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PRP For the Treatment of Cartilage Pathology

Elizaveta Kon[*,1], Giuseppe Filardo[1], Berardo Di Matteo[2] and Maurilio Marcacci[2]

[1]*Nano-Biotechnology Laboratory, Rizzoli Orthopaedic Institute, Via di Barbiano n. 1/10, 40136, Bologna, Italy*

[2]*Biomechanics Laboratory, Rizzoli Orthopaedic Institute, Via di Barbiano n. 1/10, 40136, Bologna, Italy*

Abstract: In recent years biological strategies are being more widely used to treat cartilage lesions. One of the most exploited novel treatments is Platelet-rich Plasma (PRP), whose high content of growth factors is supposed to determine a regenerative stimulus to cartilaginous tissue. Despite many promising *in vitro* and *in vivo* studies, when discussing clinical application a clear indication for the use of PRP cannot be assessed. There are initial encouraging clinical data, but only a few randomized controlled trials have been published, so it is not possible to fully endorse this kind of approach for the treatment of cartilage pathology. Furthermore, study comparison is very difficult due to the great variability in PRP preparation methods, cell content and concentration, storage modalities, activation methods and even application protocols. These factors partially explain the lack of high quality controlled trials up to now. This paper discusses the main aspects concerning the basic biology of PRP, the principal sources of variability, and summarizes the available literature on PRP use, both in surgical and conservative treatments. Based on current evidence, PRP treatment should only be indicated for low-grade cartilage degeneration and in case of failure of more traditional conservative approaches.

Keywords: Platelet-rich plasma, cartilage, regenerative medicine, osteoarthritis.

INTRODUCTION

Recent years have seen the flourishing of a completely new approach for the treatment of cartilage lesions. What we have seen is the transition from a traditional approach focusing on the concept of "repair" to the revolutionary idea of "regeneration" [1-3]. This fascinating perspective is one of the hottest topics of the current pre-clinical and clinical cartilage research: although orthopaedic practitioners have been always managing chondropathy and osteoarthritis, the most recent discoveries in the field of growth factors (GFs) have led to widespread enthusiasm about the application of innovative biological strategies for treating these conditions. A new figure is emerging: the "orthobiologist", i.e. an orthopaedic surgeon specializing in biological and bio-engineered treatments, both conservative and surgical.

In this particular field, the role played by blood derivatives, and in particular Platelet-rich Plasma (PRP), is preeminent. Platelet-derived GFs contained in PRP are the most exploited way to administer a biological stimulus to several different damaged tissues, such as cartilage, tendons and muscle that might benefit from this particular approach [4]. Being able to treat patients with a product derived directly from their own blood is an attractive proposition due to the theoretical reduced risks of intolerance and side effects than those commonly ascribed to traditional commercial drugs.

Concerning the application of this biological treatment, cartilage is one of the most targeted tissues [5]. In fact, the incidence of this kind of lesions is increasing in relation to the ever-growing interest in sport which, beyond some unquestionable beneficial effects on health, is also responsible for both traumatic and degenerative lesions of musculo-skeletal tissues [6, 7]. Young people, the most sport-active population, are often affected by these lesions. The treatment of cartilage pathology in these patients is often conservative, due to the limits of the current surgical treatments for cartilage lesions and the lack of indication for invasive joint metal resurfacing [3]. The constant research for innovative solutions has been one reason for the booming interest in biological approaches.

BIOLOGICAL RATIONAL DEFINITION OF PRP AND SOURCES OF VARIABILITY

The biological rational behind this kind of treatment is the topical administration of several important molecules normally involved in joint homeostasis, healing mechanism and tissue regeneration. First of all platelet-derived GFs, which are a group of polypeptides playing important roles in the regulation of growth and development of several tissues, including cartilage. Platelets contain storage pools of GFs [4, 8, 9] such as: platelet-derived growth factor (PDGF); transforming growth factor (TGF-β); platelet-derived epidermal growth factor (PDEGF); vascular endothelial growth factor (VEGF); insulin-like growth factor 1 (IGF-1); fibroblastic growth factor (FGF); epidermal growth factor (EGF) etc.

