Arthroscopic Injection of Platelet Rich Plasma with subchondral drilling in Cartilage Defects of the knee

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Abstract: Objective: The aim of our study was to analyse the clinical outcome after the treatment of focal cartilage defects in non-degenerative and degenerative knees with drilling augmented by injection with platelet rich plasma **Methods:** Twenty patients (18 men and 2 women) underwent drilling of cartilage defects of the knee and augmented by injection with platelet rich plasma concentrate. The mean age was 40 years range (26-55 years). All patients had focal cartilage defects on a load-bearing surface of the knee that extend down to the subchondral bone but not penetrating through it. **Results:** At 1-year follow-up, the KOOS showed clinically meaningful and significant *(P*<0.05) improvement compared to baseline **Conclusion:** platelet rich plasma is a safe, autologous, easy to prepare and to use and relative low cost procedure to deliver growth factors for cartilage healing and regeneration. Although the number of cases in my study was small and the follow up time was short, the preliminary results were promising.
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1. Introduction

Platelet-rich plasma (PRP) is a fraction of plasma in which platelets are concentrated and is reported to be utilized as a source of multiple growth factors. PRP is known to counter the catabolic mediators in order to reduce the inflammatory damage to the joint. PRP has been shown to increase the production of hyaluronic acid and hepatocyte growth factor by synoviocytes excised from arthritic patients [(1)]. Focal cartilage lesions of the knee occur frequently, are a major health problem and may progress to severe osteoarthritis in symptomatic knees. Frequently used first-line treatment options for focal cartilage defects are bone marrow-stimulating techniques, like drilling or the microfracture technique [(2,3,4)]. Platelet-rich plasma (PRP) is known as a source of autologous growths factors [(5,6)]. In cartilage repair, PRP has been shown to induce the migration and chondrogenic differentiation of subchondral progenitor cells known from microfracture [(7)].

2. Patients and Methods:

In Al-Hayat National Hospital, Khamis Mushayt , Saudi Arabia between April 2012 and October 2013, 20 patients (18 men and 2 women) suffering from focal cartilage defects in non-degenerative and degenerative knees underwent arthroscopic drilling augmented by injection with platelet rich plasma (PRP). Twelve patients had isolated cartilage defects of the knee, eight patients associated with anterior cruciate ligament reconstructions. The mean age was 40 years range (26-55 years). The right knee was involved in 14 patients and the left knee in 6 patients. The duration of follow up ranged from 9 months to 18 months with an average 13.5 months. All of the patients were informed of the nature of the operation with written consent. All patients were assessed preoperatively and at the end of follow up using the knee osteoarthritis and outcome score system (KOOS) [(8)]. Radiographs were taken pre-operatively and osteoarthritic degenerations were evaluated using the Ahlbäck classifications **(Table 1)**. The Ahlbäck classification system primarily focuses on reduction of the joint space as an indirect sign of cartilage loss. Joint space narrowing has been suggested as the best variable in assessing radiographic progression of knee OA[(9,10)] .The mean defect size was 2.7 cm^2 (1.5–5.0 cm^2). All defects were classified as ICRS class III (n=12) or IV (n=8) defects [(11)] and were located, on the femoral condyle (Medial femoral condyle, n = 12, lateral femoral condyle, n= 5 and trochlear part, n=3).

Table 1: The Ahlbäck classification of radiographic knee OA of the tibiofemoral joint

Ahlbäck grade	Ahlbäck definition
Grade I	Joint space narrowing (joint space < 3 mm)
Grade II	Joint space obliteration
Grade III	Minor bone attrition (0–5 mm)
Grade IV	Moderate bone attrition (5–10 mm)
Grade V	Severe bone attrition (>10 mm)

