Randomized Controlled Trial

The Safety and Effectiveness of Orthobiologic Injections for Discogenic Chronic Low Back Pain: A Multicenter Prospective, Crossover, Randomized Controlled Trial with 12 Months Follow-up

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Background: Chronic low back pain is one of the most common causes of disability, affecting more than 600 million people worldwide with major social and economic costs. Current treatment options include conservative, surgical, and minimally invasive interventional treatment approaches. Novel therapeutic treatment options continue to develop, targeting the biological cascades involved in the degenerative processes to prevent invasive spinal surgical procedures. Both intradiscal platelet-rich plasma (PRP) and bone marrow concentrate (BMC) applications have been introduced as promising regenerative treatment procedures.

Objectives: The primary objective of this study is to assess the safety and effectiveness of an orthobiologic intradiscal injection, PRP or BMC, when compared to control patients. The secondary objectives are to measure: patient satisfaction and incidence of hospitalization, emergency room visit and spine surgery at predetermined follow-up intervals.

Study Design: A multicenter, prospective, crossover, randomized, controlled trial.

Setting: Comprehensive Spine and Sports Center and participating centers.

Methods: Forty patients were randomized into saline trigger point injection, intradiscal PRP, or BMC. Follow-up was 1, 3, 6, and 12 months posttreatment. Placebo patients were randomized to PRP and BMC injection if < 50% decrease in numeric rating scale (NRS) scores in 3 months, while PRP and BMC patients to the other active group if < 50% decrease in NRS scores in 6 months.

Results: Both PRP and BMC demonstrated statistically significant improvement in pain and function. All the placebo patients reported < 50% pain relief and crossed to the active arm. None of the patients had any adverse effects, hospitalization, or surgery up to 12 months posttreatment.

Limitations: The limitations of our study were the small number of patients and open-label nature of the study.

Conclusion: This is the only human lumbar disc study that evaluates both PRP and BMC in the same study and compares it to placebo. PRP and BMC were found to be superior to placebo in improving pain and function; however, larger randomized clinical trials are needed to answer further questions on the comparative effectiveness of various biologics as well as to identify outcome differences specific to disc pathology.

Key words: Low back pain, disc, mesenchymal stem cells, platelet-rich plasma, biologics, back pain, degenerative disc disease, intradiscal biologics, lumbar spine, low back, regenerative treatment spine, radiculopathy

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Chronic low back pain is one of the most common causes of disability, affecting more than 600 million people worldwide with major social and economic costs (1). In the United States, approximately 27 million adults, or 11.9 percent of adults age 18 and older, reported having back problems, of which approximately 19 million adults reported receiving treatments (2). Discogenic lower back pain is the most common cause of chronic lower back pain (LBP), accounting for 39% of all cases. Current treatment options include conservative, surgical, and minimally invasive interventional treatment approaches.

The normal intervertebral disc (IVD) is an avascular structure composed of the nucleus pulposus (NP), the disc center, surrounded by the concentric lamellar fibers of the annulus fibrosus (AF). The IVD is positioned between cartilaginous endplates (CEPs) which are responsible for the metabolism occurring in the IVD (3). The center of the IVD is characterized as a low oxygen zone, creating a hypoxic environment. In addition, IVD is characterized as acidic, anaerobic, and acellular, creating a toxic milieu not conducive for cellular repair and regeneration. The etiologic and pathophysiological mechanisms underlying IVD degeneration are still being investigated.

Structural changes of proteoglycans (PG) and collagen type II degradation are considered as a final common path for IVD degeneration (4-6). Thus, IVD degeneration could be triggered by an imbalance between the anabolic and catabolic functions of the NP cells, causing a decrease in extracellular matrix (ECM) function. Moreover, age, disease, and/or injury can hasten the degenerative process and subsequently lead to microtrauma, allowing for the migration of NP contents into the outer AF. The degenerated biomechanical IVD is the leading cause of LBP. Furthermore, IVD degeneration, referred to as intervertebral disc degeneration (IDD), is responsible for calcification and thinning of the CEPs, reducing the bi-directional exchange of nutrients and metabolites to and from the NP, consequently decreasing the local pH because of lactate accumulation (7). Lower IVD pH levels (pH lower than 6.5) have been shown to reduce cell viability and increase matrix catabolism, expressed by increased proteolytic enzyme activity. Matrix metalloproteinase-3 (MMP3), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), and decreased tissue inhibitor of metalloproteinases-1 (TIMP1), and aggrecan play crucial roles in ECM degradation of the NPOM (8,9). Consequently, a lower pH reduces the rate of proteoglycan synthesis (10). It has been suggested by Gilbert et al (7) that acidity-induced aberrant mechano-transduction is a potential mechanism involved in the progression of IDD. The lower pH contributes to a catabolic shift of NP cells, as the NP encompasses high concentrations of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interferon (IFN), and interleukins-1α (IL-1α) and IL-1β (11,12). These inflammatory cytokines inhibit the synthesis of the matrix and promote the production of MMPs by macrophages entering the disc in response to injury (13,14). Noteworthy, these cytokines incite a chemical sensitization of the abundantly present nerve fibers in the outer AF, potentially leading to internal disc disruption and chronic back pain (15).

