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Cellular and clinical analyses of autologous bone marrow aspirate injectate for knee osteoarthritis: a pilot study

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Abstract

Introduction: Knee osteoarthritis (OA) is characterized by pain and functional deficits. Common conservative strategies include medications, physical therapy, and intra-articular injections. Recently, treatment using autologous cell injections has increased.

Objective: To characterize the cellular content of BMA and to evaluate the effect of intra-articular autologous bone marrow aspirate (BMA) injections in patients with mild knee OA.

Design: Prospective pilot observational study

Setting: Academic institution

Patients: Eleven patients with unilateral or bilateral mild knee OA (15 knees) were included in the cellular analysis. Ten patients (13 knees) were included in the overall (cellular and clinical) analysis.

Interventions: BMA was aspirated from patients' iliac crests and then injected intra-articularly under fluoroscopic and/or ultrasound guidance. BMA samples were analyzed using flow cytometry, colony forming unit (CFU) assays, and enzyme-linked immunosorbent assays. Questionnaires assessing pain and function were administered pre-injection and at 1, 3, 6, and 12 months post-injection. Side effects and satisfaction were assessed.

Main Outcome Measures: Total nucleated cell (TNC) concentration, mesenchymal stem cell (MSC) concentration, CFU count, and interleukin-1 receptor antagonist (IL-1Ra) concentration.

Results: BMA sample analyses revealed wide ranges in TNC concentration (173,300–4,491,050 cells/mL), MSC concentration (0–500 cells/mL), CFUs (0–19), and IL-1Ra concentration (2,806–29,394 pg/mL). Improvements in Knee Injury and Osteoarthritis Outcomes Score for Joint Replacement were observed throughout the 12-month follow-up period ($F(4,12)=12.29$, $p<0.001$).

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Additionally, current, usual, best, and worst NRS pain scores significantly decreased over time ($p<0.001$). Patient satisfaction was high (range: 8.1±2.1–8.8±1.9), and side effects were uncommon.

Conclusion: The cellular content of BMA samples varied widely between patients and was lower than the anticipated yield reported by the device's manufacturer. However, intra-articular BMA injections for knee OA in a small pilot cohort appeared to be safe with potential therapeutic value. Larger, prospective, double-blinded studies are warranted.

Clinicaltrials.gov : NCT03130335

INTRODUCTION

Knee osteoarthritis (OA) is a leading cause of disability and affects over 20 million people in the U.S. and 266 million people worldwide.[1,2,3] The prevalence of knee OA is increasing, due to greater life expectancy and risk factors such as obesity. Patients with symptomatic knee OA often experience movement limitations and are at a higher risk of death compared with the general population.[4] Knee OA is characterized by progressive articular cartilage degeneration, increased subchondral plate thickness, osteophyte formation, and inflammation; these all contribute to pain and functional impairment.[5]

The main objectives of knee OA management are to reduce pain, decrease inflammation, slow cartilage degradation, and improve function.[6] Conservative strategies include medications, physical therapy, weight loss, and intra-articular injections,[7] which are considered the final option for conservative therapy.[8] Corticosteroids, hyaluronic acid, blood-derived products, and mesenchymal stem cells (MSCs) are all used with varying success.[9,10]

Given their ability to influence the microenvironment as a signaling cell and to modulate the environment's inflammatory properties, MSCs have regenerative potential.[11] The highest concentration of MSCs from bone marrow aspiration has been found in the posterior iliac crest.[12] To increase the proportion of MSCs, many studies concentrate the bone marrow aspirate (BMA) via centrifugation to produce BMA concentrate (BMC).[13] Intra-articular injections of BMC or isolated MSCs have shown therapeutic value in the treatment of knee OA, although the biologic mechanisms underlying these treatments remain unclear.[10,14,15,16] However, no studies have yet investigated the cellular composition of BMA and the patient-reported outcomes following intra-articular injections of autologous non-concentrated BMA for the treatment of knee OA.

The primary objective of this pilot study was to analyze the cellular characteristics of BMA samples, which were harvested from iliac crests using the Marrow Cellution device. The secondary objective was to assess the effect of a single BMA injection on pain and function in patients with mild knee OA.

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METHODS

Ethics

This prospective study was approved by the Institutional Review Board. Written informed consent was obtained from all patients.

Recruitment and Baseline Procedures

Patients with radiographically confirmed knee OA of Kellgren-Lawrence grade I-II who experienced greater than 3 months of symptoms that were refractory to conservative therapies were included in the study. Patients with a history of knee surgery, meniscal injury, or loose bodies on baseline magnetic resonance imaging (MRI) were excluded, as well as patients with a history of anemia, bleeding disorders, or inflammatory joint disease. A complete list of inclusion and exclusion criteria is presented in Table 1. Radiographs, MRIs, and medical history were reviewed during eligibility screening. Patients were then referred to the study physician for confirmation of eligibility, after which informed consent was obtained.

Patients completed baseline bloodwork for complete blood counts with differentials. If hematocrit and hemoglobin levels were within the normal range (hemoglobin: 13–17 g/dL [males], 11.5–16 g/dL [females]; hematocrit: 38–52% [males], 34–46% [females]), the patient was scheduled for a baseline MRI. The MRI included sagittal inversion recovery, axial, sagittal, and coronal fast-spin echo techniques, as well as sagittal T1-rho and T2 mapping. Afterwards, the study injection was scheduled to occur within 90 days of the baseline bloodwork. For patients with bilateral knee symptoms, each knee was assessed separately for pain and function. The procedure and all related costs were covered by the study.

