Title:
Prospective, Randomized, Double-blind Clinical Trial to Investigate the Efficacy of Autologous Bone Marrow Aspirate Concentrate Post-Meniscectomy

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SPECIFIC AIMS

SPECIFIC AIM 1: To evaluate the effects of bone marrow aspirate concentrate (BMAC) on patient reported outcomes in patients undergoing meniscectomy.

SPECIFIC AIM 2: To evaluate the objective effects of BMAC injection as it relates to
A: Synovial fluid biomarkers
B: Radiographic evidence of arthritis

RESEARCH STRATEGY

SIGNIFICANCE

Both the medial and lateral knee menisci function to distribute pressure between articular surfaces and function as a secondary restraint.[1] Meniscal tears, either traumatic or degenerative, are commonly encountered clinical entities, which may cause mechanical symptoms and pain.[2-4] Between 2005-2011, over 380,000 partial or complete meniscectomies were performed in the United States alone.[5] However, this procedure can jeopardize the chondroprotective ability of the meniscus and trigger a cascade of chondral damage, which may ultimately lead to radiographic and symptomatic osteoarthritis (OA).[4,6]

Knee OA is one of the most common musculoskeletal diagnoses and a major cause of healthcare expenditure.[7,8] There is growing interest in autologous biologic interventions to potentially alter or arrest the progression of this disease, which would have major implications on healthcare cost and the burden of musculoskeletal disease.[9-11] These are advantageous over allogeneic sources that are accompanied by increased cost and decreased availability. Also, technologies that require cell expansion require FDA oversight, which is also a barrier to clinical use in the short-term. Autologous bone marrow aspirate concentrate (BMAC) is of particular interest due to the decreased cost

and improved availability, single stage nature of the procedure, the availability of pluripotent cells and the ability to limit immunogenic nucleated cells.[12-14]

BMAC injection post-meniscectomy has been shown to be a safe procedure with no major complications reported in the literature.[12-14] Furthermore, early registry data and clinical results of BMAC for the treatment of knee OA are promising.[15,16] To our knowledge, the proposed study is the first randomized clinical trial to prospectively investigate the effects of autologous BMAC administration in the post-meniscectomy patient.

INNOVATION

Recent studies have demonstrated both the safety of BMAC intra-articular injection and improvements in subjective, patient reported outcomes in patients with existing knee OA. Unfortunately these studies were largely uncontrolled, underpowered, and/or retrospective in nature.[12-16] Additionally, a recent prospective, randomized clinical study of allograft mesenchymal stem cells (MSCs) injected at a separate time point post surgical intervention has highlighted the ability of MSCs to increase meniscal volume and improve knee pain following injection.[17]

This will be the first study to examine the effects of autograft BMAC intra-articular injection in a single-stage procedure and in a prospective, randomized, double-blind fashion. The results of this study, if the null hypothesis is rejected, will have far-reaching implications for the standard of care in meniscal treatment and on OA progression in the knee. Additionally, if the results of this study are favorable in reduction of OA progression this study will change the surgical approach to all axial, synovial joints including the shoulder, elbow, wrist, hip, and ankle.

APPROACH

Patients identified as having meniscal tears needing treatment with meniscectomy will be identified and informed consent will be obtained if inclusion criteria are met. All patients will undergo the clinically indicated meniscectomy procedure, and immediately following the closure of arthroscopic portals the clinician will perform the skin incision and create the marrow access channel in the proximal iliac crest for all patients. The experimental group will then have bone marrow harvested and BMAC will be prepared using a BMAC harvesting system. The automated centrifuge system rapidly concentrates cellular contents and growth factors in bone marrow aspirate using flow cytometry. The BMAC will be injected intra-articularly. The bone marrow removal and injection will add approximately 5-10 minutes of additional time to the scheduled meniscectomy procedure. All subjects will remain under anesthesia for the duration of the meniscectomy, bone marrow aspiration, and injection. The injection will not require

any additional recovery time from the procedure for the subjects. The control group will have the an equivalent volume of saline injected into the affected knee. The addional recovery time will be added for the control group as well.