Alpha granules are also a source of cytokines, chemokines and many other proteins [4] involved in stimulating chemotaxis, cell proliferation and maturation, modulating inflammatory molecules and attracting leukocytes [4]. Besides alpha granules, platelets also contain dense granules, which store ADP, ATP, calcium ions,

*Address correspondence to this author at the Nano-Biotechnology Laboratory, Rizzoli Orthopaedic Institute, Via di Barbiano n. 1/10, 40136, Bologna, Italy; Tel: +39 051 6366567; Fax: +39 051583789;
E-mail: e.kon@biomec.ior.it

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histamine, serotonin and dopamine, that also play a complex role in tissue modulation and regeneration [10]. Finally, platelets contain lisosomal granules which can secrete acid hydrolases, cathepsin D and E, elastases and lisozyme [11], and most likely other not yet well characterized molecules, the role of which in tissue healing should not be underestimated. Several *in vitro* and *in vivo* animal studies have showed the potential beneficial effect of PRP in promoting cellular anabolism and tissue regeneration [8] and set the rational for the application of platelet concentrates in humans.

The exact definition of PRP is not clear and will probably remain so for years to come. The reason lies in the fact that blood-derived products present so many variables to be considered when making comparisons that it is not possible to establish a univocal definition of PRP. It is more correct to acknowledge that there are several PRP formulations, whose comparison in terms of potentials and limits is very difficult to test due to the large inter-product variability. A good starting point might be to clarify what the essential features are to define a blood product as PRP: it is generally regarded as a blood derivate with a higher concentration of platelets compared to basal level. It has been proposed that platelet concentration should be at least 200% of the peripheral blood PLT count [12, 13] even if in the literature PRP concentrations have been reported to range widely, up to 8 times that of basal levels [12]. Therefore, just concerning the definition of PRP, it is possible to understand the first variable involved in the comparison of different types of PRP [14]: the platelet count that can vary in such a significant way that the possible correlation between platelet concentration and clinical outcome could be a critical aspect. That is not a negligible issue, because if the biological rational of this kind of therapy is the supplementation of GFs, the number of platelets is a crucial point in determining the total amount of GFs administered. However, with regards to the scientific evidence available up to now, good clinical outcome can be obtained even with lower platelet concentration. Some studies also report correlations between outcome and platelet count [13, 15] but further investigations are needed to fully explore this topic. Furthermore, the plasma itself contains important molecules involved in the healing mechanism of connective tissues, also contributing to the platelet stimulus to tissue regeneration.

The platelet count is strictly linked to the procedures employed: there are two main preparation methods used in clinical practice [14]. The first one is by using a laboratory centrifuge and the second one a density gradient cell separator. In the first case several variables, such as number of centrifugations, their speed and timing, might influence the final product in terms of concentration of different cellular types. Furthermore, this preparation method is more technician-dependent and therefore its reproducibility is biased. Concerning cell separators, they are closed-circuit devices that allow PRP preparation without excessive manipulation of the blood. A large and constantly increasing number of these devices are available on the market, each with its own features and specifications, so it is impossible to obtain the same products and again comparison is very difficult.

Furthermore, we have to consider the overall cell types contained in PRP: even in this case the preparation method is a key factor. In fact, some PRP preparations, besides plasma and platelets, also contain leukocytes and residual blood cells. With regards to this, some disagreement has emerged among authors. In fact, whereas the antimicrobial effect of leukocyte-rich PRP has been underlined, some authors highlight the fact that leukocytes can release matrix metallo-proteinases and reactive oxygen species capable of damaging articular tissues and determining a catabolic effect. Concerning the presence of residual blood cells, it has been reported in some studies that erythrocytes have a pro-inflammatory effect in the joint [4].

Storage procedures are also a hot topic: in fact, freeze-thawing is one method but it is thought to impair platelet function and lifespan, and alter the GFs' release pattern in a negative way, besides favoring the accumulation of pyrogenic cytokines and increasing the risk of bacterial proliferation. For these reasons some authors prefer fresh administration of PRP immediately after its preparation (thus requiring blood harvesting for each injection, in case of multiple treatments) [16].

Another issue is the activation method. Several options are available even in this case: from no activation, counting on the *in-vivo* activating effect of endogenous collagen, to the use of chemical agents or biomaterials or even physical agents. Calcium chloride and thrombin are the most commonly used substances, whereas batroxobin is less often employed. These molecules contribute to PRP clot formation and increase overall GFs release even if their effect on single GF kinetic is controversial and needs further studies. In particular, thrombin, besides being a strong activator of platelets, has its own biological properties that might influence the interpretation of the effect of PRP [17].