Table 2: Patient's characteristics

Patient No.	Age	Median defect size	ICRS classifications	Localization	Concomitant surgeries	Pts. In WB x10^9/L	PRP x10^9/L	% platelets increase over WB	Platelets concentration
1	36	1.5 cm^2	III	L.F.C*	non	223	307	37%	137%
2	26	3 cm^2	IV	M.F.C**	ACL r***	149	279	87%	187%
3	28	2.7 cm^2	III	M.F.C.	ACL r.	179	206	15%	115%
4	30	2.7 cm^2	III	M.F.C	non	305	541	77%	177%
5	34	1.3 cm^2	III	L.F.C	non	244	386	58%	158%
6	29	2.3 cm^2	III	M.F.C	ACL r.	225	403	79%	179%
7	38	1.8 cm^2	III	M.F.C	non	286	508	77.6%	177.6%
8	45	4.6 cm^2	IV	Trochlea	non	261	539	106%	206%
9	40	3.4 cm^2	IV	M.F.C	non	259	356	37%	137%
10	28	2.9 cm^2	III	M.F.C	ACL r.	229	468	104%	204%
11	34	2.2 cm^2	III	L.F.C	non	218	339	55%	155%
12	34	3 cm^2	III	M.F.C	ACL r.	219	485	121%	221%
13	48	4.5 cm^2	IV	Trochlea	non	178	254	42%	142%
14	55	5 cm^2	IV	M.F.C	non	215	413	92%	192%
15	39	2.7 cm^2	III	L.F.C	ACL r.	249	406	63%	163%
16	38	3.6 cm^2	IV	M.F.C	non	314	529	68%	168%
17	36	2.3 cm^2	III	M.F.C	non	213	298	39%	139%
18	34	3.3 cm^2	IV	L.F.C	ACLr.	269	448	66.5%	166.5%
19	30	2.8 cm^2	IV	Trochlea	ACLr.	278	479	72%	172%
20	34	2.9 cm^2	III	M.F.C	non	315	423	34%	134%

* L.F.C.: Lateral Femoral Condyle. ** M.F.C.: Medial Femoral Condyle.
*** ACL r.: Anterior Cruciate Ligament reconstruction.

Surgical technique

The current study used a technique that utilizes controlled subchondral perforations that allow marrow elements (mesenchymal cells, growth factors) to accumulate in the chondral defect by doing arthroscopic drilling by manual drill with avoiding the thermal necrosis of pneumatic drill. Controlled subchondral perforations done by debridement of chondral defect until healthy cartilage all around and then drilling of chondral lesion with 3-4 mm interval between each perforation using a K-wire with a thickness of 1.8 mm. Three to four-millimeter wide bone bridges are carefully maintained between the individual holes to preserve the integrity and function of the subchondral bone plate. The release of fatty droplets from the drilling holes indicates that adequate depth of the drilling has been achieved. Injection of the prepared platelet rich plasma was done.

Protocol for PRP preparation

A 30 ml volume of autologous blood was drawn from each patient via venous puncture. Each blood sample was divided into two 5 ml vacuum tubes containing 10% sodium citrate. The separation of the blood cell elements was performed using a laboratory centrifuge (Beckman J-6M Induction Drive Centrifuge Beckman Instruments Inc., Palo Alto, CA, USA). The tubes were centrifuged at 160 G for 20 minutes at room temperature resulting in two basic components: blood cell component (BCC) in the lower fraction and serum component (SEC) in the upper fraction. A mark was made 6 mm below the line that separated the BCC from the SEC. To increase the total amount of platelets collected for the second centrifugation, all content above this point was pipette and transferred to another 5 ml vacuum tube without anticoagulant. The sample was then centrifuged again at 400 G for 15 minutes resulting in two components: SEC and PRP. The PRP then was separated from the SEC. The platelets in the whole blood and PRP samples were counted using automated cell counter coulter HMX. Two parameters, based in part on the study by Tamimi, [12] were evaluated for the PRP samples: platelet increase compared to whole blood and platelet concentration. These values were calculated using the following equations:

$$\text{\% platelet increase over whole blood} = \frac{\text{platelet count of PRP} - \text{platelet count of whole blood}}{\text{Platelet count of whole blood}} \times 100$$

$$\text{\% platelet concentration} = \frac{\text{platelet count of PRP}}{\text{Platelet count of whole blood}} \times 100$$

No drains are used so as to avoid the removal of the pluripotent mesenchymal clot from the cartilage

defect by suction or direct abrasion by the drain during postoperative joint mobilization. A compression dressing is placed, and cryotherapy is used routinely for the control of postoperative swelling.