A baseline questionnaire containing demographics, medical history, Knee Injury and Osteoarthritis Outcome Score for Joint Replacement (KOOS-JR), and numerical rating scale (NRS) pain was administered prior to the study injection. The KOOS-JR survey assesses function, pain, and stiffness, and has a minimum clinically significant difference (MCID) of 15.1 points.[17] Best, current, worst, and usual NRS pain were assessed on a scale from 0–10 (0: no pain; 10: worst possible pain imaginable). The MCID for NRS pain is 2 points.[18]

A total of 106 patients were assessed for eligibility from April 2017–December 2018; 70 patients did not meet eligibility criteria, and 1 patient declined to participate. Nineteen knees (14 patients) were enrolled in the study. Three patients (4 knees) were withdrawn due to screen failure after the baseline MRI. One patient (2 knees) was withdrawn for non-compliance during the study; as follow-up outcomes were not obtained, this patient was only included in the cellular analysis. Ten patients and 13 knees were included in the overall analysis of clinical and cellular data. Four females and three males underwent unilateral procedures. Two females and one male underwent bilateral procedures. Baseline data are presented in Table 2.

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Bone Marrow Aspiration and Injection

A single, fellowship-trained, board-certified physiatrist performed the procedure. Patients received intravenous sedation for the duration of the procedure. For the aspiration, the patient was placed in the prone position. Following anesthetization of the BMA target site, 10,000 units/mL heparin were withdrawn into a 10-mL syringe. After connecting the syringe to the introducer needle, the heparin was injected until the introducer needle was fully rinsed, and then aspirated back into the syringe. This process was repeated for the longer aspiration needle. All stylets were then rinsed with heparin. Following this, 0.5 mL heparin was added to the 12-mL collection syringe. Under fluoroscopic guidance, bone marrow was aspirated from the posterior iliac crest in accordance with Marrow Cellution Bone Marrow Harvesting Device (Ranfac Corp., Avon, MA) best practice guidelines and expert consensus technique. After the usual sterile skin preparation, draping, and local anesthesia, the access needle was inserted into the medullary space. Once proper localization was confirmed by attaching the syringe and drawing 1 mL, the syringe was removed, and the blunt stylet was inserted to drive the access needle to the necessary depth. When the outer housing reached skin level, the blunt stylet was removed, the aspiration cannula was attached to the access needle, and the syringe was attached. The physician then held the outer housing in place while rotating with the opposite hand 360° to raise the cannula tip 0.75 cm into a new location. This rotation/aspiration technique was repeated 5–6 times to obtain approximately 12 mL BMA. From this, a droplet (~50 μl) was used for marrow smear analysis, whereas 2 mL were used for flow cytometry, colony forming unit (CFU), and interleukin-1 receptor antagonist (IL-1Ra) analyses. All samples were kept on ice and were processed within 24 hours of aspiration.

For the injection, the patient was placed in the supine position. After sterile skin preparation, draping, and local anesthesia, a 20-gauge needle was placed into the medial or lateral portion of the tibiofemoral joint, depending upon where the greatest chondral loss was demonstrated on the pre-procedure MRI. Visipaque (iodiaxanol) was used as the contrast agent during the arthrogram under fluoroscopic guidance. Once intra-articular flow was demonstrated, the syringe was replaced, and approximately 10 mL BMA was injected. If the greatest degree of chondral loss was in the patellofemoral joint, ultrasound guidance was then used with a superolateral approach. For the 3 patients with bilateral knee OA, a separate, 12-mL aspiration of bone marrow was made from the contralateral iliac crest, so as not to dilute the total volume each knee received. The exact volume of bone marrow that was aspirated and then injected was recorded in the procedure notes. A sterile bandage was placed, and patients were monitored in the recovery area for 15–30 min and discharged with post-procedure instructions.

Marrow Smear Analysis

BMA droplets were placed on glass slides. Smears were prepared by placing an empty slide on the slide with the BMA and gently drawing the slides in opposite directions. This was repeated up to 15 times per sample, depending on the amount of peripheral blood. Slides were air-dried and fixed in 100% methanol for 1 min, after which they were flooded with the Wright-Giemsa stain for 1 min and then with phosphate buffer (pH 6.6) for 1 min. Slides were then rinsed with phosphate buffer (pH 6.6), air-dried, and examined

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under a microscope with oil immersion. Approximately 500 cells were counted per sample by a pathologist with expertise in marrow smears. Cell types evaluated included blast cells, promyelocytes, myelocytes, metamyelocytes, band cells, polymorphonuclear (PMN) leukocytes, erythroids, megakaryocytes, lymphocytes, and monocytes.

CFU Assay

BMA (1 mL) was plated and cultured for 2 weeks in Dulbecco's Modified Eagle Medium containing 20% fetal bovine serum, 1 ng/mL penicillin/streptomycin, and 1 ng/mL bFGF. Every 48 hours, the tissue culture plate was rinsed with phosphate-buffered saline (PBS), and the media was replaced. Cultures were inspected under light microscopy every 24 hours for the appearance of cells. CFUs were counted using previously described methods.[19] When CFUs appeared on the plate, cells were circled with a 1.8-mm-diameter, self-inking marker. After 2 weeks, CFUs (colonies 1 mm) were counted.

Eight-Plex Multicolor Flow Cytometry

Flow cytometry was performed on non-concentrated BMA and not on cultured cells. BMA (200 μL) was added to 2 mL of a 1:10 dilution of BD Pharm Lyse Solution (BD Biosciences, San Jose, CA) to lyse red blood cells. The solution was centrifuged at 300×g for 5 min to pellet cells. The cell pellet was then washed and resuspended in 1 mL PBS, and 10 μL were stained 1:1 with 0.04% trypan blue solution (VWR, Radnor, PA) to quantify live nucleated cells. This nucleated cell count was used to adjust the volume of BMA to 1×10^7 cells/mL for flow cytometry. For each sample, 1×10^7 cells were stained using the LIVE/DEAD Fixable Violet Dead Cell Stain (Thermo Fisher, Waltham, MA) and incubated in the dark for 30 min at room temperature. Cells were pelleted by centrifugation at 300×g for 5 min. The cell pellet was washed, resuspended in 1 mL BD Pharmigen Stain Buffer (BD Biosciences), and divided into 6 aliquots, which were stained as Fluorescence Minus One controls with the BD Stemflow Human MSC analysis kit (BD Biosciences). As recommended by the International Society for Cellular Therapy, MSCs were defined as viable cells possessing negative expression of CD34, CD45, CD11b, CD19, and HLA-DR, with concurrent positive expression of CD73, CD105, and CD90.[20]

