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TRATTAMENTO DEI DIFETTI DELLA CARTILAGINE ARTICOLARE DEL GINOCCHIO: STRATEGIE CHIRURGICHE, RISULTATI E ANALISI DEI FATTORI DETERMINANTI L'OUTCOME CLINICO

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GENERAL INTRODUCTION

Articular cartilage lesions, with their inherent limited healing potential, are hard to treat and remain a challenging problem for orthopedic surgeons. Despite the development of several treatment strategies, the real potential of each procedure in terms of clinical benefit and effects on the joint degeneration processes is not clear.

Aim of this PhD project was to evaluate the results, both in terms of clinical and imaging improvement, of new promising procedures developed to address the challenging cartilage pathology. Several studies have been followed in parallel and completed over the 3-year PhD, and are reported in detail in the following pages. In particular, the studies have been focused on the evaluation of the treatment indications of a scaffold based autologous chondrocyte implantation procedure, documenting its results for the classic indication of focal traumatic lesions, as well as its use for the treatment of more challenging patients, older, with degenerative lesions, or even as salvage procedure for more advanced stages of articular degeneration. The second field of study involved the analysis of the results obtained treating lesions of the articular surface with a new biomimetic osteochondral scaffold, which showed promise for the treatment of defects where the entire osteochondral unit is involved. Finally, a new minimally invasive procedure based on the use of growth factors derived from autologous platelets has been explored, showing results and underlining indicatios for the treatment of cartilage lesions and different stages of joint degeneration.

These studies shed some light on the potential of the evaluated procedures, underlining good results as well as limits, they give some indications on the most appropriate candidates for their application, and document the current knowledge on cartilage treatment procedures suggesting the limitations that need to be addressed by future studies to improve the management of cartilage lesions.

PART 1

Matrix-assisted autologous chondrocyte implantation: a prospective 7-year follow-up study

INTRODUCTION

Regenerative procedures, such as autologous chondrocyte implantation (ACI), have emerged as a potential therapeutic option for the treatment of cartilage defects, which aim to recreate a hyaline-like tissue in the damaged joint surface.[3,20,21]

The recent development of the so-called second-generation ACI techniques has further improved the potential of these treatments. By tissue-engineering technology a cartilage-like tissue in a 3-dimensional culture system has been produced. This has dispelled concerns related to the cell culture and gives marked advantages from a surgical point of view.[12,14] Essentially, the concept is based on the use of biodegradable polymers as temporary scaffolds for the *in vitro* growth of living cells and their subsequent transplantation onto the defect site. The scaffolds are easy to handle, and they can be implanted by arthroscopic techniques for some bioengineered tissues.[5,7,12,22]

However, although several papers[12,14] support the good clinical results of these treatments, new regenerative procedures have not been clearly proven to be better than the more traditional reparative techniques. Currently, there is no agreement about the effective superiority of one procedure over the others, and indications and results are still controversial.[10,11] Most manuscripts report clinical outcomes at short- to mid-term follow-up, and many different techniques may appear satisfactory at the beginning, but the higher quality of the repair tissue documented for the cell-based approach[23,24] might influence the long-term outcome.

Only a few papers report the results of second-generation matrix-induced autologous chondrocyte transplantation at follow-ups longer than 5 years.[1,6,9,13,17,19] Autologous chondrocyte transplantation on a 3-dimensional matrix was introduced in the clinical practice in 1998-1999, so it is very difficult to obtain mid- or long-term clinical results.

The main purpose of this study was therefore to document and analyze the clinical outcome obtained with the treatment of isolated knee chondral lesions by arthroscopic second-

generation ACI, to assess the durability of the results and understand its real potential over time.

MATERIALS AND METHODS

Patient selection

The treatment was indicated for patients with focal grade III-IV (ICRS evaluation package) chondral knee defects of the femoral condyles, and clinical symptoms (pain, swelling, locking, and giving way). Exclusion criteria were untreated tibio-femoral or patello-femoral malalignment or instability, diffused arthritis or bipolar ("kissing") defects, and those with other general medical conditions (diabetes, rheumatoid arthritis, etc). The patients who presented with an ACL lesion at the time of surgery underwent the combined surgical procedure of ACL reconstruction during the same surgical session with cartilage harvesting.

In this prospective study, 62 consecutive patients were enrolled, treated, and evaluated every year for 7 years. These patients included 48 men and 14 women, with a mean age of 28.1±11.4 years. The site of the defects was the medial femoral condyle in 45 cases, and in 17 patients the lateral femoral condyle was involved. The average size of the defects was 2.5±1.0 cm^2. The etiology was traumatic in 29 cases and micro traumatic/degenerative in 23 cases, and 10 patients were affected by osteochondritis dissecans (OCD). Half of the patients were well-trained or played sports at a competitive level, the others practiced sport at amateur level or did not practice any sport. Twenty-seven patients were treated surgically for the first time, and 35 patients had undergone previous surgeries. In 35 patients, other procedures were combined during the same operation.

Surgical technique

The surgical technique consists of two steps. The first one consists of a biopsy of healthy cartilage for autologous chondrocyte cell culture and subsequent seeding onto the scaffold. The second step is to implant the bioengineered tissue Hyalograft C®, according to the procedure developed by professor Marcacci.[16] A variable diameter delivery device with a sharp edge is used to evaluate the defect size. A circular area with regular margins for graft implantation is prepared with a specially-designed cannulated low-profile drill. The delivery device is then filled with a hyaluronic acid patch, that is transported and placed in the

prepared area. The graft is pushed out of the delivery device and positioned precisely within the defect area where it remains fixed to the subchondral bone.

Under arthroscopic control, the stability of implanted stamps is evaluated also during cyclic bending of the knee (Fig. 1).

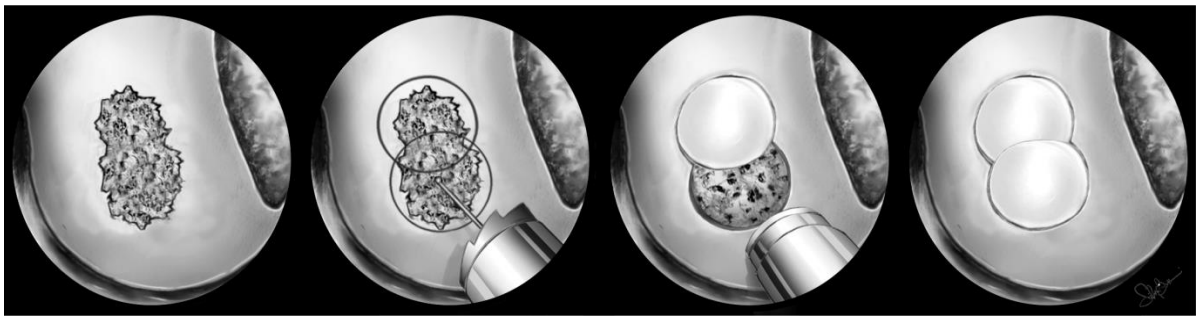

Fig. 1: Arthroscopic technique.

Rehabilitation protocol

On the 2nd post-op day, self-assisted knee mobilization or continued passive motion (CPM) 6 hours daily is recommended until 90° of flexion is obtained. Controlled mobilization exercises with reduced ROM, early isometric and isotonic exercises are performed. In the 4th week weight touch down with crutches is allowed and is usually completed within 6-8 weeks after surgery. At the beginning of the 9th week, active functional training is started to restore a correct running pathway by proprioceptive, strength and endurance exercises and aerobic training. This stage may end within 30-32 weeks after the operation. The rest of rehabilitation is dedicated to the return to previous sports activity, which is usually allowed no earlier than 10-12 month.

Follow-up evaluation

All 62 patients were evaluated preoperatively and yearly up to 7 years. The clinical outcome was analyzed using the Cartilage standard Evaluation Form as proposed by ICRS (International Cartilage Repair Society). A functional knee test was performed according to the IKDC Knee Examination Form. The lowest ratings in effusion, passive motion deficit, and ligament examination were used to determine the final functional grade of the knee (normal, nearly normal, abnormal or severely abnormal). Activity level was also recorded and evaluated with the Tegner score.

The operation was considered to have failed if the patient needed a re-operation because of symptoms due to primary defects. For patients who underwent a re-operation, the last clinical evaluation before re-operation was considered.

Statistical Methods

Continuous data are expressed in terms of mean and standard deviation of the mean. Kolmogorov Smirnov test was used to assess the distribution normality.

One Way ANOVA was used to test hypotheses about means of different groups. When the Levene test for homogeneity of variances was significant or when the variables were non normally distributed, the Mann Whitney test or the Kruskal Wallis test were used to check results. Pearson's Chi square test with Exact Method was performed to study the relationships of grouping variables. GLM for repeated measures with Bonferroni correction for multiple comparisons were used to test score differences at different follow-ups. Pearson's correlation was applied to study the relationship between continuous and normally distributed variables. Spearman rank correlation was applied to study the relationship between continuous and not normally distributed variables. $P<0.05$ was considered significant. Statistical Analysis was carried out with SPSS software version 9.0 (SPSS Inc., Chicago, USA).

RESULTS

No complications related to the implant or severe adverse events were observed during the treatment and follow-up period. A marked improvement in all scores was found after the treatment.

The IKDC objective score improved significantly at 12 months with respect to the basal level ($p<0.0005$), increasing from 21% of normal and nearly normal knees before the treatment to 90% at 12 months; results at further follow-ups were stable, reaching a final ratio of 93% of normal and nearly normal knees at the 84 months' follow-up (Tab. 1).

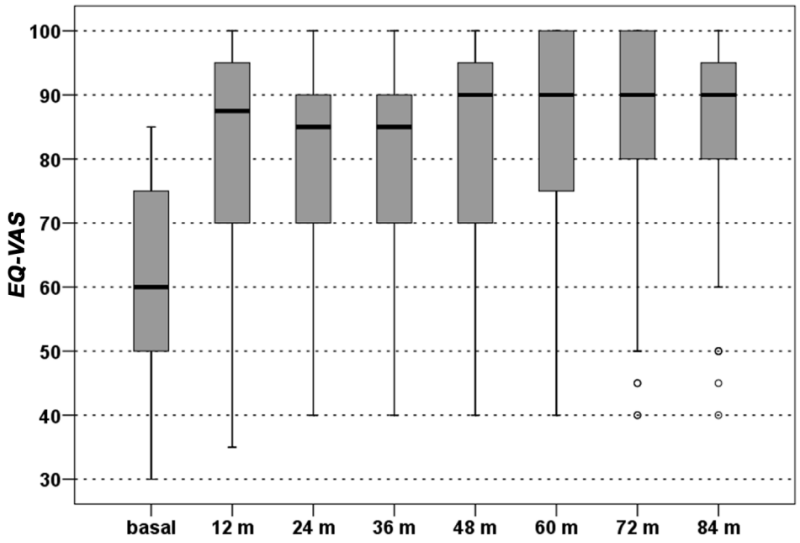

Fig. 2: EQ-VAS score: improvement from pre-op level to 12, 24, 36, 48, 60, 72 and 84 months' f-up.

	A	**B**	**C**	**D**
Basal	1	12	29	20
12 m	41	14	6	1
24 m	43	11	7	1
36 m	43	9	8	2
48 m	45	10	6	1
60 m	47	10	5	0
72 m	47	8	6	1
84 m	45	12	4	1

Tab. 1: IKDC obj score: improvement from pre-op level to 12, 24, 36, 48, 60, 72 and 84 months' f-up.

Sport activity level, evaluated with the Tegner score, showed the same trend, with a statistically significant improvement from pre-treatment level (1.7±1.3) to all the follow-up times ($p<0.0005$). However, despite the marked improvement, the highest score achieved at the 84-month follow-up (5.3±2.6) remained lower than the pre-injury activity level (6.9±2.0) ($p<0.0005$) (Fig. 3).

