COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD

COMIRB PROTOCOL

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Project Title: "Intra articular injections with platelet rich plasma in patients with Juvenile Osteochondritis Dissecans of the knee: Does it Help? A clinical and MR study. "

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1. Hypothesis:

Intra articular injections with autologous platelet rich plasma (PRP) will improve symptoms, recovery time, and imaging appearance using delayed gadolinium enhanced MRI of cartilage (dGEMRIC) or Non-contrast MRI in patients suffering from stable juvenile osteochondritis dissecans of the knee when compared with conservative therapy.

2. Specific Aims:

In adolescents with osteochondritis dissecans of the knee:

Aim 1. Compared to conservative therapy alone, determine whether intra-articular platelet injections combined with conservative therapy lead to greater cartilage healing.

> **Efficacy Endpoint** – Measurement by glycosaminoglycan (GAG) content (T1GD) on dGEMRIC, or by healing changes of MRI diagnostic cartilage sequences, using the DiPaola classification system.

Aim 2. Compared to conservative therapy, determine whether intra-articular platelet injections lead to improvements over time in:

> **Efficacy Endpoint 2.1**. Pain, as measured by the pain subscale of the KOOS-child.
>
> **Efficacy Endpoint 2.2.** Performance of activities of daily living, as measured by the ADL subscale of KOOS-child.
>
> **Efficacy Endpoint 2.3.** Sport performance, as measured by the sports subscale of the KOOS-child.
>
> **Efficacy Endpoint 2.4.** Overall function and symptom reduction, as measured by the pedi-IKDC.

Aim 3. Correlate dGEMRIC findings or MRI diagnostic cartilage sequence findings on imaging studies with measures of functional recovery and symptom reduction.

Efficacy Endpoint – statistical evaluation of outcomes between the two patient populations

3. Background:

a. Osteochondritis dissecans:

Osteochondritis dissecans (OCD) is an osteochondrosis characterized by osseous necrosis followed by reossification and healing. There are both juvenile and adult forms of this condition. The most common anatomic location for this to occur is the knee, with the most frequent involvement being the medial femoral condyle. This can also occur in the elbow, ankle, or hip. The etiology is unknown though repetitive micro trauma and or ischemia are postulated as possible inciting factors. MRI is frequently used in the diagnosis of osteochondral lesions associated with OCD. Lesions can be stable or unstable. Stable lesions in patients with open physis, juvenile form, are typically treated conservatively, while unstable lesions traditionally require surgical intervention. The adult form is similar, though the patient's physis are fused. This process affects adolescent males three times more than females.

b. Autologous platelet-rich plasma (PRP):

The therapeutic use of autologous platelet-rich plasma (PRP) constitutes a relatively new biotechnology that has been a breakthrough in the stimulation and acceleration of soft-tissue, bone and cartilage healing [1-3]. The application of PRP has been extended to many different fields, including orthopedics, sports medicine, dentistry, cosmetic and periodontal medicine, plastic, and maxillofacial surgery [4]. The efficiency of this process lies in the local and continuous delivery of a wide range of growth factors and proteins, mimicking the needs of the physiological wound healing and reparative tissue processes [4].

In this process a preparation rich in growth factors (PRGF) combines the advantage of an autologous fibrin clot that will aid in hemostasis as well as provide growth factors in high concentrations to the site of a tissue defect. The platelet-rich plasma preparation encourages the release and slow delivery of growth factors from harvested platelets, activated by endogenous thrombin, and is used as a biological enhancer in the healing of fractures, lumbar fusions, cartilage defects, muscle tears, and tendon lesions, thus promoting initiation and early maturation of bone and soft tissue formation [1, 2, 4-7] which involves a more physiologic repair with less scar tissue[7].

Growth factors offer promising treatments for enhanced regeneration of cartilagein focal articular cartilage defects or in situations of more widespread cartilage loss such as those that occur in osteoarthritis (OA). Numerous anabolic growth factors stimulate chondrocytes synthesis of proteoglycans, aggrecan, type II collagen, induce synoviocyte and mesenchymal stem cell (MSC) proliferation, drive chondrogenic differentiation of MSCs, and decrease the catabolic effects of cytokines such as interleukin-1 (IL-1) and the matrix metalloproteinases (MMP) [3].

The source of the new PRP preparation consists of a limited volume of plasma enriched in platelets, which is obtained from the patient. Once the platelet concentrate is activated a myriad of growth factors and proteins are released, progressively, into the local environment, contributing to the accelerated postoperative wound healing, tissue repair, and vascularization [3, 8].

The application of PRP in cartilage repair is relatively new and therefore limited publications are available investigating its use. Chondrocytes and MSCs exposed to PRP both have increased cell proliferation and cartilage extracellular matrix synthesis of proteoglycans and collagen type II compared with controls [9]. Synoviocytes from patients with OA cultured in PRP demonstrated increased hyaluronic acid production and secretion, suggesting that PRP could potentially serve as an endogenous source of chondroprotection and joint lubrication after intra-articular application [10]. In a rabbit model, osteochondral defects were treated with either autogenous PRP in a poly-lacticglycolic acid (PLGA) carrier, PLGA alone, or left untreated [11]. The PRP group demonstrated a higher extent of cartilage regeneration as well as an increased production of the glycosaminoglycans in the ECM.

In a cohort of 30 patients comparing injections of PRP with hyaluronic acid (HA) in the management of OA, the success rate for the pain subscale reached 33.3% for the PRP group compared with 10% for the HA group ($p = 0.004$)[12]. Additionally, the percent reductions in the physical function subscale and overall WOMAC at 5 weeks were also associated solely with treatment modality in favor of PRP with $p = 0.043$ and $p = 0.010$, respectively.

Kon et al. treated 115 knees of patients with four intra-articular PRP injections given every 21 days and followed the patients for 12 months [8]. Patients evaluated in this study included 58 with degenerative chondral lesions (Kellgren-Lawrence 0), 33 with early OA (Kellgren- Lawrence I–III), and 24 with advanced OA (Kellgren- Lawrence IV). A substantial improvement in International Knee Documentation Committee (IKDC) and EuroQol (EQ-VAS) scores was noted at the end of therapy and at both the 6- and 12-month time points. The IKDC subjective scores as well as the EQ-VAS scores also demonstrated major improvements at the end of therapy. The authors concluded that treatment with PRP is safe and effective at improving pain, function, and quality of life in patients with degenerative articular pathology.