Beyond the intrinsic differences among PRP preparations due to all the aforementioned factors, applicative variables should also be taken into account. Individual authors apply their own therapeutic protocol: the amount of PRP used for each injection, the number of the injections and the time intervals can vary widely, so study comparison is even harder than just comparing products differences. Standardization has not yet been proposed on this issue, thus the different protocols of each author introduce further confounding factors. Furthermore, authors often report their experience with PRP applied to treat different stages of disease, ranging from chondropathy and early osteoarthritis (OA) to moderate/severe OA. This is worthy of attention since PRP can produce different effects according to the particular disease phase [18] and it will be an important issue to define exactly the treatment target [19] in order to determine clearly which specific stage of cartilage pathology can benefit the most from this treatment. In the meantime, this is a further variability factor when considering the results of a trial and when comparing trials, where the lack of homogeneity in patient selection can be considered another bias.

In the light of all the issues considered, there follows a summary of the factors playing key roles in study comparison [14]:

- PRP preparation method;
- PRP formulation and cellular content, including concentration rates and cell counts;

- PRP storage modalities;
- PRP activation methods;
- PRP therapeutic protocols (amount, number of injections, intervals etc.);
- Disease phase treated.

The scenario is therefore very complex and clinicians, up till now, have made their decisions without being guided by real scientific evidence. The lack of commonly accepted guidelines and the variety of biological and procedural differences partially explain the limits of the available literature and justify the need to perform high quality trials to clarify the high number of still open questions [20].

A CLINICAL INSIGHT

Cartilage pathology has been treated both surgically (Table **1**) and conservatively (Table **2**) by the application of platelet-derived GFs. The most widely studied joint is without doubt the knee, and the majority of studies report results of the intra-articular administration of PRP.

PRP Application as an Augmentation Procedure in Surgery

Sanchez and his group [21] were the first to describe the surgical use of PRP, by treating a cartilage avulsion in a 12-year-old football player: the fragment was reattached *in situ* with the local administration of PRP both at the interface between the fragment and the surrounding healthy cartilage and in the middle of the fragment. The clinical outcome was excellent and gave full functional recovery, resumption of sport within 18 weeks, and good MRI appearance of the healed tissue. Dhollander *et al*. [22] treated 5 symptomatic osteochondral defects of the patella with microfracturing followed by the application of a collagen I/III scaffold membrane. PRP was administered beneath the membrane at the interface with the microfractured subchondral bone. The final follow-up was 24 months after the surgical procedure. Clinical results at 2 years were satisfactory both in terms of pain relief and functional improvement, and MRI evaluation showed good quality of the repair tissue. A further study by Siclari *et al*. [23] showed the efficacy of a polyglycolic acid/hyaluronan scaffold immersed in PRP for treating full-thickness chondral defects of the knee; 52 patients were treated arthroscopically by perforations and scaffold implantation. At 1 year of follow-up, a significant clinical improvement was observed in all KOOS subcategories; 5 biopsies were performed during second-look arthroscopies which revealed a homogeneous, well integrated hyaline-like repair tissue.

Talar osteochondral lesions were treated by Giannini and his group [24, 25], who used an innovative arthroscopic one-stage approach based on autologous bone marrow-derived

Table 1. PRP Application as a Biological Augmentation in Cartilage Surgical Procedures

Authors, Journal and Year	Level of Evidence	Pathology	Protocol	Combined Treatments	Control Group	Patients	Follow-Up	Outcome
SANCHEZ *et al*. Med Sci Sports Exerc. 2003	Case report	Knee osteochondral fragment avulsion	Intra-op	Fragment fixation + topical administration of PRP	No	1	10 months	Return to full sport practice as before the injury
GIANNINI *et al*. Clin Orthop Relat Res 2009	Case series	Osteochondral talar lesions	Intra-op	Scaffold made of: MSCs + PRP + HA membrane (or collagen powder)	No	48	24 months	Significant increase in all the clinical scores adopted
GIANNINI *et al*. Injury 2010	Comparative study	Osteochondral talar lesions	Intra-op	MSCs + PRP + HA membrane (or collagen powder)	Yes (historical controls)	81 (25 MSCs scaffold *vs* 10 open ACI *vs* 46 arthroscopic ACI)	24 months	Results comparable to those of arthroscopic ACI with lower costs
BUDA *et al*. J Bone Joint Surg Am 2010	Case series	Knee osteochondral lesions	Intra-op	MSCs + PRP + HA membrane (or collagen powder)	No	20	24 months	Both clinical and MRI interesting positive results
DHOLLANDER *et al*. Knee Surg Sport Traumatol Arthrosc 2011	Case series	Patellar osteochondral lesions	Intra-op	Microfractures + collagen based scaffold + PRP	No	5	24 months	Significant clinical improvement and interesting MRI findings
SICLARI *et al*. Clin Orthop Relat Res 2012	Case series	Knee osteochondral lesions	Intra-op	Pridie Perforations + polyglicolic/ HA scaffold + PRP	No	52	12 months	Good clinical outcome with hyaline-like cartilage aspect at biopsies

