Title: Bone Marrow Concentrate intradiscal Injection for Chronic Discogenic Low Back Pain: A Double Blind Randomized Controlled Trial

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3. Introduction

3A. Type of Study: A double blind randomized placebo controlled trial for an autologous bone marrow aspirate product, bone marrow aspiration concentrate, not considered by the FDA to be a drug. The preparation of this substance uses a simple concentration procedure of the bone marrow by an FDA approved Arthrex Angel flow cytometry bone marrow concentrate system through Arthrex corporation (Naples, FL). The procedure is considered to be minimal manipulation of bone marrow tissue for homologous use as per FDA draft guidance covered under Code of Federal Regulations 361 [1, 2].

3B. Purpose/Objective of the Study: To investigate the efficacy and safety of a single bone marrow concentrate injection into one or more intervertebral discs for the treatment of discogenic low back pain.

3C. Background of the Study: Low back pain is an extremely common cause of pain and disability. Although several structures within the spine have been identified as pain generators, the intervertebral disc is felt to account for 40-50% of chronic low back pain [3]. The most common conservative treatment options include activity restriction, medications, physical therapy, chiropractic treatment, and steroid injections. Many patients have an inadequate response to these conservative measures and progress to surgical treatment, either lumbar fusion or disc replacement. Lumbar fusion results for discogenic pain vary considerably, and disc replacement results do not appear to be significantly superior to fusion. While minor complications are relatively common with fusion surgery, catastrophic complications can occur. These complications are relatively rare. The larger issue with lumbar fusion for discogenic low back pain is the risk/benefit ratio. It is considered to be an extensive surgical procedure involving 3-6 months of recovery. Although there is evidence that it is superior to conservative care, the results are relatively modest [4,5]. In addition, it is not uncommon for patients to perceive a lack of effect or even worsening of symptoms.

The pathoanatomy of a painful intervertebral disc has been extensively studied [6,7]. The region of the disc felt to illicit pain is the outer portion of the disc, the annulus fibrosis. The annular region can develop tears or fissures in the collagen fibers. These fissures contain an ingrowth of vascularized granulation tissue along with extensive nerve endings. These changes are felt to contribute to the development and perpetuation of discogenic low back pain. [6,7].

Prior conservative treatments aimed at annular tear pathology have been attempted with heat or radiofrequency energy with disappointing results [8,9]. Others have investigated the use of methylene blue as a neurolytic agent within the disc [10,11]. These investigators published two quality clinical studies demonstrating the efficacy of intradiscal methylene blue. Unfortunately, further investigations have not produced their excellent results [12, 13].

Ultimately, the ideal treatment for pain induced by annular tears would be through direct healing of the tears themselves. Attempts are being directed toward this goal with research in areas such as stem cells, platelet-rich plasma, and specific growth factor injections [14-16].

Another therapeutic agent which could potentially promote disc regeneration is bone marrow concentrate (BMC). Bone marrow concentrate is obtained through a simple centrifugation of autologous bone marrow aspirate. Bone marrow aspirate / concentrate has been used extensively in orthopedic clinical settings to promote tissue healing [17,18]. Growth factors, cytokines and pluripotent stem cells within the aspirate are felt to be responsible for the ability of BMC to promote tissue healing in collagen based structures [19,20].

BMC has been used studied in animal and human trials in multiple non-spine orthopedic conditions [19-23]. Results have been promising. BMC has been used in spinal surgery to augment fusion with positive results [18].

Recently bone marrow concentrate has been used in the treatment of discogenic low back pain. The results of a single trial of BMC intradiscal injection for discogenic LBP have been published with 12 and 24 month follow-up [24,25]. Pettine et al. performed a prospective trial using autologous bone marrow concentrate in a single treatment into the intervertebral discs. The study included 26 patients who had at least moderate to severe low back pain with activity for 6 months and at least 3 months of non-operative treatment without significant changes in their symptoms.

Pain and function were evaluated by visual analogue scale (VAS) and Oswestry disability index (ODI) prior to the procedure and at 3, 6, 12 and 24 months post-injection. Average VAS and ODI scores demonstrated significant improvement at all time points. At the two year mark, 81% of the patients avoided surgery. Of the 21 non-surgical patients, the average decrease in pain was 71% at 2 years. The authors also compared pre-procedural MRI disc degeneration levels (Pfirrman grade) to MRI findings at 12 months. No discs appeared worse and in 8 patients there was actual improvement in the degree of disc degeneration of at least 1 Pfirrman grade at the level injected.

BMC appears to have an excellent safety profile. Pettine et al. reported no significant side effects from intradiscal BMC [24,25]. In addition, no significant adverse events have been attributed to this treatment in multiple animal and human studies published for a variety of orthopedic conditions [19-23, 26].