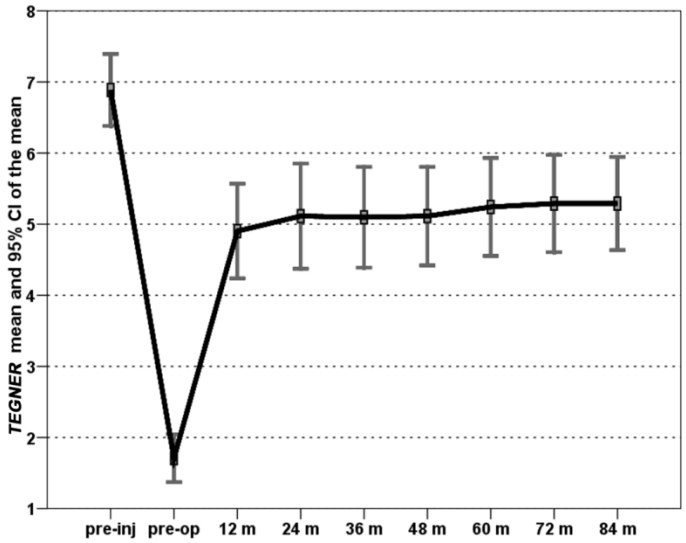

Fig. 3: Tegner score: improvement from pre-op level to 12, 24, 36, 48, 60, 72 and 84 months' f-up.

The IKDC subjective score was higher than the basal level at all the follow-ups ($p<0.0005$), increasing from 39.6±15.0 to 73.6±18.8 at 12 months; a further slight improvement was observed at 24 months' follow-up (76.5±20.7), then results were stable reaching a final 84 months value of 77.3±21.5 (Fig. 4).

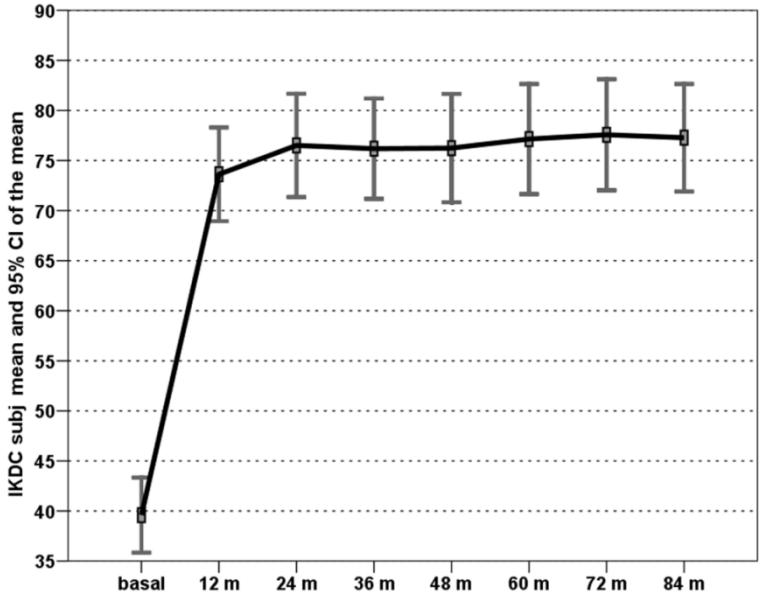

Fig. 4: IKDC subj score: improvement from pre-op level to 12, 24, 36, 48, 60, 72 and 84 months' f-up.

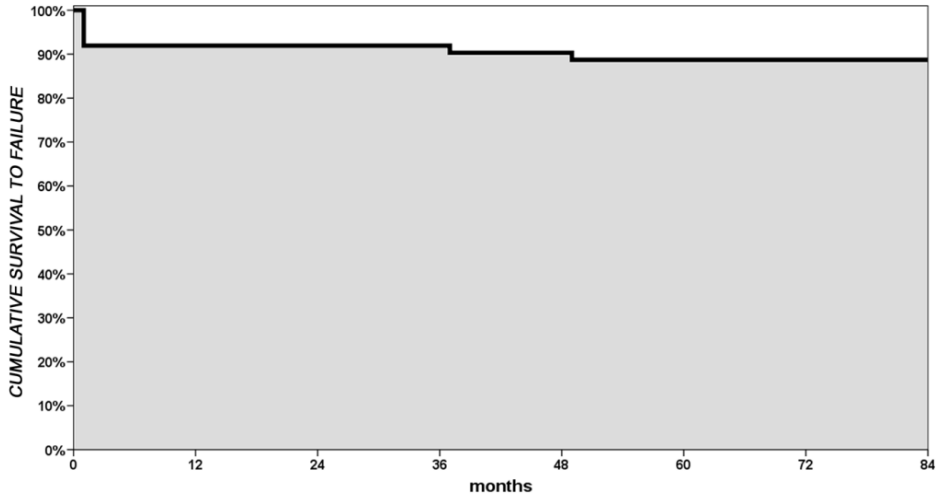

Fig. 5: Due to the failed patients, the percentage of patients with positive treatment effects decreases over time, reaching an 88.5% ratio at 84 months' f-up.

Seven cases failed (Fig. 5). Excluding the failed cases to analyze separately the results obtained in the long-term responsive patients, a tendency (even if not statistically significant) of further improvement of clinical outcome was observed at longer follow-ups: subjective IKDC reached a score of 82.7±17.5 and 82.4±16.6 at 6 and 7 years of follow-up, respectively.

Further analysis was performed to determine the parameters that influenced the clinical outcome. A greater improvement was observed at the last follow-up in active patients: 83.7±17.1 vs 70.9±23.7 IKDC subjective score (p=0.018). Better scores were also reached for men (81.8±18.5 vs 61.8±24.4, p=0.002); however, considering the lower basal scores in women, we also evaluated the improvement obtained at the last follow-up, showing a less marked gender-related difference with only a tendency towards poorer results in women (p=0.098). Younger patients (r=-0.313, p=0.013) had better IKDC subjective scores (Fig. 6) and a higher activity level at 7 years of follow-up (r=-0.361, p=0.004). Etiology influenced the final outcome: patients affected by degenerative lesions had the worst clinical results (p=0.013) (Fig. 7). Finally, poorer results were observed in patients who had undergone previous surgery (p=0.003). Other factors, such as BMI, site, defect size, and combined surgery, did not significantly influence the final outcome.

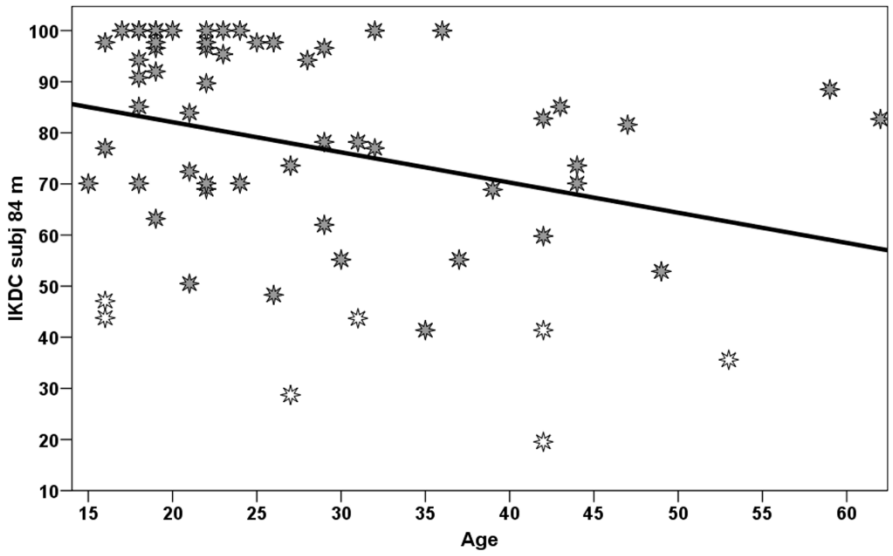

Fig. 6: Correlation between age and clinical outcome evaluated with IKDC subj score at 84 m f-up.

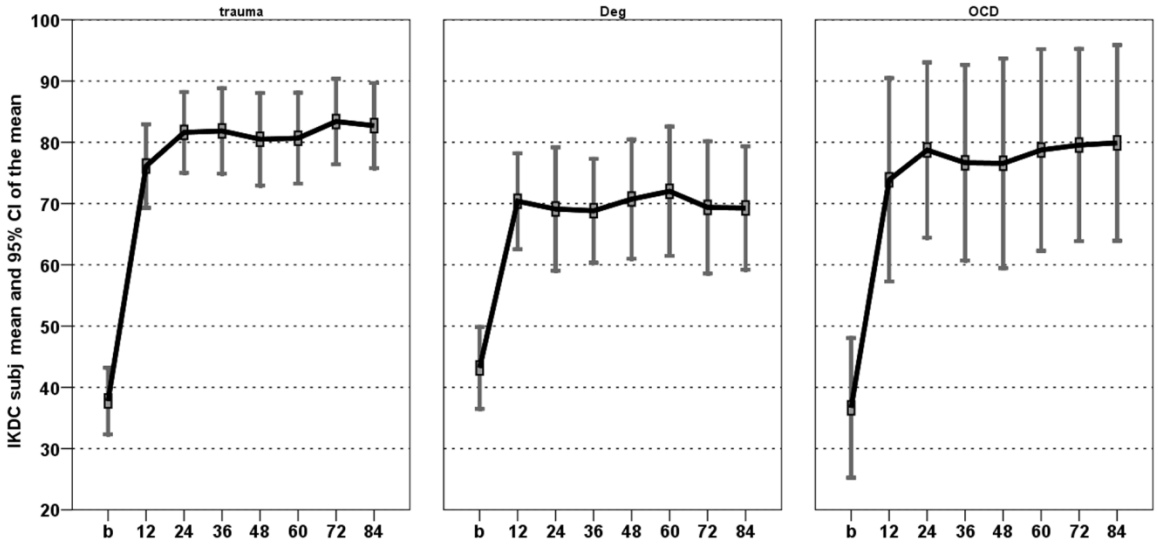

Fig. 7: Etiology influences clinical outcome evaluated with IKDC subj score at 84 months' f-up.

DISCUSSION

Various techniques have been studied and applied over the years for the treatment of cartilage defects, from classic bone marrow stimulation procedures,[9,18,25] to the more ambitious and modern regenerative techniques, which aim to produce a hyaline–like tissue

with better and durable clinical results.[4,20] Although thousands of patients have been treated and several studies published suggesting good clinical results and durability of the cell-based procedures, currently there is no agreement about their effective superiority over other treatments, and indications and results are still controversial.

Knutsen et al.[10,11] showed no differences between ACI and microfracture at 2 and 5 years' follow-up. However, he also reported that ACI biopsies tended to have a more hyaline-like appearance and that none of the patients failed after microfracture presented high-quality repair cartilage, thus suggesting that a poor-quality repair tissue might increase the risk of failure or present a poorer outcome over time.

Saris et al.[23,24] confirmed this hypothesis. Although comparable clinical outcome was found between microfracture and characterized chondrocyte implantation (CCI) at short-term, despite the superior histological score in the CCI group, the quality of the repair tissue significantly influenced the later follow-up. In fact, at 3 years CCI presented a further improvement with better clinical results compared with microfracture, that reached a plateau after 18 months. ACI procedures may regenerate a hyaline-like tissue that undergoes a remodeling process, thus leading to superior clinical results detectable only after at least 2 – 3 years. Bearing this in mind, the importance of documenting better the mid-long term outcome of the bioengineered approach appears clear. Up to now, only a few studies report mid-term follow-up results.[1,6,9,15,17] We[13] recently reported good stable results in 40 patients treated with Hyalograft C® at 5 years' follow-up, conversely to the microfracture comparative group, where a deterioration was observed over-time.

