



Level II, randomized clinical trial

Adipose tissue-derived mesenchymal stem cells (AD-MSC) versus platelet-rich plasma (PRP) in the arthroscopic treatment of chondral defects of the knee: what's the best? A histological and clinical evaluation

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ABSTRACT

The purpose of this study was to compare the clinical and histological effects of adipose tissue-derived mesenchymal stem cells (AD-MSCs) and platelet-rich plasma (PRP) in the arthroscopic treatment of chondral defects of the knee joint.

This is a randomized controlled trial including 46 patients affected by a knee monocompartmental chondral lesion (Outerbridge grade IV). All patients underwent arthroscopic microfracture and autologous PRP injection and were randomized into 2 groups: in group A they were additionally treated with autologous AD-MSCs injection whilst in group B they didn't receive any additional treatment.

Clinical outcomes were assessed with the Oxford Knee Score (OKS), the International Knee Documentation Committee (IKDC) score, and the Visual Analogue Scale (VAS) before the treatment and at 1, 6, 12, and 24 months post-operatively.

The histological evaluation was performed on biopsy specimens taken from 6 patients (needing a subsequent high tibial osteotomy and experiencing a second-look arthroscopy).

Based on the results of OKS, IKDC, and VAS, a significant clinical improvement was detected in both groups at 6, 12, and 24 months of follow-up, with no significant statistical difference in treatment effect. On the other hand, the histological evaluation of the 6 biopsy specimens confirmed better ultrastructural results in group A, with hyaline-like cartilage closely resembling native tissue.

Both PRP and AD-MSC, when associated with arthroscopic microfracture, seem to enhance clinical outcomes by promoting cartilage regeneration ability. The histological results of this study could be the premises to investigate the synergic effect of these promising techniques on the mechanisms of cartilage regeneration.

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INTRODUCTION

Articular cartilage is a thin viscoelastic specialized tissue composed of chondrocytes dispersed within an extracellular matrix. Overall cartilage homeostasis is the product of a complex interaction between growth factors, joint mechanics, hormones, and aging (1). Native articular cartilage experiences and endures a myriad of mechanical forces, such as compression, tension, shear, and hydrostatic pressure (2, 3).

Chondral defects are commonly encountered in orthopedic practice since articular cartilage has a poor self-regeneration ability and focal articular defects occur in up to two-thirds of patients experiencing knee arthroscopy, though often asymptomatic (4). Anyway, symptomatic cartilage lesions can significantly limit the common activities of daily living, with mild to severe pain or discomfort, so this situation can be truly disabling especially in health young patients. For this reason, different surgical options have been proposed for the management of isolated chondral lesions, such as microfracture, nanofracture, abrasive chondroplasty, and spongialisation (5–8). All these techniques aspire to reduce pain, restore articular function and stimulate cartilage regeneration.

The limited biomechanical properties of spontaneously formed repair cartilage contribute to its functional incompetence and deterioration; clinical results are only temporary and satisfying for low-grade cartilage defects (9). To overcome the shortcomings of these arthroscopic stimulating techniques, the concomitant support of biologic alternatives such as growth factors or multipotent cells might potentiate and ameliorate the regenerative response (10).

Platelet-rich plasma (PRP) is an important source of growth factors and it has been widely used since the 1970s as the ideal solution for cartilage defects, due to the attitude to promote cartilage healing, decrease pain and improve clinical function (11). Its effect is still temporary (being estimated to last up to 1 year) and it is still debated if PRP has a chondroprotective effect on the progression of the cartilage defect and joint degeneration.

A modern approach in the regenerative area starts from the capability of mesenchymal stem cells (MSCs) to activate the healing processes through an immunomodulatory and paracrine mechanism (12). The paracrine activity is responsible for its anti-inflammatory, anti-apoptotic, anti-fibrotic, angiogenic, and mitogenic properties (13). Adipose-tissue-derived mesenchymal stem cells (AD-MSCs), located in the stromal vascular fraction of subcutaneous adipose tissue promote tissue regeneration by secreting growth factors and cytokines and are an optimal source, given their large availability (14, 15).