Novel therapeutic treatment options continue to develop, targeting the biological cascades involved in the degenerative processes to prevent invasive spinal surgical procedures. Promising regenerative medicine approaches have been reported, both in-vitro and in vivo (16,17). The effectiveness and use of minimally invasive interventional autologous prepared orthobiologic injections have received substantial attention over the last decade, compared to surgical interventions. The application of a patient's own freshly harvested cellular tissues, like whole blood and bone marrow, to prepare point-of-care platelet-rich plasma (PRP) and bone marrow concentrate (BMC) has become a very attractive treatment option to avoid immunological complications, supply, and regulatory issues.

The rationale to use biological ingredients in the unique milieu of IDD results from the fact that numerous bio-cellular activities play key roles in the various repair processes within the disc structures. Orthobiologic therapeutic strategies are based on deploying biological events for IVD degeneration, including the delivery of molecules able to influence disk cell metabolism to biologically improve the accumulation of the ECM, by promoting IVD matrix synthesis and inhibiting ECM abnormal catabolism.

Both intradiscal PRP and BMC applications have been introduced as promising regenerative treatment procedures. Intradiscal PRP injections have been used successfully in the avascular IVD structure to release a magnitude of platelet-derived growth factors in patients with DDD and LBP (18-21). The underlying scientific rationale for PRP therapy is that an injection of concentrated platelets may initiate tissue repair or regenerate via the release of biologically active factors (platelet growth factors, cytokines, lysosomes) and adhesion proteins, contributing to matrix synthesis,

revascularization, and new connective tissue development (22). Under normal circumstances, a variety of platelet growth factors, like basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-β), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1), are involved in the renewal of matrix constituents and the synthesis process of IVD components by stimulating chondrocytes, fibroblasts, to produce IVD matrix, and contribute to the inhibition of MMPs production (23). Furthermore, PRP was demonstrated to have immunomodulatory capacities expressed via the innate and adaptive immune system (24-26) and revealed analgesic effects in patients with LBP in a dose-dependent manner (27).

BMC, more specifically, bone marrow-derived mesenchymal stem cells (MSCs), can differentiate to NP-like cells (28), secrete a range of cytokines, exert homing abilities, bring into play immunomodulatory and profound anti-inflammatory effects (29). Additionally, MSCs exhibit paracrine effects in co-cultures with AF and NP cells (30) and down-regulate the production of proinflammatory cytokines IL-1α, IL-1β, interleukin-6 (IL-6), and TNF-β in degenerated NP and AF cells (IL-1α and IL-6) and stimulated extracellular matrix deposition (31,32).

In general, PRP and BMC orthobiologic treatment options are intended to induce a biological repair of DDD (33,34). For this reason, the primary objective of this prospective randomized controlled study is to assess the safety and effectiveness of an orthobiologicintradiscal injection, PRP or BMC, when compared to control patients. The secondary objectives are to measure 1) patient satisfaction; 2) changes in disc morphology as measured by MRI scans 6-month post-procedure; 3) change in medication use, interim hospitalization, and incidence of spine surgery at predetermined follow-up intervals.

Methods

Study Design

Between March 2018 and December 2020, a prospective, multicenter, randomized, placebo-controlled crossover trial was conducted to demonstrate the safety and effectiveness of the autologous orthobiological products, PRP and BMC for degenerative disc disease.

The study was conducted in compliance with the US Code of Federal Regulations and the Declaration of Helsinki. Prior to the start of the study, the Institutional Review Board of the International Cell Surgical Society (ICSS) approved the protocol and informed consent forms. Study coordination was conducted at each center, with monitoring completed by the head research staff at the primary site, Comprehensive Spine and Sports Center.

The study is registered on Clinicaltrials.gov with NCT04102761.

The study was funded by each of the sites and supported by EmCyte Corporation, Fort Myers, FL via supply of equipment and BMA harvesting needles and procedural costs.

Subject Selection

Strict criteria were set for inclusion or exclusion to the study (Table 1a, b). Four centers participated with the goal of enrolling 60 and a minimum of 40 patients with low back or leg pain and disc pathology. All pa-

Table 1. *A. Inclusion criteria. B. Exclusion criteria.*

A. Inclusion Criteria
• A high index of suspicion for discogenic pain, i.e., painful degenerative discs with or without contained protrusions.
• Age greater than 18 and less than 70 years.
• Maintained intervertebral disc heights of at least 50%.
• Pain not generated from facet joints, sacroiliac joints, or any pathology other than discogenic origin.
• Pain is not responsive to conservative treatment measures (oral medications, epidural steroid injections, physical therapy).
• Pain persists for an extended period (i.e., at least 3 months).
• High-intensity zone (HIZ) in annular fissure detected on T2 or STIR MRI, degenerated discs, or contained disc protrusions.
• No evidence of contraindications to undergo procedure such as pregnancy, active infection, bleeding disorder, or metastatic cancer.
• English speaking.

B. Exclusion Criteria
• Disc extrusions, disc sequestrations, severe spinal stenosis, or severe disc degeneration with grade 5 Pfirmann index or with Modic 3 level change.
• Patient refusal.
• Presence of a known bleeding disorder.
• Pregnancy.
• Systemic or local infection.
• Presence of an unstable medical or psychiatric condition.
• Prior intradiscal procedure (i.e., IDET, Nucleoplasty).
• Inaccessibility to discs, such as fusion.
• Non-English speaking.
• Prior fusion surgery.

tients who were offered participation in the study did not have any interventional procedure in the discs prior to the start of the study.