4. Inclusion/ Exclusion Criteria

4A. Inclusion/exclusion criteria

Inclusion criteria:

- Chronic low back pain greater than 6 months with low back pain component greater than leg pain component if present.

- Average pain at least 40/100 on Visual analogue Scale (VAS)

- Inadequate response to conservative care including medication, physical therapy, and/or spinal injections.

- Males and females at least 18-55 years old.

    - Rationale for age limit of 55: Based upon the study by Depalma et al. [27], at age 55 and less the predicted probability of a discogenic source of pain is higher than any other source. Beyond 55 years of age, zygapophyseal joints have a higher predictive probability. Therefore, the pre-test probability for a test for discogenic pain beyond this age is considerably lower.

- Have provided informed consent.

- If female subjects are of child-bearing potential, they must be using an acceptable form of birth control.

- Advanced imaging of MRI or CT demonstrating abnormal disc morphology.

- Presumed lumbar discogenic pain based upon either A or B below:

    A. Positive provocative discography in accordance with SIS guidelines [28].

    Or

    B. Both B1 and B2

        B1. The patient must have MRI findings suggestive of discogenic low back pain including either a high intensity zone region or type 1 or type 2 end plate Modic changes [29-31].

        B2. Exclusion of other structures as source of pain (see below).

    Rationale for not requiring discography for inclusion:
    The rationale involves several factors. Although injecting the BMC at the time of discography seems most reasonable, the volume of contrast used for the discography test may reach 3ml, when performed in accordance with SIS guidelines [28]. This leaves an inadequate volume capacity to accommodate much volume of BMC. Secondly, in accordance with SIS guidelines [28] discography requires a negative control disc. Discography is invasive and may have negative detrimental effects on the disc [32]. Therefore, potential harm may exist if discography is performed. BMC is considered to be a very benign substance; it is an autologous tissue unlikely to have any detrimental effects [17, 18, 22].

    Subjecting patients to two distinct intradiscal procedures (discography prior to the BMC procedure) is difficulty to justify when the purpose of discography

(including injecting a normal control level) would be for qualification of an intradiscal injection of a very likely benign substance.

In summary, (as expressed in a prior study of a regenerative intradiscal treatment publication [15]), injecting the BMC at the time of discography would not allow enough volume for the BMC and performing a separate discography procedure "solely for determining the candidacy and level for the [BMC] injection could not be justified" [15].

- If clinical suspicion for facet pain is present, subjects must have had a negative investigation of zygapophysial (facet) joints as the primary source of pain to include diagnostic medial branch blocks or intra-articular facet injections or have failed radiofrequency ablation (assumed to have had 2 false positive blocks).

- If clinical suspicion for sacroiliac joint pain is present (Paraspinal pain below L5 or dominate buttocks pain), subjects must have had a negative investigation of the sacroiliac joint as the primary source of pain to include intra-articular sacroiliac injections or diagnostic S1-3 lateral branch blocks and L5 dorsal ramus block.

Exclusion criteria:

- Active moderate to severe lumbar radiculopathy

- Negative provocative discography. (Discography is not required for inclusion but prior discogram testing found to be negative excludes the patient from the trial.)

- Very severe decrease in disc height at a planned injection level. A disc height less than 1/3 expected normal disc height will be an exclusion.

    - Rationale for this disc height exclusion criteria: Requirements for 2/3 of normal disc height has become the norm for many intra-discal procedure trials which may have originated out of the intradiscal electrothermy annuloplasty trials as a requirement for device access [8-10]. The authors do not feel that moderate disc height loss should preclude needle access nor adequate chance for clinical success.

- Active infection

- Moderate to severe anemia, thrombocytopenia, or leukopenia

- Spinal fracture within the past 6 months

- Severe psychological illness
- Inability to consent to procedure due to cognitive issues
- Prior fusion at the level considered to be the source of pain
- Pregnant or breastfeeding females.
- Severe uncontrolled renal, hepatic, hematological, gastrointestinal, metabolic, endocrine, pulmonary, cardiac, or neurological disease or any medical condition which would make the subject unsuitable for this study.
- Inflammatory arthritis
- Any cancer within the past 5 years except basal cell or squamous cell skin cancer
- Intradural disc herniation
- Coagulopathy preventing spinal injection
- Inability to stop anticoagulants other than aspirin due to other medical issues
- Exceeds 30 mg morphine equivalent per day of opioid use.
- A history of alcohol or drug abuse within the past 5 years.
- Use of any investigational drug within the past 30 days.
- Low back surgery within the past 6 months.
- Steroid injection in the spine within the past 30 days.
- Any intradiscal injection other than contrast dye or anesthetic within the past 30 days.
- A known allergy or sensitivity to heparin or citrate (used for processing BMC)
- Pending litigation involving the subject's back pain.
- Central stenosis at the level to be injected with an AP diameter less than or equal to 5 mm
- Severe anaphylactic/anaphylactoid reaction to any of the medications used. (If a patient does have a mild or moderate allergy to any of the medications used in the procedure or prior anaphylactic/anaphylactoid reaction to any food or drug, they will be given prednisone 50 mg PO 13, 7, and 1 hour prior to the procedure and diphenhydramine 50mg PO 1 hour prior to the procedure.)

