Study Protocol:

Prospective, Randomized, Single Blind Clinical Trial to Investigate the Impact of Autologous Bone Marrow Concentrate in Knee Osteochondral Allograft Transplantation

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Principal Investigators: Brian J. Cole, MD, MBA, and Adam B. Yanke, MD

Co-Investigators: Susan Chubinskaya, PhD

Study Coordinator: Mukesh Ahuja

Abstract:
Articular cartilage injuries in the knee continue to grow in number as detection and treatment options have advanced. Treatment options, including microfracture, autologous chondrocyte implantation, osteochondral grafting and meniscus transplantation can hopefully deter the progression of degeneration and have promise to function as disease modifying solutions. Osteochondral allograft transplantation (OCA) has emerged as a preferred method of treating large focal chondral defects as it structurally replaces the cartilage and often involved subchondral bone with native hyaline cartilage and bone. The results of OCA are successful with greater than 85% survival at 5-year follow-up[1]. Failure can occur due to a lack of boney integration or low chondrocyte viability[2,3]. Therefore, anything to enhance the graft augmentation process may be useful in preventing failure. The purpose of this prospective, randomized study is to determine the effect of bone marrow aspirate concentrate (BMAC) containing bone marrow derived mesenchymal stem cells on improving graft incorporation and preventing failure. The effect of BMAC on the graft and intraarticular knee environment will be evaluated using 3 techniques: CT imaging, synovial fluid cytokine analysis, and serum biomarker analysis. Information learned from this study can be used to biochemically compare treatment response and to assess emerging therapeutic options that may positively alter the biochemical environment in patients undergoing osteochondral allograft transplantation.

Statement of Clinical Relevance:
Treatment of articular cartilage injuries in the knee is continuing to evolve as we recognize systemic and local factors that promote growth of cartilage. However, given the varying nature of these injuries, with significant differences in depth, size, and location, determination of the best treatment can be a difficult decision for the surgeon. Based on these factors, treating physicians use varying techniques including microfracture, autologous chondrocyte implantation, and osteochondral grafts as treatments for cartilage defect. These treatment options all aim to restore cartilage and healing. Osteochondral allograft transplantation involves the implantation of an allograft plug containing native hyaline cartilage and underlying bone. The cartilage survival relies on chondrocyte viability and, over time, bone integration occurs by a process of creeping substitution. Failure can occur either to a lack of chondrocyte viability or failed bone incorporation. Anything to augment these two factors, may help prevent failure and improve patient outcomes.

Additionally, the biochemical and inflammatory environment has a major effect on the development, maturation, and healing in cartilage repair. Our goal is to establish if mesenchymal stem cell augmentation improves graft incorporation and to analyze the cytokine environment of the joint after OCA with and without intraarticular BMAC injection. Information learned from this study can be used to biochemically compare treatment response and to assess emerging therapeutic options that may positively alter the biochemical environment in patients who suffer from articular cartilage disorders.

Specific Aims:

1. To evaluate the effects of BMAC on graft incorporation in patients undergoing osteochondral allograft transplantation in the knee
2. To measure the amount of cytokines and cartilage biomarkers in the synovial fluid of patients undergoing osteochondral allograft transplantation with and without BMAC augmentation at different time points, including 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 .
3. To determine changes in serum biomarkers of cartilage turnover over the course of osteochondral allograft transplantation with and without BMAC augmentation
4. To evaluate patient reported outcomes after osteochondral allograft transplantation with and without BMAC augmentation
5. To correlate changes in inflammatory and cartilage biomarkers to clinical patient reported outcomes

The goal of this study is to investigate the effect of BMAC augmentation on graft incorporation after osteochondral allograft transplantation in the knee. Additionally, using serum analysis of a cartilage biomarker, COMP, we can determine if this marker can be used in the future to assess patients following the treatment of articular cartilage disease. We hypothesize that BMAC augmentation will improve graft incorporation

Research Strategy:

Significance:

Procedures for articular cartilage lesions in the knee continue to grow in number. While the true incidence of full-thickness articular cartilage lesions is difficult to define, in athletes, studies have reported an average incidence of 14%[4]. Impaction injuries or significant injuries increasing the force in the joint may cause injury to the cartilage, leading to gross disruption or injury at the cellular level that can later lead to macroscopic change[5]. For some patients, chondral injuries cause significant pain, especially with weight bearing, and swelling.

Chondral injuries vary widely in their depth, size, and location with several treatment options available based upon various evidence-based treatment algorithms. Osteochondral allograft transplantation is typically performed for large, symptomatic chondral defects. The success of this procedure is dependent on integration of the graft into the recipient femur, which may be able to be augmented by the use of autologous bone marrow derived stem cells. Additionally, little is known about the biochemical mediators that influence cartilage healing. The biochemical and inflammatory milleu has a major effect on the development, maturation, healing, and chemical environment in the joints of patients undergoing cartilage repair. Chrondrocytes and synoviocytes in the joint both secrete substances and cytokines, which influence each other and affect the overall joint environment.

