

Treating Cervical Disc Pathology with Bone Marrow Concentrate is Equal or Better than Fusion or Disc Replacement at Two-Year Follow-up

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Abstract *Objective:* Millions of patients suffer chronic neck pain, headaches, inter-scapular pain, and radiating arm pain from degenerated cervical discs. Operative options include cervical disc fusion or cervical artificial disc replacement. Patients with more than two degenerated discs have minimal surgical options. *Study Design:* This is a prospective nonrandomized study of the two- year follow-up results of injecting bone marrow concentrate (BMC) into symptomatic degenerated cervical discs compared to five FDA studies comparing Cervical Artificial Disc Replacement (CADR) to Anterior Cervical Fusion (ACF). The BMC study is class two data. The FDA studies are class one data. *Methods:* There were 182 patients in the BMC study. The 30- minute procedure involved aspirating 55ml of bone marrow from the iliac wing, concentrating this via centrifugation to a volume of 3ml, and then injecting 0.5ml of the bone marrow concentrate into each abnormal cervical disc. The FDA studies involved 788 CADR and 671 ACF one level patients. There were 225 two-level CADR and 105 fusion patients. All the studies had a 2 -year follow-up. Inclusion/exclusion requirements were similar in all the studies. All of the studies similarly compared clinical outcomes. *Results:* The average NDI improved 63% and VAS 67% in the BMC study. All scores had a P-value of less than 0.001. There was no difference in the clinical results comparing one, two, three, four or five-disc levels injected. There were no injection complications, and no patient was made worse. The overall success in the CADR one level studies was 74% with a reoperation rate of 3%. The one level ACF had a success of 65.3%, and reoperation of 6.7%. The two-level CADR had a success of 69.7% and a reoperation rate of 3%. The ACF patients had a success of 37.4% and a reoperation rate of 11%. *Conclusions:* These results indicate a BMC injection may be a reasonable non-surgical option for patients with symptomatic degenerated cervical discs, especially in the multi-level abnormal disc patients.

Keywords Mesenchymal Stem Cells, Stem cells, Cell-based therapy, Bone Marrow Concentrate

1. Introduction

Millions of patients in the United States suffer from chronic complaints of neck pain, pain in between their scapulae, radiating pain producing headaches, and radiating pain into the arms. Epidemiologic studies have found the incidence in the general population to range from 7% to 13.8% [1, 2]. Cervicogenic headaches are considered the most common etiology for chronic headaches [3].

Non-operative treatments for patients who have degenerative discs in their cervical spine with associated neck pain, headaches, pain in between their shoulder blades, and radiating arm pain can include traction, chiropractic care, physical therapy, acupuncture, epidural steroid injections,

intermittent use of a soft collar, and ergonomic pillows for sleeping. Patients who continue to suffer severe symptoms may be candidates for a surgical approach to their problem.

The standard surgical treatment for degenerative conditions of the cervical spine is an anterior cervical fusion [4-8]. One inherent problem with any fusion of the spine is the permanent loss of spine motion and the development of adjacent level abnormalities [9-12]. Research has indicated the development of adjacent level abnormalities leading to additional surgery is between 3% and 5% per year after an anterior cervical fusion [13]. Several review articles of the literature indicate the clinical success rates of anterior cervical fusion at one level are about 70% with a reoperation rate at the two-year follow-up of 10% [14]. The clinical results of anterior cervical discectomy and fusion (ACDF) decrease the more levels that are fused [15, 16]. Another inherent problem with anterior cervical fusion is the failure of the fusion to heal. This results in what is called a pseudarthrosis or failure of fusion. Half the patients with this situation generally require a second surgery in an attempt to obtain a fusion [17, 18].

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Cervical artificial disc replacement (CADR) is a newer surgical option. This procedure underwent FDA testing beginning around 2005. McAfee *et al.*, published a meta-analysis comparing outcomes of artificial cervical disc versus anterior cervical fusion at one level. This meta-analysis reported superior reports with the use of an artificial disc versus a fusion at a single level when considering adjacent level degeneration [19]. Recently, an artificial disc has been approved for two levels. The prospective randomized study supporting this also indicated superior results with an artificial disc versus fusion at two levels [20]. There have been numerous papers published indicating the clinical superiority of artificial cervical disc, over fusion subsequent to 2005 [21-27]. One persistent problem, however, is that many patients have degenerative changes at more than two levels, which is a prognostic indicator of poorer outcomes in surgical procedures.

