BMNc group	22.0 ± 2.67				
1 week	HA group	28.4 ± 2.2	−3.429	0.001	0.365	0.999
	BMNc group	28.47 ± 2.03	−3.205	0.001		
1 month	HA group	37.67 ± 1.63	−3.414	0.001	0.548	0.925
	BMNc group	38.2 ± 1.37	−3.352	0.001		
6 months	HA group	34.93 ± 0.8	−3.417	0.001	2.008	0.001
	BMNc group	37.53 ± 1.81	−3.451	0.001		
1 year	HA group	28.07 ± 2.25	−3.422	0.001	2.373	0.000
	BMNc group	33.8 ± 1.74	−3.347	0.001		

[a] Wilcoxon signed-rank test for dependent paired data. Confidence interval: 95%. Significance level set at $p \leq 0.05$.
[b] Kolmogorov–Smirnov test for independent groups of data. Confidence interval: 95%. Significance level set at $p \leq 0.05$.

Zaki et al., 2017), while there are no studies on the use of MSCs or BMNc for the treatment of TMDs in human models.

Two important findings of this study were the safety and feasibility of BMNc injection into the TMJ. The procedure can be conducted on an outpatient-basis under local anesthesia. No complications were detected in both the donor or receiving sites in the BMNc group patients. Attention is needed in deciding the entry point on the iliac crest, which should be at least 2 cm behind the anterior–superior iliac spine in order to avoid lateral femoral cutaneous nerve injuries. The non-dominant hand of the operator must firmly hold the iliac crest, to helping ensure a proper trocar insertion through the cortical bone, into the spongiosa. After the procedure, compressive medication is placed on the donor site to prevent seromas and hematomas.

The study showed that BMNc autograft in TMD is an effective and minimally invasive treatment, which significantly reduces TMJ pain and increases maximal mouth opening in the long term. Joint injection of BMNc showed an analgesic effect similar to that seen with hyaluronic acid, but much more prolonged, providing satisfactory pain control even up to 6 and 12 months after the procedure.

Regarding joint noises, no significant differences were found between the two groups during the whole observation period. The improvement in this score just after the procedure is probably related to the mechanical effect of the lavage on the articular adhesions rather than to the type of substance injected. Nevertheless, the long-lasting analgesic effect of the BMNc resulted in significantly higher masticatory performances and MIO after 6 and 12 months.

The worst Results were recorded in three patients affected by rheumatological diseases (two patients with fibromyalgia in the HA group, and one patient with fibromyalgia in the BMNc group). Probably, patients with autoimmune disorders should be excluded from future studies.

Poor Results were also recorded in a single patient — an amateur runner — who had an amputated arm. The continued unbalanced load during prolonged workouts caused postural problems, with vertebral disk displacements, and the TMD relapsed