A separate study by our sub-investigator, Dr. Mei-Dan showed that osteochondral lesions of the ankle treated with intra-articular injections of PRP and HA resulted in a decrease in pain scores and an increase in function for at least 6 months, with minimal adverse events. Platelet-rich plasma treatment led to a significantly better outcome than HA.[9]

Recently, a randomized control trial (RCT) evaluating the effect of PRP on early osteoarthritis of the knee showed significant clinical improvement at both 6 months and 1 year. Platelet-rich plasma seemed to result in no change by MRI per knee compartment in at least 73% of cases at 1 year. No adverse effect was reported with the use of PRP [13].

c. dGEMRIC (delayed gadolinium-enhanced MRI of cartilage)

Intravenous injection of gadolinium allows for rapid penetration of contrast agent into the articular cartilage due to penetration both from the synovial fluid as well as the subchondral bone. The patient needs to move the joint after injection and the dGEMRIC imaging needs to occur within a 30–100 minute time window after injection for a reliable biochemical assessment of the articular cartilage [14, 15].

Gadolinium based biochemical MRI in the form of dGEMRIC has been established as an important modality in the imaging of articular cartilage. [14-25]. dGEMRIC is sensitive to the glycosominoglycan (GAG) content in the cartilage. When the negatively charged gadolinium contrast is administered, there is an uptake in hyaline cartilage in an inverse proportion to the GAG content [17]. Degenerated cartilage contains less overall quantity of GAG, therefore the concentration of gadolinium is high compared to the relatively low amount of gadolinium that accumulates in healthy cartilage. Consequently, T1 measurements performed post gadolinium injection can give an indirect reflection of GAG content of cartilage, due to the fact that gadolinium decreases the T1 relaxation time [16, 25, 26].

d. Non-contrast MRI of the knee/lower extremity

A non-contrast Magnetic Resonance Imaging (MRI) of the knee is a diagnostic imaging procedure which utilizes magnets to produce detailed images of the knee structure. The delivery of contrast is not required for this imaging procedure.

e. Preliminary Studies:

The application of PRP in cartilage repair is relatively new and therefore limited publications are available investigating its use. Recently, a randomized controlled study comparing HA versus PRP in the short term treatment for symptomatic knee osteoarthritis showed plasma rich in growth factors showed superior short-term results when compared with HA in a randomized controlled trial, with a comparable safety profile, in alleviating symptoms of mild to moderate osteoarthritis of the knee [27]. Another study evaluating the effects of PRP in osteoarthritis of the knee together with MRI reported significant clinical improvements at 12 months post injection. According to MRI results, there was no change in the appearance of osteoarthritis 1 year after platelet-rich plasma therapy in 83.3% of all cases when compared with baseline (N = 12) with lateral femoral and tibial compartment involvement. Similarly, there was no change in 73.3% of the cases with medial compartment involvement, although these values did not reach statistical significance. The appearance of medial compartment osteoarthritis improved in 1 knee (6.7%) after 1 year. This is in contrast to some longitudinal studies that suggest an annual decrease of up to 4% to 6% of cartilage volume in knee osteoarthritis compartments. [13]

Recently, Gobbi et al evaluated the effect of PRP in 50 patients with osteoarthritis. At 12 months follow up, patients had improved clinical symptoms with no adverse effects reported [28].

In a pilot study of 100 patients with osteoarthritis of the knee receiving intra-articular PRP injections, favorable results with pain reduction and improved function were reported. Patients were followed up at 2, 6, 12, and 24 months. Statistically significant improvement was observed in all the variables[29].

Our Sub-Investigator Dr. Mei Dan et al evaluated the effect of intra-articular injections of PRP compared to HA in osteochondral lesions of the talus. Both groups significantly improved clinical symptoms. When comparing both treatments groups, PRP produced a significantly greater improvement than HA [9]. Minor discomfort and mechanical pressure were common reports at the injection site in both groups.

Battaglia et al recently reported the outcomes of injection of PRP for OA of the hip. Twenty patients, with a mean age of 52 years, who presented symptomatic OA of the hip were treated with a total of 3 intrarticular injections of PRP. Patients were prospectively evaluated at 1, 3, 6, and 12 months follow up. All patients had significantly improved clinical symptoms. No major complications or adverse events occurred at the moment of injection or in the follow-up period. Ten patients reported a slight pain during or after the injection, which spontaneously resolved in 1 or 2 days [30].

Gillis et al evaluated autologous chondrocytee transplantation in patients with full thickness chondral lesions of the knee utilizing dGEMRIC to indirectly quantify the GAG content of cartilage at the site of transplant, adjacent to the site of transplant, and distant to the site of transplant. The study demonstrated that GAG levels in grafts that had been implanted at 12 months or greater had GAG levels comparable to adjacent and remote cartilage. [31]

Typically, patients with Juvenile Osteochondritis Dessicans will heal without treatment within a 12 month period, therefore it was recently decided that the research dGEMRIC MRI would be performed 6 months from the time of the consent instead of 12 months. [32]

4. Research Methods

We propose a randomized controlled clinical trial in children (aged 10-17) with osteochondritis dissecans (OCD) comparing the use of platelet-rich plasma (PRP) therapy and conventional therapy compared to conventional therapy alone.

The goal of this research is to test the hypothesis that intra articular injections with autologous PRP will improve symptoms, recovery time, and imaging appearance using delayed gadolinium enhanced MRI of cartilage (dGEMRIC) or MRI diagnostic cartilage sequence findings in patients suffering from stable juvenile OCD of the knee when compared to conservative therapy. Specifically, we aim to determine whether intra-articular platelet injections when compared to conservative therapy over time, lead to greater cartilage healing (measured by gadolinium accumulation or measured by healing on Non-contrast MRI, utilizing the DiPaola classification system), improvement in pain, improvement in performance of activities of daily living, improvement in sport performance, and improvement in overall function and symptom reduction.

Finally, we aim to determine if dGEMRIC findings or MRI diagnostic cartilage sequence findings on imaging studies correlate with measures of functional recovery and symptom reduction.

All protocol procedures other than patient randomization, PRP injections, and the 6 month dGEMRIC or Non-Contrast MRI are considered routine standard of care procedures. These three procedures are being conducted for research purposes in this study.

Description of Population to be Enrolled:

This study aims to enroll 24 subjects, 12 patients for each of the two treatment arms, all with documented stable juvenile osteochondritis dissecans identified via imaging. We believe 24 patients could be included during the time period allotted for the study based on the number of patients suffering from OCD seen annually at the Children's Hospital Sports Medicine Clinic.

Patient Recruitment

A diagnostic knee MRI (through CHCO or an outside facililty) will have been performed as standard of care prior to study participation, utilizing dGEMRIC, a Non-contrast MRI or a MRI with and without contrast in patients with a high clinical suspicion for osteochondritis dissecans. The study is posted on the clinicaltrials.gov website and patients and/or their parents may call and inquire about eligibility.