Table 2. PRP Conservative Application in Cartilage Pathology

Authors, Journal and Year	Level of Evidence	Pathology	Protocol	Control Group	Patients	Follow-Up	Outcome
SANCHEZ *et al*. Clin Exp Rheumatol 2008	Retrospective comparative study	Knee condropathy or OA	3 weekly injections of PRP	Yes	30 PRP *vs* 30 HA	5 weeks	Better pain control and functional outcome in PRP group
SAMPSON *et al.* Am J Phys Med Rehabil 2010	Case series	Knee condropathy or OA	3 injections of PRP one month apart	No	14 PRP	6 months	Clinical improvement at short term evaluation
WANG-SAEGUSA *et al*. Arch orthop Trauma Surg 2011	Case series	Knee condropathy or OA	3 injections of PRP two weeks apart	No	261 PRP	6 months	Clinical improvement at short term evaluation
KON *et al.* Knee Surg Sport traumatol Arthrosc 2010 Knee Surg Sport Traumatol Arthrosc 2011	Case series	Knee condropathy or OA	3 injections of PRP two weeks apart	No	100 PRP	24 months	Significant pain reduction and functional recovery. Time dependent effect of PRP injections with a mean beneficial effect of 9 months
KON *et al*. Arthroscopy 2011	Comparative trial	Knee condropathy or OA	3 weekly injections of PRP	Yes	50 PRP *vs* 50 LWHA *vs* 50 HWHA	12 months	Best results for PRP in chondropathy group, no statistical difference between treatment for higher degree of cartilage degeneration
FILARDO *et al.* Knee Surg Sport Traumatol Arthrosc 2011	Comparative trial	Knee condropathy or OA	3 weekly injections of PRP	Yes	72 leukocyte rich PRP *vs* 72 leukocyte free PRP	12 months	Comparable clinical results with higher post-injective pain in leukocyte -rich PRP group
NAPOLITANO *et al*. Blood Transfus 2012	Case series	Knee condropathy or OA	3 injections of PRP	No	27 PRP	6 months	Statistical improvement in pain and function
GOBBI *et al*. Sports Health 2012	Case series	Knee condropathy or OA	2 monthly injections of PRP	No	50 PRP	12 months	Statistical improvement in pain and function
SPAKOVA *et al*. Am J Phys Med Rehabil 2012	Prospective trial	Knee condropathy or OA	3 injections of PRP	Yes	60 PRP *vs* 60 HA	6 months	Superior results in PRP group at short term evaluation
SANCHEZ *et al*. Arthroscopy 2012	Randomized trial	Knee condropathy or OA	3 weekly injections of PRP	Yes	79 PRP *vs* 74 HA	6 months	Higher percentage of responders in PRP group but no clear superiority of the biological approach
CERZA *et al*. Am J Sport Med 2012	Randomized trial	Knee condropathy or OA	4 weekly injections of APC	Yes	60 ACP *vs* 60 HA	6 months	Superior clinical outcome for PRP in all groups of treatment
FILARDO *et al.* BMC 2012	Randomized trial	Knee condropathy or OA	3 weekly injections of PRP	Yes	55 PRP *vs* 54 HA	12 months	Clinical improvement in both groups without inter-group significant difference. Better trend for PRP in low grade cartilage pathology
SANCHEZ *et al*. Rheumatology 2012	Case series	Hip OA	3 weekly injections of PRP	No	40 PRP	12 months	Significant pain reduction and functional improvement
BATTAGLIA *et al*. Clin Exp Rheumatol 2011	Case series	Hip OA	3 weekly injections of PRP	No	20 PRP	12 months	Clinical improvement but gradual worsening up to 1 year of follow-up
MEI-DAN *et al*. Am J Sports Med 2012	Quasi randomized trial	Osteochondral talar lesions	3 injections of PRP 14 days apart each other	Yes	15 PRP *vs* 15 HA	7 months	Statistically better clinical outcome in PRP group

