Arthrosocpic Treatment of Knee Chondral Lesions: Clinical, MRI and Histologic Comparison Between the Use of PRP vs ADSCs in Addition to Marrow Stimulation Techniques

NCT ID not yet assigned

Unique protocol id 23/2019

Document date: March 25, 2019. Traslated into English on August 27, 2025

As the original documents were written exclusively in Italian, the following is the complete translation of each document into English, presented alongside the original text.

Proposing institution: MESVA Department, Residency Programme in Orthopedics and Traumatology, University of L'Aquila.

Project Title: Arthroscopic Treatment of Knee Chondral lesions: Clinical, MRI and Histologic comparisons between the use of PRP vs ADSCs in addition to Marrow stimulation techniques

Protocol version and date: version 1/19 - protocol March 2019

Project manager: Vittorio Calvisi, Associate Professor, Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio-Coppito 2, 67100, L'Aquila, Italy Tel.: +39 337 743107 e-mail:

Research location: U.O.S.D. D.U. Minimally Invasive and Computer-Assisted Orthopedic Surgery directed by Prof. Calvisi at the San Salvatore Hospital in L'Aquila.

Introduction

Today, chondral lesions are one of the most frequently diagnosed knee joint pathologies in clinical practice, as the primary cause of pain and reduced joint function in an active patient population. At a time when regenerative medicine is facing increasingly demanding expectations, by implementing the development of new surgical techniques based on tissue engineering, we have reconsidered therapeutic algorithms for the treatment of chondral lesions, which involve mesenchymal stimulation techniques proposed in the 1980s and 1990s., using chondroplasty for abrasion (L.L. Johnson 1986) or microfractures according to Steadman (1997), which are still relevant today in the treatment of such lesions, remaining the first therapeutic option given their simplicity of execution, low cost, and minimal invasiveness (1,2). Medullary stimulation through abrasion chondroplasty is based on the abrasion of the subchondral bone using a motorized instrument (ABRADER), while the microfracture technique is based on the creation of small perforations using special punches that penetrate the subchondral bone: both procedures aim to remove necrotic osteons and allow bleeding from the subchondral tissue where mesenchymal cells are present. The clot thus formed should evolve, if not subjected to an immediate load, toward the formation of a fibrocartilaginous tissue that, although with debated biomechanical and biological qualities, allows the formation of a fibrocartilaginous tissue that "repairs" the osteochondral damage (Calvisi V, 1990) (3,4).

Platelet-rich plasma (PRP) was first utilized in a surgical context in 1998 and has since become the subject of extensive research due to its potential in promoting tissue regeneration and repair. This treatment has gained significant traction in the medical field, leading to a wide range of applications (5). Its definition, a point that has been extensively discussed in the composition of the aforementioned, is generally understood to be a platelet concentrate that exhibits significant variability in its amount of mononuclear material.

The elements were found to be capable of activating degranulation, which consequently led to the release of bioactive factors (GF, PDGF, TGF-b, PEDGF, VEGF, IGF-1, FGF, EGF, ecc) with tissue-specific action, playing a key role in chemotaxis, cell proliferation, and modulation of the inflammatory response. At the cartilage level, in vitro studies have revealed the potential of

PRP to orient mesenchymal cells in a chondrogenic direction, while in vivo studies on animal models have shown histological improvement in the tissue reformed to cover the lesion after treatment with microfractures and repeated PRP injections (5,6,7,8,9).

The use of autologous mesenchymal cells has also been the subject of study and debate for years regarding their regenerative potential in cartilage lesions (10). Mesenchymal cells are present in numerous tissues of the body, but the stromal fraction of subcutaneous adipose tissue is certainly the easiest site for extraction: adipose tissue contains two main populations, mature adipocytes and stromal vascular fraction (SVF) cells; the latter comprises a heterogeneous fraction that includes preadipocytes, endothelial cells, smooth muscle cells, pericytes, macrophages, fibroblasts, and adipose-derived stem cells, which share several characteristics with subchondral bone stem cells. These cells are promising candidates for use in a wide range of innovative therapies, from regenerative medicine to tissue engineering; furthermore, the use of adipose-derived stem cells has been proposed in the treatment of autoimmune, chronic (i.e. Crohn's disease), and allergic diseases (11,12,13,14). The effectiveness in this wide range of diseases can be explained by the immunoregulatory and anti-inflammatory action of both ADSCs and non-expanded SVF cells (15). Specifically, in the context of joint pathology, these cells have been demonstrated to possess the capacity to differentiate into the osteoblastic and chondrogenic lineages, depending on the environmental conditions in which they are cultured (15,16,17). The therapeutic mechanisms of ADSCs include the reduction of cartilage degeneration and synovial inflammation, the inhibition of fibrotic remodeling and apoptosis, the enhancement of recruitment and proliferation of endogenous stem cells, and the reduction of immune responses (18,19,20,21,22).

The combination of PRP and SVF combines both the benefits of platelet-rich plasma and the potential regenerative stem cells from adipose tissue (23): this combination has been proposed as the most effective approach to cartilage repair. Supplementation of cell culture media with a system currently available on the market ("Arthrex ACP") has resulted in dose-dependent cell growth and proliferation of adipose tissue stem cells (ADSCs) in vitro (24). Promising preclinical and clinical results have been published for treating osteoarthritis with PRP and the combination of PRP and autologous ADSCs, suggesting the potential regenerative capacity of this new biological treatment option: it has been demonstrated that the synergistic effect of PRP promotes the proliferation and cellular differentiation of stem cells derived from adipose tissue (25,26). For these reasons, the combination of PRP and autologous ADSCs is frequently used in joint surgery, given the safety of the treatment (20, 21, 27, 28, 29).