Lesions will be classified according to Di Paola et al classification [33]

This uses 5 stages as follows:

Grade 1: Small change of signal without clear margins of fragment,

Grade 2: osteochondral fragment with clear margins but without fluid between fragment and underlying bone,

Grade 3: fluid is visible partially between fragment and underlying bone,

Grade 4: fluid is completely surrounding the fragment, but the fragment is still in situ;

Grade 5: fragment is completely detached and displaced.

Only patients with Di Paola stage 1 or 2 lesions will be approached for study participation and consented into the study.

Pre-Screening Visit

Study subjects will be recruited from current patient population at Children's Hospital Colorado or first time patients of Dr. Dahab/ Dr. Nagle or their colleagues at the Children's Hospital Sports Medicine Clinic. They will be identified and recruited during routine office visits. Study subjects may also come directly to the PI/Research Associate through the clinicaltrials.gov website. The initial MRI scan, initial x-rays, and any follow-up x-rays will be performed as standard of care and will follow hospital policy, and they will be used to assess and confirm eligibility. Female patients included in the study, over the age of 12 who have begun menstruation will undergo a urine pregnancy test prior to the research MRI obtained at 6 months.

Inclusion & Exclusion Criteria

Patients will be recruited based on the following eligibility criteria:

Inclusion Criteria

1. Male or female age 10 - 17 inclusive with open physis confirmed by MRI
2. Documented symptomatic stable juvenile osteochondritis dissecans of the knee based on MRI without changes of osteoarthritis and no prior history of knee surgery. The MRI diagnosis must be within a 3 month time period prior to consent.
3. The patient must be able to hold still without sedation for approximately 1 hour and must pass MRI screening evaluation for retained metal.
4. Patients with Di Paola stage 1 or 2 lesions

Exclusion criteria

1. Patients with polyarticular disease (not applicable to polyarticular disease of the knees as the most symptomatic knee will qualify for the study)
2. Patients with blood disorders (Blood disorders (thrombopathy, thrombocytopenia, anemia with hemoglobin **<**9g/dL).
3. Patients who had intra-articular treatment with steroids within 3 months
4. Patients who are pregnant or nursing at the time of consent.
5. Patients with inflammatory arthritic conditions (e.g. rheumatoid arthritis)
6. Non-English speaking patients. (Scores used for evaluation have not been validated in Spanish)
7. Patients who had previous knee surgery
8. Additional disabilities in any of the lower limbs that would interfere with any of the clinical assessments.
9. Chronic use of NSAID (defined as taking NSAID regularly every week for the last 6 months), steroids or chemotherapy drugs
10. Treatment with NSAIDs within 15 days prior to randomization in this study
11. Patients with a BMI over 30. Due to the fact that this study utilizes an injection technique which may be inaccurate in obese subjects.
12. Patients with a contraindication to MRI including: patients with cardiac pacemaker or non-approved intracranial vascular clip, and those with orthopedic hardware as the resulting artifact can complicate interpretation
13. Patients with acute or chronic renal failure
14. Patients with a previous anaphylactic reaction to gadolinium enhanced MRI.
15. Patients with Di Paola stage 3 or 4 lesions
16. Patients who received a MRI diagnosis of OCD but do not have the specific cartilage imaging sequences.
17. Clinical or laboratory evidence of septicemia

Consent/Assent

Informed consent/assent will be obtained by the PI/ Pediatric Radiologist, Michael Fadell, MD, or his research staff, Sports Medicine physicians, Dr. Dahab/Dr. Nagle or their research staff.

Consent will be obtained in the Interventional Radiology suite, Radiology Fluoroscopy suite, physician office or other private room. A signed and dated copy of the consent/assent will be given to the subject. Patients will be required to sign an assent form. The parent or legal guardian will be required to sign a consent form. Subject comprehension will be assessed by asking the subject/subject family if they understand and are willing to participate. Patients will have the option to take the consent document home and review it before consenting to participate in the study. Patients will have the option to combine the consent visit (visit 0) with the baseline visit (visit 1).

Randomization

After consent, the study personnel will open a sealed envelope containing the randomization assignment. The envelopes will also contain the appropriate patient handouts and instructions. Randomization will be balanced within the study to allow equal assignment to each treatment group. The randomization envelopes will be prepared by Dr. Deborah H. Glueck, the study biostatistician or her designated staff, using a random balanced allocation randomization protocol.

A study ID participant list in REDCap will be filled out by Dr. Dahab/Dr. Nagle, their research staff or the Radiology Research Associate, Elizabeth Carroll, and will record the treatment assignment. The study personnel will e-mail the study statistician with the patient's study id number and the treatment assignment, recorded as 1 or 2. The study personnel will keep a key file containing the patients' names and study id numbers.

The study will not be blinded to the patient, to the patient's parent, or the physician administering the PRP injections. However, the study imaging and results will be kept blinded to the PI, Michael Fadell, Pediatric Radiologist and the study biostatistician until the blind is broken, shortly before manuscript submission. Thus, treatment assignment will simply be recorded as 1 or 2 for the datasets.

The informed consent will make it clear to the patient and the patient's parents that the child will be randomized to one of two treatments: platelet-rich plasma injection with standard of care, or standard of care alone. Some patients may not like their assignment. If the patient wishes to, the patient who does not like their assignment can at that point drop out of the study and proceed with whatever care they choose. Such randomization related dropouts will be recorded.

This participant list in REDCap will contains the following information: At a minimum, empty columns are provided to record the following:

- Subject name
- Medical record number
- Patient and parent email addresses
- Initials of person enrolling the subject
- Date of enrollment.
- Treatment assignment
- List of required surveys to indicate completion

Other empty columns can be added on request.

. When a subject is enrolled in the study, s/he is assigned a confidential study identifier by adding his/her name in chronological order, to the very next empty row available on the REDCap participant list. The subject maintains this study ID for the remainder of the trial and, thereafter, the Study ID is used on all research files, randomization Lists, and other research documents.

A research assistant, Dr. Dahab/Dr. Nagle, or Dr. Mei-Dan will fill out the assignment log and randomized assignment list.

Subject Study ID Assignment Log Sample:

Assignment log will go from study ID 1 to study ID 24. Each one will correspond to a randomized number. Table 1 below is an example of the excel sheet that will be used.

Table 1 – Subject Study ID Assignment Log Sample						
Randomization #	Subject Name	MR#	Study ID	Treatment Assignment	Date Randomized	Initials
CH1			1	PRP		
CH2			2	PRP		
CH3			3	PRP		
CH22			22	Conservative		
CH 23			23	Conservative		
CH 24			24	Conservative		

Once randomization is performed, a randomized assignment list will also be filled out. Each randomization number will correspond to either of two treatments (PRP-Conservative).