As stated above, the potential benefits of bone marrow stimulation techniques could be amplified by the association with other regenerative techniques, such as PRP and AD-MSCs. Based on these premises, we defined and conducted this study to evaluate the histological and clinical effects of PRP and AD-MSCs in the arthroscopic treatment of isolated monocompartmental cartilage defects of the knee joint.

MATERIALS AND METHODS

This is a prospective randomized controlled study conducted at a single center including 46 patients (mean age: 52.6 years; range 38-73 years). Eligible patients included adults affected by an arthroscopically verified monocompartmental chondral defect (<2 cm²) classified as grade IV according to Outerbridge classification, located on the femoral condyle or tibial plate. Exclusion criteria are listed in Table I. It was not possible to blind the patients

as to what treatment they would have received, given that one treatment arm required a supplementary surgical gesture (a mini-liposuction for the harvesting of AD-MSCs).

Table I. *Exclusion criteria*

<ul style="list-style-type: none"> • Knee or hip osteoarthritis • Knee instability • Cartilage defects in the contralateral knee compartment or in the patellofemoral joint • Previous surgical treatments to the chondral defect • Systemic or local infections • Coagulopathy or hematological diseases • Neurological diseases • Pregnancy • Obesity (BMI > 30) • rheumatic/immunological/neoplastic/metabolic diseases • patients receiving immunosuppressive treatment or long-term corticosteroid therapy • patients with Hb values < 11g/dL and platelet values < 150,000/mm³ • chondral defect larger than 2 cm²
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The study was consistent with ethical principles for medical research ratified by the World Medical Association (WMA) Declaration of Helsinki, having been approved by the Internal Review Board. All patients were carefully informed about the modality and purpose of the study, gave their approval, and subscribed to a specific informed consent form. Before treatment, baseline demographic data was collected.

All patients, preliminary evaluated with knee MRI¹⁶ and clinical examination, underwent a diagnostic arthroscopy to confirm an isolated chondral lesion (Outerbridge grade IV). All the surgical procedures (diagnostic and therapeutic ones) were performed by the same skilled surgeon (senior author). The chondral defect underwent arthroscopic debridement with an arthroscopic abrader to form a stable edge of healthy cartilage and a bone marrow stimulation technique (microfracture).

After that an autologous PRP injection was performed in all patients: 8 mL of venous blood was taken from each patient and centrifugated to separate the blood into the plasma, the buffy coat, and residual red blood cells and collect the desired quantity of PRP, which was then injected into the affected knee.

At this point, patients were randomly assigned to one of the two groups (allocation ratio 1:1) by the only member of the staff with access to the allocation spreadsheet. In this way, 23 patients (group A) experienced mini-abdominal liposuction to harvest the AD-MSCs which were then injected into the affected knee. The other 23 patients (group B) did not receive any further treatment. Group characteristics at inclusion are shown in Table II.

Table II. Demographic characteristics of the cohorts of patients included in the study.

Characteristics	Group A (Microfracture + PRP + AD- MSCs)	Group B (Microfracture + PRP)
Mean age	54.4	50.8
BMI	26.4± 2.2	25.8±2. 8
Sex		
(i) Male	13	14
(ii) Female	10	9
Affected side		
(i) Left	9	7
(ii) Right	14	16

Six patients (needing a subsequent high tibial osteotomy) experienced a second-look arthroscopy to evaluate cartilage healing; a histological analysis was performed on the biopsy samples taken from the original chondral defect. Histological evaluation was conducted according to the International Cartilage Repair Society (ICRS) visual histological assessment scale (Table III).