Study Design and Power Analysis
Prospective, randomized, double-blind clinical trial.

Based on prior studies of the same primary outcome measure, IKDC,[18] a sample size of 21 in both study groups was selected. A sample size of 21 per group will insure 80% power to detect a difference between groups if the probability is .75 that an observation in one group is less than an observation in the other group with a .05 significance level. The total sample size would thus be 2 x 21 = 42. The study will enroll 50 subjects per group (100 total) to match the size of an ongoing BMAC rotator cuff repair trial and to elicit any differences that are smaller than expected.

Eligibility
All patients presenting with symptomatic meniscal pathology will be screened for potential enrollment. The following inclusion/exclusion criteria must be met:

Inclusion Criteria
- Subject is greater than 18 years old
- Written informed consent is obtained
- Subject is determined to have a symptomatic meniscal tear requiring a meniscectomy
- Meniscal pathology is confirmed through MRI and arthroscopically
- Subject agrees to all follow-up evaluations
- Osteoarthritis Kellen-Lawrence grade 1-2 on flexion PA and extension AP views

Exclusion Criteria
- Any subject lacking decisional capability
- Unwillingness to participate in the necessary follow-up
- Subjects who are pregnant or may become pregnant.
- History of diabetes mellitus
- History of rheumatoid arthritis or other autoimmune disorder
- History of solid organ or hematologic transplantation
- Diagnosis of a non-basal cell malignancy within the preceding 5 years
- Infection requiring antibiotic treatment within the preceding 3 months
- Osteoarthritis Kellen-Lawrence grade 3 or 4 on flexion posteroanterior (PA) or extension anteroposterior (AP) views
- Prior surgery on the index meniscus
- Concomitant surgery such as ligament surgery or cartilage repair or restoration
- Infection
- Prior cortisone/viscoscupplemtation/PRP injection within 6 weeks

Enrollment Process and Schedule of Events:
Any patient who consents to an arthroscopic meniscectomy for the treatment of symptomatic meniscal pathology will be initially screened for this study. The following procedure will be followed when enrolling a study subject:

1. The informed consent document will be reviewed with the patient and all questions answered.
2. Inclusion/Exclusion criteria will be met.
3. Preoperative, baseline questionnaires will be completed and physical examination will be documented.
4. Preoperative MRI will be conducted if not done so already based on clinical necessity.
5. Subjects will undergo arthroscopic meniscectomy. Articular cartilage status will be documented.
6. An incision will be made and marrow access will be obtained in the iliac crest of all subjects.
7. Once the hole is drilled the randomization envelope is opened and the surgeon will perform BMAC harvest and injection in the experimental group or perform the saline injection in the control group.
8. The examiner of objective parameters postoperatively will be blinded to the experimental group of the patient.

Endpoints:
Primary Endpoint:
1. One year post-meniscectomy: IKDC score

Secondary Endpoints:
1. Patient reported outcomes throughout follow-up period
2. Synovial fluid analysis at 6 and 12 weeks
3. 2 Year Radiographic analysis

Outcome Measures

Patient Questionnaires
The effect of BMAC administration will be measured with validated outcome measures, serial magnetic resonance imaging (MRI) and change in synovial fluid markers of OA. The patient reported outcome measures will include change in scores from preoperative to postoperative Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Visual Analog Score (VAS), International Knee Documentation Committee form (IKDC), Knee injury and Osteoarthritis Outcome Score (KOOS) and SF-12.

Radiographic Analysis
Progression of OA will be in part determined by MRI assessment of the cartilage and in part by comparison of K-L grades. The degree of OA will be examined in all patients preoperatively and at subsequent follow-up visits at 1 and 2 years. The OA grade will be

determined using the guidelines outlined by Kellgren and Lawrence, including the formation of osteophytes and joint space narrowing.[21]

Biomarkers
BMAC from each subject in the experimental cohort will be analyzed via ELISA assay to test for the presence/absence and quantity growth factors and to test for cell surface receptors, including CD 34 and CD 105.