In this study we analyzed 62 patients affected by focal grade III-IV chondral knee lesions of the femoral condyles. All patients were treated with the arthroscopic implantation of the bioengineered cartilage tissue Hyalograft C® and evaluated prospectively every year up to 7-years, to assess the durability of the results and understand its real potential over time. A significant improvement was observed at all follow-up times, thus demonstrating stable results. In this series we recorded 7 failures, which occurred mainly in the first years. Analyzing separately the clinical outcome of the not failed patients, we found a further tendency to improve over time. This suggests that, when the treatment is successful, the process of continued remodeling of the regenerated tissue after 2nd generation ACI continues after a number of years, thus allowing a better knee "homeostasis" with a slight further

improvement over-time to be achieved. Further analysis enabled the parameters that influenced the clinical outcome to be determined. Age was correlated with the clinical outcome, as previously underlined in other manuscripts.[2,10,11,24] A lower improvement was observed in less-active patients with a degenerative etiology, in patients who had undergone previous surgery, and in women. This gender-related outcome is difficult to explain, but our results are supported by some findings already reported in the literature.[8] Finally, we found that the results were not influenced by the defect dimension, at least in this range of small-medium lesions treated, as previously reported in the literature.[10,11]

The limitations of this study are the lack of a control group and histological and imaging analysis. However, other studies have already described the histological results of the cell-based approach,[9,10,11,23,24] whereas there is a lack in the literature regarding the persistency of the clinical results offered by the recently developed bioengineering technologies.

This prospective study included 62 patients affected by focal chondral defects of the condyles and treated with arthroscopic Hyalograft C implant. The analysis allowed us to show that this bioengineered approach may offer good and stable clinical results over time.

CONCLUSIONS

The results of this study show that this arthroscopic bioengineered approach may offer, with the proper indications, good and stable clinical results over-time for the treatment of knee cartilage lesions. Better results have been obtained in younger and more active patients, whereas in our series women and patients who presented degenerative etiology and previous surgery presented a lower improvement, even if still significant with respect to the pre-operative level.

Filardo G, Kon E, Di Martino A, Iacono F, Marcacci M. Arthroscopic second-generation autologous chondrocyte implantation: a prospective 7-year follow-up study. Am J Sports Med. 2011 Oct;39(10):2153-60. The final publication is available at http://journal.ajsm.org/ doi: 10.1177/0363546511415658.

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Matrix-assisted autologous chondrocyte implantation for the treatment of degenerative cartilage lesions

INTRODUCTION

The management of chondral pathology is challenging due to the distinctive structure and function of hyaline cartilage and its low healing potential.[7,8,34,42] The interaction between cells, collagen framework, aggrecan and fluid constitute the complex biomechanical feature of this tissue, making it difficult to repair.[4] An imbalance of regulatory factors, which may result from aging, disease, or injury, may hinder tissue maintenance and repair, ultimately resulting in the loss of tissue homeostasis with deleterious changes in gene expression, altered extracellular matrix, and consequently tissue degeneration.[45] The pathogenesis of cartilage pathology is multifactorial and heterogeneous but, regardless of the initial cause, lesions lead to progressive loss of hyaline cartilage. Even isolated injuries are a risk factor for more extensive articular damage. Early changes in cartilage include proteoglycan loss, increase in water content, and disorganization of the collagen network with an accelerated damage to the joint surface, thus leading to end-stage arthritis.[7,18,34]

Modern regenerative treatments can replace the damaged articular surface with a hyaline-like tissue,[40,41] and recently good mid-long term clinical results have been achieved with these techniques.[2,14,25,33,37] However, it is controversial how degenerative processes may affect cartilage repair. While some studies show inferior results in chronic lesions,[31,32] other ones show that even patients with early osteoarthritis can benefit from cartilage treatments, with : lasting clinical improvement and histological and biochemical analyses suggesting no inhibition of the regenerative process by the degenerative joint environment.[19,29,30]

More data are needed on how the perturbation of joint homeostasis may affect the intra-articular environment and the clinical outcome negatively in patients affected by degenerative cartilage lesions and treated with regenerative procedures still needs to be determined.

The main purpose of our study is therefore to analyze the clinical outcome obtained with the treatment of isolated degenerative knee cartilage lesions with second-generation arthroscopic autologous chondrocyte implantation (ACI) in patients with no clear signs of osteoarthritis, and to understand the real potential of this regenerative procedure related to degenerative etiology. The secondary outcome was to evaluate the failure rate and the complications of this treatment, and to find prognostic factors that may further influence the results expected for degenerative lesions.

MATERIALS AND METHODS

Patient selection

An arthroscopic technique has been developed and used in our Institute since 2001: experienced knee surgeons selected patients according to the defined criteria and treated them arthroscopically.

The selection criteria for the study were: micro traumatic/degenerative focal grade III-IV (ICRS) cartilage knee lesions involving femoral condyles or trochlea, complaining of clinical symptoms. Exclusion criteria were BMI higher than 30, untreated tibio-femoral or patello-femoral misalignment or knee instability, multiple lesions, diffused arthritis or bipolar lesions, and those with infective, tumor pathology or other general medical conditions. Patients who presented with an ACL lesion at the time of surgery underwent the combined surgical procedure of ACL reconstruction in the same surgical session with cartilage harvesting.

In this prospective study 58 consecutive patients were treated and evaluated prospectively at 6 years of follow-up (5-7). Of these patients, 3 were lost to follow-up and 1 was excluded because of another major knee trauma. One patients presented a bilateral lesion, making a total of 55 knees evaluated. The patients evaluated were 39 men and 15 women, with a mean age of 34.7±9.1 years and a mean BMI of 24.3±2.8. The site of the defects was the medial femoral condyle (MFC) in 32 cases, lateral femoral condyle (LFC) in 16 cases, and trochlea in 7 patients. The average defect size was 2.3±0.9 cm^2. The etiology was micro traumatic/degenerative in all cases, according to the selection criteria. The medical history showed that pain and limited function related to the cartilage pathology had a mean duration of 48 months ranged (6 months to 20 years).

Forty-three patients (78%) had undergone previous knee surgery and in 23 patients (41%), combined procedures were performed.

Surgical technique and rehabilitation protocol were described previously.[27]

Follow-up evaluation

All 54 patients underwent a prospective evaluation, preoperatively and at a mean of 6 years of follow-up (5-7). The clinical outcome was evaluated using the Cartilage standard Evaluation Form as proposed by ICRS. A functional test was performed according to the IKDC Knee Examination Form used to determine the final functional knee grade (normal, nearly normal, abnormal or severely abnormal).[20] Activity level was evaluated with the Tegner score relatively to pre-operative and pre-injury levels.[44] From 2 to 6 years after transplantation, repair of the articular surface was evaluated in 26 knees with high-resolution MRI and the MOCART scoring system was applied.[28] Surgery was considered to have failed if the patient needed a re-operation because of symptoms related to the primary lesion.

Statistical Methods

Continuous data were expressed in terms of the mean and the standard deviation of the mean. One Way ANOVA with the Scheffé post-hoc pairwise analysis was used to assess differences among groups when the Levene test for homogeneity of variances was not significant; otherwise, the Mann Whitney test or the Kruskal-Wallis test with the non-parametric post-hoc pairwise LSD test were applied. The GLM for repeated measures with Sidak's correction for multiple comparisons was used to test score differences at different follow-ups. The Friedman test with the post-hoc pairwise Wilcoxon test with Sidak's correction for multiple comparisons was applied to test differences among different follow-ups of IKDC objective score. The Spearman rank correlation was applied to study the correlation between scores and continuous variables. The Multiple Regression, via GLM, was performed to study the variables most related to the clinical scores. The Kaplan-Meier survival analysis with the Breslow test was applied to determine the influence of the variables on the failure. The Cox Regression survival analysis was performed to evaluate the influence of the continuous variables on failures. $P<0.05$ was considered significant.

Statistical Analysis was carried out by means of the SPSS software version 15.0 (SPSS Inc., Chicago, USA).

RESULTS

No complications related to the implant or serious adverse events have been observed during the treatment and follow-up period. A significant improvement in all the scores was observed.

IKDC obj score: from 40% of normal and nearly normal knees before surgery to 82% at 2-years and 85% at 6 years, with a significant improvement at both follow-ups ($p<0.0005$) and stable results. IKDC subj score: from 39.3±13.6 at the basal evaluation to 68.8±22.7 and 68.5±23.9 at the 2 and 6-year follow-ups, respectively, with a significant improvement (both $p<0.0005$) and stable results (Fig. 1).

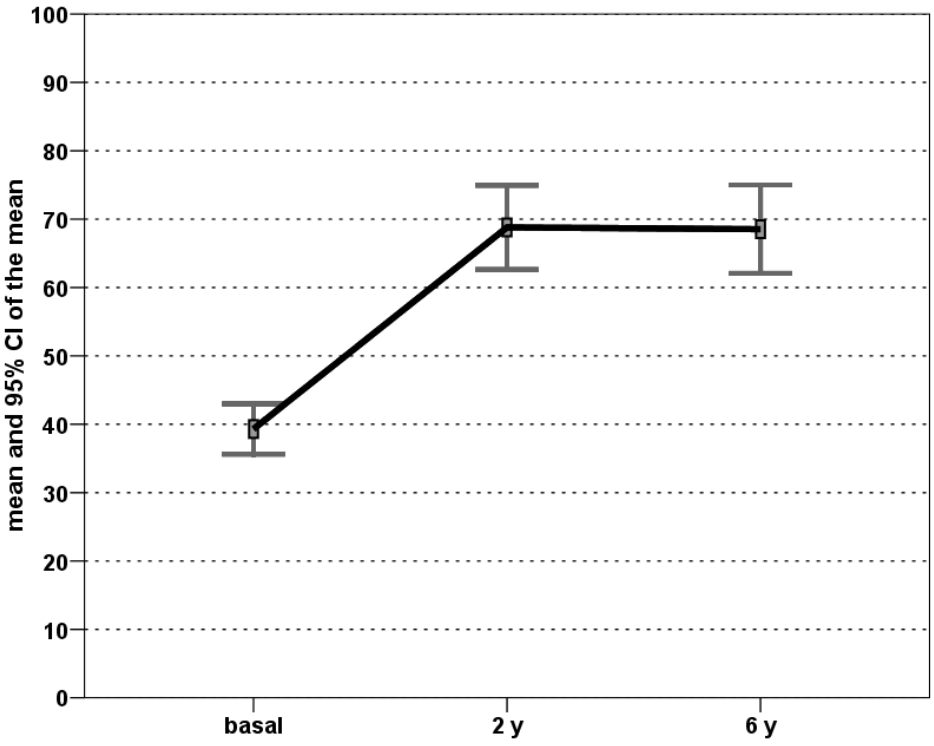

Fig. 1: IKDC subjective score: improvement from the pre-operative level to 2 and 6-year follow-ups.

Tegner score: significant improvement ($p<0.0005$) from pre-surgery level (1.7±1.4) to the 2-year and 6-year follow-ups (3.9±2.7 and 4.1±2.2, respectively) and stable results over time. Despite the marked improvement, the score achieved was lower than before the onset of symptoms (6.1±2.0) ($p<0.0005$) (Fig. 2). Ten patients failed for a total failure rate of 18.5% at 6 years.