In patients exhibiting gross deviation of the mechanical axis of the lower limbs, fibrocartilage regeneration, if obtained, upon resumption of normal activity, would be subjected to the same pre-intervention overloads. Consequently, this would result in a short lifespan for the regenerated fibrocartilage. Therefore, the promotion of effective formation of a repair cartilage with an appearance and architectural structure that can withstand the daily mechanical loads after surgery is imperative. In patients under 60 years of age who exhibit severe deformities (varus greater than 10° and valgus greater than 20° deviation), as substantiated by extant literature and extensive clinical experience, there is a compelling indication for corrective osteotomy surgery. In such cases, our established protocol entails an arthroscopic second-look procedure, planned in accordance with the patient's consent, which enables a comprehensive evaluation of the newly formed fibrocartilage. This evaluation encompasses both macroscopic analysis and, if necessary, histologic examination, ensuring a thorough assessment of the quality and integrity of the tissue (30, 31, 32, 33, 34, 35). In patients aged 70 years and older or for whom repair tissue formation has not occurred, we

will bypass the osteotomy procedure, thereby establishing the indication for knee replacement. The decision regarding the type of prosthesis, whether unipartimental or total knee, is contingent upon the particulars of each case (This approach enables the acquisition of histological preparations suitable for the evaluation of regeneration obtained after index procedure with PRP and ADSCs).

A critical review of the extant literature on the "all-style" treatment of cartilage pathology reveals several methodological shortcomings. Firstly, the lesions to be treated and the procedures used exhibit a lack of homogeneity, which calls into question the efficacy of the treatment methods. Secondly, the evidence regarding the morphology and histochemistry of the regenerate obtained in arthroscopic treatment is particularly limited, and there is a paucity of data on the comparison of PRP infiltration vs. PRP + ADSCs: Having made these premises, we would like with this project, which includes the classical clinical and imaging outcomes, to give clear and unambiguous answers that can clarify the real efficacy of a combination of these two derivatives in the treatment of chondral lesions by evaluating the macro morphology of the regenerate through an arthroscopic second look, during scheduled osteotomy and/or joint arthroplasty surgery and in this way obtain finally definitive histological and histochemical findings.

Objectives

The aim of this study is to evaluate the clinical efficacy of arthroscopic mesenchymal stimulation treatment associated with intraoperative infiltration of autologous PRP combined with adipose-derived stem cells, frequently used in clinical practice, compared to arthroscopic mesenchymal stimulation associated with infiltration of PRP alone. Over time, the objective results obtained from diagnostic imaging (i.e. follow-up MRI scans) and, if available, histological results obtained from second-look arthroscopic sampling will also be evaluated.

Description of the experimental protocol

The proposed study is a single-center, randomized, parallel-group clinical trial.

- The study will enroll patients for at least three years from the start date, with follow-up continuing for at least three years after each patient's enrollment.
- At least 80 patients recruited from the "U.O.S.D. D.U. Minimally Invasive and Computer-Assisted Orthopedic Surgery" unit at San Salvatore Hospital in L'Aquila will be involved in the study.

The inclusion criteria will be as follows:

- Patients who provide consent to participate in the research study
- Patients with a diagnosis of osteoarthritis of the knee joint
- Patients with grade III-IV Outerbridge monocompartmental chondropathy, varus < 15°, valgus <25°, grade 1-II Kellegren-Lawrence osteoarthritis, aged between 40 and 65 years of both sexes)

The exclusion criteria will be as follows: multi-compartmental chondral lesions, varus >15°, valgus >25°, grade III-IV Kellegren-Lawrence osteoarthritis, presence of rheumatic or connective tissue diseases, diabetes, heart disease, kidney disorders, thrombocytopenia <200,000, coagulation difficulties, varicose veins, phlebitis and very visible capillaries, use of anticoagulant drugs, active infections.

The study will not require any different or additional procedures to those normally used for arthroscopic treatment, except for the injection at the end of the procedure of autologous PRP or PRP + autologous adipose tissue stem cells, which have been commonly used for years in orthopedic clinical practice: this will involve a venous blood sampling, which will then be appropriately processed in the former case, and also a sampling of adipose tissue from the abdominal subcutis in the latter (which will later be appropriately processed). A specific informed consent will be obtained for the acquisition, handling, and utilization of these derivatives. As per standard protocol, patients will undergo a series of preoperative evaluations including laboratory testing, EKG, anesthesiologic examination, and any patient-specific consultations, if needed. Additionally, patients will undergo weight bering knee X-ray and MRI of the knee.

In cases where the initial arthroscopic procedure marks the preliminary stage preceding subsequent corrective osteotomies, explicit consent will be obtained for the execution of an arthroscopic second-look procedure. At the time of the subsequent procedure, an atraumatic Bordier needle (2 mm in diameter) will be used to obtain a biopsy sample from the treatment site for histologic evaluation. This will allow for the evaluation of the cartilage status and confirmation of previously planned corrective osteotomy.

As is standard practice in orthopedics, the clinical condition of the knee will also be monitored with the aid of postoperative MRI scans.

Patients will be randomly assigned to two groups:

Group A: 40 patients who are candidates for mesenchymal stimulation using chondroplasty for

abrasion and/or microfractures according to Steadman and PRP infiltration at the end of the arthroscopic procedure.