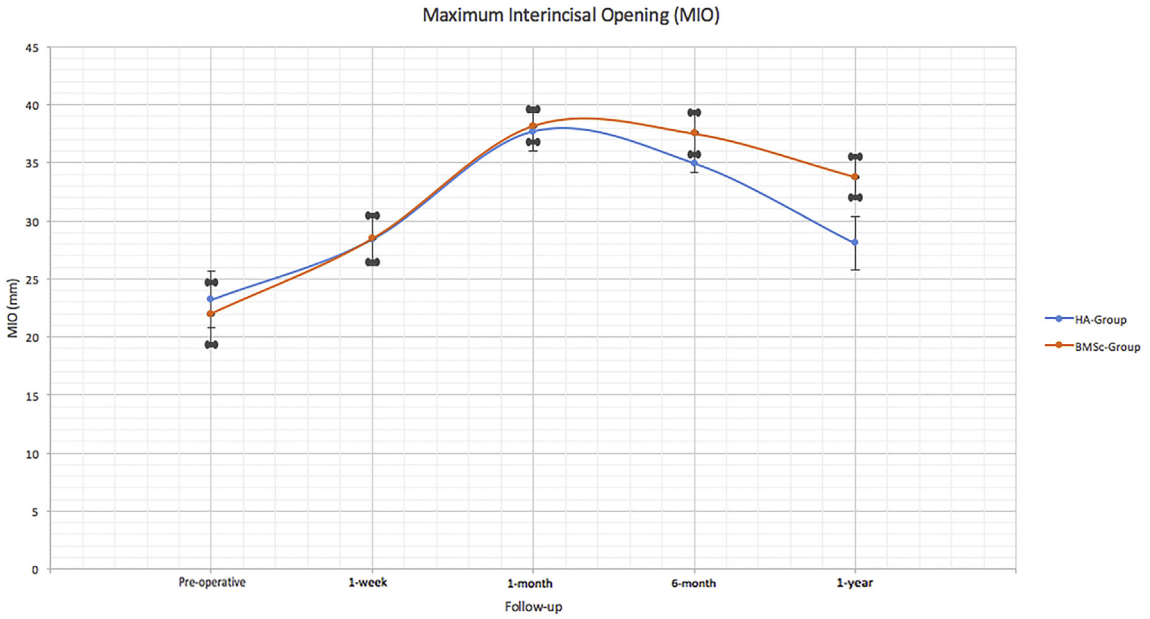

Chart 4. Chewing efficiency trend during the observation period.

Table 7
MRI findings (No. of patients (%)).

Group D				Group L				Group J			
Preop HA group	Postop HA group	Preop BMNc group	Postop BMNc group	Preop HA group	Postop HA group	Preop BMNc group	Postop BMNc group	Preop HA group	Postop HA group	Preop BMNc group	Postop BMNc group
D0 0 (0%)	0 (0%)	0 (0%)	0 (0%)	**L0** 0 (0%)	0 (0%)	0 (0%)	0 (0%)	**J0** 0 (0%)	0 (0%)	0 (0%)	0 (0%)
D1 15 (100%)	9 (60%)	15 (100%)	9 (60%)	**L1** 15 (100%)	9 (60%)	15 (100%)	8 (53.3%)	**J1** 5 (33.3%)	5 (33.3%)	7 (46.7%)	7 (46.7%)
D2 0 (0%)	0 (0%)	0 (0%)	0 (0%)	**L2** 0 (0%)	0 (0%)	0 (0%)	0 (0%)	**J2** 0 (0%)	0 (0%)	0 (0%)	0 (0%)
D3 11 (73.3%)	11 (73.3%)	10 (66.7%)	10 (66.7%)	**L3** 0 (0%)	0 (0%)	0 (0%)	0 (0%)	**J3** 8 (53.3%)	8 (53.3%)	8 (53.3%)	8 (53.3%)
D4 4 (26.7%)	4 (26.7%)	5 (33.3%)	5 (33.3%)	**L4** 9 (60%)	8 (53.3%)	8 (53.3%)	8 (53.3%)	**J4** 0 (0%)	0 (0%)	0 (0%)	0 (0%)
D5 0 (0%)	0 (0%)	0 (0%)	0 (0%)	– –	–	–	–	**J5** 0 (0%)	0 (0%)	0 (0%)	0 (0%)