Stained cells were visualized on an LSR II flow cytometer (BD Biosciences). Compensation values were set before sample analyses. The collected data was processed using FlowJo software (TreeStar Inc., Ashland, OR). Stained cells were gated first for cell size using forward and side scatter and then gated by viability. Cells were further gated to identify cells negative for expression of CD34, CD45, CD11b, CD19, and HLA-DR, followed by positive for expression of CD105 and CD73, and finally positive for CD90. This final population was identified as MSCs.

Enzyme-Linked Immunosorbent Assay (ELISA)

BMA samples were vortexed until they became homogeneous, and then mixed with diluent. After dilution, all samples were vortexed. Immediately prior to plating for the ELISA, all samples were vortexed for 30 seconds again. IL-1Ra protein concentrations in BMA samples were measured using an Invitrogen IL-1Ra human ELISA kit (#KAC1181, Carlsbad, CA),

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according to the manufacturer's directions. To simulate the state in which the BMA would be injected into a joint, samples were not centrifuged before assaying.

Follow-Up Procedures

Questionnaires including KOOS-JR, NRS pain, patient satisfaction, and procedure-related adverse events (e.g., bleeding, swelling, redness, allergic reactions, weakness) were administered at 1, 3, 6, and 12 months post-procedure. The primary outcome was the KOOS-JR score. Data were collected and stored on Research Electronic Data Capture. [21,22]

Data and Statistical Analyses

For cellular analyses, percent cell viability was calculated based on flow cytometry data for each sample, using the events defined as singlets and live cells. The number of singlets was defined as events having the forward and side scatter within the range of these values for white blood cells, as well as having a linear relationship between forward scatter height and forward scatter width. The number of live cells was defined as singlets that stained low for LIVE/DEAD Fixable Violet Dead Cell Stain. The number of MSCs/mL BMA was calculated using the following formula:

$$\mathrm{MSC/mL\ BMA} = \frac{\left[\left(\frac{\mathrm{mL\ PBS\ used\ for\ staining}}{0.1}\right) \times \mathrm{MSCs\ detected}\right]}{0.2}$$

The total nucleated cells (TNCs)/mL BMA were calculated using the same formula, but the original number of MSCs counted by the flow cytometer was replaced by the number of TNCs, which were defined as single events that were within the range of size and complexity for cells. The percentage of MSCs per TNC was then determined.

Medians and ranges are reported for TNC/mL, MSC/mL, and CFU/mL, due to the small sample size and large variation in samples. Regression analyses were performed to determine if CFU or TNC could be used to predict MSC concentration.

For clinical outcomes, continuous data are presented as means±standard deviations. Categorical data are presented as frequencies and percentages. A one-way repeated measures analysis of variance was run to assess for differences in KOOS-JR and NRS pain scores across the follow-up period. $P<0.05$ was considered statistically significant.

RESULTS

Bone Marrow Aspiration and Injection

On average, the physician aspirated 12.2±1.3 mL from the iliac crest and injected 9.9±1.2 mL into each knee joint. No complications were reported during the procedure. Procedure durations averaged 18.5±4.6 minutes for unilateral procedures and 26.7±8.5 min for bilateral procedures (range: 15–35 min).

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Marrow Smear Analysis

Marrow cells were detected in smears from all but one sample. An average of 453±52 cells (median: 447; range: 383–579) was counted per sample. PMN leukocytes were the most common cell type (mean: 51.0±10.8%; median: 49.9%; range: 35.8–72.2%). Megakaryocytes comprised an average of 1.5±1.0% (median: 1.2%; range: 0.6–3.7%) of all cells. Percentages of other cells are presented in Figure 1.

Cellular Analyses

BMA was obtained from 11 patients and 15 iliac crests (Table 2). The processed and stained cells from the first BMA sample were not analyzed on the flow cytometer within 24 hours of aspiration; therefore, this sample was excluded from analyses.

Flow cytometry was initially used to determine the cellularity of BMA samples and the viability of these cells. The median TNC concentration was 782,363 cells/mL (range: 173,300–4,491,050 cells/mL) (Figure 2A). Despite this variability, BMA cells were viable across all samples (median cell viability: 94%; range: 77–99%). Next, surface cell markers on viable cells were analyzed to determine the MSC content of each sample. The median concentration of MSCs was 238 MSCs/mL (range: 0–1,925 MSCs/mL) (Figure 2B). Additionally, the median percentage of MSCs per TNC was 0.027% (range: 0–0.24%).

CFUs were counted from cultured BMA as a proxy measurement for the number of MSCs in a BMA sample. The median number of CFUs/mL BMA was 8 (range: 0–19) (Figure 2C). When this number was plotted against the number of MSCs/mL on a scatterplot, no correlation was found ($R=0.169$) (Figure 3A). Similarly, no correlation was found when the number of TNCs/mL was plotted against the number of MSCs/mL ($R=0.147$) (Figure 3B).

Most samples contained measurable concentrations of IL-1Ra (median: 12,053 pg/mL; range: 2,806–29,394 pg/mL) (Table 3). IL-1Ra concentrations could not be measured in two samples that clotted upon thawing.