mesenchymal stem cells (BMDCs), PRP and, alternately, porcine collagen powder or hyaluronic acid (HA) membrane to create a scaffold. The first clinical trial [24] involved 48 patients affected by focal lesions and followed-up for 24 months using the AOFAS score. A significant increase in this parameter was found up to the final evaluation. The majority of patients returned to sport within 11 months. A correlation was found between clinical outcome and lesion size, and previous surgery was also shown to affect the outcome negatively. Five second-look arthroscopies were performed at 1 year and in 2 cases biopsies were taken, which revealed, after histologic and immunohistologic analysis, the presence of new cartilage tissue with varying degrees of hyaline-like tissue remodeling. The overall findings suggested that this novel approach might produce results comparable to those of autologous chondrocyte implantation (ACI), but avoiding the double surgical time and the inherent stress for the patient. A further study [25] by the same authors compared BMDCs + PRP + scaffold technique with open and arthroscopic ACI. Eighty-one patients were included in this analysis, 10 treated with open ACI, 46 with arthroscopic ACI, and 25 with the BMDCs "one-step" technique. Clinical results were compared for up to 3 years of follow-up. The clinical improvement in each subgroup was significant and no inter-group difference was observed, thus confirming the possibility of matching the effectiveness of chondrocyte transplantation by a single step procedure. X-Rays showed no signs of OA progression and MRI revealed a good rate of defect filling and integration. Another aspect worth noting is the cost: in fact, the authors claimed that their novel one-step regenerative technique costs less than an half that of traditional arthroscopic ACI.

This technique was also applied to condylar osteochondral knee lesions [26]: 20 patients were treated and followed-up for 24 months with IKDC and KOOS scores combined with MRI analysis. Besides the significant improvement in clinical scores, interesting correlations were found: combined surgery slowed down recovery although at final evaluation similar results were obtained with respect to those of patients without associated procedures; hyper-intense MRI signal of repair tissue was correlated with poorer clinical results. In general, these preliminary reports suggested good results, but the low scientific level of these papers and even more the concomitant application of different treatments hinder the possibility to understand the real role of PRP.

PRP Application as Conservative Management

Several studies are available on the conservative application of PRP but only recently some high quality trials have been published. In order to simplify discussion, these studies have been split into two different sub-sections, one dealing with case series and the second one with comparative trials.

CASE SERIES

Knee Application

In 2008, Sanchez *et al*. first reported the injective application of a platelet concentrate (PRGF) in a retrospective observational study on 60 patients [27], 30 treated with intra-articular injections of PRGF and 30 with injections of HA. Patients from both groups underwent 3 injections one week apart and were evaluated basally and at 5 weeks of follow-up focusing in particular on "stiffness", "pain", and "function". The results were encouraging, showing better efficacy in pain control, even though the short follow-up is a major weak point of the study.

In 2010, Sampson *et al*. published a study [28] on 14 patients with knee OA who received 3 PRP injections 1 month apart. Inclusion criteria were clinical and radiographical OA signs in patients with previous unsuccessful conservative management. Evaluation was carried out for up to 52 weeks using the Brittberg-Peterson Visual Analog Pain, Activities and Expectation score, VAS for pain, and KOOS score. Cartilage thickness was measured *via* ultrasonography to assess any changes between pre- and post-treatment. Concerning the clinical outcome, the authors found a statistically significant improvement in the scores examined, with a reduction in pain at rest and during physical activity. After one year, 8 patients were completely satisfied with the treatment received. No significant differences were observed in cartilage thickness after PRP injections.

In 2010 Wang-Saegusa *et al*. [29] published a prospective study on a large cohort of 261 patients treated for mono- or bilateral knee OA, who received 3 injections of PRP 2 weeks apart. Clinical evaluation was performed at 6 months of follow-up using the WOMAC score, VAS, Lequesne Index and SF-36. Statistical analysis revealed significant results with an improvement in all the scores adopted.