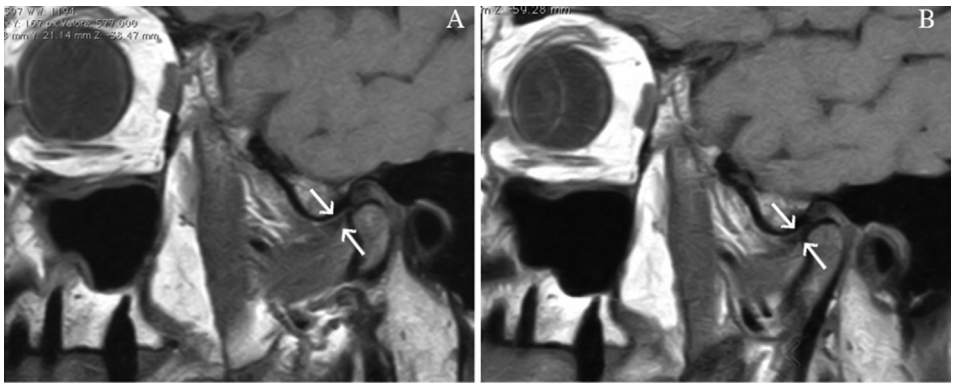

Fig. 3. Preoperative (A) and 1-year postoperative (B) MRI scans of a BMSc-group patient. No evidence of disk cartilage repair was detected 1 year after the BMSc inoculation (white arrows).

after washing and BMNc autografts. In larger groups, maybe these non-homogeneous patients should be considered for exclusion.

The exact mechanism by which BMNcs exert their therapeutic effects is not fully understood. These cells do not exhibit multilineage differentiation into osteocytes, adipocytes, and chondrocytes, but they can mediate a wide spectrum of immunoregulatory activities that usually modulate innate and adaptive immune responses. BMNcs inhibit the pro-inflammatory activities of neutrophils as well as the proliferation, cytokine production, and cytotoxic activity of resting natural killer cells, while they promote the development of regulatory T cells (Shadmanfar et al., 2018). These effects are mediated by a wide spectrum of

bioactive factors secreted by BMNcs, including growth factors, cytokines, microvescicles, and exosomes. This paracrine function seems to have had the most important therapeutic effect in this trial.

No evidence of cartilage regeneration was detected by the 12-month MRI assessment. This finding seems to confirm the fact that the presence of MSCs with chondrogenic differentiating capacities is very low in marrow concentrates. However, isolated bone marrow nucleated cells implanted into degenerated human peripheral joints have shown some promise for joint repair (Centeno et al., 2006). In contrast to the findings of this trial, a couple of animal model studies reported cartilage lesion regeneration after injection of uncultured bone marrow mononuclear cells (Zhang et al., 2012; Tiwary et al., 2014). The authors speculated that in vivo culture in the joint cavity environment may provide better conditions for MSC differentiation than in vitro culture, involving mechanical stimulation caused by the motions of the joint, the lower oxygen tension in the knee joint capsule, the many nutritive materials of the synovial fluid, and BMNc secretion of cytokines and growth factors, which provide a more favorable and constant microenvironment, consequently contributing to the proliferation and chondrogenic differentiation of MSCs (Zhang et al., 2012). However, based on our findings, cartilage healing seemed beyond the capability of this cell population.

In this study, hyaluronic acid provided satisfactory control of the symptoms until 3/6 months after the procedure, and, compared with BMSc, it is quite inexpensive. On the other hand, BMNc seemed to provide prolonged asymptomatic stabilization of TMD.

Even if these first Results are encouraging, in our opinion, larger studies with several years of follow-up, which could confirm an appropriate, prolonged pain-relieving effect or microscopic signs of cartilaginous regeneration, are now necessary to further indicate whether BMNcs should be considered as first-line therapy after joint lavage in cases of TMD.

5. Conclusions

Based on these observations, intra-articular TMJ BMSc injection is regarded as a simple and safe method, with potential beneficial effects, that proved to be successful in the treatment of TMDs. BMNc treatment presented a longer pain-relieving effect than HA, but did not induce macroscopic cartilage regeneration. The Results of this first human-model study have been promising, however, further studies are needed to determine whether BMNc could represent a valid alternative to HA in the treatment of TMDs.

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Conflicts of interest

All the authors declare that they have no conflict of interest.

Ethical standard

This investigation was conducted according to the principles embodied in the Helsinki Declaration of 1975 for biomedical research involving human subjects, as revised in 2013, and the study protocol was approved by the University of Sassari Ethical Committee (No. 297/2016).

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcms.2018.11.025.

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