Table III. *ICRS (International Cartilage Research Society) Visual Histological Assessment Scale.*

Feature	Points
Surface	
Smooth/continuous	3
Discontinuities/irregularities	0
Matrix	
Hyaline	3
Mixture: hyaline/fibrocartilage	2
Fibrocartilage	1
Fibrous tissue	0
Cell distribution	
Columnar	3
Mixed/columnar-clusters	2
Clusters	1
Individual cells/disorganized	0
Cell population viability	
Predominantly viable	3
Partially viable	1
<10% viable	0
Subchondral bone	
Normal	3
Increased remodelling	2
Bone necrosis/granulation tissue	1
Detached/fracture/callus at base	0
Cartilage mineralization (calcified cartilage)	
Normal	3
Abnormal/inappropriate location	0

Patients were prospectively evaluated basally before surgery and at 1, 6, 12, and 24 months of follow-up using OKS and IKDC for general health status, and VAS for pain evaluation. The survey of all clinical data was performed by a member of the staff not involved in the surgical procedure.

Sample size and statistical analysis

We considered this trial as a pilot study: we are aware that pilot studies should be undertaken before evaluation in a full randomized controlled trial, according to published guidelines. Sample size calculation was not possible since there is insufficient data published to date on the effects of PRP and AD-MSCs on knee chondral defects. On this basis, we included 46 participants with 23 patients allocated to each group.¹⁷ Demographic and clinical

characteristics have been presented as means and standard deviations (SD) for data with a normal distribution and median and interquartile range for non-parametric data. P-values of <0.05 were considered statistically significant and they were interpreted based on Bonferroni correction. All statistical analyses were performed using MedCalc® version 13.3.1.

Results

OKS, IKDC and VAS data were collected for all patients at 1, 6, 12, and 24 months of follow-up. Mean IKDC scores for group A and group B at baseline, at 12 and 24 months (final follow-up) are shown in Fig. 1. No significant statistical differences in mean IKDC scores were detected between the two cohorts. The mean IKDC score at 24-month follow-up in group A was 72.4 ± 1.8 compared to 70.3 ± 1.5 in group B. The increase in IKDC score from baseline to follow-up was significant for both groups.

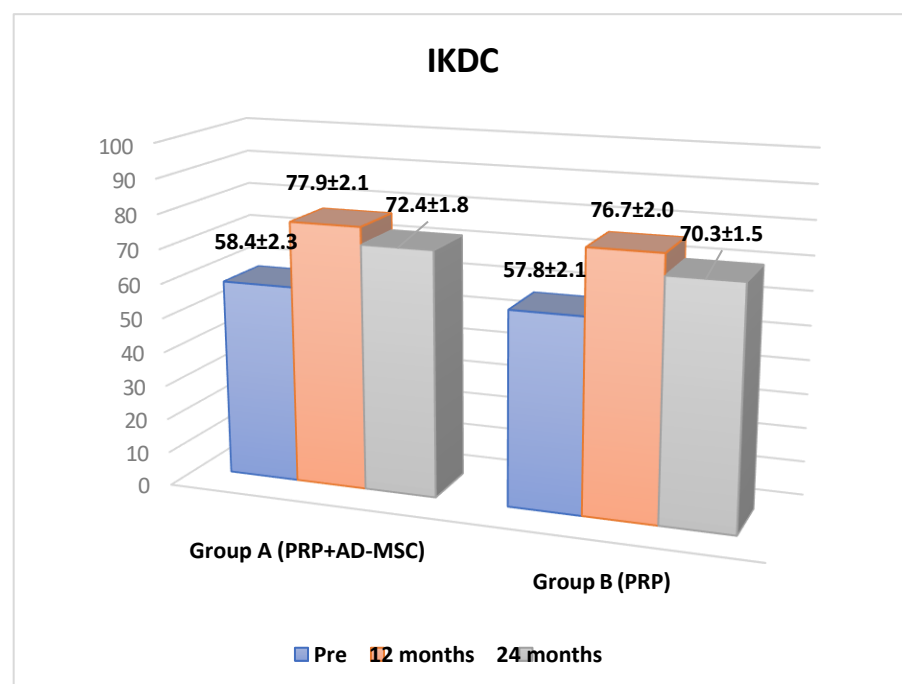


Fig. 1. IKDC score – pre-treatment, at 12- and 24-months follow-up.