At the time of diagnostic arthroscopy, a synovial fluid aspiration will be performed prior to knee arthroscopy. The patient will then undergo routine knee arthroscopy per the usual protocol. Synovial fluid comparisons of biochemical markers of cartilage inflammation and degradation will be made from preoperative (intraoperative aspiration prior to incision for arthroscopy) and postoperative aspiration performed at the time of surgery (baseline) and in the clinic at 2 weeks and 12 weeks. Synovial fluid analysis will include enzyme-linked immunosorbent assay (ELISA) to measure for approximately 20 cytokines and cartilage specific biomarkers. These cytokines and biomarkers will include: BMP-2, BMP-7, FGF-2, FGF-18, IGF-1, PDGF, TGF-β, IL-1a, IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α, Bcl-2, Bcl-XL, iNOS, SOX9, SMADs, EGF, IFN, MIP, MCP, TIMP-1, OPN, MMP-1, MMP-3, VEGF, PDGF, and RANTES[22]. Synovial fluid aspirations will be placed directly into red top tubes and be frozen for batch analysis at a future date.

Safety Measurements/Parameters:
Adverse events reporting at each clinical evaluation and time point
Physical examination at enrollment and at each time point

Schedule of Events

Event	Pre-Procedure ≤ 21 days	Procedure Day	Post-Operative Week					
			2	6	12	24	52	104
Informed Consent	x							
Demographics	x							
Confirm Inclusion/Exclusion Criteria	x	x						
Medical History	x	x						
Physical Examination	x	x	x	x	x	x	x	
Vital Signs	x	x	x	x	x	x	x	
Radiographs Standard Views (4 views)	x						x	x

Radiographs Mechanical Axis	x							
Questionnaires (WOMAC, KOOS, IKDC, VAS, SF-12, Tegner-Lysholm)	x	x	x	x	x	x	x	x
Randomization		x						
Synovial Fluid Testing		x	x		x			
BMAC Harvest		x						
Injection		x						

Table 1. Outline of pre- and postoperative timing of events and schedule of data collection.
*Note: The grace period for clinic visits for week 2 will be 4 days, for weeks 6-12 the period will be 7 days, and from 24 weeks it will be 14 days.

Statistical Analysis:
A combination of the chi-square test, paired T-test and Fisher's exact test will be implemented to compare the two cohorts depending on nominal or categorical data. For non-categorical data, the Friedman and Kruskal-Wallis tests will be used to compare the groups over time. Categorical, normally distributed data will be compared over time using the two-factor repeated-measures ANOVA. Linear univariate and multivariate regressions will be used to investigate the relationships between demographic data and outcomes. All tests will be two-tailed in nature. Statistical significance will be set at 0.05 for all testing.

PROJECT TIMELINE

Prepare a proposed timeline for each of the projects specific aims, demonstrating progress expected at 6, 12, 24, and 36 months using the table below.

	6 months	12 months	24 months	36 months
Specific Aim 1	Complete patient enrollment and preoperative data collection. Collect postoperative data to the 6-month time point. Analyze synovial fluid between preoperative and postoperative time points.	Collect all data up to 1-year as outlined in Table 1.	Complete final radiographs and patient questionnaires.	Compare all outcome measures between BMAC experimental cohort and saline injection control cohort. Complete data analysis. Submit preliminary data to conferences and write manuscript
Specific Aim 2	Complete patient enrollment and preoperative data collection.		Complete meniscal volume data analysis. Submit prelimary data to conference and write manuscript.	Complete manuscript.

BIBLIOGRAPHY AND REFERENCES CITED

Provide a bibliography of any references cited in the Research Plan. Each reference must include names of all authors (in the same sequence in which they appear in the publication), the article and journal title, book title, volume number, page numbers, and year of publication. Include only bibliographic citations. Follow scholarly practices in providing citations for source materials relied upon in preparing any section of the application. The references should be limited to relevant and current literature. While there is not a page limitation, it is important to be concise and to select only those literature references pertinent to the proposed research.

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