Patients will sign an informed consent form regarding both the arthroscopic procedure and the collection of venous blood to obtain autologous PRP. Once in the operating room, before

the surgery and spinal anesthesia, a blood sample (approximately 15 ml) will be taken using a special syringe ("Arthrex ACP® Double Syringe") which will then be placed in a special centrifuge at 1500 rpm for 5 minutes. Once centrifugation is complete, the double syringe will allow the supernatant (the PRP to be injected later) to be isolated. This will be placed in a sterile position on the sterile table for intraoperative use. At the same time as the procedure described above, spinal and/or general anesthesia will be administered. Once this is complete, patients will be placed in the arthroscopic position and the sterile surgical field will be prepared for arthroscopy: diagnostic arthroscopy will therefore be performed to confirm the therapeutic indication described above, and at the same time joint debridement (and any associated procedures) will be performed. Chondroplasty by abrasion and/or microfractures according to Steadman will then be performed at the site of the cartilage lesions, assessing the desirable blood supply. Under arthroscopic guidance, a needle will be positioned at the stimulated area, the arthroscopic fluid will be drained from the joint, and the portals will be sutured. At this point, the previously prepared PRP will be injected through the needle into the joint. If diagnostic arthroscopy does not confirm the surgical indication for the treatment of cartilage pathology, only the surgical procedures necessary for the specific pathology found (e.g., meniscectomy, synovectomy, biopsy, etc.) will be performed. The patients in question will still be monitored until the end of the follow-up period in the manner provided for, and included in the intention-to-treat analysis.

Group B: 40 patients who are candidates for mesenchymal stimulation using chondroplasty for abrasion and/or microfractures according to Steadman and infiltration of PRP + ADSCs at the end of the arthroscopic procedure. Patients will sign an informed consent form regarding

both the arthroscopic procedure and the collection and use, after centrifugation, of PRP + subcutaneous adipose tissue. Once in the operating room, before the surgery and spinal anesthesia, a blood sample (approximately 15 ml) will be taken using a special syringe ("Arthrex ACP® Double Syringe") which will then be placed in a special centrifuge at 1500 rpm for 5 minutes. Once centrifugation is complete, the double syringe will allow the sumatant (the PRP to be combined later with the stem cells obtained from adipose tissue) to be isolated, which will be placed in a sterile position on the sterile table for intraoperative use. Simultaneously with the procedure just described, spinal and/or general anesthesia will be performed, and once finished, a sterile field will be set up at the abdominal level, a sampling of loose connective tissue associated with subcutaneous abdominal adipose tissue will be performed. first a subcutaneous infiltration of 250 ml of anesthetic solution will be made as follows: 500 ml of NaCl, 30 ml of 2% Lidocaine, ¼ of a 1-mL vial of 1% Adrenaline, 1 ml of sodium bicarbonate. The tumescent fluid will be allowed to stand for 15 minutes; after this time period has elapsed, a withdrawal will be made by vacuum suction syringe and cannulated needle of the subcutaneous connective tissue (40 cc). A compressive dressing will be applied at the abdominal level. The lipoaspirate, kept in the appropriate syringes, will be centrifuged with Rotofix 32 A Centrifuge at 2500 rpm for 4 minutes. Following the centrifugation process, three distinct components will have been isolated within the syringes: the oil and the aqueous fraction, which will be disposed of, and the adipose tissue graft, which will undergo a sterile homogenization process (exchange between two appropriately connected syringes). Once homogenization is complete, the graft will be placed back in the centrifuge for 4 minutes at 2500 rpm. This step will further isolate the mesenchymal vascular stromal fraction leaving an oily supernatant that will be, in a sterile manner, discarded. The residual mesenchymal vascular fraction (approximately 2 ml) will be combined with the previously prepared PRP for subsequent infiltration.

At the same time as the centrifugation process, patients will be placed in the arthroscopic position and the sterile surgical field will be prepared for arthroscopy: diagnostic arthroscopy will therefore be performed to confirm the therapeutic indication described above, and at the same time joint debridement (and any associated procedures) will be performed; Chondroplasty by abrasion and/or microfractures according to Steadman will then be performed at the site of the cartilage lesions, assessing the desirable blood supply. Under arthroscopic guidance, a needle will be positioned at the stimulated area, the arthroscopic fluid will be drained from the joint, and the portals will be sutured. At this point, the previously prepared PRP will be injected through the needle into the joint. At this point, the previously prepared PRP and subcutaneous adipose tissue will be injected through the needle into the joint. If diagnostic arthroscopy does not confirm the surgical indication for the treatment of cartilage pathology, only the surgical procedures necessary for the specific pathology found (e.g., meniscectomy, synovectomy, biopsy, etc.) will be performed. The patients in question will still be monitored until the end of the follow-up period in the manner provided for, and included in the intention-to-treat analysis.

All patients in both groups will undergo the same postoperative rehabilitation protocol, including no weight bearing on the operated limb for 4 weeks and subsequent cautious gait re-education and isometric exercises. All patients will have to give consent for the use for research purposes of questionnaires containing outcome measures on knee function and general health status: over time (preoperative, At 2 and 6 weeks, then at 6, 12, 24, and 36 months postoperatively) patients will be monitored clinically by SF-12, subjective IKDC, VAS, and KOOS score. The study will require patients to undergo pre- and post-operative MRI scans of the knee (at 12, 24, and 36 months), a commonly used investigation to first determine the

indication for surgical treatment and then verify the state of tissue healing following surgical treatment, evaluated blindly by the radiologists involved in this research project. Patients who give their consent and who are indicated for subsequent corrective osteotomy will undergo a second-look arthroscopy to assess the healing status of the cartilage by evaluating its macroscopic appearance, compactness by palpation, and microscopic appearance (blindly evaluated by the pathologist involved in this research project) through biopsy sampling with a Bordier needle (2 mm in diameter), using Safranin O and type II collagen staining.
Over time, the following will also be monitored adverse events such as swelling, fever, reduced joint mobility, systemic effects, any abnormal request for analgesics, date of discharge from the surgical ward, satisfaction with the technique, any unexpected events (severity or frequency), events related to participation in the research, the incidence of postoperative side effects related to the techniques.
The following will also be monitored: postoperative side effects/complications and other side effects related to the use of the techniques described in the protocol (deep or superficial infections, allergies or other reactions to device materials, hematoma, damage to blood vessels and nerves resulting in pain or numbness following autologous sampling, delayed wound healing).
For reasons of informed consent in the surgical field, it will not be possible to perform blinded treatments either for patients (who will therefore know which group they belong to) or for the individual surgeon.
However, the questionnaire evaluator and the statistician who will process the relevant data, the radiologist, and the pathologist who will express an opinion on the results obtained in the MRI and histological preparation, respectively, will be blinded.