The OKS profiles with mean scores before surgery, at 12 and 24 months-follow-up for group A and group B are shown in Fig. 2. There were no differences between the two groups in any of the OKS data collection, with a progressive improvement at 12 months (Group A: 44.0 ± 1.2 ; Group B: 42.9 ± 0.6) and a subsequent reduction in the performance scores at 24 months (Group A: 42.0 ± 0.8 ; Group B: 40.6 ± 0.4).

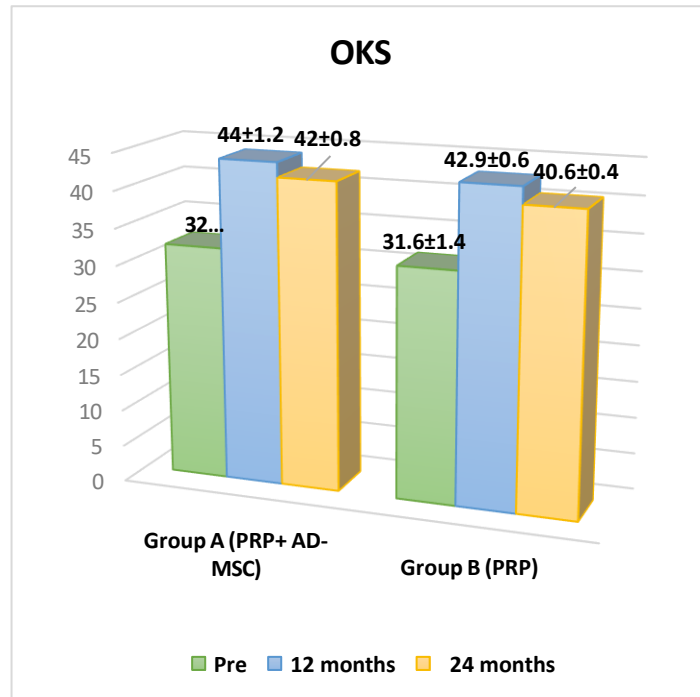


Fig. 2. OKS score – pre-treatment, at 12- and 24-months follow-up.

In both groups we appreciated a similar progression from baseline in VAS (Fig. 3). VAS score dropped from 6.4±1.33 before treatment to 3.32±1.55 at 12 months follow-up and to 4.0±1.6 at 24 months follow-up for group A and from 6.12±1.27 to 3.12±2.10 for group B at 12 months and to 4.8±1.6 at 24 months.