Patients who have three or more degenerated discs in the cervical spine present a challenging surgical treatment situation. The surgical results of three- and four-level anterior cervical fusions are certainly less than the 65% success rate reported with fusion at a single level. Thus, patients with more than two levels of degenerative changes in their neck have very poor surgical options [15, 16]. Performing CADR at more than two levels is unusual and would very rarely be covered under insurance benefits.

The use of biologics to treat disc abnormalities is a possible non-surgical option which potentially can bridge the gap between traditional non-surgical treatments for cervical degenerative disc abnormalities and surgery. There is mounting evidence to support the use of biologic and cell-based therapy for chronic discogenic low back pain, a condition with similar etiology [28, 29]. The authors of this paper have published one, two and three- year follow-up from a study assessing the safety and efficacy of bone marrow concentrated cells as an alternative to surgery for discogenic back pain at one or two levels [30, 31]. There have been numerous studies utilizing mesenchymal stem cells to enhance tissue repair and decrease inflammatory damage in both in vitro lab studies and in vivo clinical models [32-36]. It is known that bone marrow concentrate (BMC), the treatment used in this study, contains mesenchymal stem cells as well as a number of other cell types including but not limited to: hematopoietic stem cells, endothelial progenitor cells, and platelets. Studies have shown both the mesenchymal stem cell population and other nucleated cell types have healing properties and may contribute synergistically to the healing seen in studies on the spine [37-44].

This is the first study to evaluate the clinical results of intradiscal BMC to treat patients who have symptomatic degenerated cervical discs and associated chronic axial neck pain, headaches, and radiating arm pain, compared to anterior cervical fusion and artificial cervical disc replacement.

2. Materials and Methods

Study Design

This study is a prospective open-label non-randomized evaluation of patients having an injection of BMC (class two data) compared to ACDF and CADR. The ACDF and CADR studies were prospective randomized studies (class one data). Follow up in all the studies was two years. The patients enrolled as subjects in all these studies presented clinically with symptomatic moderate to severe chronic axial neck pain. Axial neck pain was also associated with inter-scapular pain, headaches, and radiating arm pain. Abnormalities were present on cervical MRI scanning and plain radiographs. These abnormalities include anterior and posterior osteophyte formation, disc space narrowing on plain radiographs, and nucleus pulposus desiccation on MRI scanning.

Pre-treatment baseline neck disability index (NDI) was a minimum of 30mm/100mm and pre-treatment baseline axial neck pain was at least 40mm/100mm on visual analog scale (VAS) pain scores. The patients were required to sign and fully comprehend an informed consent document before participating in the study. All patients underwent a pre-injection medical history and physical examination along with the neck disability index and visual analog scale pain scores.

These questionnaires were repeated at six weeks, three months, six months, 12 months, and 24 months post injection of BMC. The patients' primary physical complaint in this study was one of axial neck pain with associated inter-scapular and headaches and may or may not have included radicular arm pain. Standard exclusion criteria included evidence of a symptomatic herniated disc. Patient demographics and treatment levels for the BMC study are listed in Table 1. The Inclusion/exclusion requirements were similar in all FDA IDE studies, and this study except the FDA studies required a neurologic deficit and accepted patients with a herniated disc.

Table 1. Patient demographics in the BMC Study

Number of enrolled patients		182	
Number of levels injected	1 level	33	
	2 levels	60	
	3 levels	45	
	4 levels	44	
Average number of levels injected		2.44	
Age range		54.5	median

Bone Marrow Collection and Processing

Bone marrow aspirate (BMA, 55ml) was collected over acid citrate dextrose-anticoagulant (ACD-A, 5ml) from the patient's posterior iliac crest. The procedure was performed with IV sedation consisting of Versed and Fentanyl. Fluoroscopy confirmed the positioning of the Jamshidi needle in the iliac wing. BMA was collected in a 60ml syringe in a series of discrete pulls on the plunger (targeting a

collection of 5-10ml per pull) with repositioning of the needle tip between pulls based on the reported enrichment of progenitor cells by (Hernigou et al. 2013) [45]. The BMA was processed using the ART21 system (Celling Biosciences, Austin, TX) to produce a bone marrow concentrated cell preparation. The 55ml of BMA were centrifuged for 12 minutes to produce 3ml of BMC. The 3ml of BMC were drawn from the processed device, and then 0.175cc of 50% glucose and 0.175cc of bicarbonate were added to the 3ml of BMC which was then immediately transferred to the physician for injection [46].