Clinical Outcomes

Significant improvements in KOOS-JR scores were observed over time ($F(4,12)=12.29$, $p<0.001$) in this cohort. Clinical significance was achieved at 3, 6, and 12 months post-procedure (Table 4). Decreases in current, usual, best, and worst NRS pain scores were also statistically significant over time ($F(4,12)=14.5$, $p<0.001$; $F(4,12)=17.5$, $p<0.001$; $F(4,12)=2.9$, $p=0.003$; and $F(4,12)=35.5$, $p<0.001$, respectively) (Table 5). Improvements in usual and worst NRS pain were clinically significant at all follow-up time points. Improvements in current NRS pain were clinically significant at 3, 6, and 12 months post-procedure. Best NRS pain was low at baseline (1.3±1.6), and any decreases did not meet the 2-point definition of clinical significance.

Patient satisfaction was high (Table 6). All patients noted that they would refer a friend for the procedure, and 85% noted that they would repeat the procedure. Side effects were uncommon and were reported by 4 patients and 5 knees, with a total of 7 documented instances out of 225 possible instances. These included weakness in the knee (2 knees), pain around the injection site (1 knee), minor bleeding from the aspiration site (1 knee), and

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minor redness or swelling around the injection site (3 knees). The most common side effects were redness/swelling and weakness with movement, especially within the first couple weeks following the procedure. No infections, allergic reactions, or flares were reported.

DISCUSSION

In this pilot study, BMA samples were collected from 11 patients and 15 iliac crests using the Marrow Cellution device. The BMA was not concentrated via centrifuge. Results of cellular analyses revealed large variations in TNC, MSC, and IL-1Ra concentrations, as well as CFU numbers. These concentrations were lower than those of BMC samples, as reported in previous studies, and were also lower than those advertised by the manufacturer of the device.[23]

The median number of MSCs found by 8-plex flow cytometry in the current study was within previously reported limits for the number of MSCs/mL characterized by flow cytometry in BMA.[14] However, this was also three times lower than the median number of MSCs/mL reported in BMC, based on multiplex flow cytometry for the lack of surface cell markers CD34, CD45, CD19, CD11b, and HLA-DR, with concurrent expression of surface cell markers CD90, CD73, and CD105.[14,24] Interestingly, the number of MSCs in the current study was lower than what was advertised by the BMA device manufacturer. While this may be attributed to the learning curve for the advanced technique of harvesting bone marrow, as well as patient variability, the exact reason behind this discrepancy is unknown. In addition, analysis of marrow smears revealed low percentages of megakaryocytes and large amounts of peripheral blood, which may have reduced the number of MSCs in the sample.

Similarly, the number of CFUs/mL found in this study was within normal limits of the reported number for BMA but was lower than that for BMC.[19,24,25,26] It is important to note that the methods for determining and presenting the number of CFUs/mL in published studies are not standardized, and the methods used to identify CFUs can vary greatly.[19,26,27] Although CFUs are often used to determine the number of MSCs in a sample,[27] the lack of a clear definition for counting and reporting CFUs suggests that CFUs may not be a reliable proxy for MSC determination. In the current study, the number of CFUs/mL was not predictive of MSCs/mL.

TNC concentration has also been used as a proxy for MSC concentration. Similar to a study by Cassano et al., which found that two different BMC methods generated similar CFU counts regardless of TNC count,[19] the number of TNCs/mL was not predictive of MSC/mL in the current study. This suggests that TNCs/mL cannot be used to predict the concentration of MSCs in BMA when MSCs are enumerated using flow cytometry. Similar to MSC and CFU counts, TNC concentrations were lower than the reported values for both BMA and BMC.[14,19,24,25,26,27] This is likely due to different methods of counting TNCs, as several studies used automated counting. The low number of TNCs/mL may also have been due to the amount of debris in the samples, resulting in less events meeting the criteria of a nucleated cell.

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Few studies have reported IL-1Ra concentrations in BMA; in these studies, the mean IL-1Ra concentration ranged from 2,324–4,510 pg/ml.[19,28] In our study, we measured much higher IL-1Ra concentrations, with a median of 12,053 pg/mL ranging from 2,806–29,394 pg/mL. It should be noted that there may be technical challenges associated with IL-1Ra measurement in biologically complex fluids, such as BMA. Similar to blood, BMA contains many proteins, such as growth factors and cytokines.[29] This may confound antibody-based methods such as ELISAs, which depend on the specificity of the antibody binding to only the target molecule of interest to produce an accurate assessment of the protein concentration. Therefore, any non-specific binding to other proteins present in BMA may be responsible for the range of values seen in the literature. Additionally, Oliver et al. reported that factors such as hematocrit can influence the concentration of IL-1Ra found in BMC.[24] Therefore, a high hematocrit in BMA samples may cause major differences in reported values of IL-1Ra in BMA. Finally, simple biological processes, such as clotting, before measurement may affect the measured levels of IL-1Ra. Clotting was a technical issue encountered in this study, despite BMA being drawn into anticoagulant due to the up-to-24-hour transport time between aspiration and storage of BMA for ELISA. Because coagulation can sequester proteins, partial clotting of the BMA may be responsible for the high values of IL-1Ra seen in some samples. Nevertheless, these high levels of IL-1Ra may have been responsible for the observed clinical benefit seen with intra-articular injections of BMA.

It is unknown whether BMA or BMC is more effective when used for clinical applications. Similarly, whether the number of MSCs matters clinically remains unclear. BMA requires less bone marrow, as centrifugation systems discard approximately 80% of the aspirate due to the high concentration of peripheral blood that infiltrates the marrow aspiration. Furthermore, desirable healing cells may be lost during the centrifugation process due to their higher density. Depending on the system, centrifugation to obtain BMC could add 15–20 minutes to the procedure, which may increase the risk of infection due to the processing of the BMC sample off the sterile field.[30]

Because MSCs modulate the joint environment and decrease inflammation, it is thought that a greater number of MSCs would lead to more clinical benefits, such as pain reduction. Interestingly, despite the lower number of MSCs/mL observed in our samples, patients reported significant improvements in pain and function and were generally satisfied with the results of their treatments. These improvements were observed up to 12 months post-procedure and were clinically significant at most follow-up time points. Patient satisfaction was high overall. Cellular analyses of the BMA samples demonstrated wide variations in TNC, MSC, and IL-1Ra concentrations, as well as CFU numbers.