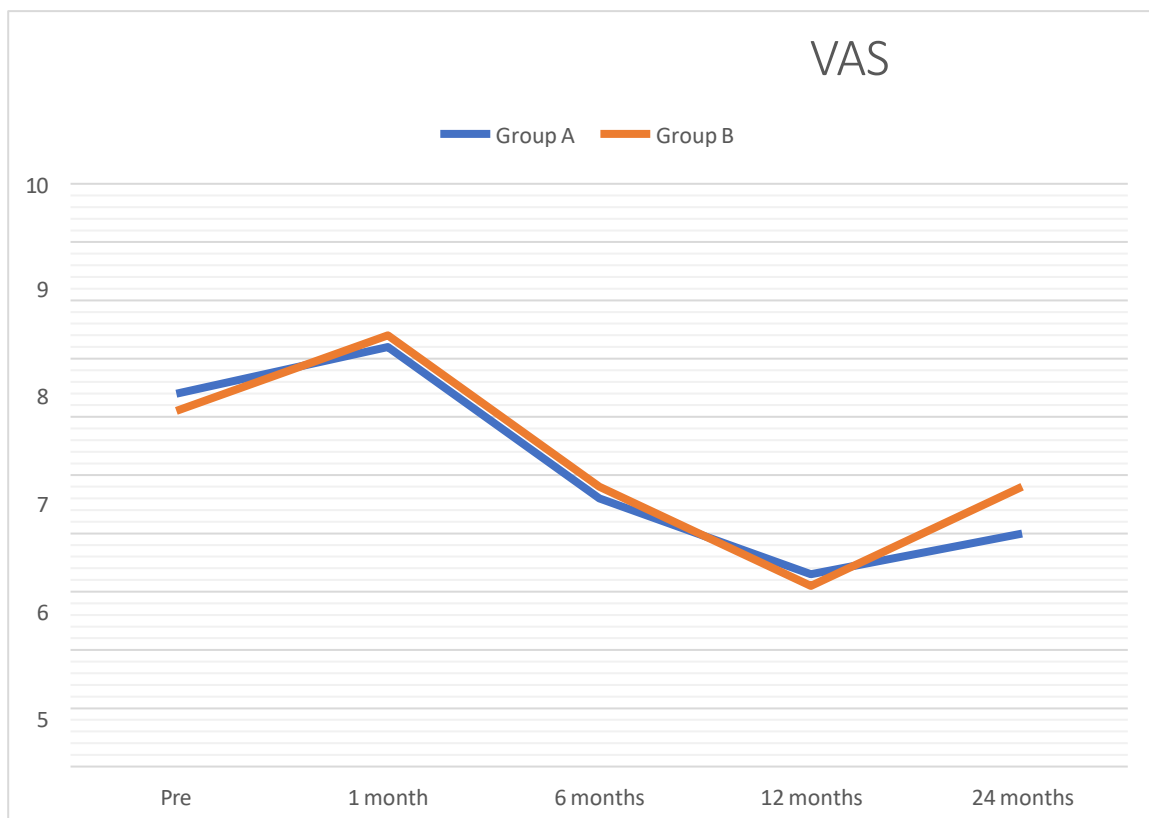


Fig. 3. VAS score evolution over time in group A and Group B. We note a comparable reduction of pain intensity for both groups from one month to 12 months after surgery with a rebound effect of pain perception at 24 months follow-up.

Eight patients (5 for group A and 3 for group B) experienced adverse events (swelling and pain with a longer recovery time). They were treated with oral nonsteroidal anti-inflammatory drugs, ice, and rest, with no functional limitations, once passed the acute phase (lasting a few days).

We performed a second-look knee arthroscopy (3 patients for group A and 3 patients for group B) one year after the regenerative/arthroscopic treatment in 6 patients eligible for knee corrective osteotomy (high tibial osteotomy in genu varum): smooth hyaline cartilage was appreciated in all patients, thus justifying the good clinical outcomes. Specifically, a thick smooth hyaline-like cartilage with normal mineralization and predominantly viable cell population (comparable to native articular cartilage) was detectable in group A (fig. 4) whereas a smooth resurfacing fibrocartilage with a predominantly viable cell population and abnormal cartilage mineralization was present in group B.