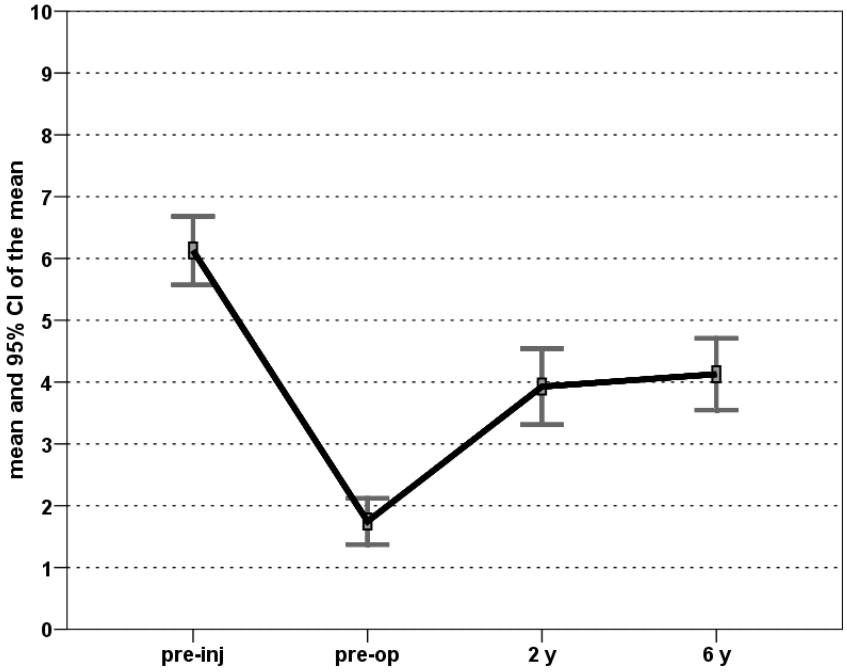

Fig. 2: Tegner score: improvement from the pre-operative level to 2 and 6-year follow-ups with respect to the pre-injury level.

Parameters that influenced the final outcome: a higher IKDC subj improvement in sport-active patients at 6 years (68%±32 vs 36%±41, $p=0.005$). Better scores were also achieved by men (55%±33 vs 21%±48, $p=0.005$). Site and previous surgery markedly influenced the results. The worst outcome was observed in medial femoral condyle lesions, the best one in lesions of the trochlea (36%±42 MFC, 51%±30 LFC, 81%±37 trochlea, $p=0.023$). Previous surgery: IKDC subj improvement of 39%±41, significantly lower ($p=0.008$) than the other patients 73%±29. Age, BMI, defect size, combined surgery, and duration of symptoms did not significantly influence the final outcome in this series.

Failures: 6 cases in men and 4 in women. Due to the low number of failures, we did not find any statistical significance, with the exception of the activity level: in fact, all the failures happened in the group of not active patients (p=0.018) (Fig. 3).

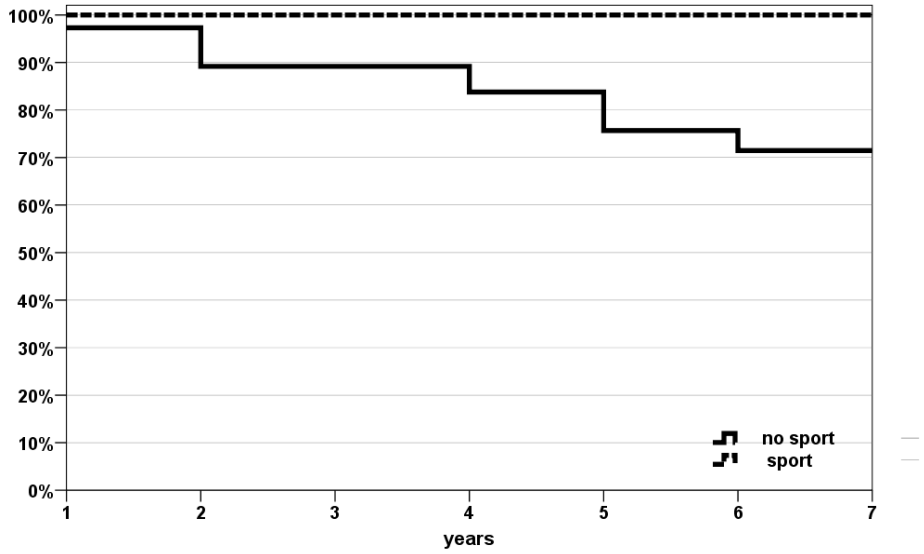

Fig. 3: Survival curve of sport and no-sport groups: all the failures occurred in the not active group.

To determine which parameters were more important in influencing the clinical outcome a multiple regression analysis was performed, confirming the role of sport (p=0.003, β=0.36), previous surgery (p=0.007, β=0.328), and gender (p=0.046, β=0.243).

Of the MRIs (Fig. 4) evaluated with the MOCART scoring system, a complete filling of the cartilage was shown in 50% of the lesions, complete integration of the graft in 58% of cases, the repair tissue surface was intact in 38%, the structure of the repair tissue was homogeneous in 46% of the cases, and the graft signal intensity score was iso-intense in 54% and 58% of the cases in dual T2-FSE and 3D-GE-FS sequences, respectively. The subchondral lamina was considered intact in 58% of the cases, and subchondral bone changes were observed in 65% of the cases. Adhesion and effusion were present in 4% and 23% of the cases, respectively (Tab. 1).

Tab. 1 MRI evaluation with the MOCART scoring system.

Variables	Point Scale
1) Degree of Repair and filling of the defect	
Complete (on a level with adjacent cartilage)	13/26 (50%)
Hypertrophy (over the level of the adjacent cartilage)	4/26 (15%)
Incomplete (under the level of the adjacent cartilage; underfilling)	
>50% of the adjacent cartilage	7/26 (27%)
<50% of the adjacent cartilage	2/26 (8%)
Subchondral bone exposed (complete delamination/dislocation and/or loose body)	0/26 (0%)
2) Integration to border zone	
Complete (complete integration with adjacent cartilage)	15/26 (58%)
Incomplete (incomplete integration with adjacent cartilage)	
Demarcating border visible (split-like)	6/26 (23%)
Defect visible	
< 50% of the length of the repaired tissue	4/26 (15%)
> 50% of the length of the repaired tissue	1/26 (4%)
3) Surface of the repair tissue	
Surface intact (lamina splendens intact)	10/26 (38%)
Surface damaged (fibrillations, fissures and ulcerations)	
<50% of repair tissue depth	15/26 (58%)
>50% of repair tissue depth or total degeneration	1/26 (4%)
4) Structure of the Repair tissue	
Homogeneous	12/26 (46%)
Inhomogeneous or cleft formation	14/26 (54%)
5) Signal intensity of the repair tissue	
Dual T2 FSE	
Isointense	14/26 (54%)
Moderately hyperintense	11/26 (42%)
Markedly hyperintense	1/26 (4%)
3D-GE-FS	
Isointense	15/26 (58%)
Moderately hyperintense	11/26 (42%)
Markedly hyperintense	0/26 (0%)
6) Subchondral lamina	
Intact	15/26 (58%)
Not intact	11/26 (42%)
7) Subchondral Bone	
Intact	9/26 (35%)
Edema, granulation tissue, cysts, sclerosis	17/26 (65%)
8) Adhesions	
No	25/26 (96%)
Yes	1/26 (4%)
9) Effusion	
No	20/26 (77%)
Yes	6/26 (23%)

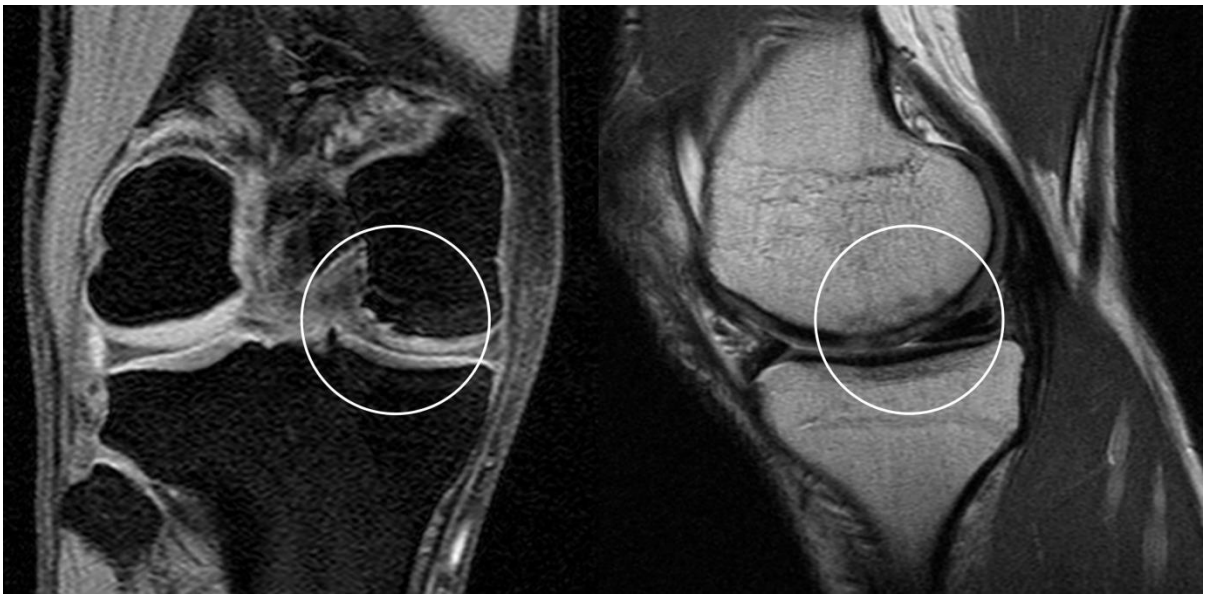

Fig. 4: MRI evaluation at 4-year follow-up of an MFC implant.

DISCUSSION

The modern regenerative procedures aim to produce a hyaline–like tissue and therefore achieve good and durable clinical results for the treatment of cartilage lesions. Since the introduction 20 years ago[5] of the cell-based approach, both the production of a hyaline-like articular surface and a satisfactory clinical outcome have been reported.[6,37] The development of tissue bioengineering has led to the second generation ACI: the use of 3-dimensional matrix for chondrocyte transplantation has shown to offer similar results while overcoming most of the biological and surgical concerns related to the first generation procedures.[23,24] Despite some controversies about the effective superiority of one procedure over the others,[21,22] good results have been shown for different types of scaffold,[2,14,16,26,33] and the higher quality of the repair tissue obtained with the regenerative techniques seems to allow a better long-term outcome.[25] However, worst results are expected in degenerative joint disease.[23]

Tissue engineering for degenerative pathology presents some additional problems: chondrocyte therapy is suitable for cartilage lesions surrounded by healthy cartilage in order

to have stable shoulders for the implant, whereas in a degenerative process the adjacent areas can be involved, leading to loosening of the graft, and the cytokines produced by the chondrocytes around the implant might also cause apoptosis of the implanted cells.

A multitude of interactions among different structures, such as synovium, synovial fluid, ligaments, menisci, cartilage, and subchondral bone, concur to the delicate physiological joint equilibrium.[7,8] Once this complex highly-regulated environment is disturbed, many intra-articular changes, such as inflammatory, cellular or molecular factors, come into play.[39] In case of degenerative defects, cartilage damage may initiate long before the onset of symptoms, and often a further delay is due to the slow increase in the magnitude of clinical problems before patients seek treatment and undergo surgical cartilage replacement. At this point, joint homeostasis has already been disturbed, with synovitis, matrix degradation, and very likely even subchondral bone changes,[17,39] which may provide an unfavourable environment for tissue regeneration. Some preclinical studies support this hypothesis.[36,38,39] Saris et al.[39] evaluated the effect of metabolic alterations in joint homeostasis on the outcome of cartilage repair using tissue engineering in goats. In the presence of a disturbed intra-articular environment, histological, biochemical, and macroscopic parameters showed a significant decrease in the outcome of tissue engineering. These findings were confirmed by Ozsoy et al.:[36] in an experimental study on the treatment of osteochondral lesions in rabbits they showed that the ongoing degenerative activity of the joint influenced the results. Treatment in early stages resulted in a better outcome, probably due to the more prolonged period of disturbed homeostasis and more prominent negative effects in chronic degenerative stages. Finally, Rodrigo et al.[38] analysed the effect of synovial fluid and showed that, whereas it contains factors that can stimulate chondral healing in the acute period following traumatic injury, once the lesions become chronic its effect can be inhibitory, thus suggesting that a negative healing environment may impair chondrogenesis.