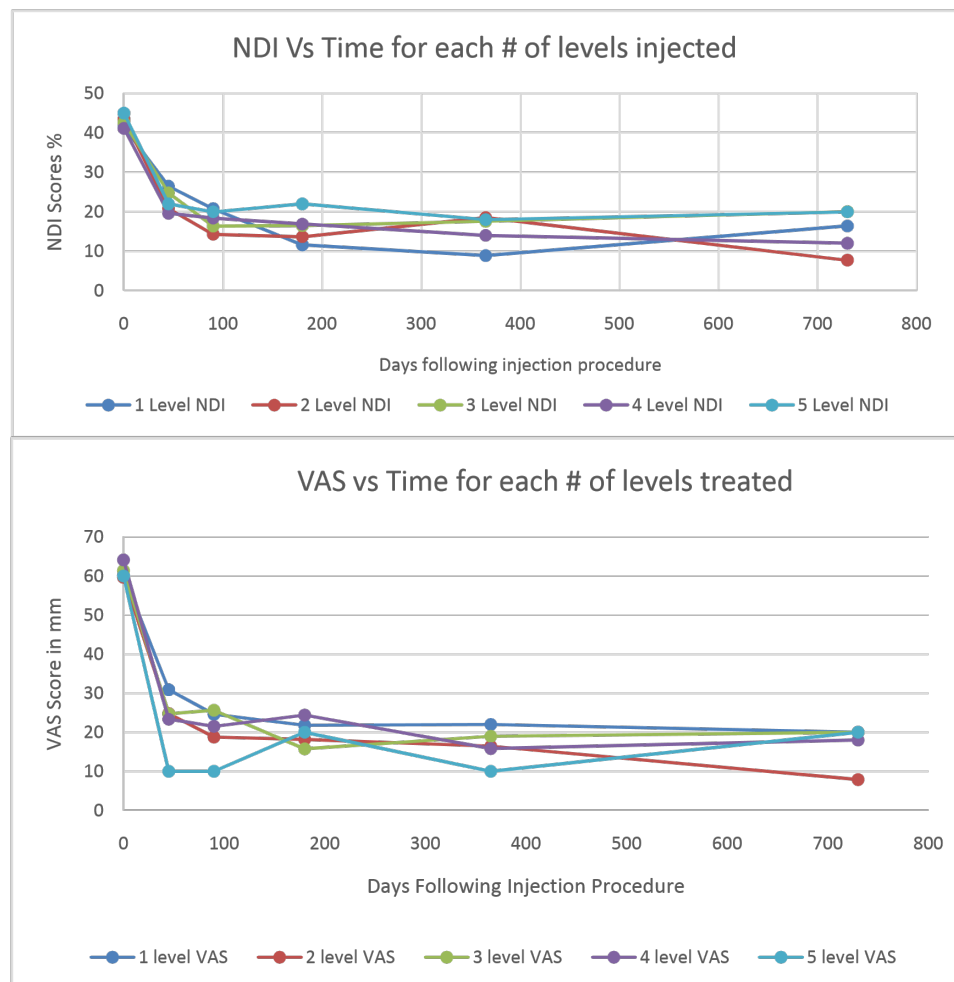
Intradiscal Injection

With the patient in a supine position, the skin overlying the disc to be injected was anesthetized with 1% buffered Lidocaine. Bone marrow concentrate was percutaneously injected into the symptomatic cervical discs through the standard anterolateral approach on the patient's right side of the cervical spine. Digital pressure was utilized to separate the carotid sheath and sternocleidomastoid laterally, and trachea and esophagus medially and then a 20-gauge needle was placed into the disc space and centered on the anterior-posterior and lateral fluoroscopy.

Approximately 0.5ml of bone marrow concentrate was used per symptomatic cervical disc. The entire procedure averaged less than 45 minutes. Patients were prescribed pain medication as needed for three days and put on restricted physical activity for two weeks.