- In order to mitigate any economic risk, a patient without adequate medical insurance coverage for any subsequent tests or procedures deemed clinically necessary will be excluded. BMC is an autologous blood product with multiple clinical uses. Intradiscal administration should not preclude insurance coverage for any subsequent medical issues that might develop pertaining to the intradiscal injection itself or the BMC

4B. There is no gender discrimination
Pregnant women will be excluded as above.
Women of child bearing capacity will need an acceptable form of birth control.

4C. There is no racial discrimination

4D. Persons considered to be of a vulnerable population will not be included in the study.

4E. Age: 18 years – 55 years old

4F. Number of participants: 60 participants expected to be enrolled. Duration of patient enrollment is 3 years.

Power analysis:

With two treatment patients for each placebo patient, 40 treatment and 20 placebo patients will be required to achieve 80% power to detect a difference of 0.35 in the proportion of patients with successful improvement between groups with a chi-squared test at a level of significance of 0.05, assuming the proportion of patients achieving success in the control group is 0.2 (20%) This is based upon the preliminary data calculated success rate (>50% improvement in Pain) from Pettine et al. study [24] was 61%. (Our calculation differs from Pettine et al. as we included surgical patients as failures.) The 95% CI are 44-75% using an n of 40 in our proposed treatment group. The placebo rate is a very well done spinal explanatory study was 13% [33]. The placebo rate from an intradiscal study using intradiscal normal saline was 12.5% and 23.5% using hyaluronic acid (the carrier for the treatment). We therefore used the conservative estimate of a placebo response rate of 20% in this study.

5. Study design/methods/procedures

5A. Summary of research design

A total of 60 patients with randomization 2:1 ratio (treatment : control). Pain and function will be assessed using VAS and ODI. Primary outcome will be percentage of patients in the treatment group compared to the control group at 6 months categorized as a clinical success, at least 50% relief of pain. Secondary outcomes will be comparison of success rates (at least 50% pain relief) at 3 and 12 months. Other secondary outcome will be percentage of patients in the treatment group vs control group with greater than 30% improvement in ODI at 3, 6, and 12

months. Global Perceived Effect (GPE) will also serve as a secondary out come at 3, 6, and 12 months. Medication log and adjunctive treatments will also be recorded and analyzed.

Randomization process: computer randomization program
Randomization shall be performed through a computer randomization program. https://www.random.org/#numbers (Randomness and Integrity Services Ltd. Dublin, IR.) A random number shall be generated between 1 and 60. For all randomly generated number from 1 to 40, the patient will be assigned to the treatment group. For all randomly generated random number from 41 to 60, the patient shall be assigned to the placebo group.

The patients will be blinded to treatment assignment. The patient will follow up for assessment with an investigator who remains blinded to the treatment assignment. Only the physician performing the procedure will be aware of the group assignment.

Based upon previous recommendations for the assessment of blinding success in clinical trials [34] the patients will be queried at 2 weeks whether they believe they have received the active treatment, the placebo, or if they do not know to which group they were assigned.

Treatment: a single intradiscal injection of autologous bone marrow concentrate into suspected painful discs based upon history, image findings and the exclusion of other anatomic structural sources of pain such as z-joints and SIJ.

Placebo: Intramuscular injection of normal saline directly above the transverse process at each suspected discogenic pain level. (The physician performing the procedure will not be blinded, but the assessor for follow up evaluation will be blinded).

A sham bone marrow aspiration will also be performed involving fluoroscopic guidance and anesthetic to the iliac crest. The patient will hear the noise of the bone marrow drill (unattached), while a 21 gauge needle is tapped against the periosteum.

Patients will be screened from a pool of existing patients at a community outpatient spine and musculoskeletal practice. These patients will have had an inadequate response to conservative treatments, some of whom will be considering fusion surgery. Some of these patients will be considering provocative discography through the recommendation of a local surgeon. One of the options discussed will be bone marrow concentrate intradiscal injection under the context of the study. If the patient has any interest in participating in this study, the general risks, benefits and literature will be discussed. The patient will be informed that their status as a patient in the principle investigator's practice and the physician-patient relationship will not be altered regardless of their participation in the study. They will be given a written summary of the study. If they have continued interest, an appointment will be arranged for the verbal and written consent process. Once the consent form process has been completed, the patient will perform the VAS as well as the ODI which will serve as the baseline measurement data [35]. A pain medication query shall be performed as well.