However, despite the awareness of the difficulties related to a disturbed articular homeostasis, other authors suggested that regenerative techniques may be also successfully applied to joints affected by degenerative pathology. Hollander et al.[19] found tissue regeneration even when implants were placed in osteoarthritic joints, thus demonstrating that tissue engineering can initiate a regeneration process that is not inhibited by the

degenerative environment. Laboratory studies confirmed the potential of regenerative procedures in joints with degenerative lesions: Tallheden et al.[43] showed that osteoarthritic chondrocytes have a good proliferation potential and are able to redifferentiate in a 3-dimensional pellet model, when loaded into a scaffold based on hyaluronic acid, producing cartilage specific matrix proteins. These findings have been widened by Cavallo et al.,[9] who showed how the cells seem to benefit from the presence of hyaluronan, which is able to recreate an ideal environment. The growth of chondrocytes onto Hyaff 11® membrane seems to erase the differences between the cells derived from normal and degenerated cartilage, and histological and biochemical analyses also showed that osteoarthritis does not inhibit the regeneration process,[9] thus indicating that bioengineered tissue may be used to facilitate tissue repair even in the treatment of joints with degenerative processes.

The clinical literature also shows interesting findings,[1,29] and recently promising results have been reported for the modern regenerative procedures. Minas et al.[30] followed 153 patients after treatment with ACI for early-stage osteoarthritis, and reported that at 5 years 92% of patients were able to delay joint replacement. Ossendorf et al.[35] reported an improvement in symptoms and quality of life treating with polymer-based autologous cartilage graft implantation mild degenerative cartilage lesions, as well as focal osteoarthritic knee defects. Kreuz et al.[26] confirmed these findings reporting stable results at 4 years, with a significant improvement in the patients' condition and a good defect filling at MRI.

In this study we focused on the clinical outcome obtained with the treatment of isolated degenerative knee cartilage lesions with arthroscopic second generation autologous chondrocyte implantation (ACI) in patients with still no clear signs of osteoarthritis, in order to understand the real potential of this regenerative procedure related to degenerative etiology. No complications related to the implant or severe adverse events were observed, and a statistically significant improvement in all the scores was observed at mid-term follow-up. However, results were lower with respect to the outcome reported in different study populations,[13,14,26] and the number of failures was also markedly higher, with a total failure rate of 18.5% at 6 years.

Further analysis allowed parameters to be determined that can influence the results expected for degenerative lesion. A lower improvement was observed in less-active patients, thus confirming our previous findings on the importance of sport activity and an active lifestyle

for the outcome after MACT.[13] The worst results were also observed in women, confirming some findings already reported in the literature.[15] In this group of small and medium lesions we observed that the results were not influenced by the lesion dimension, as previously reported.[21,22] The analysis of the defect location showed opposite findings with respect to the literature on traumatic lesions,[12] with worst results in lesions of the MFC. It is likely that mechanical loading, higher in the MFC, plays a more important role in the regeneration of degenerative lesions, thus affecting the final outcome. A history of previous surgery also markedly influenced the clinical outcome, whereas other factors, such as combined surgery, BMI and age, were not correlated to the scores measured. The absence of age influence is in contrast with most of the literature.[3,21,22,40] The difference can be likely explained by the lower importance of age-related articular changes in joints already affected by a disturbed homeostasis due to the chronic degenerative processes. Duration of symptoms was not correlated with the results obtained, conversely to the better clinical outcome observed in early versus late-treated cartilage defects with traumatic etiology.[12] Whereas the treatment of traumatic fresh lesions may benefit form limited or localized cartilage matrix disturbance or degeneration in the surrounding tissues,[39] in degenerative lesions the pathologic process may initiate long before the clinical onset of symptoms, and therefore even the treatment of patients with a shorter history of pain and limited function can be affected by a chronically altered negative environment.

A limitation of our study is the lack of histological evaluation. However, the high number of homogeneous patients treated and evaluated at 6 years allowed us to highlight the clinical improvement obtained treating degenerative lesions with MACT, but at the same time the inferior results and the higher failure rate compared to those reported in the literature for other treatment indications.

Although results are expected to be inferior, we still recommend this treatment approach. Untreated degenerative defects double the rate at which cartilage is lost compared to intact knees,[10,11] and therefore MACT may potentially decelerate the disease progression. Moreover, many young active patients suffer from cartilage degeneration, and these patients lack good therapeutic options and are too young for joint replacement, thus making the biological reconstruction a valid choice. When all the environment factors involved, such as synovitis, meniscal damage, ligament stability, and limb alignment, are recognized and

addressed, favorable results can be obtained with second-generation ACI also for chondral degeneration. The improvement of bioengineered tissues with better biomechanical properties, as well as the development of therapies to normalize the chondral metabolic activity and joint homeostasis, will allow further a improvement in the potential of this regenerative procedure for the treatment of degenerative lesions.

CONCLUSION

Degenerative cartilage lesions present a negative joint environment, which may have a negative effect on the regeneration process. We observed a clinical improvement at mid-term follow-up, but we also found inferior results and a higher failure rate compared to those reported in the literature for other treatment indications.

Tissue-engineered cartilage implant is a promising approach for the treatment of degenerative chondral lesions, but graft properties, besides mechanical and biochemical joint environment have to be improved.

Filardo G, Kon E, Di Martino A, Patella S, Altadonna G, Balboni F, Bragonzoni L, Visani A, Marcacci M. Second-generation arthroscopic autologous chondrocyte implantation for the treatment of degenerative cartilage lesions. Knee Surg Sports Traumatol Arthrosc. 2012 Sep;20(9):1704-13. The final publication is available at Springer via http://dx.doi.org/ doi: 10.1007/s00167-011-1732-5.

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Matrix-assisted autologous chondrocyte implantation in patients older than 40 years

INTRODUCTION

Treating cartilage lesions is a multidimensional task for surgeons in the operating room and basic scientists in the laboratory, due to the complex highly ordered structure that makes it difficult to replace or reproduce.[14,15,19,36]

Modern regenerative procedures can replace the articular surface with a hyaline-like tissue,[10,32] and recently good mid-long-term clinical results have been reported.[2,23,30] Most surgical treatments for cartilage are commonly indicated in young patients,[13,17,20,21,25,35] whereas these procedures are generally not indicated for older patients.[24,35]

Age is responsible for degenerative changes of all cartilage elements, thus impairing properties and healing potential. Structure and composition of proteoglycans change with age:[5] the keratan sulfate and protein content of monomers increases, the chondroitin sulfate content decreases, chondroitin sulfate chains become shorter, average monomer size decreases and the variability in monomer size increases. Link proteins fragment, the concentration of functional link protein decreases, aggregates become smaller and hyaluronate filament length decreases.[7,33] These changes may be caused by age-related alterations in chondrocyte synthetic function, which may justify the contraindication for treating older patients using regenerative techniques.

However, some studies show interesting findings, which may suggest a potential usefulness of regenerative procedures also for the treatment of cartilage lesions in older patients.[8,16,34]

There is no agreement in the literature on the real healing potential of regenerative procedures for cartilage lesions in older patients, and apparently for the time being old age cannot be considered an absolute contraindication criterion.

The main aim of our study was therefore to analyze the clinical outcome in the treatment of cartilage lesions using second-generation autologous chondrocyte implantation (ACI) techniques in patients over 40 years old with no clear signs of osteoarthritis, and to understand their real potential in relation to aging. Our secondary aim was to determine the

failure rate and the complications of these procedures in patients over 40 years old, and to compare these results with those achieved in younger patients.

MATERIALS AND METHODS

Patient selection

Sixty-one patients with symptomatic ICRS grade III-IV cartilaginous defects on the weight-bearing surface of the medial or lateral femoral condyles but nor clear signs of osteoarthritis (Kellgren-Lawrence grade 0-1), and a minimum age of 40 years were consecutively treated with MACT and prospectively evaluated at mid-term follow-up. Exclusion criteria were BMI higher than 30, cartilage lesions greater than 5.0 cm^2 or less than 1.0 cm^2, trochlear, patellar or tibial plateau chondral defects, diffused arthritis or bipolar lesions, and non-corrected axial deviation or knee instability. Furthermore, patients with infective, tumor, metabolic, and inflammatory diseases were excluded from the study.

The male to female ratio was 40:21. The mean age at surgery was 45.5±4.9 (40-62) years and the mean BMI was 25.3±2.7. The number of patients affected by chronic lesions was 55 (90%), whereas 6 presented acute lesions (10%). In 54 patients (89%) lesions were situated on the medial femoral condyle (MFC), and in 7 (11%) they were on the lateral femoral condyle (LFC). The mean defect size was 2.9±1.2 cm^2. Twenty-six patients (43%) had undergone previous knee surgery and in 26 patients (43%), associated procedures were performed.

Twenty-two patients were treated with Hyalograft C® (Fidia Advanced Biopolymers Laboratories, Padova, Italy) implantation in one hospital and 39 underwent MACI® procedure (Verigen Transplantation Servic, Copenhagen, Denmark) another hospital.

The two groups were homogeneous for age, sex, BMI, size, combined surgery, etiology, and time of follow-up. Differences were related to previous surgery, and LFC site, which were more frequent in the Hyalograft C® group ($p<0.0005$ and $p=0.038$, respectively), and osteotomies, which were more frequent in the MACI® group ($p<0.0005$).

Scaffolds

The scaffold used for arthroscopic 2nd generation ACI is based entirely on the benzylic ester of hyaluronic acid (HYAFF 11®). The cells harvested from the patient are expanded for 3

weeks and then seeded for 1 week onto the scaffold to adhere, proliferate, and deposit the typical extracellular matrix within the biomaterial to create the tissue-engineered product Hyalograft C®.[28]

The scaffold used for open 2nd generation ACI is based on a porcine collagen I/III matrix (Chondro-Gide®). Autologous chondrocytes are cultured for 4 weeks before being seeded on the rough side of the porcine collagen type I/III matrix. The loaded matrix is subsequently cultured with autologous serum for the remaining 3 days and then delivered as a cell scaffold construct for implantation.[2]

Surgical technique

The surgical technique for Hyalograft C® has been described before. For MACI, in the second operative step the chondrocyte-loaded matrix is transplanted into the defect area through a mini-open approach (Fig. 1).

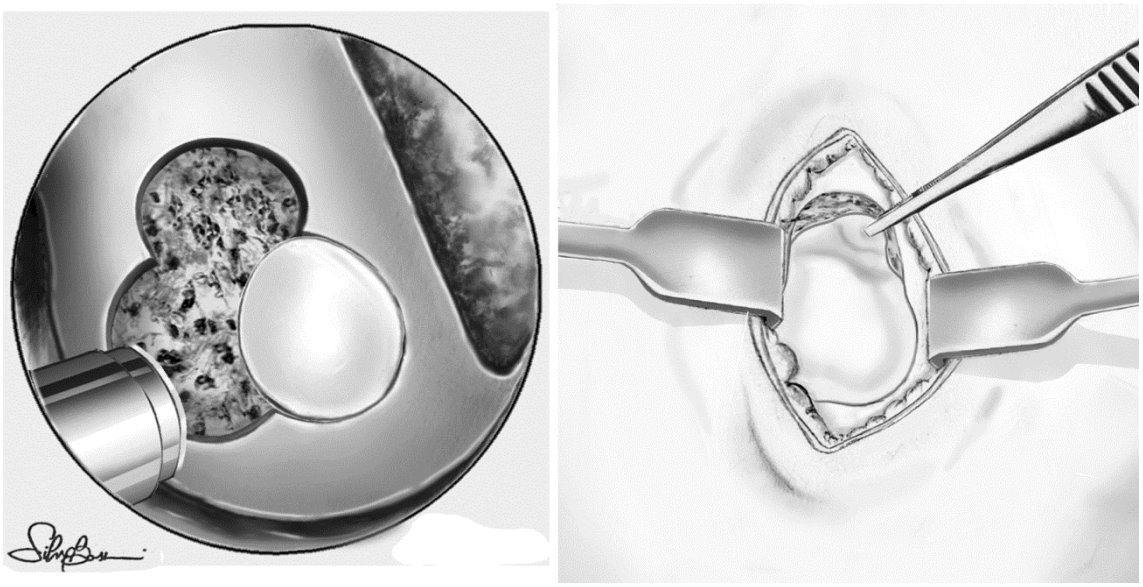

Fig. 1: Schematic representation of the surgical implants.
Left: arthroscopic Hyalograftc C implantation; Right: open MACI implantation.