In the same year Kon *et al*. also published a prospective study [30] on 91 patients (a total of 115 knees) treated with 3 injections of 5 mL PRP (1 every 3 weeks). Inclusion criteria were: clinical history of knee pain or articular swelling lasting more than 4 months, radiographic or MRI signs of OA. Patients underwent clinical evaluation at basal level and at 2, 6, and 12 months of follow-up through IKDC objective, IKDC subjective, and EQ-VAS (general health status evaluation) scores. No major complications were seen, except for a case of marked post-injective pain and swelling which resolved spontaneously after 2 weeks. Eighty percent of the patients expressed satisfaction with the treatment received. The clinical improvement in all the variables at 2 months was later confirmed at 6 months of follow-up, whereas a tendency to worsen was reported at 12 months of follow-up. Despite the decrease reported after 1 year, the clinical scores were still higher than the basal level. Some factors were also identified to influence the clinical efficacy: young male patients were the best responders to PRP application, and also the grade of articular cartilage degeneration correlated with clinical outcome. Patients with chondropathy alone without signs of OA presented better and more lasting results compared to patients with early or severe OA. A subsequent evaluation [31] at 24 months of follow-up confirmed the trend that emerged after the 12 months' follow-up: a further and marked decrease in the clinical outcome was evident, thus confirming the time-dependency of intra-articular therapy with platelet-derived GFs. The authors estimated the median duration of the PRP effect to be 9 months, and the influence of age and grade of

degeneration was shown again to be correlated with clinical results also at 2 years of follow-up.

Napolitano *et al*. [32] treated 27 patients, either affected by simple chondropathy or initial OA, with 3 injections of 5 ml PRP performed one week apart from each other, and followed up for 6 months with the NRS scale for pain and WOMAC score. Significant results were obtained after treatment without the occurrence of adverse events. Similar results were also reported by Gobbi *et al*. [33] who treated 50 patients with 2 monthly injections of PRP and evaluated them up to 1 year, showing a positive outcome both in patients who had undergone previous cartilage surgery and those who had not.

Hip Application

Two studies on this particular topic have been published. The first one, authored by Battaglia *et al*. [34] reported the results of PRP ultra-sound-guided injective treatment in 20 patients affected by hip OA (Kellgren-Lawrence Score from I to III): 3 intra-articular injections 2 weeks apart were performed and patients were followed-up for 1 year. The clinical outcome was positive but a worsening occurred after 3 months up to the final evaluation, thus confirming the time-dependent effect of PRP.

The second one was recently published by Sanchez *et al*. [35], who treated 40 patients affected by OA with 3 weekly ultrasound-guided injections of PRP. Evaluation was carried out for up to 6 months using the WOMAC, Harris, and VAS scores for pain. Satisfactory results were reported with a significant reduction in pain level at the first evaluation after 6 weeks, which was confirmed even at the final 6 months' follow-up. Functional recovery was encouraging as evaluated through a specific subscale of the WOMAC score. However, 11 out of 40 patients did not have any beneficial effect after injective treatment: in these cases, a metal resurfacing was required.

COMPARATIVE STUDIES

Knee Application

A multi-center study was published by Kon *et al*. [18] in 2011. They compared the clinical efficacy of PRP with low molecular weight HA (LWHA) and high molecular weight HA (HWHA). For this purpose, 3 homogeneous groups of patients were studied. Treatment consisted of 3 weekly injections of PRP, LWHA, or HWHA. Subjective IKDC and EQ-VAS were used for evaluation at 2 and 6 months of follow-up. The results showed a better performance for the PRP group at 6 months of follow-up in both scores. In particular, subgroup analysis according to the grade of articular cartilage degeneration revealed that PRP gave better results than HA at 6 months of follow-up in the chondropathy group. Conversely, in the early OA group the gap with HA was not significant and in the severe OA group no difference in clinical outcome was observed between HA and PRP. Another finding underlined in this study was that patients under 50 years old had a greater chance to benefit from this biological approach, whereas in the case of older patients there was no advantage with respect to HA. Another comparative study by the same authors explored the clinical efficacy of high concentrate leukocyte-rich PRP compared to low concentrate leukocyte-free PRP: 144 patients affected by knee cartilage pathology presented comparable positive clinical effects with both treatments; the leukocyte-rich PRP group suffered from more swelling and pain reaction immediately after the injections [36].