Statistical analisys
Randomization will be performed by assigning each patient a random number corresponding to a list of numbers, randomly associated with one of the two treatments, developed by the statistician. The mean values + standard deviation of the results of each questionnaire (SF-12, subjective IKDC, KOOS Score, and VAS) will be evaluated, after logarithmic transformation, using analysis of variance with two factors, time and type of treatment. Statistical significance will be set at $p<0.05$.
The study will be based on an estimated value of 80 subjects, with a 1:1 ratio for the two treatment groups that was calculated to be sufficient to achieve a power greater than 95% in order to test an Effect Size of 0.25 and a of 0.05 on the values of the questionnaire averages among the three treatment groups. Statistical power was calculated using G*POWER Version 3.1.9.2.
An intention-to-treat analysis will be performed, including all subjects randomized into the two groups, and a per-protocol analysis will be performed, excluding subjects in whom exploratory arthroscopy did not confirm the indication for treatment in the study.

Storage of collected data and research project results
During the study, only the medical staff will have access to patients' personal data.
The results of the questionnaires, MRIs, and histological preparations will be associated with a unique code for each patient, which only Prof. Calvisi and Dr. Goderecci will be able to access, and which will remain anonymous. The results of this study will be published or presented at conferences by the promoters. The identity of the study participants will, in any case, remain anonymous in accordance with privacy laws.
Sensitive data will be stored both in paper archives and in computerized format in dedicated

archives (in a locked room of the U.O.S.D. D.U. of Minimally Invasive and Computer-Assisted Orthopedic Surgery directed at the San Salvatore Hospital in L'Aquila), in
compliance with current regulations. The person responsible for data processing and storage is the department director.
Identification data will not be stored for more than 5 years after the start of the study.

Risks related to research
As far as the scientific research aspect of this project is concerned, patients will not be exposed to any greater risks than those normally associated with arthroscopic surgery, for which they will sign the usual informed consent form. The infiltration of PRP and/or ADSCs may rarely, like all infiltrations of any substance, cause self-limiting synovial irritation and inflammation or easily treatable with anti-inflammatories, ice, and arthrocentesis if necessary. The removal of subcutaneous abdominal connective tissue, given the small amount of lipoaspirate (approximately 40 cc), does not pose any real risk of thromboembolism, for which the usual antiplatelet prophylaxis prescribed for arthroscopic surgery is administered in any case. Any hematomas at the aspiration site, the risk of which is reduced by the administration of the anesthetic cocktail combined with a minimal dose of adrenaline, are prevented by maintaining a compressive abdominal dressing for 7 days post-surgery and, if necessary, limited by the use of ice and anti-inflammatories.

Bibliography

1. Johnson LL. Arthroscopic abrasion arthroplasty-historical and pathological perspective: present status. Arthroscopy 1986 2: 54-69.
2. Steadman, J. Richard, et al. Microfracture technique for full-thickness chondral defects: Technique and clinical results. Oper Tech Orthop 1997; 7 (4): 300-304.
3. Mithoefer K, Williams RJ, Warren RF et al. The microfracture technique for the treatment of articular cartilage lesions in the knee: a prospective cohort study. J Bone Joint Surg Am 2005; 87:1911-1920.
4. Calvisi V, Pappalardo S, Niceforo A, et al. Evoluzione morfologica ed istologica del rigenerato fibrocartilagineo dopo condroabrasione. GIOT. 1990;XVI (suppl)(1):155-162.
5. Marx R. Platelet-Rich Plasma (PRP): What Is PRP and What Is Not PRP? Implant Dentistry: December 2001 - Volume 10 - Issue 4 - p 225-228
6. Kon E, et al. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. Knee Surgery, Sports Traumatology, Arthroscopy 2011; 19 (4): 528-535
7. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR: Platelet-rich plasma growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85: 638-646.
8. Mishra A, Tummala P, King A, Lee B, Kraus M, Tse V, et al. Buffered platelet rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. Tissue Eng Part C Methods 2009; |15:431-5.
9. Milano G, Sanna Passino E, Deriu L, et al. The effect of platelet rich plasma combined with microfractures on the treatment of chondral defects: an experimental study in a sheep model. Osteoarthr Cartil 2010; 18: 971-80.
10. Chen Y et al: Mesenchymal stem cells: a promising candidate in regenerative medicine. Int J Biochem Cell Biol, 2008;40(5):815-20
11. The MSC: An Injury Drugstore. Arnold I. Caplan, and Diego Correa. Cel Stem Cel 9, July 8, 2011