Postoperatively, patients were instructed for using the continuous passive motion. Its use is highly recommended especially on day one and continued throughout the hospital stay (2-3 days). Continuous passive motion provides cartilage nutrition and stimulates mesenchymal stem cell differentiation in the clot while avoiding the creation of detrimental compression and shear forces on the fragile initial clot. Gradual increase in the range of motion but not beyond 70º during the first 3 days. Weight-bearing is avoided for at least six weeks, and then the patient is gradually advanced to full weight-bearing, depending on the size and location of the lesion and the symptoms.

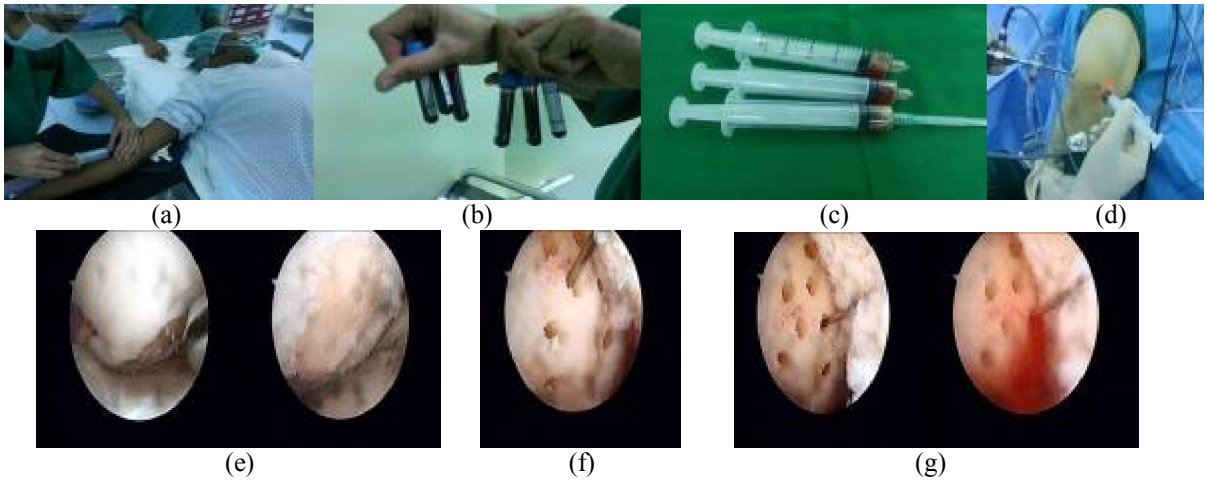


Fig.1. Intraoperative photos showing:

a) Taking the blood sample from the patient before arthroscopy.
b) Collection of the samples in multiple tubes.
c) PRP after preparation in syringes before arthroscopic injection.
d) Insertion of the syringe during arthroscopy to inject the PRP.
e) Cartilage defect with preparation by shaver.
f) Drilling of the cartilage defect by using manual drill bit.
g) Insertion of the needle in the predrilled cartilage defect with release of PRP.

3. Results:

For evaluation of clinical results after arthroscopic injection of platelet rich plasma combined with subchondral drilling, the KOOS (Knee injury and Osteoarthritis Outcome Score) [8] was applied preoperatively and at12-month post-operatively. The pre-operative data as well as the 12-month follow-up data were used for comparison and were reported previously. The KOOS is a patient-administered score and is divided into subcategories such as pain, symptoms, activities of daily living (ADL), sports and recreation function (sport& recr), and knee-related quality of life.