Spakova *et al*. [37] compared the efficacy of PRP versus viscosupplementation on 120 patients evaluated by the WOMAC score and a pain numeric rating scale at 3 and 6 months of follow-up; an increase in the clinical score was reported in both groups but statistically superior results were found in the PRP group.

Recently, three randomized controlled trials have been published. The first one was authored by Sanchez *et al*. [38], who investigated the efficacy of single-spinning leukocyte-free PRP compared to HA in 153 patients evaluated with the WOMAC score at 6 months of follow-up. Despite an overall interesting clinical outcome, a clear superiority of PRP emerged only in the percentage of responders (50% of pain reduction: primary outcome measure) which was statistically higher in PRP group. Furthermore the study confirmed that this biological treatment produces inferior results in moderate/severe OA.

The second randomized trial was performed by Cerza *et al*. [39]. They treated 120 patients, divided in two groups, the first one receiving 4 weekly injections of Autologous Conditioned Plasma (ACP) and the second one 4 injections of HA. The patients were followed up for 24 weeks and the ACP group showed a significantly better performance than did the HA group: the clinical gap between treatments increased over time in favor of ACP. Surprisingly, these authors reported a significantly better clinical outcome in the ACP group even in patients affected by grade 3 knee OA.

The last published paper on PRP was authored by Filardo *et al*. [40]. It was a double-blind randomized controlled trial comparing leukocyte-rich PRP and HW HA. The authors reported the preliminary results of a cohort of 109 patients (55 PRP and 54 HA) who reached the 1 year follow-up evaluation, which was performed using IKDC objective and subjective scores, Tegner score, KOOS score and VAS for general health status. Conversely to what was suggested in the previously-mentioned papers, and although a significant increase in clinical scores was observed in both treatment groups, no statistical inter-group difference was reported and just a tendency toward better results for the PRP group at 6 and 12 months follow-ups was found only in patients affected by low grade cartilage degeneration (Kellgren Lawrence up to 2). In the light of these preliminary findings, the authors concluded that no superiority of PRP over HA emerged so it could not be considered as a first line treatment in moderate/severe OA in middle-aged patients. Therefore, to avoid indiscriminate use, PRP should be limited the less degenerated cases, until better evidence is available to identify the best indications for this biological approach.

Talar Osteochondral Lesions

A prospective study by Mei-Dan *et al*. [41] compared the efficacy of HA and PRP in 30 patients (15 per group) not responsive to other previous conservative treatments. The patients were allocated to receive 3 weekly intra-articular injections of HA (2 ml each) or PRP (2 ml each) and were followed for up to 28 weeks. Investigators used AHFS,

AOFAS, and VAS scores for pain, stiffness and function. Results were statistically significant and PRP proved to be more effective in controlling pain and re-establishing function when compared to viscosupplementation.

PRP THERAPY: POSSIBILITIES, LIMITS AND FUTURE IMPLICATIONS

Just a few months ago the scientific literature on clinical PRP application consisted mainly of reviews or experts' opinions rather than trials [42]. The debate has always been fervent but, without the support of robust clinical trials, it was inconclusive. Besides the large amount of *in vitro* and animal studies, only a few papers discuss clinical results and their average quality was poor, being mostly case series. In the last year, and especially in very recent months, a step forward in the literature has begun, especially with regards to conservative therapy, and hopefully this quality improvement of the new data will help to answer some of the open questions on PRP.

The present authors critically reviewed the available literature. For what concerns the current understanding on the potential and feasibility of applying PRP in the management of cartilage pathology, first of all, looking at the surgical application it is not possible to draw definite conclusions about the efficacy of this approach. It is a consequence of this particular kind of treatment: it is very difficult to identify how much PRP might contribute to determine the clinical outcome with respect to the surgical treatment performed alone. Comparative studies aimed at assessing the specific role of PRP are needed. Furthermore, in many cases PRP is administered together with other biological augmentation methods, such as mesenchymal stem cells [24-26] or bio-engineered scaffolds [22, 23], so it is even more difficult to determine the contribution of PRP. The studies available are just case series treating disparate conditions in biomechanically very different joints (knee and ankle). Maximum follow-up evaluation is 24 months, so further studies are needed to determine the persistence of the good clinical outcome of these particular procedures. In the near future PRP will be more and more widely used in cartilage regenerative techniques but, despite being safe, according to the present evidence there is still no recommendation for using PRP in such procedures.