All procedures will be performed by two of the investigators (DL and SH). Both are board certified in Physical Medicine and Rehabilitation, experienced discographers, fellowship trained in spine injections and serve as instructors for spinal injection procedures at the national level (Spine Intervention Society). Both investigators have supplemental training and years of experience with bone marrow aspiration procedures.

Bone marrow aspiration and intradiscal BMC injection procedure:

Pre-procedure:
The patient will be offered a P.O. sedative of diazapam 5 to 10 mg or alprazalam 0.5 to 2 mg to be taken 30-60 minutes prior to the procedure. Alternatively, patients will be offered conscious sedation in the form of 2 mg to 6 mg midazolam IV with the possible addition of 50 to 150 mcg fentanyl IV. Alternatively, 50-100 mcg fentanyl may be injected IM for pain control. Continuous cardiac, pulse oximetry, and clinical monitoring will be performed throughout the procedure. Oxygen by nasal cannula 2 to 4 L may also be used. Emergency equipment will be immediately available. The investigators (DL and SH) performing the procedure are ACLS certified by the American Heart Association. Conscious sedation, if utilized, will be performed in accordance with state requirements including the assistance of a nurse, physician's assistant or a second physician.

Bone marrow aspiration procedure: The patient will be placed in a prone position in our outpatient fluoroscopy suite. Strict sterile technique will be observed. The lower back will be cleansed with a solution of chlorhexadine mixed with alcohol and covered with a sterile drape.

The posterior superior iliac spine will be located by fluoroscopy. A 25 gauge needle will be placed down to the periosteum, which will then be anesthetized with 3ml of lidocaine 2%. An additional 2ml will be used to anesthetize the superficial tissues and skin. The needle, syringes and BMC filter are primed with heparin 500 units/ml. A total of 9 ml of anticoagulant citrate dextrose-formula, (ACD-A) is placed in the syringes to prevent clotting of the aspirate and divided equally into the 6, 10ml syringes. A 15g needle with stylet will be directed manually down to the periosteum under intermittent fluoroscopic guidance. The Arrow Oncontrol (Teleflex, Morrisville, NC) drill aspiration system will be used with a 15g aspiration needle. The drill, within the sterile cover, shall then be placed and "locked" onto the aspiration needle. The aspiration needle is then advanced by drill into the cavity of the ilium. The stylet is then removed and the syringe attached. 50 ml of bone marrow aspirate is slowly withdrawn by negative pressure from 4-6 sites via 10ml syringes. The stylet is then replaced into the needle. The drill reattached to the needle and the needle is slowly withdrawn.

The aspirate is then placed through a bone marrow filter to remove any calcific fragments. Using the Angel processing system, the bone marrow is concentrated by means of flow cytometry in a sterile closed system.

Immediately after processing of the aspirate, it is relabeled with patient information then withdrawn into sterile syringes, and placed onto the sterile field in preparation for the intradiscal injection.

Placebo Bone Marrow Aspiration: The patient shall be draped in a manner that injection is not visible even if the patient looks behind to observe. The above set up will be identical as the actual bone marrow aspiration. The area will be anesthetized in a similar manner. The needle shall not be attached to the drill but the drill trigger shall be squeezed so the patient hears the noise of the drill. Simultaneously, a 21g needle will be tapped against the periosteum. To simulate the pressure of aspirating the bone marrow, 3 ml of normal saline shall be injected just superficial to the periosteum.

Determination of disc levels to be injected:

Levels to be injected will be determined by A or B below.

A: Disc level(s) determined to be positive on prior discography performed within the preceding 6 months using Spine Intervention Society standards [28].

B: As discography is not required in the inclusion criteria, disc levels will be determined by clinical and image findings. MRI findings of high intensity zone, modic changes type 1 or 2, and decreased T2 disc signal will be used [29-32] in conjunction with the general location of pain and tenderness on exam (upper, middle or lower lumbar location).