Randomized Assignment Sample List

Table 2 – Randomized Assignment List Sample	
Enrollment	Randomization

Study ID #	Subject Name	Subject MR#	Date Enrolled	Initials	Randomization #	Date Randomized	Initials
01-A					CH1		
02-B					CH2		
03-C					CH3		
04-D					CH4		
05-E					CH5		
06-F					CH6		

Subject ID Assignment Log

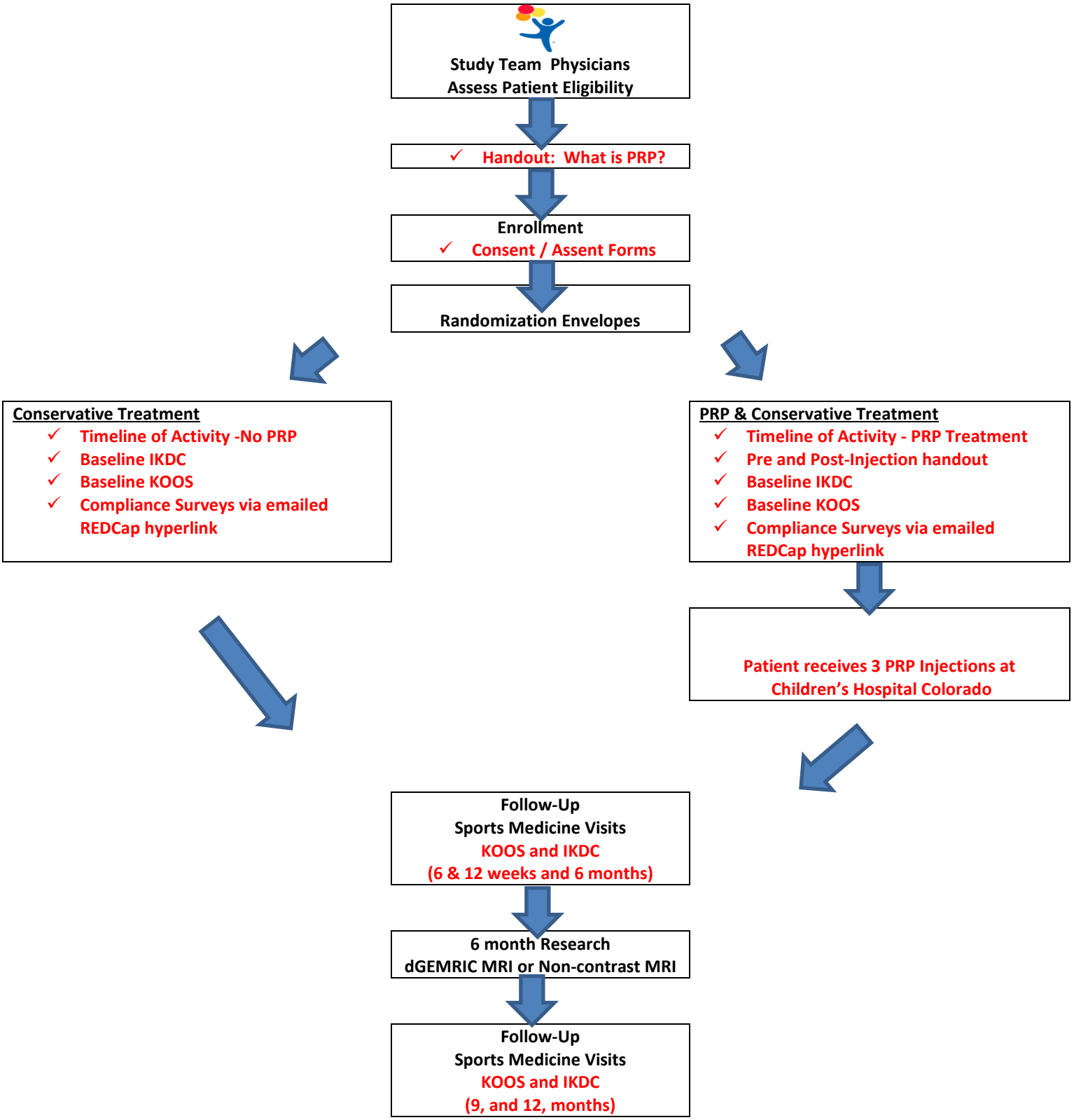
Randomization #: CH-F-1 Treatment Assignment: PRP

Study ID #: _____

Date envelope opened: __ __ /__ __ / __ __

Time opened: __ __ : __ __ (24 hour clock)

Signature of the person who is randomizing the subject: _____


Study Team Physicians
Assess Patient Eligibility
✓ Handout: What is PRP?
Enrollment
✓ Consent / Assent Forms
Randomization Envelopes
Conservative Treatment
✓ Timeline of Activity -No PRP
✓ Baseline IKDC
✓ Baseline KOOS
✓ Compliance Surveys via emailed REDCap hyperlink
PRP & Conservative Treatment
✓ Timeline of Activity - PRP Treatment
✓ Pre and Post-Injection handout
✓ Baseline IKDC
✓ Baseline KOOS
✓ Compliance Surveys via emailed REDCap hyperlink
Patient receives 3 PRP Injections at Children's Hospital Colorado
Follow-Up
Sports Medicine Visits
KOOS and IKDC
(6 & 12 weeks and 6 months)
6 month Research
dGEMRIC MRI or Non-contrast MRI
Follow-Up
Sports Medicine Visits
KOOS and IKDC
(9, and 12, months)

Treatment Regimens and Dosing

All eligible patients will be randomized to a treatment or conservative therapy group. Those within the conservative therapy group will be fitted with a hinged brace locked in extension, with weight bearing as tolerated and crutches for those with painful to weight bear, complete rest from impact activities, progressing to weight bearing as tolerated for 6 weeks, and then followed according to the Orthopedic department's protocol for OCD.

The PRP treatment group will receive three injections per randomization table into the symptomatic knee, they will also be fitted with a hinged brace locked in extension, with weight bearing as tolerated and crutches for those with pain upon weight bear, complete rest from impact activities with progression to weight bearing as tolerated for 6 weeks, and then followed according to the Sports Medicine department's protocol for OCD. Study subjects in the PRP treatment arm will receive a total of 3 intra-articular injections, one week apart +/- 10 days. All subjects will have a physical exam performed to assess their knee function.

If a patient has bilateral symptomatic knee osteochondritis dissecans, the more symptomatic side will be injected while the less symptomatic side will not be injected, the patient will then follow the same treatment regimen as the treatment group on the treated side.