12. Maumus M, Jorgensen C, Noël D: Mesenchymal stem cells in regenerative medicine applied to rheumatic diseasesi role of secretome and exosomes. Biochimie, 2013;95(12):2229-34
13. Zuk PA et al: Human/adipose tissue is a source of multipotent stem cells. Molecular Biology of the Cell, 2002;13(12):4279-95
14. Kilroy GE et al: Cytokine profile of human adipose-derived stem cells: expression of angiogenic, hematopoietic, and pro-inflammatory factors. J Cell Physiol, 2007;212(3);702-9
15. Michalek et al: Autologous adipose tissue-derived stromal vascular fraction cells application in patients with osteoarthritis. Cell Transplant, 2015
16. Conese M et al: Paracine effects and eterogeneity of marrow-derived stem/progenitor cells: relevance for the treatment of respiratory diseases. Cells Tissues Organs, 2013;197(6):445-73
17. Pers YM et al: Adipose Mesenchymal Stromal Cell-Based Therapy for Severe Osteoarthritis of the Knee: A Phase I Dose-Escalation Trial. 2016;5(7):847-56
18. Djouad F et al: Mesenchymal stem cells: innovative therapeutic tools for reumatic diseases.Nat Rev Rheuatol, 2009;5:392-9
19. Im GI: Regeneration of articular cartilage using adipose stem cells. Journal of Biomedical Materials Research, 2016;104(7):1830-44
20. Yong-Gon Koh, Oh-Ryong Kwon, Yong-Sang Kim, Yun-Jin Choi, and Dae-Hyun Tak Adipose-Derived Mesenchymal Stem Cells With Microfracture Versus Microfracture Alone: 2-Year Follow-up of a Prospective Randomized Trial. Arthroscopy: The Journal of Arthroscopic and Related Surgery, Vol 32, No 1 (January), 2016: pp 97-109
21. Naomasa Yokota, Masayuki Yamakawa, Tomohiko Shirata, Tetsuya Kimura, Hideto Kaneshima Clinical results following intra-articular injection of adipose-derived stromal vascular fraction cells in patients with osteoarthritis of the knee. Regenerative Therapy 6 (2017) 108-112.
22. Y.S. Kim, Y.J. Choi, S.W. Lee, O.R. Kwon, D.S. Suh, D.B. Heo, Y.G. Koh Assessment of clinical and MRI outcomes after mesenchymal stem cell implantation in patients with knee ostéoarthritis: a prospective study. Osteoarthritis and Cartilage 24 (2016) 237-245
23. Loibl M et al: The effect of leukocyte-reduced platelet-rich plasma on the proliferation of autologous adipose-tissue derived mesenchymal stem cells. Clin Hemorheol Microcirc, 2016;61(4):599-614
24. Bianchi F, Maioli M, Leonardi E, Olivi E, Pasquinelli G, Valente S, Mendez AJ, Ricordi C, Raffaini M, Tremolada C, and Ventura C. A New Nonenzymatic Method and Device to Obtain a Fat Tissue Derivative Highly Enriched in Pericyte-Like Elements by Mild Mechanical Forces From Human Lipoaspirates. Cell Transplantation, Vol. 22, pp. 2063-2077, 2013
25. Yong-Gon Koh, M.D., Oh-Ryong Kwon, M.D., Yong-Sang Kim, M.D., and Yun-Jin Choi, M.D. Comparative Outcomes of Open-Wedge High Tibial Osteotomy With Platelet-Rich Plasma Alone or in Combination With Mesenchymal Stem Cell Treatment: A Prospective Study. Arthroscopy: The Journal of Arthroscopic and Related Surgery, Vol 30, No 1(November), 2014: pp 1453-1460
26. Khanh Hong-Thien Bui, Triet Dinh Duong, Nhan Thanh Nguyen, Thanh Duc Nguyen, Vien Tuong Le, Viet Thanh Mai, Nhan Lu-Chinh Phan, Dung Minh Le, Ngoc Kim Ngoc, Phuc Van Pham Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet-rich plasma: a clinical study. Biomedical Research and Therapy, 2014 (1):02-08

27. Lee, G. W., Son, J. H., Kim, J. D. & Jung, G. H. Is platelet-rich plasma able to enhance the results of arthroscopic microfracture in early osteoarthritis and cartilage lesion over 40 years of age? Eur J Orthop Surg Traumatol 2013; 23: 581-587
28. Mancò, A., Goderecci, R., Rughetti, A., De Giorgi, S., Necozione, S., Bernardi, A., & Calvisi, V. (2016). Microfracture versus microfracture and platelet-rich plasma: arthroscopic treatment of knee chondral lesions. A two-year follow-up study. Joints, 4(3), 142-147.
29. Koh et al: Adipose-Derived Mesenchymal Stem Cells With Microfracture versus Microfracture alone: 2-Year Follow-up of a Prospective Randomized Trial. Arthroscopy, 2016;32(1):97-109
30. Mainil-Varlet P, Van Damme B, Nesic D, Knutsen G, Kandel R and Roberts S. A New Histology Scoring System for the Assessment of the Quality of Human Cartilage Repair: ICRS II. The American Journal of Sports Medicine. Vol. 38, No. 5, 2010
31. Pascale W, Simone Luraghi S, Laura Perico L, Valerio Pascale V. Do Microfractures Improve High Tibial Osteotomy Outcome? Orthopedics. 2011;34(7):e251-e255
32. Akizuki S, Yasukawa Y, Takizawa T. Does Arthroscopic Abrasion Arthroplasty Promote Cartilage Regeneration in Osteoarthritic Knees With Eburnation? A Prospective Study of High Tibial Osteotomy With Abrasion Arthroplasty Versus High Tibial Osteotomy Alone. Arthroscopy: The Journal of Arthroscopic and Related Surgery. Vol 13, 1, 1997: pp 9-17
33. Saw KY, Anz A, Jee CS, Ng RC, Mohtarrudin N, Ragavanaidu K. High Tibial Osteotomy in Combination With Chondrogenesis After Stem Cell Therapy: A Histologic Report of 8 Cases. Arthroscopy. 2015 Oct;31(10):1909-20.
34. T. T. NiinimäKi, A. Eskelinen, B. S. Mann, M. Junnila, P. Ohtonen, J. Leppilahti. Survivorship of high tibial osteotomy in the treatment of osteoarthritis of the knee. FINNISH REGISTRY-BASED STUDY OF 3195 KNEES. J Bone Joint Surg Br 2012;94-B: 1517-21.
35. Sterrett WI, Steadman JR. Chondral Resurfacing and High Tibial Osteotomy in the Varus Knee. Am J Sports Med 2004;32:1243-1249.