Treatment:

Intradiscal injection: The patient will be placed in a prone position in an outpatient fluoroscopy suite. Strict sterile technique will be observed. The lower back will be cleansed with chlorhexidine mixed with alcohol and covered with a sterile drape. A standard posterolateral extrapedicular discogram technique will be used under intermittent fluoroscopic imaging for each level previously determined to be a presumptive pain generator either clinically or through prior discography. The skin and superficial tissues will be anesthetized with 2-5 ml of lidocaine 1%. The needle tip of a 22-gauge or 25-gauge needle with stylet, single or double needle technique, will be directed toward the disc into Kambin's triangle. Depending upon the patient's tolerance of the procedure, one ml lidocaine 2% may be injected just outside the disc annulus. The stylet will then be replaced and directed into the disc nucleus. A volume of 0.6 ml of contrast solution consisting of 0.4 ml Omnipaque 240 or 300 contrast agent with 0.2 ml of cefazolin 330 mg/ml (for a total of 66 mg at a final concentration of 18 mg/ml based upon 3.5 ml total volume) (or in cefazolin allergic patients 0.4 ml of 40 mg/ml gentamicin) will be injected to confirm intranuclear location and for discitis prophylaxis.
Then, 2-3 ml of previously prepared autologous BMC will be injected into the disc. Thus, a total volume of 2.6-3.6 ml will be injected into each of the presumed pain generating discs during the treatment. There may be instances when the disc cannot accommodate the full volume of BMC due to high pressure (based on manual pressure estimation) or leakage into the epidural space. It may be necessary for the clinician to inject less than the 2ml of BMC. At least 1ml of BMC will

need to be injected into a disc to be considered a completed treatment. If the patient is experiencing a great deal of discomfort during the BMC injection, the physician may add 0.2 to 0.5 ml of ropivicaine 0.2%-1%. After the solution is injected, the needle will be removed. The alcohol and chlorhexadine will be cleansed from the patient's back and the drape will be removed. The patient will then either ambulate with assistance or be transported via wheelchair to the recovery room.

Placebo treatment procedure:

As it was not felt ethical to puncture a disc for a placebo treatment [32], it was elected to use an intramuscular injection of 3ml of normal saline on the dorsal surface of the corresponding proximal transverse process. For example, if the L5/S1 disc was the "treatment" disc, the corresponding placebo will be an injection of 3ml of normal saline just dorsal to the L5 transverse process. This will be performed for each disc level to be "treated".

The patient will be placed in a prone position in an outpatient fluoroscopy suite. Strict sterile technique will be observed. The lower back will be cleansed with chlorhexidine mixed with alcohol and covered with a sterile drape. The patient shall be draped in a manner that injection is not visible even if the patient looks behind to observe. The skin will be anesthetized with 2ml lidocaine 1% at each level to be injected. A 22 g spinal needle will be advanced to the transverse process at the superior level of each disc to be "treated". One ml of contrast agent, omnipaque 240, will be injected. Then, assuming an appropriate intramuscular contrast pattern, 3ml of normal saline shall be injected just dorsal to the transverse process. After the normal saline is injected, the needle will be removed. The alcohol and chlorhexadine will be cleansed from the patient's back and the drape will be removed. The patient will then either ambulate with assistance or be transported via wheelchair to the recovery room.

Rationale for non-intradiscal placebo: In light of theoretical risk of needle injury to the disc [32], it was not felt to be appropriate to have the placebo involve a direct disc injection of normal saline. A normal saline injection into the epidural space using an infraneural (retro-discal) transforaminal needle position as a potential placebo was also considered but felt also to be in appropriate due to the risk of unintentional needle position. The risk of disc puncture with this technique is 5% as well the risk of intrathecal injection is 3% [36]. In addition, a normal saline injection into the epidural space might have some dilutional therapeutic benefit as inflammatory cytokine have been documented in the epidural space [37] and therefore, normal saline may have a dilutional therapeutic benefit.

The patient will be observed clinically for 30 minutes with vital signs obtained upon initially arriving to the recovery room and then every 15 minutes. The patient will be discharged to a companion with written and verbal instructions to include no driving or operating machinery for 12 hours. They will be instructed to call for any concerning symptoms such as redness, swelling, fever, or increase in pain. They will also be instructed to call for any bowel or bladder problems, worsening of low back symptoms beyond day 2, post-treatment severe low back pain at any time, new onset fever, or new or worsening of any lower extremity symptoms. They will be

given clear written and verbal instructions on how to contact our providers with 24 hour availability. They will be offered a prescription for post-procedure pain medication, unless they currently have such medication, such as hydrocodone/ acetaminophen 5/325 mg or oxycodone/ acetaminophen 5/325 mg to take 1-2 tabs q 4-6hrs prn post-procedure pain, #10-20. They will be instructed not to engage in any strenuous activity for two weeks after the treatment.

Anticipated procedure time 60-90 minutes.

The patient will be contacted by telephone during post-treatment week one to monitor for any concerning symptoms or adverse events. The patient will be seen in our office for routine follow-up at post-treatment week 2, 4, and 8 weeks to assess for any adverse events and to monitor progress. At baseline 3,6,and 12 months the patients will complete the VAS, ODI and Global Perceived effect (GPE not performed at baseline) [35,38]. Patients will be seen more frequently if clinically indicated.