The participant's eligibility to participate in the study was determined by the Clinical Investigator in each center based on the Inclusion and Exclusion Criteria (Table 1a, b). General information related to the patient, including age, gender, medical history, duration of symptoms, was obtained from patient charts. Informed consent was obtained upon enrollment.

Study Protocol

Once the participants were enrolled, informed consent was obtained and randomization using a computerized custom random number generator was completed to assign patients to one of the 3 study arms namely, placebo, PRP, or BMC (Fig. 1). They were allowed to cross over if the numeric rating scale (NRS) score was less than 50% after 3 months from placebo and 6 months from PRP or BMC. Patients in the placebo group received deep trigger point injection with

Fig. 1. *Study flow chart.*

normal saline into the muscle. The PRP group patients received an injection of approximately 1-2 mL of PRP into the painful disc/s until resistance to further injection was felt by the operator. In the BMC group, approximately 1-2 mL of BMC was injected intradiscally again until resistance to further injection was felt by the operator. Patients in the PRP and BMC groups were allowed to cross to the other active group if there was a < 50% decrease in NRS at 6 months from the injection.

At the end of each treatment follow-up period, data related to pain outcome NRS, patient satisfaction (North American Spine Surgery, NASS scores), and physical function and disability specific for spinal conditions (Oswestry Disability Index, ODI) were collected. Follow-up was completed at 1, 3, 6, and up to 12 months from last treatment. Independent statistician was used to analyze and interpret the data using SAS/STAT software version 9.4 (SAS Institute Inc., Cary, NC).

Outcome Measures and Follow-Up

Our main research questions and goals were 1) to evaluate improvement in [a] pain and [b] function with the use of intradiscal PRP or BMC compared to control; 2) to compare the safety and efficacy of BMC and PRP; 3) to study patient satisfaction related to the use of PRP and BMC compared to placebo; and 4) to evaluate effectiveness of the biological therapy via post therapy hospitalization and surgery.

Outcome data were collected at baseline prior to the intervention and at the end of each period. NRS Pain scores, ODI, and modified NASS data were collected from the patient at pre-injection and post- injection at 1, 3, 6, and 12 months. Questionnaires were completed at the time of a normally scheduled office visit on paper by PIs and stored in a HIPAA-compliant secure online questionnaire portal.

Biological Preparations

PRP Preparation

The EmCyte GenesisCS PurePRP® System technology (EmCyte Corporation, Fort Myers, FL) was used at point of care in all patients. Fresh blood was obtained from each donor by inserting a 19-G intravenous line into the antecubital vein, following institutional phlebotomy guidelines, for the sterile preparation of PRP by using the GS60-PurePRP II® autologous PRP system. A 60 mL syringe was pre-loaded with 8 mL of sodium citrate as anticoagulant prior to the collection of 52 mL of whole blood. The whole blood specimen was processed by a 2-spin procedure with an Executive Series Centrifuge II (EmCyte Corporation, Ft. Myers, FL), according to the manufacturer's instructions for use. Briefly, the 60 mL anticoagulated whole blood was loaded into the concentrating device and centrifuged for 1.5 min at 3,800 RPM (2,300 RCF). Platelet plasma suspension (PPS) was then aspirated until RBC filled the aspirating pipe, and the PPS was then transferred into the concentrating accessory and centrifuged for 5 min at 3,800 RPM (2,300 RCF). Platelet-poor plasma (PPP) was then aspirated off, leaving 7 mL of PPP behind to resuspend the platelet buffy coat, which was then extracted from the concentrating accessory, yielding the final neutrophil-poor PRP fraction.

BMC Preparation

In all patients, a unilateral bone marrow extraction technique was executed. A subcutaneous tissue tract to the periosteum was injected with 1% lidocaine. Once local anesthesia was obtained, a single cutaneous entry site was used to access the posterior superior iliac spine (PSIS). A Jamshidi™ BMA needle, included in the EmCyte PureBMC® kit, was used. All syringes, BMA needle, and other accessories were pre-rinsed with a heparin solution (1,000 IU/mL) to avoid clotting. Using fluoroscopic and/or ultrasound imaging, the BMA trocar was introduced just beneath the PSIS bone cortex, seated in the subcortical bone marrow, and the trocar was removed. Subsequently, 60 mL of BMA was aspirated and centrifuged at bedside with an EmCyte GenesisCS PureBMC®-60 mL kit (EmCyte Corporation, Fort Myers, FL). Following a first 2.5-minute centrifugation procedure at 3,800 RPM (2,300 RCF), the BMA was sequestered in a BM plasma fraction (BMPF) containing the buffy coat layer and RBCs. The BMPF was aspirated, immediately followed by a separate collection of 2 mL of RBCs, following the manufacturer's instructions of the PureBMC® concentration device. Both volumes were then transferred for a second centrifugal spin cycle to the concentration accessory device. During the second spin (7 minutes at 3,800 RPM), the concentrated BM cells were attached to the bottom of the device. The top layer consisting of BMPF was manually aspirated and removed until 7 mL was left. This volume was used to resuspend the buffy coat BMC cells. The final PureBMC® volume was approximately 7 mL. If patients were randomized for laboratory analysis, 1 mL of BMA and PureBMC® was reserved for laboratory analysis.