Arthrosocpic Treatment of Knee Chondral Lesions: Clinical, MRI and Histologic Comparison Between the Use of PRP vs ADSCs in Addition to Marrow Stimulation Techniques

Informed consent for the processing of sensitive data

NCT ID not yet assigned

Unique protocol id 23/2019

Document date: March 25, 2019. Traslated into English on August 27, 2025

As the original documents were written exclusively in Italian, the following is the complete translation of each document into English, presented alongside the original text.

Patient Information Note

In accordance with Legislative Decree No. 196 of June 30, 2003 (Personal Data Protection Code) and Authorization to Process Sensitive Data for Observational Studies

TITLE	Arthroscopic Treatment of Knee Chondral lesions: Clinical, MRI and Histologic comparisons between the use of PRP vs ADSCs in addition to Marrow stimulation techniques
ID NUMBER	
VERSION AND DATE OF THE PROTOCOL	version 1/19 - protocol March 2019
HEAD OF THE PROJECT	[redacted], Associate Professor, Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio-Coppito 2, 67100, L'Aquila, Italy Tel.: [redacted] e-mail:
RESEARCH LOCATION	U.O.S.D. D.U. Minimally Invasive and Computer-Assisted Orthopedic Surgery directed by [redacted] at the San Salvatore Hospital in L'Aquila.

PURPOSE OF TREATMENT

The "U.O.S.D. Minimally Invasive and Computer-Assisted Orthopedic Surgery" of the San Salvatore Hospital of L'Aquila, as Study Center and Data Controller, in accordance with alleresponsibilities provided by the rules of good clinical practice (d.l. 211/2003) and to the current legislation on the processing of personal data (Dlgs 196/2003 and subsequent amendments and/or integrations), will process your personal data, in particular health data and, only to the extent that they are indispensable in relation to the objective of the study, other data relating to date of birth, sex, weight, height, according to the implementation of the study. Processing your personal data related to general and specific clinical conditions in the field of orthopedics is essential to conducting the study. Refusing to provide this data will prevent you from participating. [redacted], the study initiator, will be identified as the data controller.

The study's scientific staff includes: [*omissis*]

This staff will process your personal data—in particular, data concerning your health and medical history—and other sensitive data only to the extent that it is indispensable in relation to the objective of the study, exclusively for the purpose of conducting the study itself.

NATURE OF DATA

The doctor who will follow you in the study will identify you by a code. The data collected about you in the course of the study, with the exception of your name, will be transmitted to the departments of diagnostic imaging, pathological anatomy, and statistics. The investigators working in these departments will score the quality of the cartilage found in the control MRIs and biopsies performed during the second-look arthroscopy. They will also statistically analyze

the results obtained from the questionnaires you will fill out. This data will be recorded, processed, and stored together with this code, your date of birth, gender, weight, and height. Only the doctor and authorized persons will be able to link this code to your name.

TREATMENT MODALITIES

The data will be processed using paper and electronic tools.The data concerning you, collected during the study will be recorded, processed and storedfor 5 years along with this code. The data will be kept in a locked room pressol UOSD University Knee Surgery of San Salvatore Hospital in L'Aquila.The person in charge of data processing and storage is The Director of the Department, [redacted].

Your participation in the study implies that, in accordance with clinical trial regulations, the staff conducting the study and the Internal Review Board may have access to data concerning you, including identifying information, which may include data contained in your original clinical documentation. The data may be disclosed, for example through scientific publications, statistics, and scientific conferences, only in strictly anonymous form.

EXERCISING RIGHTS

You may also exercise your rights under Article 7 of the Privacy Code (e.g., accessing your personal data, supplementing them, updating them, rectifying them, objecting to their processing for legitimate reasons, etc.) by contacting the testing center directly ([redacted], Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio-Coppito 2, 67100, L'Aquila, Italy Tel.: [redacted] e-mail: [redacted].

You may withdraw from the study at any time without providing justification. In this case, your biological samples will be destroyed. No additional data will be collected about you, without prejudice to the use of any data already collected to determine the results of the research without altering them.

AUTHORIZATION FOR THE PROCESSING OF SENSITIVE DATA OF PARTICIPANTS IN OBSERVATIONAL STUDIES

Place and Date

I, (First and last name of participant in block letters) _____, accept the information provided by the data controller pursuant to art. 13 of Legislative Decree No. 196/2003. I am aware that the processing will concern the "sensitive" data referred to in Art. 4, Paragraph 1, Letter d), as well as Art. 26 of D.lgs. 196/2003. This includes data "capable of revealing racial and ethnic origin, religious, philosophical, or other beliefs; political opinions; membership in parties, trade unions, associations, or organizations of a religious, philosophical, political, or trade union nature; as well as personal data disclosing health and sexual life."

-I give my consent to the processing of data necessary for the performance of the operations indicated in the privacy policy.

Signature .....

- gives consent for the communication of data to the subjects indicated in the privacy policy. (in the event that the communication of sensitive data of the data subject is also envisaged)

Signature ....

- gives consent for the dissemination of data within the scope indicated in the privacy policy. (if the dissemination of sensitive data other than that which may reveal the state of health of the data subject is also envisaged; the latter, in fact, cannot be disseminated).