A medication query shall be performed at baseline 3, 6, and 12 months. In addition, any additional treatments obtained during the follow up period shall be recorded. Other treatments during the study period will be discouraged but not forbidden.

Alternatives to bone marrow concentrate treatment:

- These patients have typically had extensive conservative treatment and may be considering lumbar fusion surgery (or disc replacement) as a possible treatment option. The risks of this type of surgery in the lumbar spine include infection, bleeding, nerve injury, paralysis and death. The most common adverse event or outcome encountered, however, is perceived lack of efficacy or worsening of symptoms after this extensive surgery and recovery period.

- Although these patients have had inadequate pain relief and functional improvements from their prior conservative treatments, they certainly have the alternative of continuing with their current treatment regimen, which may be of some benefit. These treatments often include medications, physical therapy, chiropractic care, and spine injections. The risks of spinal injections, although rarely occurring, include infection, bleeding, nerve injury, paralysis, and death. The risks of the various medications used for pain are medication dependent and are numerous.

- Other types of intradiscal injections are an option. Intradiscal methylene blue has shown early promise in a quality RCT [11] but investigations to replicate their results have been disappointing [12, 13]. Intradiscal steroid may be of benefit for individuals with certain end plate changes on MRI [39] but these positive results have been viewed with extreme caution based on poor results from prior studies [40]. Other regenerative type treatments such as platelet-rich plasma may be also be beneficial [15, 16]. The risks of intradiscal injections, although rare, include infection, bleeding, nerve injury, paralysis, and death.

- "Alternative" or "complementary" medicine treatments such as acupuncture, prolotherapy or homeopathic remedies could also be considered. Serious risks of prolotherapy include paralysis and severe risks of acupuncture include pneumothorax.

- The option of no further treatment may also be a reasonable alternative for some patients.

5B. Analysis of study results:
The data from the pre and post VAS (0-100 mm) and Oswestry disability index 0-100 will be analyzed from baseline to 3, 6, and 12 months [35]. Categorical data will be used. Primary outcome for success will be considered at least 50% improvement in pain by VAS at 6 months and secondary outcome at 3 and 12 months. Additional secondary outcomes will include 3, 6, and 12 month minimum of 30% improvement in ODI. Further secondary outcomes will also include a minimum of 30%, and 70% improvement in pain at 3, 6, and 12 months. All time point categorical success rates will be compared to placebo group with 95% CI determined. Group data mean pain scores will also be analyzed for each time point using *t*-tests. Global perceived effect will be analyzed for 3,6,12 months on a 5 point scale using categorical data analysis.

Pain medication log at baseline, 3, 6, and 12 months will be analyzed by a simple categorical decrease, increase, or unchanged compared to baseline. Morphine equivalents will not likely be helpful as the majority of the subjects will not likely be on opiate medication. Those patients not on medication at baseline shall not be included in the medication analysis.

Adjunctive treatments: All other therapeutic treatments for LBP during the study period shall be recorded at 3, 6 and 12 months.

5C. Monitoring:
Patients will be contacted by telephone during post-treatment week one to monitor for any concerning symptoms or adverse events. The patient will be seen in follow-up at approximately 2 weeks and 4 weeks post-treatment to assess for any adverse events. Patients will also be seen 2 months, 3, 6, and 12 months post-treatment for evaluation and data collection or more frequently if clinically necessary. Patients will be made aware by verbal and written instructions that they have phone access to the study physicians at any time and are instructed to contact the practice for any concerning symptoms. These include redness at the injection site, swelling, fever, bowel or bladder problems, worsening of low back symptoms beyond day 2 post-procedure, severe low back pain at any time, and new or worsening of any lower extremity symptoms. Any new or worsening lower extremity symptoms such as pain, numbness, or weakness will be evaluated clinically, and if needed, further diagnostic tests such as MRI or electrodiagnostic testing will be ordered. Any significant increase in low back pain or fever, without an identified source in the 7-30 days post intradiscal treatment shall be considered suspicious for discitis. CBC with differential, C-reactive protein, sedimentation rate, as well as MRI shall be performed (CT or bone scan if contra-indications to MRI exist).

Any severe adverse event deemed specifically related to the bone marrow concentrate injectate shall be reported within 24 hours to the IRB (see adverse events 6C).

5D: Storage of data

The research data will be stored in paper files accessible only to the investigators as well as research clinical staff under direct supervision of the investigators. The data will be kept secure in a locked cabinet when not in use.

5E. Confidentiality of data
Data will be kept in a secure location following all HIPAA standards.
Patients will be assigned a subject number and data will correspond to such number and not any identifiable demographic data.