Laboratory Analysis

BMA and BMC preparations were meticulously agitated; aliquots were then taken and shipped for analysis to an independent, Good Laboratory Practice accredited laboratory (Bio Sciences Associates, Cambridge, MA). Complete blood counts (CBCs) were performed using a 3-part differential hematology analyzer to quantify the platelets, RBCs, and calculated HCT. CBCs were measured according to the BSR TM-076 Coulter Ac-T diff 2 Hematology Analyzer. Total nucleated cell counts were performed using a Beckman Coulter AcT diff2 hematology analyzer (Beckman Coulter, Brea, CA) for baseline BMA samples and BMC concentrates. Cell counts were performed in open sample mode according to the manufacturer's and laboratory's standard procedures. Prior to sample cell counts, the analyzer passed all system setups, calibration, and daily quality control testing.

Samples for flow cytometry were prepared and analyzed as recommended by the International Society for Hematotherapy and Graft Engineering (11). Total Nucleated Cells (TNCs) (1 × 106 cells/sample) were incubated with PE anti-human CD34 and anti-human CD45 Alexa Fluor 647 for 15 min at room temperature. To validate the specificity of the CD34 antibody, a control sample was also prepared with an isotype control. Lysis buffer was added to each sample and incubated for 10 min at room temperature. Cells were washed with PBS, 0.2% BSA before adding cell viability solution and counting beads. Stained samples were protected from light and analyzed using an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA) immediately following processing. The CD34 positive population, implemented as a hematopoietic stem cell (HSC) marker, determined using a single platform methodology, was defined as the CD45 'dim' and CD34 'bright' population. Cell viability was assessed by dye exclusion of 7-AAD solution. The 7-AAD negative population was reported as a percentage of viable cells. Spectral compensation between fluorescent channels was set using beads labeled with the respective fluorophores for corresponding channels. (Anti-human CD34, PE IgG1 k Isotype Ctrl, lysing buffer, cell viability solution – BD Biosciences, San Jose, CA; Anti-human CD45 Alexa Fluor 647 – BioLegend, San Diego, CA; counting beads – Spherotech, Lake Forest, IL). Colony-forming units-fibroblasts (CFU-f) were used to study MSC quality. Samples were adjusted to a density of 2 × 106 nucleated cells per mL and cultured with supplemented mesenchymal stem cell growth media (Stem Cell Technologies, Cambridge, MA) at 37°C in 5% CO_2. Following 10-14 days of incubation, nonadherent cells were removed by washing with PBS. Adherent cells were stained with Giemsa stain at room temperature (Ricca Chemical Company, Arlington, TX). Excess stain was washed away with distilled water. Colonies containing > 50 cells with fibroblast morphology were counted using a Nikon Diaphot 300 microscope and reported as CFU-f per mL of sample. Isolation and expansion of MSCs were quantitatively and qualitatively assessed between testing and control culture conditions using 2-tailed t tests.

Statistical Analysis

Statistical analyses utilized SAS/STAT software version 9.4 (SAS Institute Inc., Cary, NC). Descriptive statistics are reported as mean and standard deviation (±). Statistically significant differences between groups were determined using independent sample or paired t tests as appropriate, with a 95% confidence level on each principal effect meant to account for multiple comparisons. All statistical tests were 2-tailed, a *P*-value < 0.05 was determined to be statistically significant. Pearson product-moment correlation was calculated to ascertain the parametric measure of a linear relationship between pairs of variables.

Results

Fifty-seven patients were initially selected from the 4 study sites to participate in the study based on the inclusion and exclusion criteria. A total of 43 patients completed the trial, and data were collected up to 12 months posttreatment. Patients were randomized into a placebo group (n = 12, group 1), a PRP group (n = 15, group 2), and a BMC group (n = 16, group III). The placebo group received saline solution injection of approximately 1-2 mL into the paraspinal trigger point muscles, while the study groups received 1-2 mL of intradiscal PRP (Grp 1) or BMC (Grp 2). Patients in the placebo group were randomly crossed over to either the PRP or BMC group after 3 months, when a < 50% decrease in NRS was observed. Patients in the active group were allowed to cross over to the other active group after 6 months, when a < 50% decrease in NRS was observed. Forty patients completed evaluations at 12 months posttreatment, and complete data points were obtained.

Table 2 shows the patient characteristics for 40 patients that were in the study at 12 months posttreatment. No statistical differences were found in age and gender between groups. Briefly, at 12 months posttreatment there were 18 men (45%) and 22 women

(55%), with an average age of 45.3 years (SD ± 9.4). The distribution of these variables in the 3 groups was similar, with no statistical differences. Age and gender are not significantly related to treatment outcomes for the studied parameters. Twelve patients from the initial placebo group crossed over to either PRP or BMC groups, 7 and 5 patients, respectively. Two patients from the PRP and BMC crossed over to the other therapy arm after 6 months because pain and function did not improve after the initial treatment.