Procedure durations were short and ranged from 15–35 minutes. KOOS-JR scores increased significantly starting at 3 months post-procedure, with clinically significant improvements up to 12 months. Current, usual, and worst NRS pain also improved significantly up to 12 months post-procedure. Because best NRS pain scores were low at baseline, no clinically significant differences were observed, but scores decreased throughout the follow-up period. Outcomes were similar in patients with bilateral or unilateral symptoms.

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Similar results with BMC injections have been reported in patients with knee OA. When used in conjunction with artificial cartilage scaffolds, autologous BMC may significantly improve pain outcomes and function over time.[31,32] Patients who received BMC also showed greater cartilage filling of the artificial scaffold.[32] In another study, treatment with peripheral blood, rather than BMC, led to better patient outcomes for those with large chondral lesions.[33] Additionally, treatment with BMC alone, without a cartilage scaffold, was not effective. Multiple studies have demonstrated improvements in pain and function after treatment with BMC injections alone.[14,34,35,36] Injections of MSCs from adipose tissue and umbilical cord blood have also shown functional improvement and pain reduction in patients with knee OA.[37,38] To the best of our knowledge, no study has assessed the clinical outcomes of non-concentrated BMA injections in this patient population.

Limitations

This study has several limitations. There was no control group, and only patients with mild knee OA were eligible for the study. Patients with moderate-to-severe knee OA were excluded, so therapeutic efficacy of BMA injections in advanced stages of OA remains unknown. Additionally, due to the high amount of peripheral blood present in some samples, the number of red blood cells was not counted in the marrow smear analysis. However, blast cells, promyelocytes, myelocytes, metamyelocytes, band cells, polymorphonuclear leukocytes, erythroids, megakaryocytes, lymphocytes, and monocytes were all counted as part of the marrow smear analysis. Marrow smears from one sample contained zero cells; this was likely due to a technical error in sampling, as MSCs were detected in the sample via flow cytometry. IL-1Ra levels could not be assayed in four samples, due to clotting. In addition, previous studies have shown similar efficacy of PRP in low-grade knee arthritis.[39] Lastly, the sample size was small. Future studies in larger populations to elucidate the cellular components of BMA and the clinical efficacy of BMA injections on a greater scale are warranted.

Conclusions

Overall, this pilot study evaluated the cellular components and clinical outcomes of using direct BMA without processing for the treatment of knee OA. BMA samples varied widely with regard to MSC numbers, TNC concentrations, CFU counts, and IL-1Ra levels. MSC numbers in the BMA samples were also lower than the anticipated yield reported by the device manufacturer, and were lower than the numbers reported in BMC samples in previous studies. Despite the cellular data, clinically significant improvements in pain and function were observed over a 12-month period in this case series of patients with mild knee OA. Perhaps this can be attributed to the increased amount of IL-1Ra, rather than the number of MSCs. Studies examining the cellular composition and clinical effects of a single BMA injection on moderate-to-severe knee OA are warranted, as are prospective double-blinded controlled studies.

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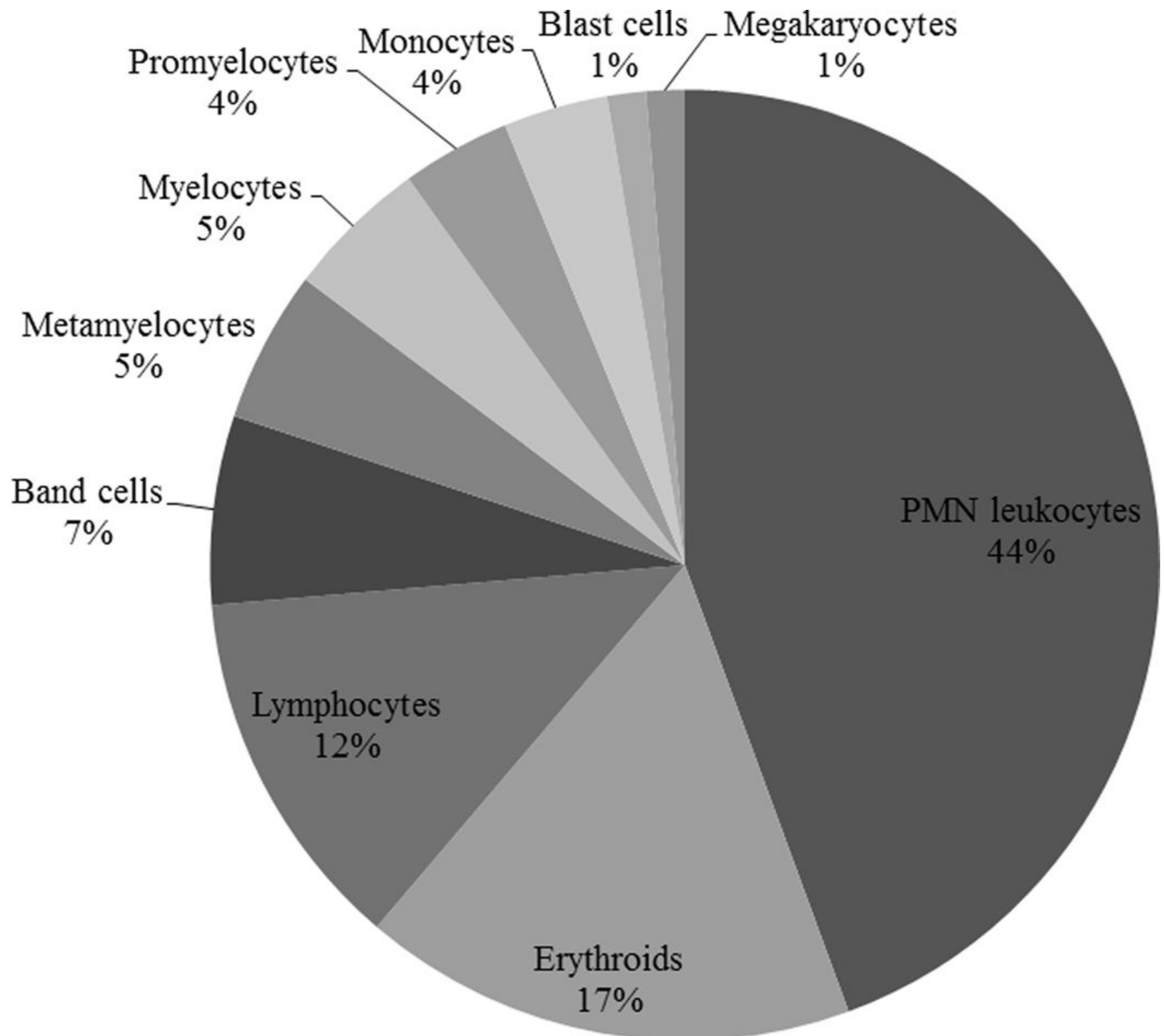