6. Risk/Benefit assessment

6 A. Risks of participation in the study
Physical risks:

Risks specific to bone marrow aspiration:
These risks include bleeding, infection and damage to nearby tissues, vessels, nerves or organs. Other very unlikely risks include injury to the bone itself including fracture and continued pain in the region. Extremely unlikely risks include: paralysis, cardiac arrest, brain damage and/or death. Estimated risk of minor complication is less than 1%. Estimated risk of a catastrophic complication is near zero.

Risks specific to placebo bone marrow aspiration:
These risks include bleeding, infection and damage to nearby tissues, vessels, nerves or organs. Estimated risk of minor complication is less than 1%. Estimated risk of a catastrophic complication is near zero.

Risks of intradiscal delivery of the BMC, i.e. the injection procedure itself, are considered equivalent to the risk of discography and are as follows:

- Nerve injury, in particular the exiting ventral ramus. Risk is considered extremely low in an awake patient either with no sedation or appropriate conscious sedation [28]

- Discitis with a risk of 0% to 1.3% per disc [41]

- Theoretical risk of bowel injury and nerve root injury are considered an anatomic impossibility unless a grossly inappropriate technique is used. Estimated risk is near zero. [28]

- Theoretical risk of needle passage through the dura may be estimated at 3% based upon data from a retrodiscal transforaminal epidural steroid injection approach [36]. The risk of this being clinically significant is less than 1% [28].

- The risk of clinically significant bleeding during discography in a non-anticoagulated patient is exceedingly rare and is estimated at less than 1%.

- Severe allergic reactions to the contrast dye, local anesthetic, or antimicrobial skin prep are rare and are estimated at less than 1%.

- Vaso-vagal reactions in a non-sedated patient are estimated at 3-4%. In an awake but sedated patient, the risk is estimated at 0-10% [42].

- Risks of the use of needles include local muscle pain [28].

- The risk of needle puncture to a disc includes acceleration of disc degeneration and increased risk of disc herniation over a 10 year period. Risk is considered minimal but statistically significant over control [32].

- The risk of accelerated disc degeneration secondary to contrast medium placed into the disc. Possible injury to intervertebral disc cells has been demonstrated experimentally [43]. Clinical implications are questionable. Risk is considered very minimal and is estimated at less than 1%.

- General risks of disc injections include the exceedingly rare risk of paralysis or death. Estimated near zero.

- General risks of conscious sedation (if utilized) include respiratory depression, cardiac arrhythmia or arrest, stroke, or death. Estimated risk is far less than 1%.

- Risk of venipuncture for IV sedation for the BMC injection procedure (if utilized): The risks of IV access include infection, bleeding, and nerve injury. The risks of the sedation medication itself include low blood pressure, low oxygen level, depression of the respiratory drive, cardiovascular compromise, stroke, severe allergic reaction, and death.

Risks of bone marrow concentrate within the disc:

- The injectate is an autologous aspiration of bone marrow minimally altered through a centrifuge process. No reported side effects beyond local soreness have been reported in other tissues or the spine. Bone marrow concentrate does contain stem cells. The theoretical risk of the development of a neoplasm does exist however unlikely. These are non-cultured stem cells and therefore, should hold the same risk for tumor development as those naturally occurring. A registry surveillance study which included follow-up MRI's did not reveal any evidence tumor development [22]. However, we do not assume the risk of injury to the disc tissue is zero. Risk is unable to be quantified but assumed much less than 1%.

- Risk of BMC in the epidural space. With disc injections, leakage may occur into the epidural space. Whole blood is commonly injected into the epidural space for the

purpose of treatment of spinal headache. Although we would not anticipate a tissue response different from whole blood, we do not assume risk of injury to the neural structures and dural tissue is zero. Although there is not extensive data on the effect of BMC on nervous tissue, bone marrow has been used extensively during spinal fusion procedures without any reports of complications attributed to the bone marrow itself [17,18]. Risk is unable to be quantified but assumed much less than 1%.

- The risk of anticoagulant acid citrate dextrose (ACD) used in the processing of the BMC: ACD is an FDA approved anticoagulant most often used to prevent clotting of blood samples. It is commonly used in blood transfusions as well as hemodialysis in heparin intolerant patients. The amount of ACD remaining available within the BMC to be injected is very small. Risks are as follows: hypocalcemia, bleeding, severe allergy. Risk is unable to be quantified but assumed to be much less than 1%.

The risk of heparin used to prime the needle, syringes and BMC filtering and processing containers of the BMC: heparin is an FDA approved anticoagulant most often used to prevent clotting. It is commonly used clinically at much higher dosages.

Risks of placebo treatment procedure: an intramuscular injection of normal saline.