PRP Dosing and Protocol

Several phlebotomy and processing kits are FDA approved for safe and rapid preparation of PRP, and are commercially available. Dose, preparation and regimen of intra-articular injections of PRP have been variable in the literature. Doses vary between one to three injections, given consecutively within one or two week intervals [30].

For this protocol, we will use the FDA approved Harvest PRP Procedure Pack kit (BK000037) from Terumo-Harvest. The Harvest PRP system is designed to be used for the safe and rapid preparation of autologous platelet-rich plasma (PRP) from a small sample of blood at the patient's point of care. Each Harvest PRP kit consists of components necessary to collect whole blood from the patient into a syringe containing anticoagulant and to separate the centrifuged sample into fractions (including the one containing platelet-rich plasma).

Each PRP preparation requires a total of 30 mL of peripheral blood. Blood will be obtained via direct venipuncture by radiology nursing staff, Michael Fadell or one of the study team phyisicians using the provided tourniquet and butterfly needle. Blood will be collected into a 30mL syringe containing 3mL ACDA. 30 mL of acid-citrate-dextrose Formula (ACD-A) anticoagulated whole blood will be processed according to the manufacturer's directions. In brief, the anticoagulated whole blood will be transferred into the first chamber, which has a floating shelf of a proprietary density. Following an initial centrifugation of 4 minutes, the supernatant, containing plasma and the cellular elements less dense than the floating shelf, will be decanted into the second chamber of the disposable and centrifuged for 10 minutes. The

PRP volume of 3 or 4 mL will be prepared using the appropriate spacer to remove the desired volume of the supernatant platelet-poor plasma (PPP). The PRP is resuspended in the remaining volume of PPP.

This PRP preparation process takes place in the Interventional Radiology suite, Flouroscopy suite or a Co-Investigator's clinic office.

All sites/clinics are amenable for sterile procedures.

Immediately prior to PRP injection into the study subject, calcium chloride is drawn up from the activator ampoule and will be added to the PRP. The activated PRP will then be injected in its entirety into the knee joint. All the preparation is performed under strict aseptic technique and follows the appropriate manufacturer instructions.

Harvest PRP kits and centrifuge are provided free of charge from the vendor, Harvest TERUMOBCT . The PRP kit/device is not intended to provide data to support the claim for the device but rather to study the clinical effect of the device.

PRP and Conservative Treatment Schedules Following Randomization

Patients will be assessed during a standard of care visit for basic eligibility to participate in this study or via a telephone call with one of the Study Team Physicians. Patients who meet the basic inclusion criteria will be given the option of participating in the study either at this first visit, or a subsequent visit after they are given the opportunity to review the informed consent documents.

PRP study participants: A total of 8 visits (9 visits, if consent is done as a separate visit) are expected during a 12-month time period for patient follow up. All initial visits will include a patient assessment using basic standard of care to determine eligibility for study enrollment. This first visit, if the patient decides to enroll, may also include patient consenting. The second through fourth visits, one week apart +/- 10 days, will include treatment with PRP injections. After the 3rd injection, subjects will be seen for clinical evaluation and survey assessment per schedule of assessment below as the standard of care. MRI obtained prior to enrollment for initial evaluation of the knee is performed as standard of care in the management of knee OCD. The research MRI performed at 6 months post treatment will be obtained to assess cartilage healing utilizing physiologic imaging of cartilage with the dGEMRIC technique or a Non-contrast MRI of the knee.

The three injection visits (1 through 3) will take approximately 30-45 minutes per visit. This includes time for the baseline patient reported outcomes and blood draw.

Follow up visits (5 through 9) last around 30 minutes and will include patient reported surveys, physical examination, and x-rays to assess the injury in accordance with the standard of care at CHCO.

Conservative treatment participants: A total of 5 visits (6 visits, if consent is done as a separate visit) are expected during a 12-month time period for patient follow-up. The first visit will include initial assessment and possible recruitment. The second through fifth visits will include patient reported surveys, physical examination and x-rays to assess the injury in accordance with the standard of care at CHCO. MRI obtained prior to enrollment for initial evaluation of the knee is performed as standard of care in the management of knee OCD. The research MRI performed at 6 months post treatment will be obtained to assess cartilage healing utilizing physiologic imaging of cartilage with the dGEMRIC technique or a Non-contrast MRI of the knee. **Please refer to the patient schedule matrix, Table 3, below.**

Informational patient brochure/Compliance Surveys/ Baseline and Follow-up KOOS and IKDC surveys/Clinical Assessments

An informational brochure (see attached) will be used to assist in the recruitment of eligible patients. The brochure will describe the autologous platelet rich plasma treatment, process, risks, appointment information and post procedure information.

Patients randomized to the PRP Treatment arm will receive a Pre and Post-Injection Instruction handout (see attached). The handout describes how the patient should prepare for the PRP injections and what to expect after the injection.

The study personnel who consents the patient will request the patient's and parent's email addresses which will be used to send out the weekly and monthly surveys.

For the first 6 weeks of the study, all patients will receive a weekly Compliance Survey (see attached) created by the Sports Medicine physicians to validate compliance with 1. non-weight bearing, 2. wearing a brace, 3. at home PT exercises, and 4. no high impact activity.

KOOS/IKDC surveys: All patients will receive a baseline survey and repeat surveys at 6 wks, 12 wks, 6 m, 9m, and 12 months, which is considered standard of care, on the CHCO main campus or one of the following satellite locations (South, Parker, Centennial, Uptown/St Joseph's or Broomfield. The surveys will include the following outcome scales: Pedi IKDC and KOOS-child.

The Pedi-IKDC is a modified version of the widely used International Knee Documentation Committee (IKDC) Subjective Knee Form, which is a knee specific measure of symptoms, function and sports activity. The Pedi-IKDC was developed for use with children and adolescents ages 10-17. The reliability and validity of the Pedi-IKDC has been demonstrated to be acceptable in young patients with a variety of knee disorders.

Similarly, the KOOS – child is a pediatric version of the Knee Injury and Osteoarthritis Outcome Score (KOOS). The KOOS-child score is subdivided and scored in five categories: Pain, Other Disease-Specific Symptoms, Activities of Daily Living Function (ADL), Sport and Recreation Function and Knee-related Quality of Life (QOL).

http://www.actaorthop.org/sup_files/5292.pdf

http://www.sportsmed.org/uploadedFiles/Content/Medical_Professionals/Research/Grants/IKDC_Forms/Pedi-IKDC%20Form.pdf

Clinical follow up will also be performed following injections or the commencement of conservative therapy at 6 weeks, 12 weeks, 6 months, 9 months, and 12 months.by Dr. Dahab/Dr. Nagle or their colleagues on the CHCO main campus or one of the following satellite locations (South, Parker, Centennial, Uptown/St Joseph's or Broomfield). The clinical findings documented will include presence or absence of a joint effusion, overall range of motion, presence or absence of joint line tenderness and positive or negative Wilson's test 6 week follow up for both groups will also include radiographic evaluation, which is standard of care. If

the patient is clinically or radiographically improving, physical therapy will be started as standard of care, following the same treatment protocol initially with closed kinetic chain, non-impact exercises. Patients will then slowly return to impact activities as tolerated over 3 to 4 months.