Figure 1. Bone marrow cell differentials.

Percentages of polymorphonuclear (PMN) leukocytes, lymphocytes, erythroids, band cells, metamyelocytes, myelocytes, promyelocytes, monocytes, megakaryocytes, and blast cells are shown. Approximately 500 cells were counted per sample by a pathologist with expertise in marrow smears.

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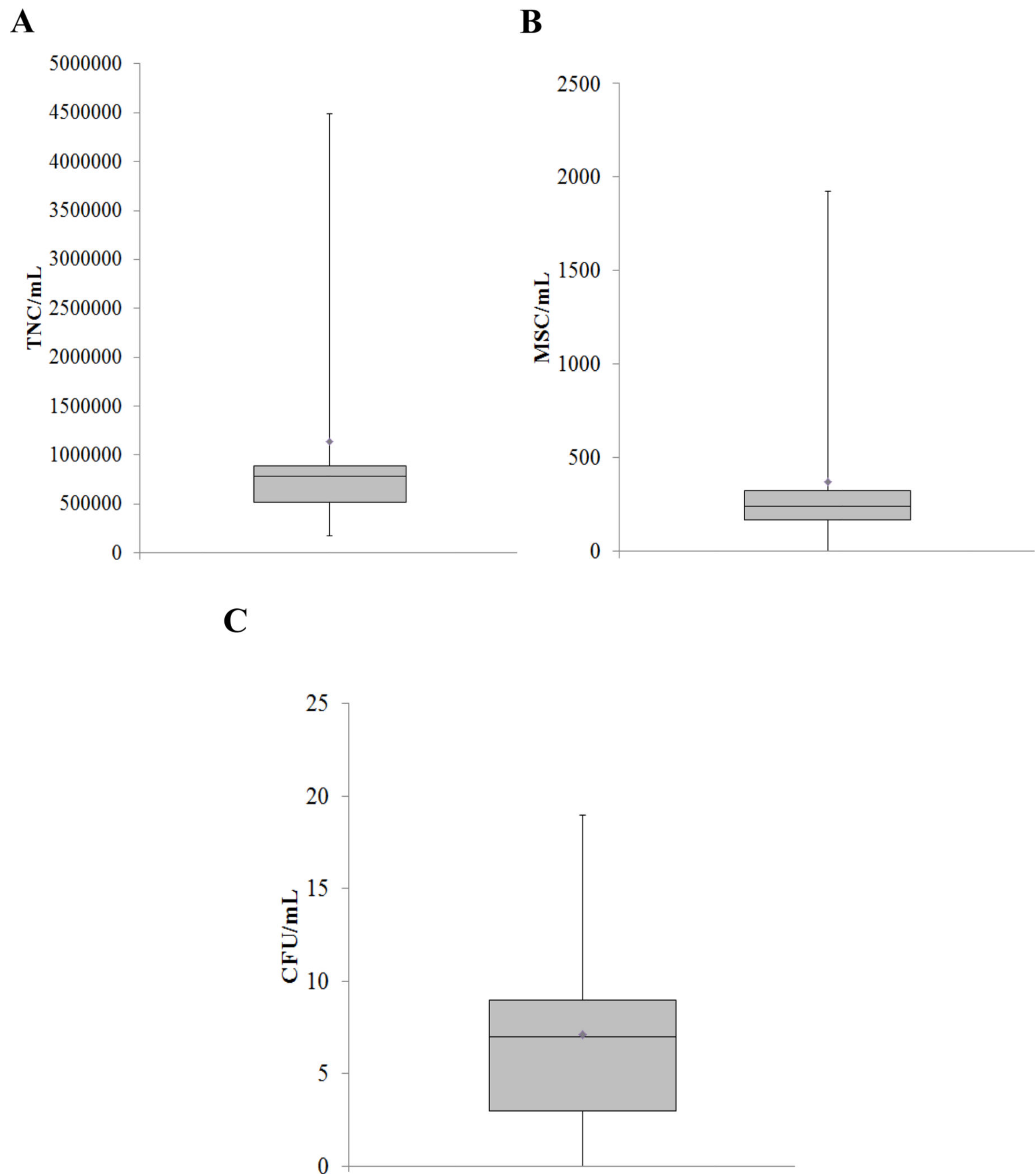

Figure 2. Cellular analysis results.