An extensive laboratory analysis was performed on 6 randomly assigned patients from the BMC group, measuring a variety of typical laboratory parameters for BMA and BMC tissue samples. HCT, RBC, and platelet concentrations: the HCT percentage was reduced by more than 3-fold in the BMC vials to 11%, compared to an average HCT of 36% in the extracted BMA ($P < 0.0001$). Similarly, the average RBC count was 1.12 × 109/mL, compared to 3.8 × 109/mL in the BMA. The platelet count in the BMC injectate was 6.5 times higher than in the BMA specimen. CD34+ cell concentrations: Generally, the average HSC content (measured as CD34+ cell concentrations) was significantly lower in the total collected BMA, compared to the BMC product (70,376/mL and 400,628/mL, respectively). TNC concentration: Concentrations of TNCs were higher in centrifugated BMC samples (77.1 x 106/mL) compared to the aspirated BMA (18.6 × 106/mL), representing a 4.3 increase from baseline. CFU/f cultures: The CFU/f counts, after culturing, were 9.6 times greater in the BMC than the BMA vial (771 vs. 80/mL, respectively). Patient variability was noted by the large standard deviation in BMC vials (409/mL). Cell viability: the cell viability after BMC processing was 92%, and 98% in BMA volume. Hemolysis and plasma free hemoglobin: centrifuging BMA resulted in a 59% reduction of the hemolysis percentage, with an associated decrease in plasma free hemoglobin concentration in the BMC (155 mg/dL) when compared to the collected BMA (1,424 mg/dL) prior processing.

Adverse effects were monitored closely throughout the entire duration of the study. There were no adverse events of local injury from bone marrow extraction, local or systemic infection, neurologic injury, or hospitalizations relating to the injection of placebo, PRP or BMC. The most cited adverse event was temporary low back pain related to the disc injection, which resolved after 3-5 days.

Improvements in NRS scores of both the PRP and BMC groups were statistically different over the 12-month observation period, compared to control patients ($P < 0.001$). In Fig. 2, the pain now for all 3 groups is illustrated. We were not able to show a statistical difference between PRP and BMC-treated patients.

Table 2. *Patient demographics and crossovers.*

	Placebo (n:12)	PRP (n:13)	BMC (n:15)
Gender, N (%) Male / Female	6 (50) / 6 (50)	6 (45) / 7 (55)	6 (35) / 9 (66)
Age, (mean ± SD)	44.8 ± 11	47.1 ± 10.5	44.1 ± 7.3
Crossed Over, (N/%)	12/ 100	1/5.8	1/6.3

PRP, platelet-rich plasma; BMC, bone marrow concentrate

The pre-treatment NASS outcome questionnaire was not significantly different among the treatment groups. Significant differences in NASS score occurred at 6- and 12-months posttreatment for BMC and 12 months for PRP, compared to the previous period. There was no significant difference between PRP and BMC over a 12-month period, $P = 0.094$, and 0.039, respectively, demonstrated in Fig. 3. The ODI scale scores are illustrated for all groups in Fig. 4. The pre-treatment ODI % was not significantly different among treatment groups. At 6 months posttreatment, the lowest ODI score was achieved ($P < 0.001$) for both biological groups. Six months thereafter, the ODI score increased for BMC-treated patients only from 27% to 31%. The PRP and BMC groups were significantly decreased at 12 months follow-up, $P = 0.0144$ and $P = 0.0109$ respectively. There was no significant difference between PRP, and BMC-treated groups ($P = 0.3$). The data for NRS, NASS, and ODI scores for all patients who crossed-over after 3 months from their initial randomization group are illustrated in Fig. 5a-c. The changes in pain scores over 12 months were statistically significant ($P = 0.0043$). The ODI score significantly improved after crossing over ($P = 0.0137$). The NASS score did not improve one year after crossing, $P = 0.607$.

In summary, our study was able to demonstrate that PRP and BMC were equally effective in treating discogenic low back and/or leg pain after 12 months of a single injection. All placebo patients crossed to a biologic interventional procedure after 3 months. All crossed patients showed significant improvements of NRS pain score, ODI functional score, and NASS score up to 12 months. A secondary biological intervention was not indicated in any of the patients. None of the patients underwent a surgical procedure for back pain or hospitalization due to the biological interventional procedure or pain associated with the area of treatment. There were no complications in any patients.

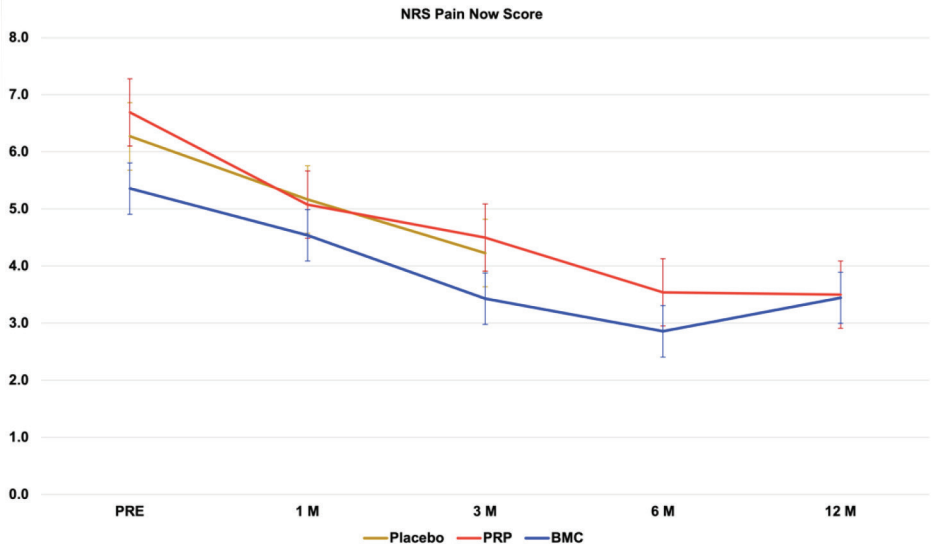

Fig. 2. *Changes in NRS scores for Placebo, PRP, and BMC groups over a 12-month period. Values are mean values and SD. A significant decrease in pain scores was observed for the PRP and BMC groups ($P < 0.001$), compared to the placebo group. The decrease in the placebo group was statistically significant, $P = 0.006$. There was no difference between the 2 biological treatment groups ($P = 0.2$).*