3. Results

BMC Injection Results: Pre-procedure neck disability index (NDI) median was 44.5, and visual analog scale (VAS) median was 62. Two-year follow-up NDI and VAS were 16.5 and 20.7. All scores had a p-value of less than 0.001. This represents a 63% improvement in NDI and a 67% improvement in VAS at the two-year follow up. There was no difference in the clinical results comparing one, two, three, four or five-disc levels injected. There were no injection complications, and no patient was made worse by the procedure. No patient had surgery during the study. Figure 1 details the pre-procedure and post-procedure changes in NDI and VAS through two-year follow-up. The results in **Figure 1** represent all 182 patients.



Overall Success of BMC injection was 63% in NDI and 67% in VAS p<0.001

Figure 1. Clinical results of the BMC Injection

SURGICAL RESULTS: Four of the studies were one level CADR or ACF comparisons. There was one two-level study. Success required no additional surgery, no neurologic worsening, improvement in NDI, and absence of implant adverse events. Thus, success was the same comparing these studies to the BMC study. Table two details patient numbers, demographics, over-all success, and additional surgery.

Table 2. Demographics and Results of one level disc surgery

	Number of Patients	Age	Success	Additional Surgery
Prestige	276	43	79.3%	11
Bryan	242	44.4	82.6%	8
Prodisc-C	106	42.1	72.3%	2
Mobi-C	164	43.2	61.9%	2
Totals	788	43.2	74.025%	3%

	Number of Patients	Age	Success	Additional Surgery
Prestige Fusion	265	44	67.8%	15
Bryan Fusion	221	44.7	72.7%	9
Prodisc-C Fusion	104	43.5	68.3%	9
Mobi-C Fusion	81	42.9	52.2%	12
Totals	671	43.8	65.3%	6.7%

Results of two-level disc surgery

	Number of Patients	Success	Additional Surgery
Mobi-C	225	69.7	3%
Fusion	105	37.4	11%

Analysis of the Bone Marrow Concentrate

This section is included from a previously published paper to detail the BMC cell analysis expected in these patients [30]. The demographics of those patients were similar to this study of patients with the same diagnosis in the cervical spine. This information is included to detail the method of cell analysis and MSC cell counts expected in this group of 182 patients.

An aliquot (1ml) of each subject's BMC was packed in a shipping container with 5°C cold packs and shipped overnight to the cell analysis laboratory (Celling Biosciences, Austin, TX). The samples were received and processed immediately to determine total nucleated cell (TNC) count and viability using a Nucleo-Counter NC-100 (Chemo-metric, Denmark). The BMC was diluted in phosphate buffered saline (PBS, Invitrogen, Grand Island, NY) with 2% fetal bovine serum (FBS, HyClone human mesenchymal grade, Thermo Scientific, Waltham, MA) and

subjected to a Ficoll-Paque (GE Healthcare Life Sciences, Piscataway, NJ) gradient separation (1:1 cell solution to Ficoll ratio by volume) in order to deplete red blood cells. Analysis of the recovered cells included performing colony-forming unit-fibroblast and osteogenic (CFU-F and CFU-O, respectively) assays and phenotypic analysis by flow cytometry. For phenotype analysis, fresh (non-cultured) BMC cells were stained with a series of rabbit anti-human monoclonal antibodies for a hematopoietic lineage-committed (non-progenitor) panel of markers including CD2, 3, 8, and 11b (APC-Cy7), CD34 (PE), CD90 (FITC), and CD105 (APC) as well as appropriate isotype controls. Isotype, single color stain, and four-color stain samples were analyzed by a Guava Easy-Cyte 8HT (Millipore, Billerica, MA). The CFU-F assay was performed by creating a dilution series (in culture medium with 5% FBS and 1% antibiotics) of each cell preparation at concentrations of 50,000-500,000 TNC per well in standard 12-well plates. The plates were placed in an incubator at 37°C, 5% CO₂, and 100% humidity for 72 hours when the medium was replaced. The medium was replaced every three days. After nine days in culture, cells were gently washed with PBS, fixing the colonies/cells with methanol, staining the attached cells with Crystal Violet, rinsing with water, and air-drying the plates. Visualization and counting of the colonies were done with an inverted microscope. Colonies containing 20 or more cells were scored as a CFU-F. The CFU-O assay was performed identically as CFU-F, but after nine days the medium was changed to an osteogenic induction medium (Advance STEM Osteogenic Differentiation Kit, Hy-Clone, Logna, UT) for an additional nine days with complete medium change every three days. On day 18, the wells were washed with PBS, then fixed for 15 minutes in 2% formalin solution, and contained for alkaline phosphatase activity (Vector Blue ALP, Vector Labs, Burlingame, CA) and calcified extracellular matrix (0.5% Alizarin Red solution, Sigma-Aldrich, St. Louis, MO).