- Risk of infection for an intramuscular patient estimated at much less than 1%.
- Risks of the use of needles for an intramuscular injection of contrast and then normal saline include local minor skin puncture and muscle pain [28].
- Severe allergic reactions to the contrast dye, local anesthetic, or antimicrobial skin prep are rare and are estimated at less than 1%.
- Risk of venipuncture for IV sedation for the injection procedure (if utilized): The risks of IV access include infection, bleeding, and nerve injury. The risks of the sedation medication itself include low blood pressure, low oxygen level, depression of the respiratory drive, cardiovascular compromise, stroke, severe allergic reaction, and death.

Vaso-vagal reactions in a non-sedated patient are estimated at 3-4%. In an awake but sedated patient, the risk is estimated at 0-10% [42].

The psychological risk does involve the emotional and physical discomfort of undergoing a bone marrow aspiration and an intradiscal procedure or placebo procedure.

Social and legal risks are considered minimal.

Economic risk: Considered minimal. There will be no cost to the patient except for routine follow-ups including co-pays and deductibles from commercial insurance.

6 B. Prevention of risks:
The risks of the delivery of the BMC into the disc will be reduced by following strict sterile technique as well as procedural technique as described in SIS guidelines [28]. Prophylactic antibiotic will be placed into the disc to minimize the risk of discitis.

The discomfort of the procedure shall be minimized with by oral or IV sedation unless the patient prefers no sedation. Intravenous or IM fentanyl may be given for intra-procedural pain control. Local anesthetic, 2-5ml lidocaine 1%, shall be infiltrated in the skin and superficial tissues. Local anesthetic, 1ml 2% may be placed outside the disc to minimize the discomfort of the disc injection. Ropivicaine 0.2- 1% may be placed within the disc as well.

The patients will be monitored for adverse events as previously described.

6C. Adverse events:

Adverse events include:

- Infection including discitis
- Bleeding, in particular, an epidural hematoma
- New and/or increased pain, numbness, dysesthesia, or weakness in one or both lower extremities
- Increased low back pain beyond normal flares typical for the patient
- Allergic reaction
- Fever
- Redness at injection site
- Bowel or bladder dysfunction
- Paralysis
- Death

Any minor adverse event will be reported to the IRB within 10 days. Any serious adverse event will be reported to the IRB within 24 hours of being brought to an investigator's attention.

Our medical staff will be available at all times for any adverse event. If subsequent diagnostic tests or procedures are necessary they will be in accordance with current standard of care. All patients will have medical insurance coverage for any subsequent tests or procedures. BMC is an autologous tissue product with multiple medical uses. Intradiscal administration should not preclude insurance coverage for any subsequent medical issues pertaining to its use.

6D. Benefits:

The potential benefits of participating in the study are significant. The patient has an opportunity to significantly reduce or alleviate his or her pain and disability with a relatively minimally invasive procedure compared to fusion surgery. The benefits to the community as a whole could be significant as well, potentially decreasing or even eliminating the need for surgical fusion for discogenic pain.

7. Participant recruitment and informed consent

7A. Recruiting: Patients will be screened from a pool of existing patients at a community outpatient spine and musculoskeletal practice. Patients referred by outside clinicians may be considered. These patients will have had an inadequate response to conservative treatments, many of whom will be considering fusion surgery. Some of these patients may be considering provocative discography through the recommendation of one of the physicians in the principle investigator's practice or from a local surgeon. One of the options discussed will be Bone marrow concentrate intradiscal injection under the context of the study. If the patient has any interest in participating in this study, the general risks, benefits and results from the previously published study for intradiscal BMC will be discussed [24,25]. Some of these patients may wish to undergo discography. The results of the discography help determine their candidacy for fusion surgery. Patients who have undergone discography at the request of an outside physician, typically a community neurosurgeon or orthopedic surgeon, will also be screened with the surgeon's approval.

It will be explained to all potential subjects that their participation in the study is strictly voluntary. Their participation will have no effect on their standing as a patient within their current practice. Nor will it have any effect on the physician-patient relationship. Their treatment course will remain unaffected if they choose not to participate in the study.

7B. Consent:
Consent form as a separate document submitted with this document.

7C. Obtaining and documenting consent
One of the investigators will perform the consent process. This will be verbal and written with ample time for any and all questions. The consent form will be signed by the patient and witnessed by one of the research assistants and/or one of the investigators. The document will be stored with other research materials in a locked cabinet when not in use.

7D. Participant comprehension and capacity
Measure to ensure appropriate understanding:
Prior to signing the consent form, the patient will be asked to briefly explain the study risks and benefits to one of the investigators.

7 E. Cost to participant
As previously mentioned in economic risk section:

This will be a funded study without cost to the participant other than co-pays and deductibles from commercial insurance for routine follow up visits.

Participants will be compensated for their time at the 3, 6, and 12 month visits with 30 dollars at each of the 3 visits.

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