Innovation:
Bone marrow aspirate concentrate is known to contain osteoprogenitor cells and osteoconductive proteins which may augment bone healing. Previous animal studies have shown that BMAC accelerates bone integration as an adjuvant therapy[6,7]. Additionally, a recent study of a retrospective cohort used radiographs to evaluate graft incorporation and found a significant improvement at 3 months post-operatively OCA patients who received a BMAC injection[8]. Augmentation of OCA with BAMC may help prevent failure due to a lack of graft incorporation.

Numerous studies have analyzed synovial fluid after anterior cruciate ligament (ACL) injuries focusing on the cytokine environment after injury and following surgery[9-11]. Synovial fluid has also been analyzed during diagnostic arthroscopy before any cartilage intervention. Vasara et al. found an increase in matrix metalloproteinase-3 (MMP-3) and insulin like growth factor-I (IGF-I) associated with cartilage lesions[12]. Within 1 year after ACI, levels of MMP-3 and IGF-I remained elevated, even though the lesions were filled with repair tissue[12]. Cuellar et al. similarly evaluated the correlation of synovial fluid markers with symptoms in patients undergoing knee arthroscopy[13]. They found a strong correlation between MCP-1 and IL-6 in patients with severe cartilage lesions, whereas IL-1Ra was inversely related[13]. Schneider et al. assessed the presence of molecular markers following ACI surgery including pyridinium crosslink (PY), deoxypyridinolin (DPD), n-telopeptide (NTX) from type I collage, MMP-1, MMP-3, TIMP-1, PICP, proteoglycan, and YKL-40 at 4 times points[14]. They found that DPD continuously increase after surgery, however all molecular markers for cartilage degradation increased initially then dropped off around 3 months[14]. To our knowledge, the impact of BMAC in conjunction with cartilage restoration procedures on synovial fluid contents has not been investigated.

Our goal is to investigate the enhancement on osteochondral allograft incorporation with the use of BMAC. Additionally, we want to define the cytokine environment of the joint before and after OCA with and without BMAC augmentation. With this information, consideration for therapeutic options such as BMAC augmentation or the exogenous addition of hyaluronic acid, IL-1Ra, IL-4, or IL-10 might positively impact the post-operative outcome of patients undergoing osteochondral allograft transplantation.

Approach:

Inclusion Criteria:

1. Patients aged 18-50 with a cartilage defect indicated for treatment with osteochondral allograft

Exclusion Criteria:

1. Patients with known rheumatoid arthritis, any other inflammatory arthropathy or synovial tissue disorder.
2. Patients with known bipolar osteoarthritis of the knee as determined by the treating physician, greater than Kellegren-Lawrence Grade 3 on xray imaging
3. Patient with a known infection or history of infection in the affected knee

All patients meeting inclusion and exclusion criteria will be offered enrollment. This study will include patients undergoing surgery with Drs. Brian Cole and Adam Yanke. All patients will be evaluated in the office setting. Upon clinical evaluation and MRI suspicion of a cartilage defect, patients will be offered enrollment in the study. Patients will then undergo routine care, including a diagnostic arthroscopy followed by a cartilage restoration procedure at a later date. A synovial fluid aspiration will be performed prior to knee arthroscopy.

The patient will then undergo their definitive osteochondral allograft treatment based on standard clinical protocol. At that time, a synovial fluid aspiration will be performed prior to beginning the surgical procedure. Defect size and location will be recorded. Patients will be randomized to the BMAC or control group. If in the BMAC group, a bone marrow aspiration will be performed from the iliac crest or proximal tibia depending on surgeon preference. The bone marrow aspirate will be processed using a BMAC harvesting system which concentrates the aspirate using flow cytometry to isolate the mesenchymal stem cells and growth factors. The osteochondral allograft plug will then be soaked in the BMAC for a minimum 2 minutes prior to implantation. The remaining BMAC will be placed in the defect site prior to plug implantation. The control group will receive a 0.5cm sham incision over the iliac crest, but bone marrow aspiration will not be performed.

Bone marrow aspirate and bone marrow aspirate concentrate samples will be collected for analysis by flow cytometry to evaluate stem cell concentrations.

Patients will then be seen in the office setting at the normally scheduled follow-up visits at 2 weeks, 6 weeks, 6 months, and 12 months and routine radiographs will be obtained at those time points. Synovial fluid aspirations of the knee will be repeated at the following time points: 2 weeks, 6 weeks, 6 months, and 12 months after surgery.

Patients will also be asked to have a computed tomography (CT) scan of their operative knee 6 months post-operatively to evaluate graft integration.