For clinical grading the results were graded into 4 grades, by using the Brittberg Score [13] :

Excellent: No pain, swelling, or locking with strenuous heavy knee-loading activity (90-100 points).

Good: Mild aching with strenuous activity, walking on flat ground without pain, no swelling or locking (80-89 points).

Fair: Moderate pain with strenuous activity, occasional swelling but no locking, (70-79 points).

Poor: Pain at rest, swelling and locking, (less than 70 points).

Patients have been subgrouped into, eight patients with cartilage defects associated with torn ACL and did arthroscopic injection of PRP with drilling with ACL reconstruction using the double looped semitendinosus and gracilis grafts with rigid fix system (subgroup1) and twelve patients with cartilage defects only and did arthroscopic injection of PRP with drilling (subgroup 2). Patients have been followed for an average of 13.5 months, ranged 9 to 18 months. The right knee was involved in 14 patients and the left knee in 6 patients. The mean age was 40 years range (26-55 years). All patients had focal cartilage defects on a load-bearing surface of

the knee that extend down to the subchondral bone but not penetrating through it. The mean defect size was 2.7 cm^2 (1.5–5.0 cm^2). All defects were classified as ICRS class III (n=12) or IV (n=8) defects [(11)] and were located, on the femoral condyle (Medial femoral condyle, n = 12, lateral femoral condyle, n= 5 and trochlear part, n=3).

Statistical Analysis

Results were analyzed statistically using SPSS 10 for windows version; the test was Student t- test for paired data (SPSS Corporation, Chicago, IL). The differences were considered statistically significant when *p* values were <0.05.

There was a significant improvement in pain in all patients with a mean of (41.97) and with standard deviation (11.69). Other symptoms like swelling, grinding or catching of the knee improved significantly but less than in pain with a mean of (33.25±17.47). Function in daily living (ADL) significantly improved more than in pain with a mean (52.81±18.576). Function, Sports and Recreational activities like squatting, running and kneeling especially during praying showed significant improvement less than in pain and ADL and slightly lower than in symptoms with a mean (34.63±21.27). knee-related quality of Life (QOL) significantly improved but less than in other parameters of KOOS with a mean (30.67±18.89) **(Table 3)**. There is marked improvement in subgroup 1 in comparison with subgroup 2 with all the parameters of KOOS evaluation and explained by the young age of the patients of that group and relatively small size of the cartilage defects.

Size of the cartilage defect have no significant effect on the results because in all sizes the aim is to reach to contained lesion after debdridement, but only affect the postoperative follow up as we delay walking and sports with more time for physiotherapy.

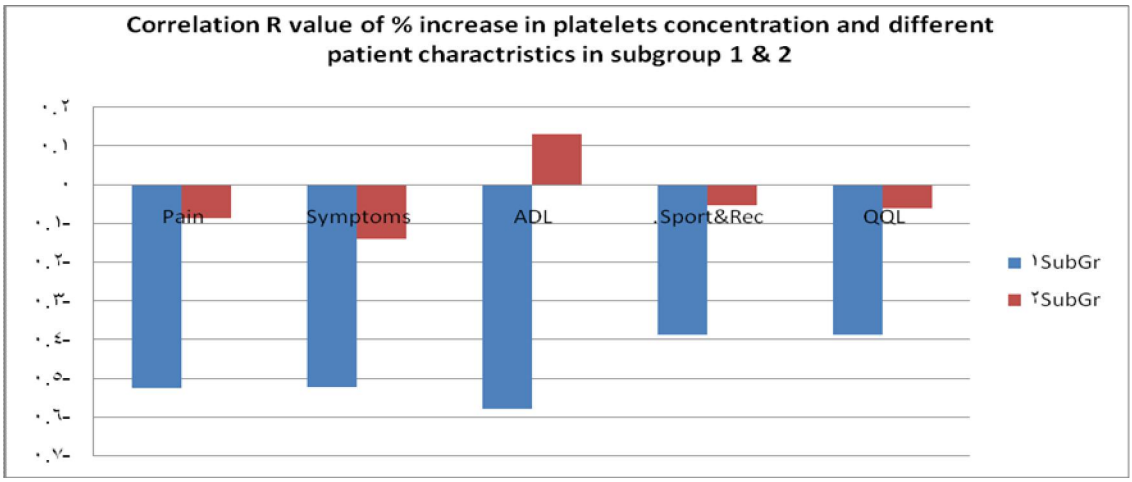
Follow up of the eight patients (subgroup 1) after one year showed that 4 patients were graded as excellent, 2 patients good, 2 patients fair and one patient poor according to the brittberg scoring system. Preoperative score averaged 47.5 points (range 35-60). Postoperative score increased to 84 points (range 68-100).