Following parapatellar arthrotomy, the defect area is debrided down to the subchondral bone. Afterwards, using a foil template with size and geometry of the defect, the Chondro-

Gide® matrix loaded with chondrocytes is cut to size and fitted into the defect with the cell-loaded surface facing the subchondral bone. Fibrin glue is used to secure the implant directly to the base of the defect. Some degrees of flexion-extension of the knee are used for stability test.[2]

The same rehabilitation protocol, described before, was used for both treatment groups.

Follow-up evaluation

All 61 patients were evaluated preoperatively, at 1 and 2 years, and at a mean final follow-up of 5 years (4-6). The clinical outcome of all patients was analyzed with the Cartilage Standard Evaluation Form as proposed by ICRS.[18] A knee functional test was performed by the surgeon according to the IKDC Knee Examination Form used to determine the final functional grade of the knee (normal, nearly normal, abnormal or severely abnormal).[18]
Surgery was considered to have failed if the patient needed a re-operation because of symptoms due to primary defects. For re-treated patients, the last clinical evaluation before re-operation was considered.

Statistical Methods

Continuous data were expressed in terms of the mean and the standard deviation of the mean. One-Way ANOVA was used to determine IKDC subj differences between groups. When the Levene test for homogeneity of variances was significant, the Mann Whitney test was used to check ANOVA results. GLM repeated measures with the Bonferroni post hoc pairwise analysis was applied to study differences of IKDC subj among follow-ups. Friedman's test with the Wilcoxon post hoc pairwise analysis with the Bonferroni correction was used to study differences of IKDC obj among follow-ups. GLM repeated measures with treatment as a fixed effect was applied to evaluate the influence of the treatment on the IKDC subj follow-up. The non-parametric Fisher Chi-square test was applied to study the relationships between dicotomic variables. Pearson's correlation was applied to study the correlation between continuous variables. Multiple regression with Backward method was performed as multivariate analysis to determine factors that independently influenced the final outcome. Logistic regression with Backward Wald method was applied to identify factors that independently influenced failures. $P<0.05$ was considered significant. Statistical

Analysis was carried out by using the SPSS software version 15.0 (SPSS Inc., Chicago, USA).

RESULTS

No severe implant-related adverse events were observed.

IKDC obj score: from 20% of normal and nearly normal knees before the treatment to 54% at 1 year, 79% at 2 years, and 80% at the final follow-up, with a significant improvement ($p<0.0005$) at all follow-ups. The improvement at 1 year further increased at 2 years ($p=0.001$), then results were stable from to the final evaluation. IKDC subj score: marked improvement from the basal evaluation (36.8±8.4) to the different follow-ups ($p<0.005$). The improvement achieved at 1 year (60.1±22.4) further increased at 2 years (66.5±21.8) ($p=0.008$), then results were stable up to the final evaluation (68.1±21.8) (Fig. 2).

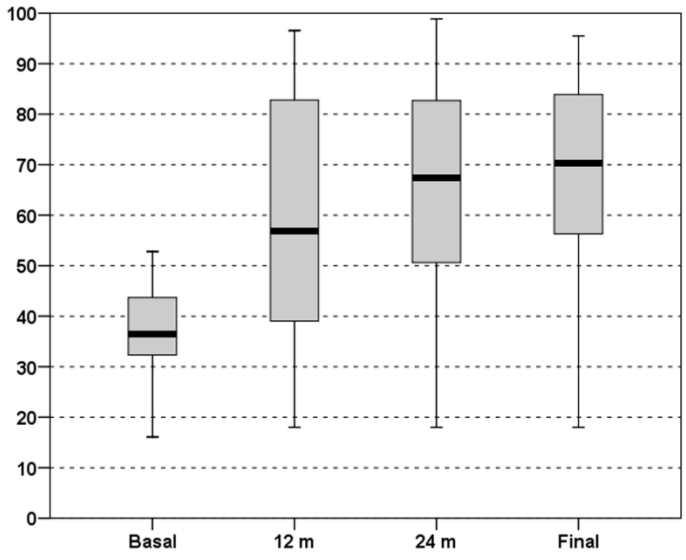

Fig. 2: IKDC subjective score: improvement from the pre-op level to 12, 24 months, and final f-up.

Failures: 12 patients failed, for a total failure rate of 20% at the 5 years. Four patients failed in the Hyalograft C® group, 8 in the Chondro-Gide® group, with no statistically significant

difference between treatments. Nine patients were re-operated with other cartilage reconstructive procedures, whereas 3 underwent prosthetic joint replacement.

Parameters that influenced the clinical outcome: the improvement was lower in women (23.6±19.9 vs 35.2±23.6, p=0.037), also with respect to the specific sex and age-matched scores of the healthy populations[1]. Site, etiology, combined and previous surgery, size, and BMI did not significantly influence the final outcome. Lower subjective outcomes at 1 and 2 years were correlated with more failures (p<0.0005).

When comparing the Hyalograft C® and Chondro-Gide® groups, lower IKDC subj scores were obtained at 1 year in the group of patients treated with the mini-open Chondro-Gide®, whereas a faster improvement was documented in the group treated with the arthroscopic Hyalograft C® procedure (p=0.049) (Fig. 3).

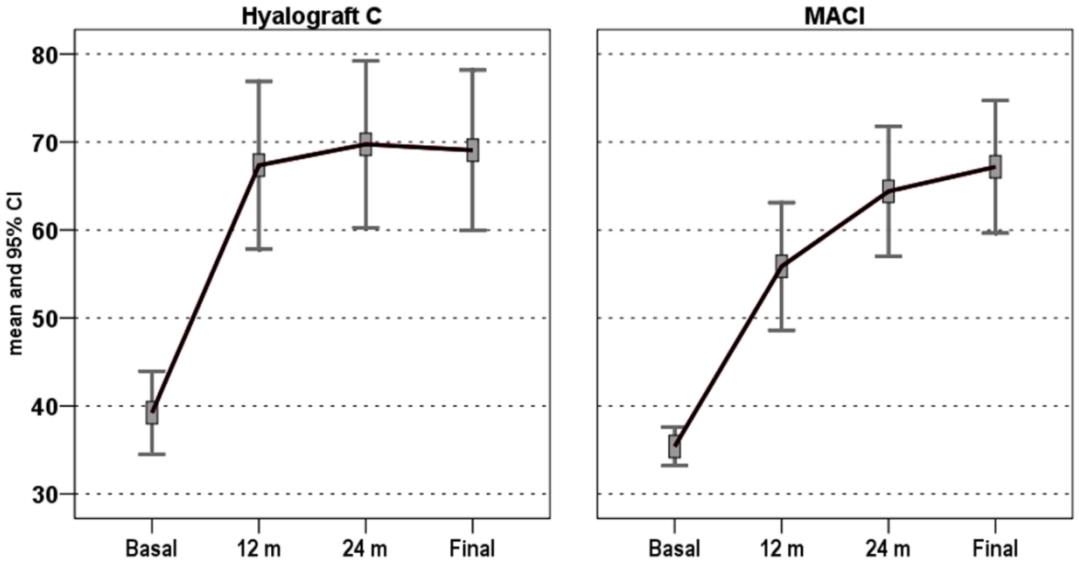

Fig. 3: IKDC subjective results obtained at the different follow-ups with the two treatments: whereas similar results were obtained at medium-term follow-up, the arthroscopic Hyalograft C technique allowed a faster clinical improvement with significantly better results at 12 months.

DISCUSSION

Various techniques have been studied and applied through the years for the management of chondral lesions, from the classic bone marrow stimulation procedures,[1,3,30] to the more ambitious and modern regenerative techniques, which aim to produce a hyaline–like tissue with better and durable clinical results. ACI was developed in the 1990's,[31] and both the production of a hyaline-like articular surface and a satisfactory clinical outcome have been shown at mid-long follow-up.[4,31] Second-generation ACI procedures have been developed to offer similar results while overcoming most of the biological and surgical concerns related to the first-generation ones.[2,6,23,28]

The literature shows that these procedures may be successfully applied for the treatment of the young active population, whereas the results in older patients are few and still very controversial.[23] Most authors prefer to reserve cartilage treatments only for patients younger than 40-50 years.[13,17,20,21,25,35] In fact, partial or total knee replacement is thought to provide faster recovery, greater patient satisfaction and more reliable outcomes in the elderly. However, a growing number of not young but still active patients are presenting for consultation to maintain their active lifestyles and hobbies, and more and more of these patients are still too young to undergo prosthetic replacement.

Another factor has to be taken into consideration: chondrocytes cannot migrate to the site of injury from an intact healthy site.[6] Once started, even small degenerative injuries in the knee cartilage can initiate a vicious circle, leading to more extensive joint damage[12] and may speed the age-related degenerative process of the entire joint. In this perspective, the treatment of cartilage lesions in older patients may not only be useful for pain relief, but it can also avoid or at least delay progressive joint degeneration.

Moreover, unlike bone marrow stimulation techniques, which are less effective in older patients because they depend on the patient's own healing potential which decreases with aging,[24] some findings indicate that regenerative techniques may be applied to these patients and give better results. Cell culture on scaffolding may trigger the activation of anabolic factors which induce the differentiation of chondrocytes and reduce the expression and production of molecules involved in cartilage degenerative processes.[8,16] Finally, the effectiveness of the cell-based approach has also been highlighted recently in older patients,

with outcomes comparable to those reported in the literature for younger patients if all articular comorbidities are recognized and treated: Rosenberger et al.[34] analyzed 56 patients with an average age of 48.6 years (45-60). They documented 14% of failures, and at their latest available follow-up 72% of patients rated themselves as good or excellent and 81% would again choose ACI treatment.

We analyzed the clinical outcome obtained with the treatment of cartilage lesions of the femoral condyles with MACT in patients over 40 years old with no clear signs of osteoarthritis, to understand the real potential of this surgical approach related to aging. No severe implant-related adverse events were observed. We observed a statistically significant improvement in both subjective and objective scores at mid-term follow-up. However, results were inferior with respect to the outcome reported for younger study populations. Moreover, the failure rate at mid-term follow-up was markedly higher, too, with poor short-term outcome being predictive of failure. These findings were consistent in the two treatment groups. The only difference found was the faster recovery when the arthroscopic approach was used, thus confirming the results previously obtained by Ferruzzi et al..[10] We previously reported[23] the results obtained in a younger population treated with Hyalograft C® implantation and showed a higher IKDC score at 5 years follow-up. The literature also confirms the better results obtained in younger patients, regardless of the applied treatment strategy.[13,20,24,27,35,24] Krishnan et al.[26] evaluated 199 patients treated with collagen-covered ACI analyzing 3 age groups: older patients had worse results, with the proportion of patients with excellent and good clinical results varying from 85.7% for those aged < 20 years, to 64.2% for those aged 21 to 40, and 55.9% for those aged >41 years. More recently, age-dependency for cartilage procedures was confirmed by De Windt et al.:[9] the analysis of a mixed group of patients treated with microfracture or first/second generation ACI for traumatic knee lesions showed a greater improvement in patients < 30 years old.

The reported age thresholds are inconsistent and vary a lot among studies. However, most of them agree on the age-dependency for the clinical outcome as confirmed by this study focusing on patients treated with MACT.