Data collected preoperatively will include age, gender, laterality, inciting event, body mass index, smoking status, operative history on the operative extremity, and any history of trauma to the affected knee. The synovial fluid aspirates taken from the times points (during staging knee arthroscopy, pre-cartilage restoration procedure, 2 weeks postoperatively, 6 weeks postoperatively, 6 months postoperatively, and 12 months postoperatively) will then be analyzed for approximately 20 cytokines and cartilage specific biomarkers using biomarker assays. 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[15].

Additionally, at 2 time points (cartilage restoration procedure and 6 weeks postoperatively) serum will be collected for analysis of cartilage oligomeric matrix protein (COMP)[16-18]. COMP is a component of the articular cartilage extracellular matrix that is synthesized by chondrocytes. It has been suggested to be associated with cartilage turnover and may be sensitive biomarker of chondrocyte differentiation and cell turnover.

Patients will be administered validated clinical questionnaires including a visual analog scale (VAS), Knee Injury and Osteoarthritis Outcome Score (KOOS), and Lysholm scale at 7 clinical time points: diagnostic staging knee arthroscopy, cartilage restoration procedure, 2 weeks postoperatively, 6 weeks postoperatively, 6 months postoperatively, 12 months postoperatively, and 24 months postoperatively. Patients will also be administered IKDC, Marx activity scale, and VR/SF12 at 4 clinical time points: cartilage restoration procedure, 6 months postoperatively, 12 months postoperatively, and 24 months postoperatively.
All portions of this study will be part of conventional care with the following exceptions: (1) Synovial fluid aspirations at 6 time points, 2 intraoperatively while the patient in under anesthesia and 4 in the outpatient office setting, (2) serum collection at 2 time points, (3) and CT imaging at 6 months post-operatively. Knee aspirations and injections are routinely performed in the outpatient office setting using sterile technique.

Schedule of Events

Table 1. Outline of pre- and postoperative timing of events and schedule of data collection.

Event	Pre-Procedure ≤ 21 days	Diagnostic Arthroscopy	Cartilage 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	x	
Vital Signs	x	x	x	x	x	x	x	x	
Radiographs Standard Views (4 views)	x			x	x	x	x	x	
Radiographs Mechanical Axis	x								
CT							x		
MRI	x								
Questionnaires (KOOS, VAS, Lysholm)	x	x	x	x	x	x	x	x	
Questionnaires (IKDC, Marx, VR/SF-12)			x				x	x	
Randomization			x						
Synovial Fluid Testing		x	x	x	x		x	x	
Serum Testing			x		x				
BMAC Harvest			x						
Injection			x						

*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.

Sample Size Determination:
Sample size determination will be based off past retrospective studies that osteochondral allograft integration. Oladeji et al. used radiographs to compare retrospective sample populations of 17 osteochondral allografts without BMAC and 29 osteochondral allografts with BMAC[8]. Brown et al. used CT 6 months post-operatively to evaluate graft incorporation in 34 patients receiving osteochondral allografts.[19] As this is a pilot study looking at the effect of BMAC on graft incorporation evaluated by CT, we will aim for a target population of 20 patients in each group.

Timelines:

The primary outcome of this study will by synovial fluid analysis of cytokines and biomarkers at 5 time points. Our target sample size will be 40 patients (20 in each group). At our institution, approximately 5 cartilage restoration procedures are performed monthly. Given a 75% recruitment into the study, we would be able to achieve the target sample size within 12 months.

Specific Aim 1: To establish the effect of graft incorporation of BMAC on osteochondral allograft transplantation in the knee.

Specific Aim 2: To assess the level of inflammatory cytokines and cartilage biomarkers in the synovial fluid of patients undergoing osteochondral allograft transplantation with and without intraarticular BMAC injection

- 6 months: Recruit and collect data from 20 patients, half the target population
- 12 months: Recruit and collect data from an additional 20 patients, completing the study

Specific Aim 3: To assess changes in serum biomarkers of cartilage turnover in the perioperative period in patients undergoing osteochondral allograft transplantation with and without intraarticular BMAC injection

- 6 months: Recruit and collect data from 20 patients, half the target population.
- 12 months: Recruit and collect data from 20 patients, completing the study

Specific Aim 4: To evaluate patient reported outcomes after osteochondral allograft transplantation with and without BMAC augmentation

- 6 months: Recruit and collect preoperative data from 20 patients, half the target population
- 12 months: Recruit and collect preoperative data from 20 patients, completing the study target population

Specific Aim 5: To correlate changes in inflammatory and cartilage biomarkers to clinical patient reported outcomes in patients undergoing osteochondral allograft transplantation with and without intraarticular BMAC injection

- 12 months: Once all patients have been recruited and synovial samples analyzed, the final portion of the project will be to correlate changes in inflammatory and biomarkers to patient reported outcomes collected at the 5 time points.

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