Boxplots of (A) total nucleated cell (TNC) concentration, (B) mesenchymal stem cell (MSC) concentration, and (C) colony forming units (CFUs) are shown. Upper and lower whiskers represent maximums and minimums, respectively. The upper and lower lines of the box represent third and first quartiles, respectively. The line within the box represents the median, and the star represents the mean.

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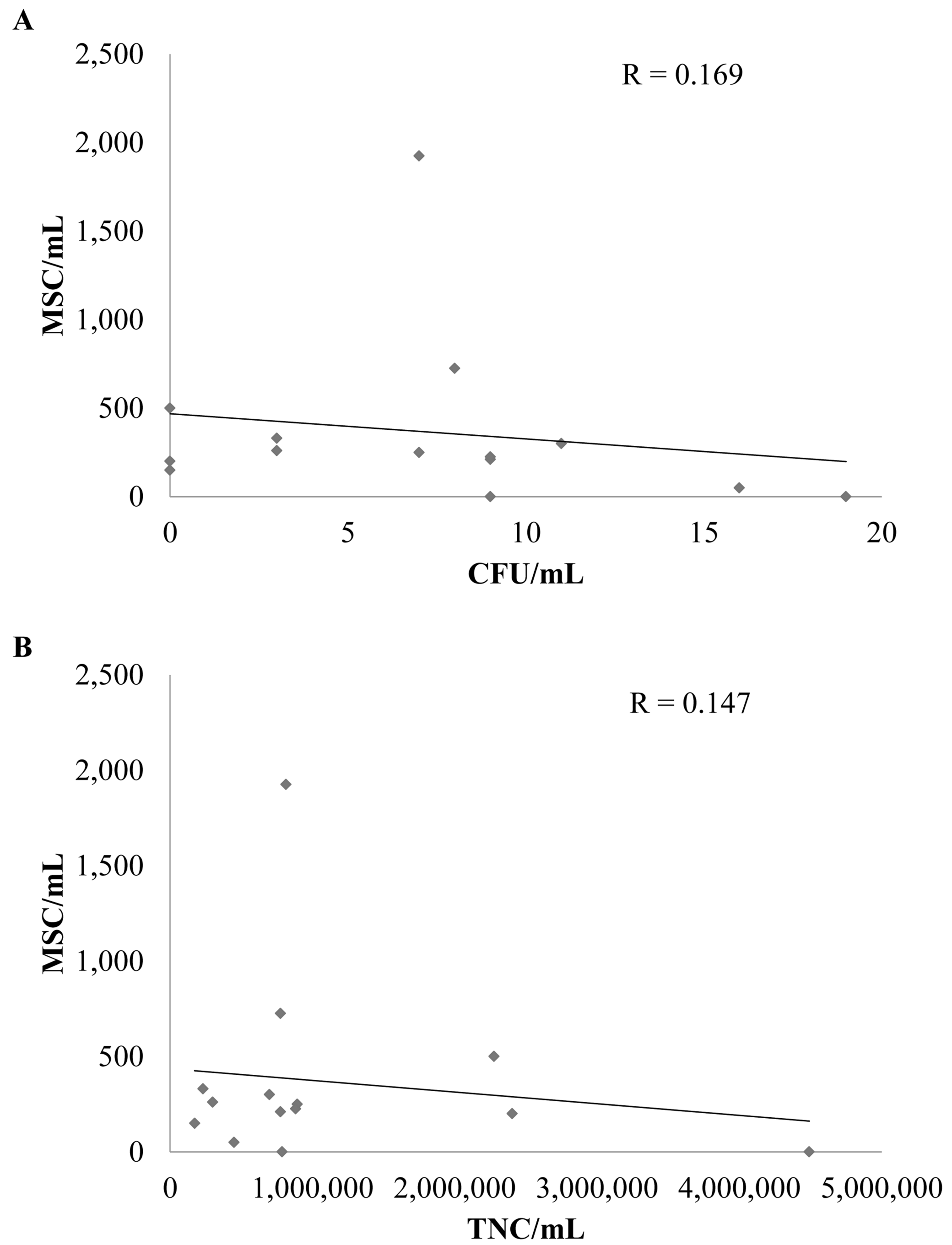

Figure 3. Correlation plots.

Correlation plots between MSC/mL and (A) CFU/mL (R=0.169) or (B) TNC/mL (R=0.147) are shown.

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Table 1:

Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
• Radiographically confirmed KL I-II knee OA within 1 year of initial screening	• History of meniscal injury other than degenerative meniscal tears
• Age 18–79 years	• Previous knee surgery
• 3 months of symptomatic knee OA unresponsive to conservative therapies, including NSAIDs, PT, and steroid or hyaluronic acid injections	• Presence of a degenerative meniscal tear causing mechanical symptoms such as locking, buckling, or give-way
	• Presence of loose bodies on baseline MRI
	• Clinically and radiologically confirmed anterior/posterior cruciate ligament deficiencies
	• Intra-articular injection to affected knee within 3 months of intra-articular BMA injection
	• Mechanical axis deviation >7 degrees
	• Intolerance to acetaminophen or hydrocodone
	• Use of NSAIDs <1 week prior to BMA
	• History of drug/alcohol abuse
	• Current cigarette smokers
	• Current use of systemic steroids
	• History of anemia, bleeding disorders, or inflammatory joint disease
	• Active infection
	• Active malignancy per medical or surgical history
	• Inability to refrain from statin regimen from 1 month pre-injection to 1 month post-injection
	• Pregnancy or breastfeeding at time of treatment
	• Participating or planning to participate in a worker's compensation program
	• Pending or planned legal action pertaining to knee pain
	• Non-English speaking

BMA: bone marrow aspirate; KL: Kellgren-Lawrence; MRI: magnetic resonance imaging; NSAID: non-steroidal inflammatory drug; OA: osteoarthritis; PT: physical therapy

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Table 2:

Baseline Information

	Cellular and Clinical Dataset (N=13)†		Cellular Dataset Only (N=15)	
	Mean or n	**SD or %**	**Mean or n**	**SD or %**
Age (years)	55.3	7.0	58.0	9.5
Sex, n (%)				
Male	5	38	7	47
Female	8	62	8	53
Laterality, n (%)				
Right	8	62	9	60
Left	5	38	6	40
Duration of symptoms (months)	23.8	18.0	22.3	17.1

KOOS-JR: Knee Injury and Osteoarthritis Outcome Score for Joint Replacement; NRS: numerical rating scale; PROMs: patient-reported outcome measures; SD: standard deviation

†Patients who were included in both cellular and clinical outcomes analyses.