The limitations of our study include the lack of imaging and histological evaluations. However, the high number of homogeneous patients treated and evaluated at 5 years allowed us to highlight good results with a marked clinical improvement also in patients

over 40 years old, who in most cases benefited from MACT at mid-term follow-up. The high number of combined surgical procedures (43%) may blur the effect of scaffold implantation. However, the analysis of patients with isolated cartilage lesions has also shown a significant improvement in clinical outcome. Another weak point is the use of two different techniques. However, the evaluation of two different procedures of the same generation is helpful to confirm and strengthen the results obtained, because both treatments presented the same trend, and also allowed us to observe interesting findings with a different short-term improvement. The status of the joint is probably the main contributing factor for a good clinical outcome, not age, as suggested also by the interesting results in older patients obtained by other authors.[29] Thus, instead of the patient's chronological age, the "joint age" should be considered.

However, as demonstrated by the inferior scores achieved and by the high failure rate, not all older knees may be indicated for cartilage treatment. In fact, in some of these joints degenerative processes may have already started, thus compromising the treatment outcome of the focal lesion. In fact, although laboratory studies show interesting findings which may suggest a potential usefulness of regenerative procedures even in osteoarthritic joints,[8,16] the clinical use of existing tissue-engineered grafts in degenerated knees still presents some unresolved problems. Progress in bioengineering, with the development of tissues with better biomechanical properties, and increasing knowledge of the biochemical environment of the aging and degenerating joint, will allow a better selection of the candidates for these procedures, improve the clinical outcome, and extend the potential of this regenerative treatment also to joints doomed to more invasive procedures.

CONCLUSIONS

A clinical improvement was found in patients over 40 years old, who, in most cases, benefited from MACT with good results at a mid-term follow-up. However, the results were inferior with respect to those previously found for younger populations, and the failure rate was also higher. These findings were consistent in the two treatment groups. The only difference was the faster recovery when the arthroscopic approach was used.

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PART 2

Matrix-assisted autologous chondrocytes implantation combined with bone grafting for the treatment of knee osteochondritis dissecans: results at 6 years

INTRODUCTION

Osteochondritis dissecans (OCD) is a lesion of the joint surface characterized by separation of an osteochondral fragment. Multiple causes have been suggested: repetitive microtrauma correlated with a possible vascular insufficiency is the most credited theory; however, for the time being the etiology of OCD is still unknown.[1,2] The resulting clinical condition may require surgical treatment. In particular, whereas juvenile OCD has a better prognosis and may heal with nonoperative management,[3] adult OCD typically follows a clinical course that is progressive and unremitting. Unstable defects must be treated surgically; adult OCD therefore usually requires surgical repair. In fact, the biomechanical perturbations due to the osteochondral lesion have the potential to contribute to the development of osteoarthritis, as underlined by Linden et al.[4] in a retrospective study: patients with adult OCD developed osteoarthritis about ten years earlier than primary knee osteoarthritis, thus confirming that the natural history of this osteochondral joint pathology is an earlier degeneration process.

Several surgical options have been proposed for the treatment of OCD.[5] The choice depends largely on patient age, lesion size, and stability of the osteochondral fragment. A stable lesion can be treated by drilling, an unstable but intact fragment can be treated with curettage and internal fixation, but unsalvageable fragments require a more complex procedure to restore the joint congruity.[6,7]

The various procedures developed to reconstruct the articular surface include bone marrow stimulation, autologous periosteal or perichondral grafts, autologous osteochondral transplantation, and allografting.[8,9,10,11] All these options present advantages and disadvantages, and none has emerged over the others. More recently, MACT procedures have been introduced into clinical practice, aiming to promote healing with a hyaline-like tissue, relieve symptoms and halt disease progression.[12,13,14,15,16]

The purpose of this study was to explore the potential of the arthroscopic implantation of the bioengineered tissue Hyalograft C to treat this pathology. The hypothesis was that second-generation ACI supported by autologous bone grafts may regenerate the damaged osteochondral unit, thus restoring the articular surface and improving symptoms and function in patients affected by knee OCD.

MATERIALS AND METHODS

Patient selection.

Thirty-three consecutive patients (2 bilateral, for a total of 35 knees treated), affected by symptomatic knee OCD grade III or IV on the ICRS (International Cartilage Repair Society) scale,[17] were enrolled and treated with arthroscopic second-generation ACI and autologous bone grafts. One patient was lost at follow-up. The patients evaluated were 23 men and 9 women, with a mean age of 21.0±5.6 years. Fifteen (47%) of the patients were very fit or played sports at a competitive level, whereas 17 (53%) practiced sport at amateur level or did not practice any sport. The site of the defects was the medial femoral condyle in 24 cases, whereas in 10 patients the lateral femoral condyle was involved. The average size of the defects was 2.9±1.0 cm^2. Twenty-four patients were treated for the first time, whereas 10 patients had undergone previous surgeries. In 2 patients, other associated procedures were performed during the same operation.

Surgical technique

The technique consists of two steps. During the first surgical stage of chondrocyte harvesting, a small incision is made at the metaphyseal area of the tibial head medial side, and a bone window is cut for harvesting a cancellous bone graft. The cortical flap is then closed, and the autologous cancellous bone chips are impacted artrhoscopically into the base of the osteochondral defect to reach the level of the surrounding subchondral plate and restore the bony surface. Blood clotting promotes the stability of the implanted grafts, which is assessed by knee flexion-extension movements. The second step, performed after 4-6 months, consists of the arthroscopic implant of the bioengineered tissue Hyalograft C, according to the previously described procedure (Fig 1).

The rehabilitation protocol has been previously described.[19]

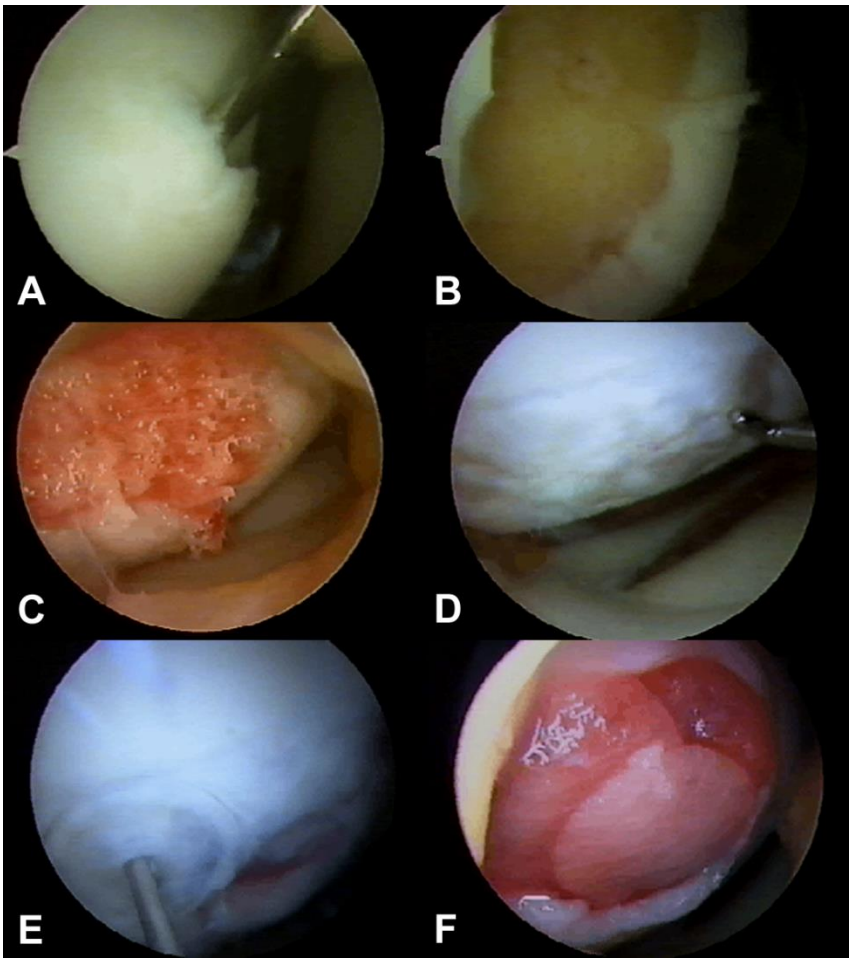

Fig. 1: Arthroscopic technique.

A: OCD lesion of the LFC. B: the defect area is debrided. C: Cancellous bone chips are impacted in the lesion. D: Second surgical step after 4-6 months: the bony level is restored. E: a circular area with regular margins is prepared with a specially designed cannulated drill. F: the hyaluronic acid-based bioengineered tissue is positioned in the prepared area to cover the lesion.

Follow-up evaluation

Patients were prospectively clinically evaluated before treatment, at 12 and 24 months of follow-up, and at minimum final follow-up of 4 years. The mean final follow-up was 6.0±1.2 years. The clinical outcome of all patients was evaluated using the Cartilage standard Evaluation Form as proposed by ICRS.[20] The subjective clinical outcome was

assessed with the IKDC subjective score. A functional knee test was performed by the surgeon according to the IKDC Knee Examination Form. The lowest ratings in effusion, passive motion deficit and ligament examination were used to determine the final functional grade of the knee (normal, nearly normal, abnormal or severely abnormal). Activity level was also recorded and evaluated with the Tegner score relative to pre-operative and pre-injury levels at the different follow-ups.[21] Surgery was considered to have failed if the patient needed a repeat operation because of symptoms due to primary lesion. For patients undergoing a repeat operation, the last clinical evaluation before this new operation was taken into consideration. From 2 to 6 years after transplantation, repair of the articular surface was evaluated in 17 knees with high-resolution MRI and the Henderson scoring system was applied.[22]

Statistical analysis

Continuous data were expressed in terms of the mean and the standard deviation of the mean. One-Way ANOVA was used to identify differences among groups when the Levene test for homogeneity of variances was not significant; otherwise, the Mann Whitney test was performed. The GLM for repeated measures with Bonferroni's correction for multiple comparisons was applied to evaluate score differences at different follow-ups. The influence of grouping variables on scores at different follow-ups was studied by the GLM for repeated measures with the grouping variable as fixed effect. The non-parametric Pearson's Chi square test was used to study the relationships between grouping variables. Pearson's correlation was applied to evaluate the correlation between continuous variables. The Wilcoxon test with Bonferroni's correction was applied to assess differences among different follow-ups of IKDC obj. The Pearson correlation was performed to study the relationship between age and measures; the Spearman rank correlation was applied to study the relationship between size/plugs and scores. $P<0.05$ was considered significant. Statistical Analysis was carried out by the SPSS software version 15.0 (SPSS Inc., Chicago, USA).

RESULTS

No implant-related complications or severe adverse events were observed.

IKDC subjective score analysis: all the follow-up times showed a significant improvement with respect to the basal level of 38.1±12.8 ($p<0.0005$), and the results obtained at 1 year further improved from 72.8±20.1 to 80.6±19.9 at the final follow-up ($p=0.09$) (Fig 2). The IKDC objective score significantly improved at all the follow-ups ($p<0.0005$), passing from 14.7% of

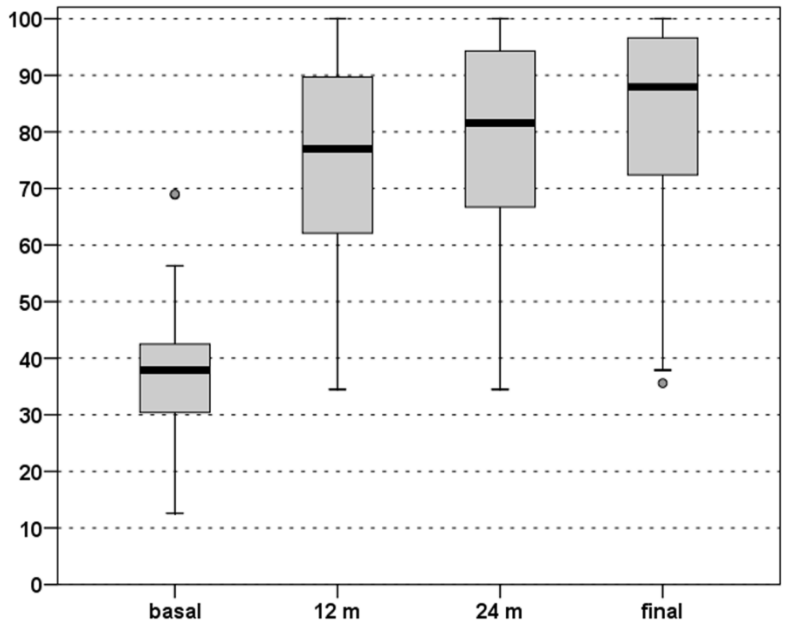

Fig. 2: IKDC subjective score: improvement from pre-operative level to 12 months, 24 months, and final evaluation at mean 6 years of follow-up.

normal and nearly normal knees before the surgery to 85.3% at 12 months, 94.1% at 24 months, and 91.2% at the final evaluation. Results further improved from 1 to 2 years ($p=0.04$) and then remained stable. Tegner score: statistically significant improvement ($p<0.0005$) from pre-surgery level (1.6±1.2) to 2 years and final evaluation levels (4.9±2.6 and 5.3±2.6, respectively), even if with a tendency to reach a lower score with respect to pre-injury (6.6±1.9, $p=0.07$) (Fig 3).