A patient Timeline of Activity has been created for each arm of the study (see two attachments). The Timeline describes the weekly and monthly events that will occur during this study and can be handed to the patient following the consent and randomization process.

6 month Research MRIs

- If the patient received their initial diagnostic MRI by a Non-contrast MRI, the patient will receive a Non-contrast MRI at their 6 month research MRI visit.
- If the patient received their initial diagnostic MRI by a dGEMRIC MRI, the patientwill receive a dGEMRIC MRI at their 6 month research MRI visit.
- If the patient recived their initial diagnostic MRI by a MRI with and without contrast, the patient will receive a Non-contrast MRI at their 6 month research MRI visit.

Research MRI utilizing dGEMRIC or Non-contrast MRI

Once the intrarticular PRP injections have been administered, a follow-up MRI will be performed utilizing dGEMRIC or Non-contrast MRI at 6 months from the initial visit. Mean T1 values on dGEMRIC will be calculated by drawing a region of interest at the site of hyaline cartilage pathology, adjacent to the site of pathology within the hyaline cartilage, and distant to the site of pathology within the hyaline cartilage on initial and follow up imaging. If the follow-up imaging is with a Non-contrast MRI, the DiPaolo classification system will be used to quantify interval change.

Knee MRI with dGEMRIC will be performed in the standard manner. Upon arriving to the radiology department for the MRI, a pregnancy test will be administered for all female participants of child bearing potential. An institutional MRI screening form will be given to each patient and parent and reviewed with the MRI technologist. If there is any retained metal (pacemaker, metallic bb, orthopedic hardware), MRI will not be performed. If there is no contradiction to MRI based on the institutional screening form, the patient will have an IV placed. Using weight based dosing, .04 ml/kg, injection of gadolinium will be administered. The patient will then be asked to exercise for 30 minutes, which will be performed by walking around the hospital. The patient will then return to the MRI suite in the department of radiology. Standard screening for retained metal will be performed prior to entering the MRI scanning area. If the parent/guardian wishes to accompany the child for the MRI scan, they will be screened in identical fashion. In the MRI scanner, the patient will be required to lie still for approximately 1 hour, the duration of the scan. The patient will have the option of watching a

movie or listening to music on ipod or similar device (plugged in outside the scanner in technologist area) during imaging. No sedation will be given. There is no ionizing radiation associated with MRI.

Each patient will have four values of GAG (T1GD) measured at the baseline MRI and again at the 6 month MRI, via dGEMRIC. The locations of the measurements will be designated using the lateral view of the femoral condyle as a clock face with the medial femoral condyle superior location being designated as 12 o'clock, the inferior location being designated as 6 o'clock and the anterior location being designated as 3 o'clock; the lateral view of the femoral condyle as a clock face with the lateral femoral condyle superior location being designated as 12 o'clock, the inferior location being designated as 6 o'clock and the anterior location being designated as 9 o'clock. The values will be measured at four locations: the medial side at a weight bearing surface, the medial side at a non-weight bearing surface of the posterior condyle (4 o'clock), the lateral side at a weight bearing surface, and the lateral side at a non-weight bearing surface of the posterior condyle (8 o'clock). The side with the lesion will be designated as the affected side, and the side without the lesion will be designated as the non-affected side. GAG (T1GD) will be measured on the affected weight bearing surface at the site of the lesion. GAG (T1GD) will be measured on the non-affected side at the corresponding weight bearing surface of the femoral condyle.

Research MRI utilizing Non-contrast MRI

A Non-contrast MRI will be performed in the standard manner. Upon arriving to the radiology department for the MRI, a pregnancy test will be administered for all female participants of child bearing potential. An institutional MRI screening form will be given to each patient and parent and reviewed with the MRI technologist. If there is any retained metal (pacemaker, metallic bb, orthopedic hardware), MRI will not be performed.

Table 3 – Patient Schedule of Assessment												
Procedure Step	**Pre-Screen Visit**	**Week 1 +/- 10 d**	**Week 2 +/- 10 d**	**Week 3 +/- 10 d**	**Week 4**	**Week 5**	**Week 6 +/- 1wk**	**Week 12 +/- 1wk**	**Month 6* +/- 1wk**	**Month 9 +/- 1wk**	**Month 12 +/- 2wk**	
Non-contrast MRI, MRI with and without contrast, or dGEMRIC for OCD lesion (**standard of care**)	X[A]								X[R]			
Initial & Follow-up x-rays dependent on symptoms (**standard of care**)	X							X[D]	X[D]	X[D]	X[D]	
Informed Consent (**research**)	X[B]											
Inclusion / Exclusion Criteria confirmation (**research**)		X[R]										
Patient Reported Outcomes IKDC, KOOS-child (**research**)		X[R]					X[R]	X[R]	X[R]	X[R]	X[R]	
Randomization (**research**)		X										
Compliance Survey (research)		X[R]	X[R]	X[R]	X[R]	X[R]	X[R]					
Physical exam (**research** for PRP arm patients weeks 1, 2, & 3 and **standard of care** visits for both treatment arms for weeks 6, 12 and months 6, 9, 12)		X[C]	X[C]	X[C]			X	X	X	X	X	
Blood draw & processing using the Harvest PRP kit(**research**)		PRP only	PRP only	PRP only								
Autologous PRP Injection (**research**)		PRP only +/- 7days	PRP only +/- 7days	PRP only +/- 7days								
Hinged Brace in extension for 6 wks (**standard of care)**		X	X	X	X	X	X					

X = occurs for both patient groups;

A = Standard of care visit to pre-screen for minimal inclusion criteria; all patients will undergo the same standard of care MRI procedure

B = Patient consenting can occur at either the initial screening visit, or when given the option to take the consenting material home for review they will be consented at their 2nd visits if they choose to enroll in the study.

C = PRP Arm – Physical Exam will be performed for research only and will be take place prior to the injection of PRP.

D = X-rays are performed as standard of care depending on the conditions of each individual patient.