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Table 3.

Interleukin-1 receptor antagonist protein concentration in bone marrow aspirate

Sample ID	IL-1Ra concentration (pg/mL)
1	2,591
2[†]	N/A[‡]
3[†]	N/A[‡]
4	29,394
5	26,965
6	13,265
7	8,967
8	15,384
9	N/A[‡]
10	16,223
11	2,806
12	4,405
13	7,801
16	N/A[‡]
17	10,840

IL-1Ra: interleukin-1 receptor antagonist

[†]The patient corresponding to samples 2 and 3 was included in cellular analyses only.

[‡]Samples clotted upon thawing and could not be assayed.

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Table 4.

Knee Injury and Osteoarthritis Outcome Score for Joint Replacement scores.

ID	Baseline	1 month†	3 months†	6 months†	12 months†
1	76.3	**100.0**	**92.0**	**92.0**	**92.0**
4	68.3	66.0	79.9	**84.6**	79.9
5	66.0	44.9	70.7	**100.0**	76.3
6	57.1	**100.0**	**92.0**	**100.0**	**100.0**
7	66.0	73.3	**100.0**	**100.0**	**100.0**
8	63.8	**79.9**	73.3	70.7	**84.6**
9	73.3	76.3	84.6	84.6	**100.0**
10	70.7	79.9	**92.0**	**100.0**	66.0
11	54.8	66.0	68.3	**70.7**	**73.3**
12	57.1	68.3	70.7	**84.6**	**73.3**
13	66.0	70.7	**92.0**	**92.0**	79.9
16	36.9	44.9	47.5	**57.1**	42.3
17	63.8	70.7	**79.9**	73.3	76.3
Mean	63.1	72.4	80.2	85.4	80.3
SD	10.1	16.5	14.2	13.9	16.1
N			13		
P			<0.001		

† Scores meeting the minimum clinically important difference of 15.1 points from baseline are bolded.

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Table 5:

Numerical Rating Scale Pain Scores

	Current pain‡					Usual pain‡					Best pain‡					Worst pain‡				
ID	**B**	**1**	**3**	**6**	**12**	**B**	**1**	**3**	**6**	**12**	**B**	**1**	**3**	**6**	**12**	**B**	**1**	**3**	**6**	**12**
1	6	**0**	**0**	**0**	**0**	4	**0**	**0**	**0**	**0**	1	0	0	0	0	6	**1**	**0**	**0**	**0**
4	4	3	**1**	3	3	5	**3**	**1**	**2**	**3**	1	1	0	0	1	7	**4**	**2**	**3**	**3**
5	3	8	4	**0**	3	4	6	3	**1**	**2**	2	5	1	**0**	1	7	8	**5**	**2**	**3**
6	4	**0**	**0**	**0**	**0**	4	**0**	**0**	**0**	**0**	2	**0**	**0**	**0**	**0**	6	**1**	**1**	**1**	**0**
7	2	**0**	**0**	**0**	**0**	2	1	**0**	**0**	**0**	0	0	0	0	0	5	**3**	**1**	**0**	**0**
8	2	1	**0**	**0**	**0**	2	**0**	1	1	1	0	0	0	0	0	3	2	3	3	2
9	1	0	0	0	1	1	1	1	0	0	1	0	0	0	0	4	3	**1**	**1**	**1**
10	2	2	**0**	**0**	**0**	4	**2**	**0**	**0**	**0**	0	0	0	0	0	6	**3**	**1**	**0**	**0**
11	2	2	**0**	1	**0**	2	2	2	2	1	1	0	0	0	0	8	**5**	**4**	**3**	**3**
12	4	**1**	**1**	**0**	3	4	3	**1**	**1**	3	2	**0**	**0**	**0**	1	7	6	6	**2**	**5**
13	3	2	**0**	**0**	**0**	7	**1**	**1**	**1**	**1**	0	0	0	0	0	5	**2**	**2**	**2**	**1**
16	7	**4**	**4**	**2**	**4**	7	**4**	**3**	**3**	7	6	**2**	**2**	**1**	**3**	9	8	**7**	**5**	8
17	2	1	1	1	**0**	4	**1**	**1**	**1**	**1**	1	0	0	0	0	5	**3**	**2**	**3**	**2**
M	3.2	1.8	0.8	0.5	1.1	3.8	1.8	1.1	0.9	1.5	1.3	0.6	0.2	0.1	0.5	6.0	3.8	2.7	1.9	2.2
SD	1.7	2.2	1.5	1.0	1.6	1.8	1.8	1.0	1.0	2.0	1.6	1.4	0.6	0.3	0.9	1.6	2.4	2.2	1.5	2.3
N					13					13					13					13
P†					<0.001					<0.001					0.003					<0.001

Abbreviations: B: baseline; M: mean; 1: 1 month; 3: 3 months; 6: 6 months; 12: 12 months.

†Data were analyzed using one-way repeated measures analysis of variance.

‡Scores meeting the minimum clinically important difference of 2 points from baseline are bolded.

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Table 6:

Patient Satisfaction

	Satisfaction (N=13)
1 month	8.1 ± 2.1
3 months	8.3 ± 2.8
6 months	8.2 ± 2.9
12 months	8.8 ± 1.9

Data are reported as means and standard deviations.