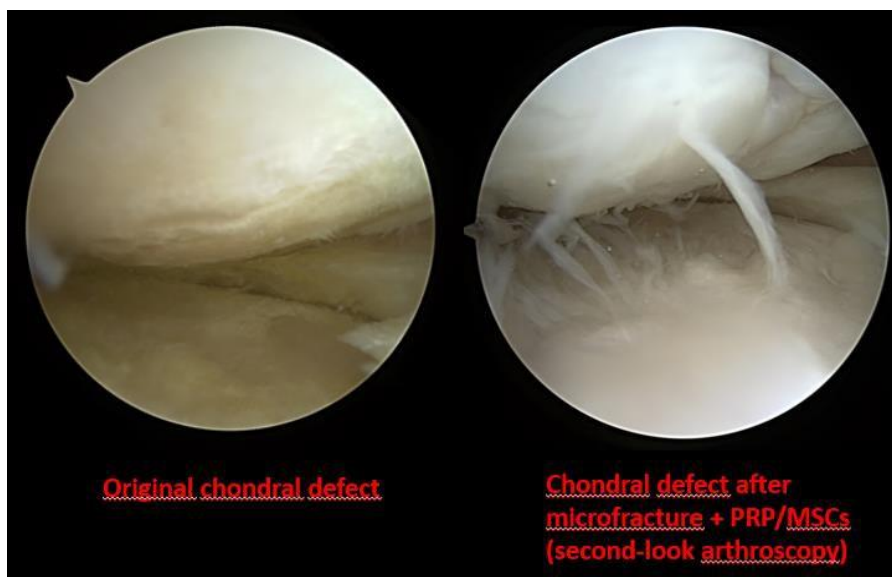


Fig. 4. Macroscopic evaluation of the cartilage lesion – original chondral defect compared with the same lesion after microfracture + PRP/MSCs (as detected during a second-look arthroscopy). A biptic sample was harvested for histological evaluation.

Macroscopic and microscopic histological evaluation of biopsy samples referred to ICRS visual histological assessment scale; all data are reported in Table IV.

Table IV. *Histologic features of the biopsy samples according to the ICRS Visual Histological Assessment Scale (Group A: PRP+ AD-MSC; Group B: PRP).*

	Group A			Group B		
Surface	3	3	3	3	3	3
Matrix	3	3	3	2	1	2
Cell distribution	2	3	2	1	2	2
Cell population viability	3	3	3	1	1	1
Subchondral bone	3	2	2	2	2	2
Cartilage mineralization	3	3	3	0	0	0

DISCUSSION

The results of this study focus on the clinical and histological evolution in the treatment of knee chondral defects. No significant statistical difference emerged from data collected in IKDC, OKS, and VAS. A similar progression is detectable in the 2 cohorts since a progressive improvement is present from baseline to 1 year and subsequent deterioration of clinical performance in both groups (though the clinical parameters at 2 years were better than pre-operative situation). The most interesting aspect of this study (though the evident statistical limits), to our opinion, concerns the histologic scope. The synergic effect of two regenerative procedures, such as AD-MSCs and PRP (in association with bone marrow stimulation such as microfracture) can lead to a more performing neo-cartilage, with predominantly viable cell population and normal mineralization (thus mimicking the native articular cartilage).

Different surgical techniques have been developed and refined over the last years to stimulate a spontaneous repair reaction in cartilage defects but none of them has been able to restore the desired hyaline cartilage.^{18–20} For this reason, these treatments are usually unsatisfactory in the long term. Knee microfracture (a bone marrow stimulation technique) is one of the most popular solutions in orthopedic practice, since this technique is cheap, doesn't require specific skills, and doesn't hinder potential future surgical treatments.

Cartilage deterioration is characterized by a mechanical basis, due to the ultrastructural damage, but also by an inflammatory process, as a consequence of pro-inflammatory cytokines activation, such as tumor necrosis factor α (TNF α), interleukins (IL), aggrecan, and metalloproteinases. Regenerative options investigated and developed over the last decades have focused on this inflammatory aspect, aiming to defuse these catabolic factors and promote a regenerative path. Based on these strategies, PRP and MSCs can be ideal candidates for this teamwork and can play a key role.

PRP is a safe non-surgical treatment for cartilage disease and, considering its potential role in tissue healing, has been widely investigated both in preclinical and clinical studies. Progressive implementation of the scientific production on this focus has been found in the last 15 years (21).

One of the actual limitations is the lack of standardization in PRP preparation and application, with no consensus on a definitive protocol. It is known that PRP preparation has a direct impact on the final composition, but the relative implications are not clarified. Different classification systems for PRP have been proposed (22–25) but none of these has been approved and validated by an international consensus.