R=Research * 6 months from initial visit

Participant Discontinuation Criteria

Patients may be withdrawn from the trial for one of the following reasons:

1. Noncompliance with the protocol procedures
2. The patient has been given other or additional treatment, which may interfere with the study treatment (such as intra-articular steroid injection).
3. The patient wishes to withdraw from the trial
4. A severe adverse reaction by treatment applied is evident. Such as joint infection which requires IV antibiotics and possible surgical irrigation and drainage.
5. Patient becomes pregnant prior to completion of the third injection
6. Patients receiving conservative treatment whose lesion becomes unstable, requiring surgery during the study period will be excluded from participating in the remainder of the study
7. The investigator may withdraw patients from the trial if it is in the best interest of the patients. The reason for withdrawal shall be clearly described and the patient shall, whenever possible irrespective of the reason for withdrawal, as soon as possible be called for a final visit. All relevant assessments shall be completed, preferably according to the schedule for the final visit.

Risks associated with study

a. Risks associated with PRP

Intra-articular injections have been proposed to be a safe procedure, with low complication rate, if performed while taking adequate precautions [34]. The risks related to the intra-articular injections are infection, with an incidence of 1 in 50,000 inoculations, pain at the injection site, and erythema.

Intra-articular injections with PRP, as with every intra articular injection (and not due to the PRP itself) can result in cutaneous rashes or urticaria, pain at the injection site, or erythema, all of which are considered very rare based on previous level 1 studies [3, 8, 9]. Pain, swelling, redness, warmth, and bruising at the injection site or headache may occur, all which are rather rare.

As with every joint injection, a joint infection could potentially occur.

b. Risks Associated with dGEMRIC MRI and Non-contrast MRIs

The initial MRI performed for this study is considered standard of care in the management of knee osteochondritis dissecans. The follow-up MRI at 6 months is for research purposes.

If a patient receives a dGEMRIC MRI, the gadolinium based contrast agents used for this type of imaging can result in nausea, vomiting, cutaneous rashes, shortness of breath, or chest pain, all of which are considered very rare [35]. Death attributed to gadolinium based contrast agents is extremely rare, "The overall rate of gadolinium-based contrast agent deaths reported to the FDA Medwatch database is less than 1 in a million (40 deaths in 51 million administrations), similar to the risk of death from a chest radiograph, drinking one half liter of wine, smoking 1.4 cigarettes (tobaccodocuments.org/lor/03732381-2387.html), or traveling 86 miles by car (www-nrd.nhtsa.dot.gov/Pubs/811291.PDF)."

The specific gadolinium based contrast agent used at Children's Hospital Colorado, gadobenate dimeglumine (MultiHance), was recently evaluated for safety in children without any adverse effects observed during the first twenty-four hours after contrast administration [36].

Nephrogenic systemic fibrosis is a fibrosing disorder associated with exposure to gadolinium-based contrast agents in people with severely compromised renal function [36]. There have been seventeen documented cases in patients 18 years or younger with documented exposure to gadolinium-based contrast agents [37]. Proposed risk factors are dialysis patients with GFR below 30 ml/min, high-dose gadolinium, proinflammatory conditions, acute renal failure, postoperative state, and hyperphosphataemia. The American College of Radiology recommends that people with severe chronic kidney disease (eGFR <30 mL/min/1.73 m2) or acute kidney injury should avoid exposure to gadolinium-based contrast agents. The American College of Radiology recommends that people with severe chronic kidney disease (eGFR <30 mL/min/1.73 m2) or acute kidney injury should avoid exposure to gadolinium-based contrast agents [37].

Risk which apply to either dGEMRIC or Non-contrast MRIs: There are no major risks associated with the magnetic fields and radio waves associated with extremity MRI. Rarely, one in thousands of exams, a sunburn-like skin burn might occur over a small area of the body, but special precautions are taken to avoid this. The patient must lie still in a small space which could create stress or anxiety. The patient might also feel cold because the MRI scanner room is kept cool, but blankets can be provided. The scanner makes a loud knocking noise when it is operating, but earplugs or headphones can be worn. Expected adverse events include stress/anxiety, claustrophobia, discomfort, or having a non-diagnostic study as a result of motion artifact. Some patients are unable to hold adequately still resulting in motion artifact on the images which could deem them uninterruptable. The amount of motion artifact for this to occur would have to be extensive, and will therefore most likely occur in few study participants, if any.

c. Complications and Pain associated with Study

Follow-up visits will take place on the CHCO main campus or one of the following satellite locations (South, Parker, Centennial, Uptown/St Joseph's or Broomfield). All PRP injections will be performed at Children's Hospital Colorado. The clinic, being a well-equipped outpatient facility, has additional rescue devices. Adverse events and pain medications used will be reported and monitored by Dr. Dahab/Dr. Nagle or their colleagues during each follow-up visit using the electronic medical records clinical encounter notes. Patients are reminded to contact Dr. Dahab/Dr. Nagle or Dr. Michael Fadell with questions or to discuss adverse events they might sustain.

We will assist in arranging care for any injuries patients may sustain by participating in this study. However patient's insurance will be billed for any care that is provided.

d. Recording and Reporting Adverse Events

The study investigators will monitor all subjects enrolled for any potential adverse effects (AE) associated with the treatments provided. Clinically significant results will be reported as adverse events and will be graded on a three- point scale (mild, moderate, and severe)[38]. In the event that AE occurs the following will be documented and reported to the IRB:

- Nature of adverse effect.
- Clinically significant adverse events will be graded on a three- point scale (mild, moderate, severe)
- Statement as to why it is considered unanticipated or anticipated but with change in nature or severity or frequency of occurrence from baseline
- Statement as to the degree to which it is considered treatment related (unrelated, remote, possible, probable), and why.
- Results of any diagnostic tests that were performed
- Description of any treatment implemented
- Statement of subject's current clinical status
- Investigator's signature and date.

Local side effects and irritations such as cutaneous rashes or urticaria, painful injection site, local heat, swelling, painful knee, and erythema will be recorded.

The investigator will supply a report for COMIRB if the adverse effects are unanticipated or if anticipated adverse effects change in intensity, frequency, or duration.

Safety Measurements

The nature, onset, duration, severity, and outcome of all adverse events, as well as any association of an adverse event related to the study medication, will be assessed and documented at each visit. Patients will be asked to avoid intake of NSAIDs. If symptoms require pain medications during the initial follow up period (6 months after last injection), NSAID consumption will be recorded, as well as other pain medications taken. Patients will be allowed to have non-NSAIDs rescue medication as needed to treat pain. This includes acetaminophen and in select cases opioids such as Vicodin or Percocet. To evaluate the safety profile of the treatments, all complications and/or adverse events will be reported with their level of severity and their causal relationship to the study treatment.