Follow up of the twelve patients (subgroup 2) after one year showed that 2 patients were graded as excellent, 4 patients good, 3 patients fair and 3 patients poor according to the brittberg scoring system. Preoperative score averaged 38.5 points (range 23-54). Postoperative score increased to 79 points (range 64 -94).

Table 3: Postoperative results of all patients including the two subgroups.

	Pain	Symptoms	ADL	Sports&Recreation	QQL	% increase of platelets
Minim.V.	15	5.71	12.4	0	-5.5	15
Maxim.V.	61.7	61.43	82	80	64.45	121
MD	41.9655	33.2465	52.805	34.63	30.668	66.505
±SD	11.69104	17.46818	18.57586	21.27227	18.88198	27.57927

* **Minim.value:** Minimum Value. ** **Maxim.value:** Maximum Value. *** **MD:** Mean Deviation. **** **SD:** Standard Deviation.



Histogram 1: Correlation "R" value between % increase of Platelets Concentration and different chractrestics of the patients in subgroup 1 and 2.

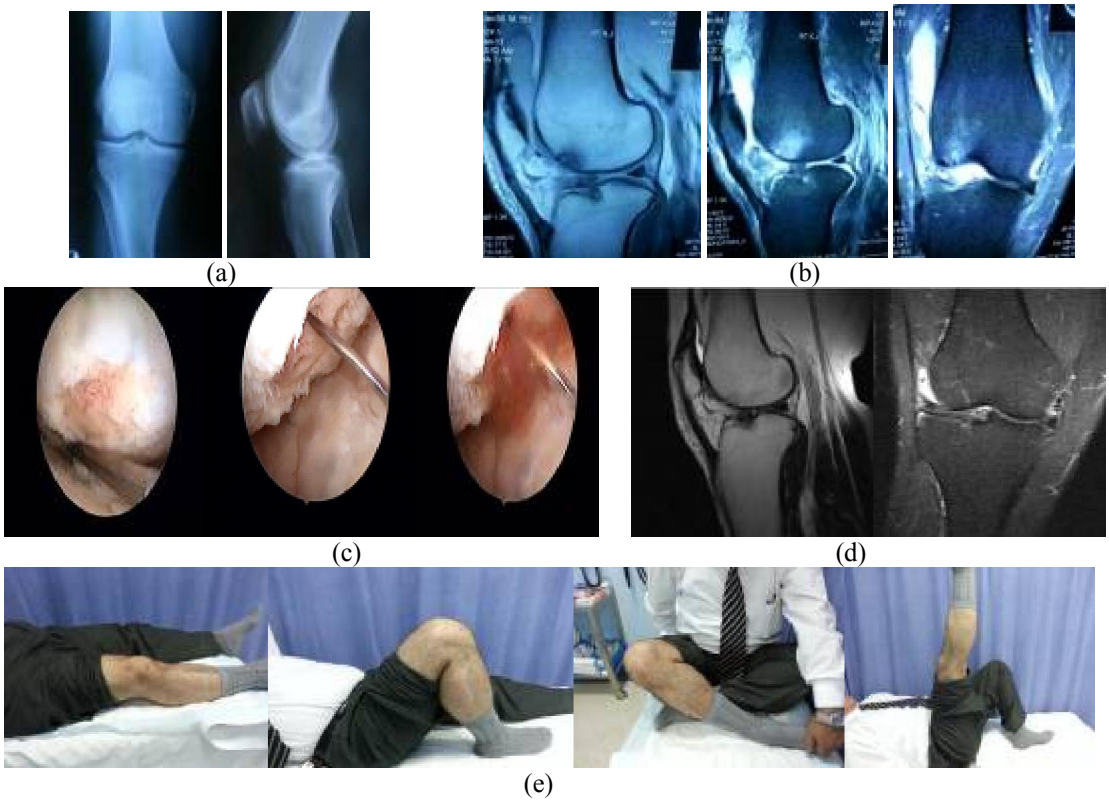