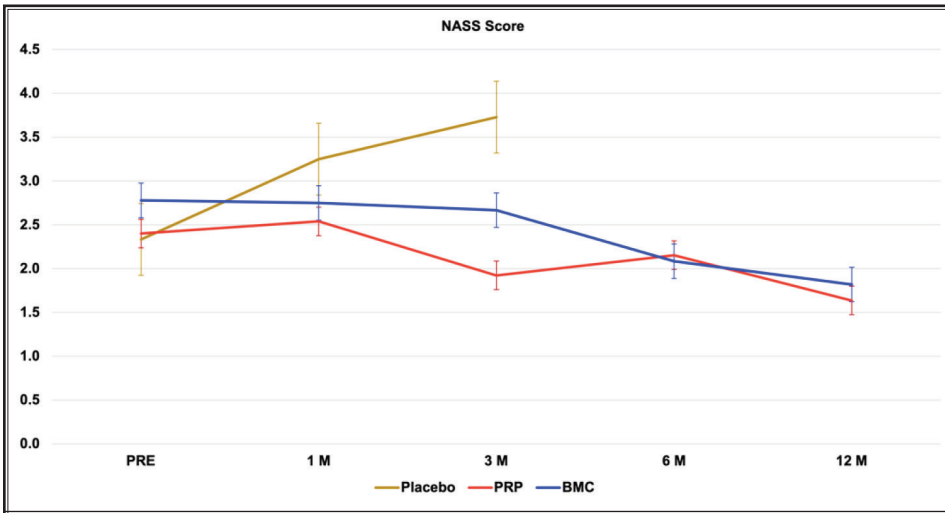

Fig. 3. *The NASS Score for the 3 groups, with no statistical difference in the treatment groups.*

Discussion

The majority of published clinical trials on intradiscal cellular therapies are prospective case series with a paucity of randomized clinical trials (RCT). According to the American Society of Interventional Pain Physicians (ASIPP) 2019 guidelines, the qualitative evidence for intradiscal PRP and MSCs has been assessed as Level III, (on a scale of Level I through V) using a qualitative modified approach to the grading of evidence based on best evidence synthesis (19). There are a few published RCTs on intradiscal biologic injection to date using PRP (35,36), BMC (37), and several others using disc cells and disc tissue (38). This study is the first multicenter, prospective, randomized, controlled clinical trial to evaluate and directly compare the individual use of PRP and BMC against placebo for discogenic pain.

Tuakli-Wosornu et al's (35) double-blind RCT of 46 patients showed that intradiscal PRP resulted in better functional outcome than intradiscal contrast injection. The treatment group showed significant improvements in Functional Rating Index (FRI), NRS for pain, and the modified NASS outcome questionnaire at 8 weeks. A follow-up of the participants who received PRP injections showed statistically and clinically significant improvements in pain and function at 2 years (39) and at 5-9 years post-injection (40). The treatment group showed significant improvements in FRI, NRS for pain, and the modified NASS outcome questionnaire at 8 weeks. No complications were observed. Noriega et al's (41) RCT showed that allogeneic BMC resulted in a significant improvement in VAS and ODI at 3 months that was sustained through 12 months, when compared to a sham paravertebral muscle injection. On the other hand, Pettine et al (42) used autologous BMC

in a prospective non-randomized study of 26 patients. The results were that 77% of the patients who received the injection treatment had significant improvement in their VAS and ODI sustained through 36 months. In addition, MRI at one year showed improvement in the Modified Pfirmann grade by one level in 18/20 patients. There was no worsening of MRI found in the treated patients.

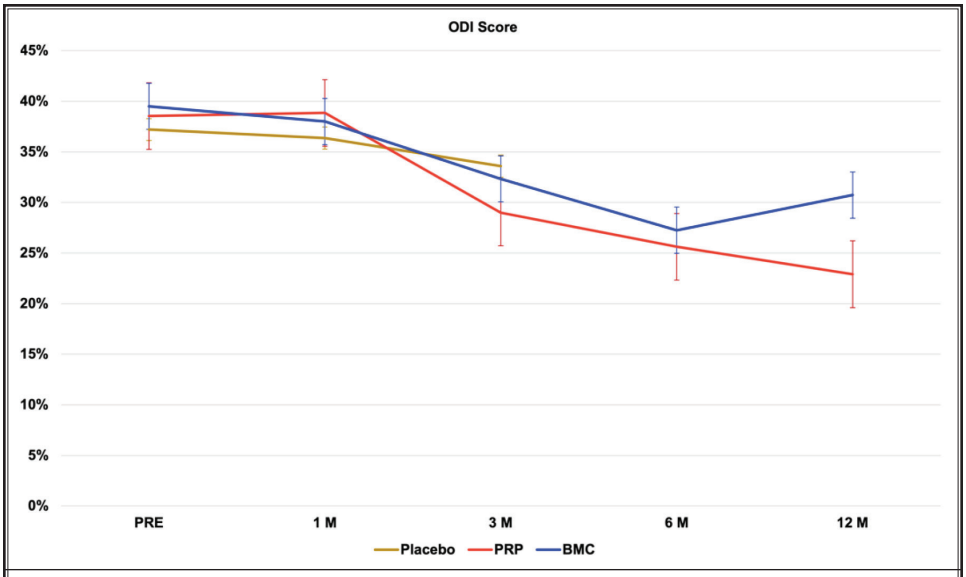

Fig. 4. *ODI scores for the 3 patient groups, represented as mean values and SD.*