Signature .....

Arthrosocpic Treatment of Knee Chondral Lesions: Clinical, MRI and Histologic Comparison Between the Use of PRP vs ADSCs in Addition to Marrow Stimulation Techniques

Informed consent for partecipation in the Study

NCT ID not yet assigned

Unique protocol id 23/2019

Document date: March 25, 2019. Traslated into English on August 27, 2025

As the original documents were written exclusively in Italian, the following is the complete translation of each document into English, presented alongside the original text.

INFORMATION SHEET AND INFORMED CONSENT FOR PARTICIPATION IN A RESEARCH STUDY TO SUPPLEMENT CONSENT FOR TREATMENT

TITLE	Arthroscopic Treatment of Knee Chondral lesions: Clinical, MRI and Histologic comparisons between the use of PRP vs ADSCs in addition to Marrow stimulation techniques
ID NUMBER	
VERSION AND DATE OF THE PROTOCOL	version 1/19 - protocol March 2019
HEAD OF THE PROJECT	[redacted], Associate Professor, Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio-Coppito 2, 67100, L'Aquila, Italy Tel.: [redacted] e-mail:
RESEARCH LOCATION	U.O.S.D. D.U. Minimally Invasive and Computer-Assisted Orthopedic Surgery directed by [redacted] at the San Salvatore Hospital in L'Aquila.

Dear Sir/Madam,
You have been invited to participate in a research project. In order for this study to take place, we need the cooperation of patients who possess the characteristics necessary for the study, which we will explain. Professional ethics require the fully informed consent of each participant in any medical research. This statement and consent form contain information that may help you decide. The document describes the purpose of the research, the procedures that will be used, and the names of physicians (and/or researchers) who can answer your questions. If you agree to participate in the study, you will be asked to sign the consent form. Your decision to participate in this study is voluntary. This means that you may participate if you wish, or not participate if you do not wish to. Your decision to participate or not participate will not affect your medical care. You may also withdraw from the study at any time without explanation and without affecting your disease management.
Please read the information in this document carefully. If you wish, you may discuss it with your family members and your family doctor, taking as much time as you need. Please ask us for clarification if any of the information is unclear.
The doctors at the U.O.S.D. of Minimally Invasive and Computer-Assisted Orthopedic Surgery are available to answer any questions you or your doctor may have.

STUDY DESCRIPTION
Our study aims to evaluate the efficacy differences between two autologous products.
(i.e., obtained from your own body) for treating cartilage defects (chondropathies) of the knee.
We aim to evaluate the effectiveness of platelet-rich plasma (PRP) infiltrated alone or in combination with adipose-derived stem cells at the end of arthroscopic treatment for knee chondropathies, using clinical, diagnostic, and histological methods.
The study will last approximately three years.
About 80 male and female subjects, aged 30 to 60 years, with severe knee chondropathy are planned to participate in the present single-center study. Treatment effect monitoring will last for at least three years.

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

You have been invited to participate in this study because you have been diagnosed with chondropathy of the knee, for which surgical treatment by arthroscopy has been recommended. You have signed the relevant informed consent form for this procedure. If you decide to participate in the study, you will be randomly assigned to one of two groups: The "control group" or the "study group." You will be offered the option of adding mesenchymal stimulation (cartilage repair stimulation) to the arthroscopic treatment, along with PRP infiltration, with or without adipose-derived stem cells. Specifically in the operating room, prior to arthroscopic surgery, whether you are in the control or study group, a venous blood sample (approximately 15 mL) will be taken and centrifuged to obtain PRP (platelet-rich plasma). If you are in the study group, a sample of subcutaneous abdominal adipose tissue (approximately 40 mL) will be taken using minimally invasive needles. This sample will then be centrifuged to extract the fraction containing stem cells, which will be combined with the previously obtained PRP. At the end of the surgical treatment, these derivatives (PRP or PRP + Stem Cells) will be injected into the joint. After the operation, you will be monitored continuously for up to 3 years of follow-up. In particular, we will evaluate you clinically at regular intervals, asking you to complete specific questionnaires on the functionality of your knee independently. As is standard practice for treating your condition, you will be required to undergo an MRI scan of your knee before surgery and again at 12, 24, and 36 months after surgery. These scans will help assess the healing of your injury. If your condition indicates the need for a subsequent knee osteotomy (the resection of a wedge of bone at the tibial level and its subsequent synthesis with a specific plate), this will be proposed as a solution to correct the mechanical axis deviation of your limbs. If you agree, you will undergo a second-look arthroscopy on this occasion. This procedure involves observing, palpating, and taking a microscopic biopsy of the cartilage with a minimally invasive needle. The goal is to assess whether the joint surface has "healed" enough to support the effects of the osteotomy. These results will also be used to evaluate the effectiveness of the two treatments in this study.

The following cooperation is required on your part: consent to arthroscopic mesenchymal stimulation and infiltration at the end of the PRP or PRP + Stem Cells procedure, depending on the group you are assigned to. In the post-operative period, you will be asked to:

- undergo free check-ups by filling out specific questionnaires
- Undergo follow-up MRI scans at 12, 24, and 36 months, as routinely required when monitoring the progress of cartilage repair.
- If necessary, and if specifically indicated and if you agree, undergo an arthroscopic second look after 12 months and subsequent corrective osteotomy.

WHAT ARE THE FORESEEABLE RISKS OF THE STUDY?