Outcome Assessment and Analysis

There were no serious complications from harvesting the bone marrow concentrate or the disc injections. The most common events were transient pain at the harvest site and discomfort at the injection site, both of which typically resolved within 48 hours of treatment. Not every patient improved significantly, but no patient reported increases in visual analog scale or neck disability index from pretreatment scores. Patient follow-up outcomes were obtained by independent reviewers who were not investigators with the study. The reviewers were paid, senior pre-med students. Uni-variable data comparisons of baseline to follow-up were analyzed using a two-tailed student's t-test with a 95% confidence interval (Alpha=0.05, Microsoft Excel).

Demographic comparisons were made using paired sample tests

Table 3. Average cell viability, TNC, total and frequency of CFU-F/CFU-O, and CD marker phenotypes in fresh bone marrow concentrate

Cell viability at 24 hours	98.1 (±1.2)%	TNC/ml in BMC 121 (±11) x 10 ⁶
Cell phenotype subpopulation	% of TNC	Subpopulation Concentration in BMC (cells per milliliter)
CFU-F	0.0025%	2,713 (±491) per ml
CFU-O	0.0027%	2,913 (±418) per ml
Lineage ⁻ cells (CD 2 ⁺ /3 ⁺ /8 ⁺ /11b ⁻)	25.89%	31.5 x 10 ⁶ /ml
Lineage ⁻ /CD34 ⁺	1.397%	1.69 x 10 ⁶ /ml
Lineage ⁻ /CD34 ^{High} /CD90 ⁺ /CD105 ⁺	0.0007%	802/ml
Lineage ⁻ /CD34 ^{Low} /CD90 ⁺ /CD105 ⁺	0.0040%	4,832/ml
Lineage ⁻ /CD34 ⁻ /CD90 ⁺ /CD105 ⁺	0.0049%	5,914/ml

4. Discussion

Several human studies have recently been published documenting the clinical results of utilizing biologics to treat symptomatic chronic discogenic pain. The Coric, Pettine study was an FDA phase one evaluation of utilizing expanded juvenile cartilage cells to treat disco-genic low back pain [47]. Fifteen patients were injected at one lumbar level with 10 million cells and followed for one year. ODI went from 53.3 to 20.3 (p-value<0.0001) and SF-36 improved from 35.3 to 46.9 (p-value<0.0002). MRI improvement of at least one Pfirrmann grade was observed in 77% of patients. No patient had surgery. Pettine et al., have published one, two, and three-year follow-up studies on 26 patients injected with BMC for disco-genic low back pain. The average improvement in ODI was 64%, and VAS was 71%. Only five of the 26 patients had surgery [30, 31].

This study is a prospective non-randomized open-label evaluation of 182 patients followed for two years to obtain preliminary data on the safety and efficacy of utilizing BMC to treat symptomatic cervical degenerated discs compared to surgery. The BMC study included injections into one to five discs. There were four FDA studies of one level disc surgery and one study of two-level surgery.

The results in this group of 182 patients undergoing a single injection of BMC into 1 to 5 discs in the cervical spine was unexpected. The two-year follow-up showed an average improvement in NDI of 63% and VAS of 67% (p<0.001). No patient was made worse, and no patient underwent surgery during the follow-up.

Limitations of the BMC study include no randomized control, no follow-up MRI scan data, and no cell count data. The author has published MRI follow-up data and cell count data in a similar group of patients in the lumbar spine [30, 31]. Limitations of the FDA studies include a low percentage

of follow-up from the original patients to the two -year follow-up.

Patients with more than two levels of symptomatic disco-genic cervical pain have limited treatment options. There is minimal literature reporting the long-term efficacy of any non-operative treatment. Two-year follow-up data in treating multilevel discogenic cervical pain with the BMC showed an improvement in NDI of 63% (p<0.001) and VAS of 67% (p<0.001). No patient was made worse by the procedure, and there were no complications from the percutaneous injection of BMC into the disc. Utilizing MSCs derived from BMC, based on these preliminary results, may offer patients with multilevel disco-genic cervical pain a viable treatment option to surgery.

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