In our study, improvements in NRS scores of both the PRP and BMC groups were statistically different over the 12-month observation period, compared to control patients. This confirms the findings of Tuakli-Wosornu, Noriega, and Pettine's study as well as several other prospective case series studies of intradiscal biologics in the literature (43).

Our study was not able to show a statistical difference between PRP and BMC-treated patients in NRS, ODI and NASS scores. However, both PRP and BMC demonstrated significant benefits over placebo, resulting in all placebo patients crossing over to a biological treatment group in 3 months. The NRS, ODI, and NASS scores showed statistically significant improvement after the orthobiologic treatment compared to the placebo injection. The modified NASS outcome questionnaire and the ODI scale scores revealed no differences between PRP and BMC over a 12-month period. However, the crossed-over patients had a significantly better ODI score at 12 month follow-up (17%) when compared with the moment of crossing-over (32%). Similarly, the NASS score decreased from the moment of actual crossing-over to 12 months postintervention from 3.1 to 1.8, and the NRS pain now score decreased from 4.4 to 1.8.

There were no complications or adverse effects. None of the patients underwent hospitalizations, emergency room visits, or surgery related to the study indication up to 12 months posttreatment. The limitations of our study were the small number of patients and open-label nature of the study.

There are several factors that may have contributed to the cell yield variability and low numbers. Bone marrow harvesting technique, aspiration syringe size, and aspiration needles have been investigated for their effects on MSC yield. In Hernigou's study (44), the use of 10 mL syringes for BMA aspiration had a higher yield of TNCs compared to a 50 mL, ranging from 20.2-65.6 million/mL to 8.6-14.4 million/mL, respectively. This is attributed to the smaller syringe having a stronger negative pressure, and it was easier to draw the plunger of the aspiration syringe at a higher speed (44). Oliver et al (46) and Li et al (47) have shown that the single-insertion method produced final cellular concentrations and culture results that were not significantly different from those of a multiple-insertion method. On the other hand, Peters et al (45) commented that multiple insertions (up to 4) resulted in a higher concentration of BMC cellular components. Although it has been opined by some that the type of harvesting needle may affect MSC yield, this published study has shown otherwise (48). Tanikawa et al (49) evaluated the yield of nucleated cells in bone marrow harvested by aspiration needles with or without side holes and also showed no difference between the 2 kinds of needles. Further studies on the various aspects of bone marrow concentrate preparation are needed to evaluate how they affect the final biologic product composition.

There are participant factors that need to be considered as possibly contributing to the results. There was a variable amount of cell yield among the partici-