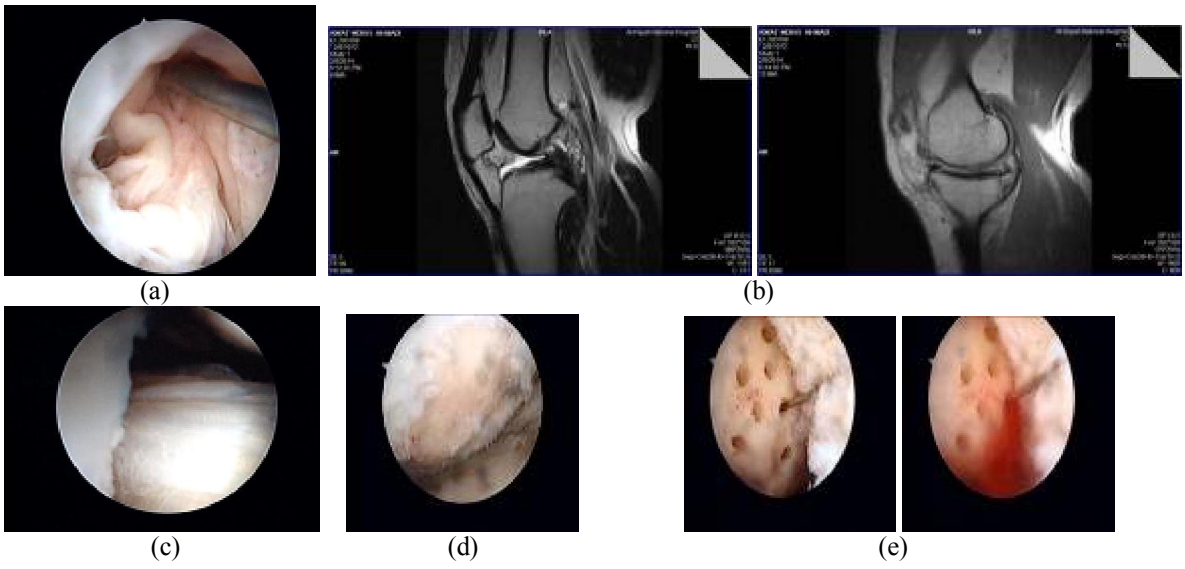


Fig. 2. Case No. 1 showing:

a) Preoperative x-ray showing Grade I Ahlbäck early osteoarthritis .
b) Preoperative MRI showing cartilage defect of the medial femoral condyle.
c) Arthroscopic photo showing cartilage defect with drilling and injection of PRP.
d) Postoperative MRI after 3 months from arthroscopic injection of PRP.
e) Follow up photos with excellent results after 4 months.



(f)

Fig.3. Case No. 2 showing:

a) Preoperative arthroscopic picture showing torn ACL.
b) Preoperative MRI showing torn ACL and cartilage defect of the medial femoral condyle.
c) Intraoperative photo showing reconstruction of ACL by DLSG with fixation by rigidfix.
d) Intraoperative arthroscopic photo showing cartilage defect of the medial femoral condyle.
e) Insertion of the needle in the predrilled cartilage defect with release of PRP.
f) Follow up photos with excellent results after 4.5 months.