Lacko et al. (26) investigated the metabolic effect of intra-articular applications of PRP in cartilage disease: according to their findings, a decrease in pro-inflammatory

biomarkers associated with an increase in specific anti-inflammatory and pro-anabolic biomarkers was detectable 3 months after PRP treatment. A recent review by Ip et al. (27), analyzing 1093 patients among 23 trials, focused on the safety, efficacy, and indications of intra-articular injections of PRP and MSCs in knee osteoarthritis. According to this study, the effectiveness of PRP injection in knee osteoarthritis is undoubtedly positive, with better clinical outcomes in patients with low-grade articular consumption (Kellgren-Lawrence grade I-II) and better results with increased dosages of PRP by repetitive injections. No significant adverse effects have been appreciated (the most common adverse reaction was a mild local self-limiting tenderness at the site of injection).

MSC plays an anti-inflammatory role in a different pattern by inhibiting the maturation of immune cells (monocytes and lymphocytes), suppressing natural killer cells, and preventing cell apoptosis and cartilage damage. MSC, acting in a paracrine way, can stimulate chondral differentiation and regeneration of hyaline joint cartilage (with similar contents of type II collagen and aggrecan if compared to native cartilage). The large availability (from a wide array of tissues, including adipose tissue), the absence of significant adverse effects, and the readiness to use of MSCs make interesting their use in the management of cartilage diseases. The most collateral morbidity effects were bruising and local discomfort after liposuction and injection procedures.

Wong et al. in a randomized controlled trial in 2013 analyzed the results of the use of intra-articular MSCs injections in conjunction with microfracture and medial opening-wedge high tibial osteotomy (HTO) (28). The primary outcome measure was the IKDC score at 6, 12, and 24 months postoperatively. Secondary outcome measures were Tegner and Lysholm clinical scores and 1-year postoperative Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scores. The effect of the treatment showed an improvement in all the scores confirming that Intra-articular injection of cultured MSCs is effective in improving both short-term clinical and MOCART outcomes in patients undergoing HTO and microfracture for varus knees with cartilage defects.

In a recent study published in 2021, Yang et al. analyzed the therapeutic value of arthroscopic microfracture in combination with PRP injection for knee cartilage injury (29). By administering VAS, IKDC, Tegner, and Lysholm at 1, 2, and 3 months the authors concluded that the microfracture technique combined with PRP injection in the treatment of knee joint cartilage injury was significantly more effective if compared with microfracture treatment alone, to reduce postoperative complications and improve the joint function.

Many studies in the last decades have investigated the therapeutic potential of PRP and AD-MSCs in the management of knee cartilage defects. The results of this pilot study indicate that both modalities are safe and effective to modify the natural history of disease progression and enhance overall function. In both groups, a significant reduction of pain in association with enhanced functional abilities has been appreciated. In light of the data collection of our study, we confirm that age is a key element in the regenerative ability of cartilage disease: better results have been found in younger patients (< 50 years). Another important element is BMI since it is largely responsible for stress forces loading on knee cartilage: overweight subjects unavoidably show worse outcomes. Undoubtedly the biological potential of PRP and AD-MSC is related to individual variability among patients, needing further in-depth research.

There are some limitations in this study; first of all, the limited number of patients in both groups. A larger-scale study would be necessary (especially for an in-depth histologic evaluation) for clinical application. Second, the follow-up should extend to assess clinical outcomes in the long term. Third, we cannot be certain that multiple PRP/AD-MSC injections are more effective. For all these reasons, we consider this trial a pilot study, preliminary for broader and thorough research.

CONCLUSION

On the clinical side, it is not yet incontrovertible if the association “PRP and MSCs” could improve the outcomes of bone marrow stimulation techniques. Anyway, the histological results of this trial address the light to the regeneration of higher-performance cartilage, with a form closely similar to native tissue. Additional research needs to be done and undoubtedly a great effort is required at both the basic and clinical research fronts.

This study represents a preliminary step to get evidence on cartilage defects treatment on which orthopedic surgeons could make important treatment selection decisions.

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Conflict of interest

The authors declare no conflict of interest.

Ethical standards

All the procedures performed in the study have been conducted in accordance with the ethical standards of the institutional and/or national research committee and the Helsinki Declaration of 1964 and its subsequent amendments or comparable ethical standards.

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