Considering PRP as conservative management, things look a bit different, especially when treating the knee region. In fact, whereas a few studies have been published both on hip and ankle cartilage lesions, several trials focus on knee treatment. As a consequence, few indications can be proposed for hip and talar osteochodral lesions. In the first case we have just two case series [34, 35] confirming the safety of PRP and a promising clinical outcome but also a gradual worsening over time: in moderate or severe OA a beneficial effect has not been observed or expired so fastly that patients then required a prosthetic solution. Therefore, at present there are no studies to support an indication for PRP as first line treatment in hip degenerative pathology. For what regards talar osteochondral lesions, only one comparative quasi-randomized trial has been published [41]: despite presenting statistically superior results for the PRP group, the low number of patients treated and the short follow-up evaluation do not allow, even in this case, the use of PRP to be endorsed.

Going back to the knee, it is possible to have more detailed indications. A lot certainly still needs to be clarified, but the considerable amount of studies published allows us to draw some conclusions. The first consideration regards safety of the procedure, which was confirmed by all trials that reported only minor adverse events with just some differences linked to the particular PRP formulation used: in fact, leukocyte-rich PRP seems to determine increased pain and swelling reaction when compared to leukocyte-poor PRP. With regards to the clinical outcome it can be said that this kind of conservative approach is time dependent, since a gradual worsening occurs over time. Filardo *et al.* [28, 29] were the first to calculate the average duration of the PRP effect, which is estimated at about 9 months. Analyzing efficacy, the encouraging results reported in case series cannot be the backbone to support the clinical application of PRP since each study faces an important bias related essentially to the lack of a control group. On the other hand, comparative trials found that PRP responsiveness might be linked to the stage of disease, where better results, superior to HA formulations, are obtained in young patients affected by early degree of cartilage degeneration, whereas moderate or severe OA show less favorable outcome without difference compared to viscosupplementation.

All the randomized controlled trials available compared the efficacy of PRP versus HA. Actually it should be pointed out that each trial employed a different type of PRP. Two out of three studies [36, 37] revealed better results in the PRP group at 6 months of follow-up. The third one led by Filardo [38], which had the longest follow-up evaluation of patients treated (1 year), reports no overall difference between PRP and HA in terms of clinical outcome; just a tendency towards better results in patients affected by a lower degree of cartilage degeneration was found. Therefore, in the case of more advanced signs of OA, PRP does not seem to be superior to viscosupplementation, a conclusion that was also reached in the study by Sanchez *et al*. Surprisingly, Cerza *et al*. reported significantly better results for ACP even in grade III OA: worthy of consideration is the fact that they used a different blood-derived products with evaluation limited up to 6 months of follow-up.

All the randomized trials deal with the application of PRP in patients affected by very different stages of disease, from chondropathy to severe OA. Therefore no conclusions can be drawn about the possibility of applying this approach to a specific phase of cartilage degenerative pathology: sub-group analysis does not allow, in any of the trials published, sufficient statistical strength to provide a real clinical indication. What emerges can be considered just a "suggestion" to avoid the indiscriminate use of PRP, which seems to offer a clinical benefit but cannot yet be considered a first line treatment for this pathology. Moreover, its use should be limited to those patients who can take most advantage from this approach, i.e. young patients with less articular degeneration, or those not responsive to other more traditional treatments.

CONCLUSIONS

A lot has still to be understood about PRP for the treatment of cartilage lesions. The only aspect that seems to have enough scientific evidence is the safety of this

procedure, which is the first prerequisite for endorsing this approach for clinical practice. However, at present there is no conclusive indication for the use of PRP in this kind of pathology, due to the fact that only a few high quality, randomized trials have been published, mainly about knee pathology. A clear superiority with respect to other traditional treatments has not been fully proven: the great enthusiasm about biological treatments, initially justified by encouraging preliminary results, now needs real support. For the moment PRP cannot be considered as first line treatment for cartilage pathology, and its application should be reserved to patients who, based on the current scientific evidence, can obtain the best results from this approach. Further studies are needed to clarify some fundamental aspects such as the best PRP formulation, the best protocols of administration, and also the patients' and lesions' characteristics correlating with the clinical outcome.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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