4. Discussion:

In spite of the major advances of modern orthopedic surgery, the surgical correction of osteochondral defects, mainly of the knee, represents a challenging problem to the specialist. Cartilage free grafts may be a possibility, but the sources of autogenous grafts are not appropriate, since they are not always available in the desired shape and size, while homogeneous grafts are still just a theoretical option because of the unresolved immunological problems. Therefore, the search for new treatment alternatives to promote articular cartilage regeneration has become imperative, giving rise to a variety of options, most of which involve some kind of surgical procedure. Ideally, the treatment should involve a minimally invasive procedure and the regenerated tissue should present histological and biochemical characteristics and biomechanical properties that are similar to those of the normal cartilage, including adherence to the surrounding normal cartilage and to the subchondral bone [(14)] .The first aspect is already possible through arthroscopy, but the second is far from ideal, as the regenerated tissue induced by most methods developed so far is invariably inferior to normal cartilage, by any comparison parameter. In spite of some controversial findings, PRP has been used to repair defects in different tissues, especially in maxillofacial surgery and dentistry, but also in the cortical bone of the musculoskeletal system [(15)] and, theoretically, the articular cartilage could benefit from its regeneration stimulation properties.

PRP is defined as a sample of plasma with a twofold or more increase in platelet concentration above baseline level or greater than 1.1×10^6 platelets /μL [(16)]. Platelets' physiological role in healing has led to the concept that PRP may improve cartilage restoration. Additionally, the multitude of growth factors stored within the platelets' alpha granules are believed to improve the biological environment within which cartilage may heal [(17)]. Multiple *in vitro* and *in vivo* studies are present in the literature delineating the potential of PRP to improve chondrogenesis in ankle cartilage repair [(18-21)]. Recently, one-step cartilage repair procedures have been developed for the treatment of chondral defects and the improvement of the microfracture procedure [(22-24)]. The main concept of these procedures is that subchondral progenitor cells are 'activated' and released into the defect by bone marrow stimulation and that the defect is covered with different types of matrices augmented with blood derivatives for cartilage repair tissue formation. In microfracture, the clinical outcome may be variable due to, for example, the size of the lesion and age/activity of the patient as well as due to uncertain long-term functional improvements [(25-27)]. We used the subchondral drilling procedure to produce deep perforations that allow for having good access to the subchondral bone marrow and in turn to the subchondral progenitor cells. Recent basic studies have shown that allogenic PRP has a promotive effect on chondrocyte metabolism. For the clinical application of PRP to cartilage defects, it is essential to use autologuous prepared PRP in each case.

Up till now, the clinical effectiveness of one-step procedures is shown mainly in pilot studies with low to moderate number of patients. In a pilot study with five patients, microfracture and a cell-free resorbable polyglycolic acid–hyaluronan (PGA-HA) implant matrix immersed with autologous serum were used that showed noticeable clinical improvement in KOOS (mean overall KOOS of 33 pre-operatively to 79 at 2-year follow-up). However, magnet resonance imaging (MRI) showed different percentages of incomplete filling, subchondral irregularities and intralesional osteophytes [(28)]. The same group showed in another five patients that the good clinical outcome obtained with the AMIC technique combined with a PRP gel did not match

with the MRI improvement. At 2-year follow-up, the authors reported persistence of subchondral bone abnormalities, incomplete filling or hypertrophy of the repair tissue and intralesional osteophyte formation. The AMIC (autologous matrix-induced chondrogenesis) technique, which uses a porcine collagen type I/III membrane with blood-fibrin glue to cover the microfractured defect, has been shown to lead to significant clinical improvement in 27 patients with moderate to complete defect filling, at an average of 37-month follow-up [(23)]. Our study is different because it included 2 subgroups of cartilage defects with ACL reconstruction added in the treatment in subgroup 1 with moderate number of patients. We did drilling by controlled perforations using manual drill bit and then injection of the prepared PRP. We have a short follow up period only 1 year with assessment by KOOS, Brittberg scoring system and MRI only in 5 cases which showed complete filling only in 2 cases.