Participation in the planned treatments and/or investigations does not involve any additional risks beyond those considered for the surgical risk of knee arthroscopy and PRP infiltration and purified autologous adipose tissue grafting; all of the above procedures have been practiced worldwide for several years, and there have been no significant complications reported in the literature associated with these techniques. Specifically, for each of the three procedures (arthroscopy, obtaining and infiltrating autologous PRP, and obtaining and infiltrating stem cells purified from autologous adipose tissue) you have been informed of the purposes, characteristics, and possible complications through specific consent forms. This document is an addition to those forms. It should be noted that both the PRP and the stem cells that will be

collected and centrifuged are entirely autologous (i.e., derived from the patient's own body) and therefore there is no external contamination with tissue derived from other human beings. If corrective osteotomy is necessary during treatment, you will be offered an arthroscopy to evaluate the healing of the injury in real time through observation and palpation. During this procedure, a microscopic biopsy will be performed on the repair site. Given the microscopic size of the needle, this procedure will not damage the repair area. These procedures are necessary to confirm the need for corrective osteotomy and will also provide data for this study. If you have any questions or concerns during the study, the medical staff will be available to assist you, either directly or with the help of other specialists.

WHAT ARE THE EXPECTED BENEFITS OF THE STUDY?

The results of this study will further knowledge of innovative techniques for treating cartilage lesions, a challenge that orthopedic surgeons still face today. Conservative treatments based on physiokinesitherapy and viscosupplementation do not always allow for complete healing and resolution of symptoms in this specialized connective tissue, which has great difficulty healing intrinsically. Platelet-rich plasma (PRP) and adipose-derived stem cells represent the latest frontier in treating this condition; they have shown promising results in laboratory and clinical studies on humans. This study aims to determine whether adding stem cells to PRP (an autologous derivative rich in growth factors) enhances its effect by increasing its reparative capacity on cartilage. We will observe the patients' clinical condition, as well as the images obtained by magnetic resonance imaging. Where indicated, we will also perform histology.
The desired benefits for you could result from implementing, through the infiltration of autologous substances (i.e., derived from your own body), the results of established arthroscopic techniques that will be performed in any case.

FREE CHOICE TO PARTICIPATE IN THE STUDY

Whether or not to participate in the study is entirely up to you. If you choose to participate, you will be given an informed consent form to sign. You will also be given specific consent forms for the autologous blood collection necessary to prepare PRP and for the transplantation of purified autologous tissue. If corrective osteotomy is indicated and you give your consent, you will be offered a second-look arthroscopy to evaluate the healing of the cartilage, take a microscopic sample of the repaired tissue, and make a final decision on the osteotomy.

WHAT HAPPENS IF YOU DECIDE NOT TO PARTICIPATE OR TO WITHDRAW FROM THE STUDY

You are free to withdraw from the study at any time by informing the investigating physician. You do not need to provide a reason, and this will not affect the quality of healthcare services you receive from this Center. If you withdraw your consent and wish to discontinue contact with us, we will delete all information concerning you. You have the right to be informed of any changes to the study protocol that may affect you. In this case, you will be asked to sign a new informed consent form.

EXPENSES AND FINANCIAL COMPENSATION RESULTING FROM PARTICIPATION IN THE STUDY

Participation in the study is free of charge. You will not receive monetary compensation for participating. You will not be reimbursed for any travel expenses to the center/hospital where the study is being conducted.

CONFIDENTIALITY OF INFORMATION COLLECTED

If you choose to participate in this study, please know that all data collected will be processed and stored anonymously, in accordance with Legislative Decree 196/2003. This decree went into effect on June 30, 2003, and addresses the protection of individuals with regard to the processing of personal data, as well as subsequent amendments and additions.

Only personnel involved in the study will have access to this information. The researcher will protect access to this data. Your signature confirming your participation will guarantee confidentiality.

If you wish, you may be informed of the results of the experiment. The results of the study will be used exclusively for scientific research purposes. They may be published, but your identity will always remain confidential.

NEW INFORMATION AND/OR RESULTS REGARDING THE STUDY

If new information or results about the study become available, we will promptly provide you with any relevant information that could affect your decision to continue participating. If we find information about the condition being studied that could affect your condition or health, we will inform you.

WHO SHOULD I CONTACT FOR MORE INFORMATION OR IF I NEED HELP?

If you have any questions about the research or communication related to the study, or if you experience any damage related to the study, please contact the U.O.S.D. D.U. Minimally Invasive and Computer-Assisted Orthopedic Surgery at San Salvatore Hospital in L'Aquila and speak with the study doctor or one of his colleagues. Below is a list of all the people at this center who provide assistance to study participants and their telephone numbers. You will be informed verbally if this list changes during the course of the study.

[*omissis*]

INFORMED CONSENT FORM

for participation in scientific research to supplement the informed consent to treatment

I, (First and last name of participant in block letters) _____,

By signing this form, I declare the following:

• I have read and understood the contents of this information sheet, which have been explained to me clearly and comprehensively.

• I was given the opportunity to ask questions and received clear and comprehensive answers.

• Participation in the study is completely voluntary, and I may withdraw at any time by informing the study doctor of my decision without losing any benefits, medical treatment, or legal rights to which I am otherwise entitled.

• I was informed of the reasonably foreseeable risks and benefits and was given sufficient time to decide.

• I am aware that my clinical data may be used for scientific publications and will remain strictly confidential in accordance with current legislation and subsequent amendments and additions.

• I understand that I will be informed of any new information or findings that may affect my participation in the study.

• I understand that I must sign two identical copies of this informed consent form. One copy will be kept by the doctor, and I will receive the other.

Participant's First and Last Name (in block letters): _____

Place and Date: _____

Participant's Signature: _____

DOCTOR WHO PRESENTED THE INFORMATION TO THE PATIENT

Doctor's First and Last Name (in block letters): _____

Place and Date: _____

Doctor’s Signature: _____