Data Analysis Plan

The anatomical location of osteochondritis dissecans will be documented for each patient on each of two MRI scans. Mean T1 values with dGEMRIC imaging **will be measured by two separate pediatric radiologists** at the site of cartilage pathology, adjacent to the site of pathology within the cartilage, and distant to the site of pathology within the cartilage on both initial and follow up imaging. Di Paola classification [33] will be used on the diagnostic imaging sequences to score the lesion pre-inoculation and on post-inoculation imaging.

Proposed statistical analysis for Aim 1: Cartilage healing

The T1GD value on dGEMRIC measures GAG content in cartilage. Higher T1GD values are associated with higher GAG content in the tissue, a positive measure of cartilage health. We assume that the value is approximately normal.

We plan to fit a general linear mixed model [39] for the outcome of T1GD. Predictors will include the visit at which the T1GD test was performed (initial MRI and 6 months), whether the site was weight bearing or not, and whether the side was affected or not, and the treatment group. Age will be included as a covariate. An unstructured covariance will be used to account for correlation between T1GD measurements on the same participant. Jacknifed-studentized residuals will be examined to assess normality, and transformations will be applied as appropriate.

We will test the null hypothesis of no time by treatment by weight bearing surface by affected side interaction. Given a non-significant interaction, we will test the main effects of time and treatment, comparing the difference between the weight-bearing and non-weight bearing surface for the affected and non-affected sides. Estimates, 95% confidence intervals, and p-values will be produced. All hypothesis tests will be performed using the Wald test with Kenward and Roger degrees of freedom[40] . Hypothesis tests will be performed at the Bonferroni corrected alpha of 0.05/3 = 0.017.

Proposed statistical analysis for Aim 2: Functional Recovery and Symptoms

We will analyze scores for each subscale of the KOOS-child, and the overall score produced by the pedi-IKDC range from 0 to 100, with higher scores indicating improved knee function. Each measure is approximately normal.

We describe the analysis for Aim 2, Sub-aim 2.1. The analyses for the other sub-aims are exactly parallel.

We plan to fit a general linear mixed model [39] with the pain subscale of the KOOS-child as the outcome. Predictors will include the visit at which the survey was given (6 weeks, 12 weeks, 6 months, 9 months and 12 months) and treatment group. Age will be included as a covariate. An unstructured covariance will be used to account for correlation between measurements on a single participant. If the resulting model fails to converge, a spatial exponential covariance will be used. Jacknifed-studentized residuals will be examined to assess normality, and transformations will be applied as appropriate.

We will test the null hypothesis of no polynomial time trend by treatment interaction. If the time-by-treatment test is non-significant, we will step down to test the main effects of time and treatment. Estimates, 95% confidence intervals, and p-values will be produced.

All hypothesis tests will be performed using the Wald test with Kenward and Roger degrees of freedom [40] . Three hypothesis tests may be performed for each outcome, for a total of 12 tests. To control for multiple comparisons, all hypotheses will be tested at the Bonferroni corrected alpha level of 0.05/12=0.004.

Proposed statistical analysis for Aim 3: Correlation of MR Findings and Measures of Functional Recovery and Symptoms

We plan to fit four general linear unviariate models with 6-month T1GD as the outcome. The primary predictor for each model will be the 6 month measurements of the pain subscale of KOOS-child, the ADL subscale of the KOOS-child, the sports subscale of the KOOS-child, and the pedi-IKDC, respectively. In each model, age will be included as a covariate. We will test the null hypothesis of no association between the functional measure and T1GD findings on MR and/or no decrease in DiPaolo score, using an F test. All hypotheses will be tested at the Bonferroni corrected alpha level of 0.05/4 = 0.0125.

Interim analysis and planned interim power analysis.

Because this study has never been done in children, the estimates for power analysis may be incorrect, and the effect of the treatment may be better or worse than that reported in the literature. Thus, after the first six study participants have been recruited, treated, and followed, the investigators will report blinded data to the study statistician, who will perform an interim power analysis using the methods of Gurka et al., 2007 [41].

Power Analysis

We conducted a power analysis for Aim 1, the primary endpoint of the study. The maximum sample size for the proposed trial is fixed at 12 patients per arm (24 participants total). The sample size is limited due to costs associated with dGEMRIC.

We calculated power for the test of no difference in T1GD between the treatment groups. We assumed a standard deviation of 50 ms for T1GD values, and a correlation of 0.2 between the baseline MRI and the 12-month T1GD measurements. Estimates were based on T1GD values reported by Neuman et al[42]. We expect T1GD values to be 85 ms higher in the injection group when compared to conservative therapy at the 6 month time point with no difference at baseline. We calculated power for 10, 11, or 12 participants per arm. Power was calculated using GLIMMPSE version 2.0.1.[43]

To detect a mean difference of 85 ms in T1GD between the treatment groups, power will be 0.93 with a Type I error rate of 0.05, for 12 participants per treatment group. To ensure a conclusive study, we will target an effective sample size of 10 participants per group. Over the 12 months, we expect to lose up to two participants per treatment group. To account for the attrition, we have set an enrollment goal of 24 participants, or 12 participants per treatment group.

Data Security

Data collection and management will be performed by Drs. Michael Fadell, , Research Associate Elizabeth Carroll and/or a Sports Medicine Research Associate utilizing "REDCap." REDCap's internet registration and data collection system has a built-in security feature that encrypts all data for transmission, preventing unauthorized access to confidential participant information. Access to the system will be controlled by a sequence of identification codes and passwords. Pertinent radiologic images will be archived in the Children's Hospital Colorado PACS system.

A hard copy file of each study subject will be kept by the research coordinator at Children's Hospital Colorado and will include the signed consent/assent. The files will be kept in a locked cabinet in the primary investigator's private office which has a locking door, and saved in Children's Hospital Colorado's Redcap.

9. Summarize Knowledge to be Gained

The study is designed to objectively evaluate whether intra articular injections with autologous platelet rich plasma (PRP) will improve recovery time and cartilaginous healing in patients suffering from (stable) osteochondritis dissecans of the knee when compared with conservative therapy.

Radiation-free MRI with dGEMRIC or a non-contrast MRI will be used to assess osteochondritis dissecans healing by comparing mean T1 values obtained at the site of pathology on initial and follow up exams in comparison to mean T1 values of cartilage adjacent to the site of pathology and distant to the site of pathology on initial and follow up imaging and/or interval change in DiPaolo score with Non-contrast MRI.

The benefit of more rapid healing of osteochondritis dissecans is reduction in morbidity, as well as potentially eliminating surgical treatment. Dissemination of results will occur via publication in a peer reviewed journal in the fields of radiology, orthopedic surgery, or both.

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