The treatment of focal cartilage defects in non-degenerative and degenerative knees with bone marrow stimulation and subsequent covering with a cell-free resorbable polyglycolic acid–hyaluronan (PGA-HA) implant immersed with autologous platelet-rich plasma (PRP) led to significant clinical improvement in a cohort of 52 patients with focal cartilage defects in a non-degenerative and degenerative environment, as assessed by KOOS (overall mean KOOS at baseline 50.3–82.9 at 2-year follow- up). Histological analyses showed the formation of hyaline-like to hyaline cartilage repair tissue at 18–24- month follow-up [(29)]. Our study is different because I addition to the above differences , we didn't make any histological analyses, very limited numbers of patients (20 patients) and also short period of follow up (1 year only).

Human clinical studies about cartilage repair by using PRP only are few in the literature. One of the first studies was performed by Sanchez *et al.* with an observational retrospective cohort study using hyaluronan injections as a control; at final follow up there were better results in the group treated with intra-articular injections of an autologous preparation rich in growth factors [(30)]. Kon *et al.* in a study that compared injection in early degenerative knee arthrosis with HA versus PRP demonstrated that autologous PRP injections could provide more and longer efficacy than HA injections in reducing pain and symptoms and recovering articular function. Better results were achieved in younger patients with a lower degree of cartilage degeneration [(31)]. Sampson et al. in a perspective, uncontrolled study, administered 3 PRP injections at 4-week intervals to 14 patients with knee OA. Significant improvements were found, with relief of pain and symptoms [(32)]. Wang-Saegusa *et al.* in a second perspective, nonrandomized, longitudinal study, 261 patients with knee OA (Outerbridge grades I–IV) were given 3 intra-articular injections of platelet concentrate suspended in plasma from autologous blood at 2-week intervals. Participants had statistically significant improvements in pain and function at 6 months [(33)]. Our study is different because we added drilling of subchondral bone before injection of PRP. We did it by using the arthroscopy, not as an outpatient clinics but our results more or less had the same effect by reducing pain and symptoms and recovering articular function with better results were achieved in younger patients with a lower degree of cartilage degeneration.

The limitations of our prospective study have been the moderate number of patients, the missing of comprehensive MRI data for the evaluation of defect filling, the clinical follow-up is limited to patients with a short-term 1-year follow-up as well as that no biopsies obtained for histological analysis are taken from any patient. The most important finding of the present study was that the validated KOOS score showed clinically meaningful and significant improvement 1 year after the injection of PRP in drilled full-thickness cartilage defects, compared to the pre-operative situation. Better results were achieved in younger and more active patients with a low degree of cartilage degeneration, whereas a worse outcome was obtained in more degenerated joints and in older patients.

Conclusion:

In conclusion platelet rich plasma is a safe, autologous, easy to prepare and to use and relative low cost procedure to deliver growth factors for cartilage healing and regeneration. The clinical results of comparative studies suggest that this procedure may be useful for the treatment of degenerative articular pathology of the knee as a treatment for articular cartilage degeneration in humans, in which produces a significant improvement in the clinical outcome of most patients especially in the short term. Furthermore recent studies support the use of PRP intra-articular injections, not only as a conservative treatment, but also after surgical procedures as many researchers attempted to enhance cartilage repair by combining surgical procedures such as microfractures with growth factors (GFs). Significant additional research is needed to define the role of PRP and to determine in which settings it might—or might not—be valuable. This may also involve defining a means of ensuring that a given PRP preparation is biologically active, by determining its critical component(s) and

developing assays that can provide this information to the surgeon in a timely manner.

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