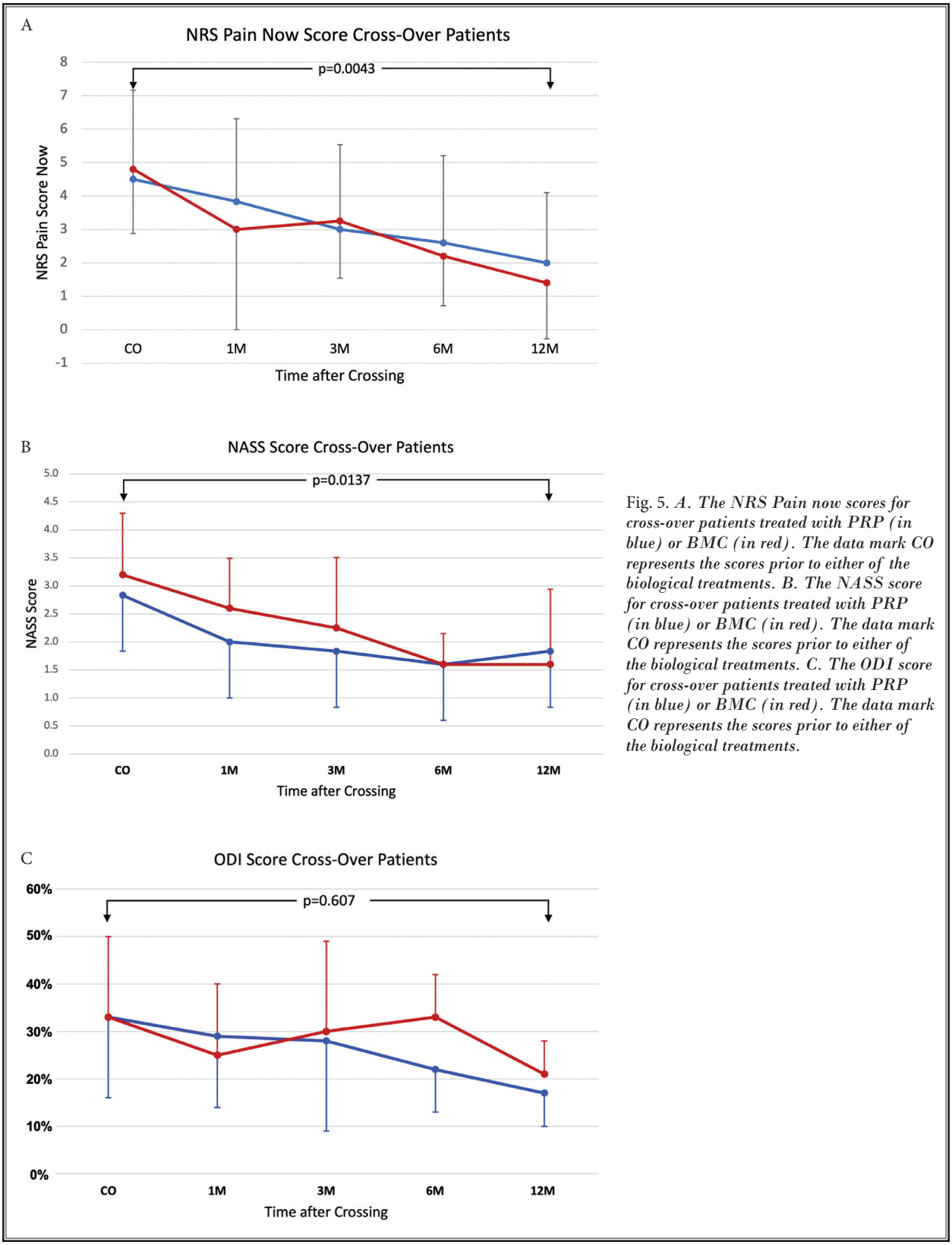

Fig. 5. *A. The NRS Pain now scores for cross-over patients treated with PRP (in blue) or BMC (in red). The data mark CO represents the scores prior to either of the biological treatments. B. The NASS score for cross-over patients treated with PRP (in blue) or BMC (in red). The data mark CO represents the scores prior to either of the biological treatments. C. The ODI score for cross-over patients treated with PRP (in blue) or BMC (in red). The data mark CO represents the scores prior to either of the biological treatments.*

pants coming from the 4 different clinical centers that participated in the study. Gender, age, and nutritional status are factors that have been shown in published studies to affect MSC survival and proliferation (50-53).

The diversity of harvesting skills of various investigators despite uniformity in protocol harvesting technique parameters in combination with patient variance may explain the ranges of cell yield in this multicenter study. In addition, we recognize the limitations related to the open-label nature of the study and the reporting bias introduced because of that.

The question of MSC dosing and whether MSC count matters is still under debate. The concentration of MSCs in BMAC is minimal, with a percentage from 0.001% to 0.01% of mononuclear cells after centrifugation (54). Further investigation is needed to evaluate the effect of MSC count in the clinical outcomes after intradiscal BMAC therapy. In the same manner, the differences in cell composition of PRP and BMAC and how it affects symptoms of disc degeneration need to be further explored. PRP and BMAC are composed of a variety of cells, growth factors and bioactive molecules important to promote tissue healing and repair. The MSCs present in BMAC are multipotent stem cells that have the capacity for self-renewal and differentiation into musculoskeletal lineages, with immunomodulatory, anti-inflammatory, and antiapoptotic properties (55). There may be some variability in growth factor composition between these two biologics, although some studies have found no statistically significant difference (56).

Reiterating the need for further studies into the composition of the different biologics, the association of cytokines with clinical outcomes needs to be further investigated, thereby strengthening the translation between basic and clinical research. BMAC has a significantly higher concentration of Interleukin (IL)-1ra, a potent anti-inflammatory cytokine, compared to PRP (56). This could have accounted for the better numeric pain score of the participants in this group.

Lastly, it is important to note that in the understanding of the degenerative process in the spine and the inter-relationship of the different spine structures, the majority of the patients with chronic low back pain have multiple pain generators. A more comprehensive approach that addresses the functional spinal unit using multi-target biologic injection, that is injecting the facet joints, epidural space, interspinal ligaments, and other relevant structures contributing to spinal stability, may yield better functional outcomes than addressing the disc alone (57).

Conclusions

Orthobiologic lumbar disc treatment has been an area of keen interest, inspiring promising and novel possibilities. However, current scientific support is at best, modest. There are limited randomized controlled trials to study the effects of biologics in human discs and no study that directly compares PRP and BMC to each other and placebo. This study demonstrated statistically significant improvement in pain (NRS), function (ODI), and patient satisfaction in the biologic group compared to placebo. This pilot trial was designed to ask the basic question of the safety and efficacy of PRP and BMC but was not powered enough to compare the efficacy between PRP and BMC. This study paves the path for future, large, well-designed studies to study the comparative safety and efficacy of biologics and their effect on a specific spine, orthopedic and musculoskeletal conditions.

Author Contributions

Conceptualization of the research and Methodology is by A. Navani. Execution and investigation by A. Navani, M. Ambach, A. Calodney, R. Rosenthal, G. Li, and P. Everts. Validation by P. Everts. Formal statistical analysis by C. Brown Mahoney. Data curation by each site, central collection and C. Brown Mahoney. Manuscript writing by A. Navani, M Ambach, and P. Everts. Review and editing by A. Navani, M. Ambach, A. Calodney, R. Rosenthal, G Li, and C. Brown Mahoney.

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