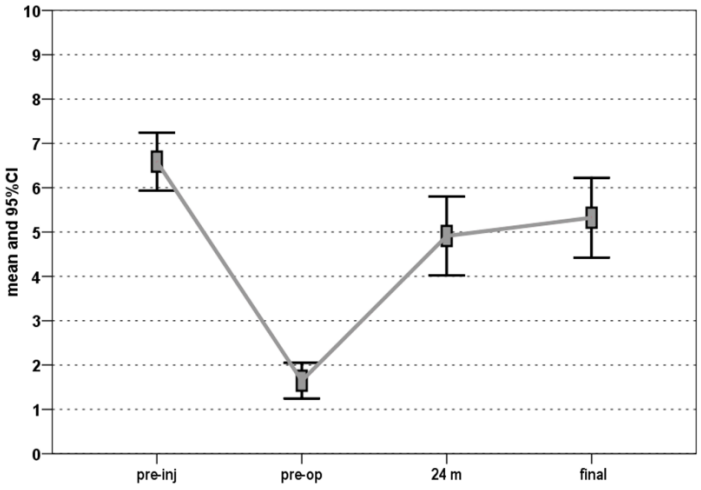

Fig. 3: Tegner score: improvement from pre-treatment level to 24 months and final follow-up levels, even if with a tendency to reach a lower score with respect to the pre-injury level.

We observed 4 failures (11.8%), all treated with bone grafting followed by implantation of 4 Hyalograft C patches.

Parameters that influenced the final outcome: a higher subjective IKDC improvement was observed in men at all the follow-ups ($p=0.007$ at 1 year, $p=0.04$ at 2 years, and $p=0.05$ at the final evaluation). Defect dimension markedly influenced the results, with a correlation between size and IKDC subj at 2 years and final evaluation (rho=-0.363, $p=0.035$, and rho=-0.479, $p=0.004$, respectively) (Fig 4). Larger defects also correlated with a reduced performance in sport activity at 2 years (rho=-0.341, $p=0.048$) and at the last follow-up (rho=-460, $p=0.006$). Sport-active patients had lower basal EQ-VAS scores but similar final results, thus showing an increased improvement with respect to less active patients ($p<0.0005$). Age, BMI, site, combined and previous surgery, did not significantly influence the final outcome in this series.

The analysis of the 17 MRIs available at final follow-up (Fig 5) showed that 88.2% (n = 15) had >50% or complete fill, 70.6% (n = 12) had a normal or nearly normal signal, 82.3% (n = 14) had mild or no effusion, and 82.3% (n = 14) had mild or absent subchondral edema.

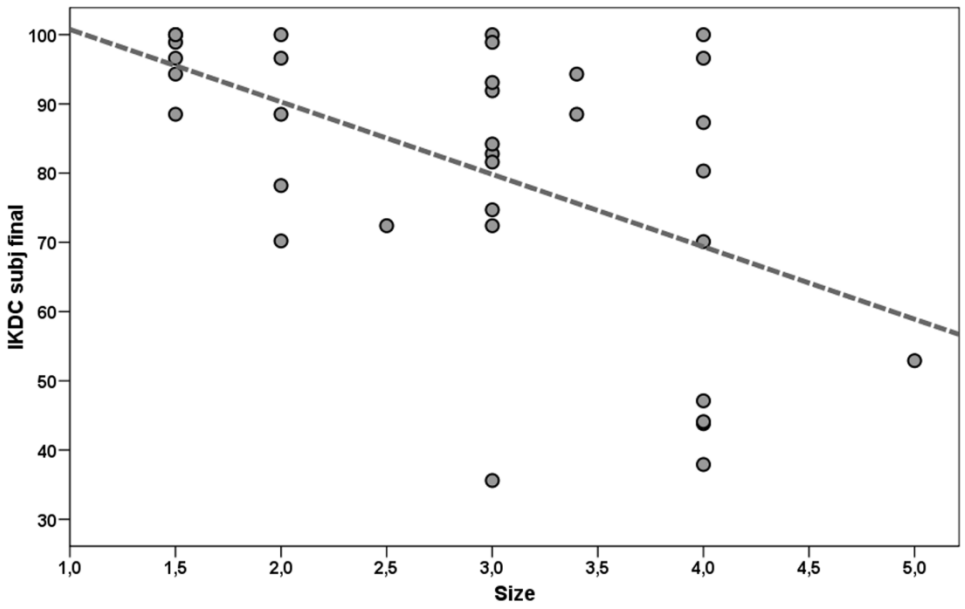

Fig. 4: Correlation between defect size and IKDC subjective results at the final evaluation at mean 6 years' follow-up.

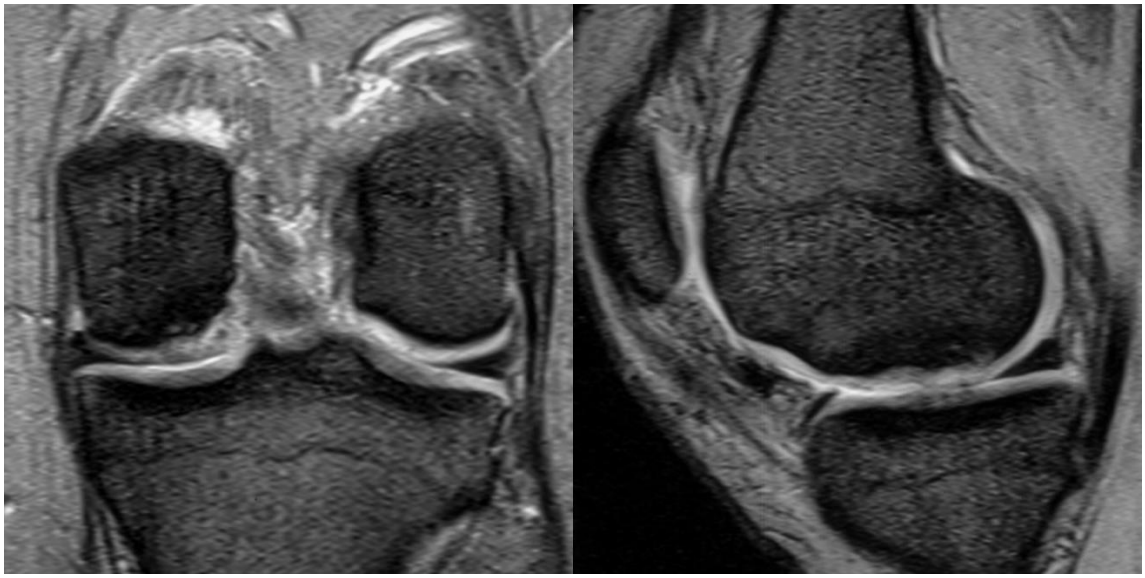

Fig. 5: MRI evaluation at 2 years' follow-up of a MFC knee lesion treated with bone grafting and Hyalograft C implantation in a 18-year-old woman.

DISCUSSION

OCD is an acquired subchondral bone lesion that may result in separation and instability of the overlying articular cartilage resultsing in pain, dysfunction, and progressive osteoarthritis.[23,24,25]

Regenerative procedures have been successfully proposed to restore the damaged joint surface obtaining integrated hyaline-like repair tissue with good clinical and histological results.[12,13,26,27] However, the treatment of OCD is more complex. An MRI study[28] showed an edema-like signal in bone marrow and incomplete repair of subchondral bone at the surgical site, and the use of ACI resulted in a delayed maturation in osteochondral defects deeper than 8-10 mm.[29] In fact, articular cartilage and its supporting bone are closely related and the biomechanical perturbations caused by osteochondral alterations substantially alter pattern and magnitude of contact pressures in the joint.[30]

Surgical goals should always try to re-establish the articular surface in the most anatomical way possible: to restore the physiological properties of the entire osteochondral unit, the ACI technique has been perfected. Peterson et al.[14] developed the "sandwich technique" for treating OCD lesions deeper than 10 mm involving significant subchondral bone loss: cancellous bone is used to fill the defect and closed with periosteal flap, the grafted chondrocytes are then suspended in between the first periosteal flap and are closed with a second periosteal flap. Krishnan et al.[31] described a modified procedure for the treatment of OCD, using a porcine collagen membrane instead of periosteum.

Despite some promising results, numerous problems have been observed with the ACI procedures. Besides the problems related to the complex surgical technique, there are also the technical problems of the culture and transplantation procedure, such as maintenance of chondrocyte phenotype, non-homogeneous cell distribution in the 3-dimensional spaces of the defect, and cell loss using liquid suspension.[15]

To address these problems, MACT has been developed.[16] A two-step technique has been described also for this matrix-induced ACI[32,33,34,35] for deep lesions, preceding bioengineered tissue transplantation by an autologous bone grafting, to restore the entire osteochondral structure and obtain a more anatomical joint surface. Bartlett at al.[32] reported

preliminary results in five patients: at one year follow-up 80% of the patients had good or excellent results. Ochs et al.[33] treated 22 patients: the evaluation at 16 months showed a marked improvement with a mean Lysholm score which increased from 50.1 to 84.5. Maus et al.[34] treated 13 patients and reported 83.4% of excellent or good results at 3 years' follow-up. Steinhagen et al.[35] used the same treatment for 21 patients: the evaluation at 3 years' follow-up confirmed the potential of this procedure, with 85% of good or excellent results. An analysis of the literature on the use of bone grafting and second generation ACI for the treatment of OCD shows good results at short-term follow-up, with a better outcome in smaller lesions and younger patients.[31,32,33,34,35]

In our study we evaluated 32 patients with a mean age of 21.0±5.6 years, affected by OCD and treated with Hyalograft C implantation and bone grafting. We observed good results in all scores after the treatment, with a significant linear trend of improvement over-time. Further analysis allowed us to determine the parameters that influenced the clinical outcome: defect dimension markedly influenced the clinical results, with poorer results in bigger lesions, and the worst results were observed in women. Finally, sport-active patients achieved similar final results, but starting from lower pre-operative scores, thus suggesting that the knee pathology affected more active patients to a larger extent. However, despite the more demanding functional activities, they were able to recover and reach the same level as the other patients. In our series we observed 4 failures, for a total failure rate of 11.8%.

The limitations of this study are the lack of control group and histological analysis. However, whereas other studies have already described the results of the cell-based approach,[15,16] there is a gap in the literature regarding the clinical results achieved by arthroscopic MACT plus bone grafting for the treatment of knee OCD, and the strong points are the number of homogeneous patients, treated by the same surgical equipe with the same combined bioengineered approach, and prospectively evaluated at mid-term follow-up.

There is an increasing awareness of the importance to reconstruct both cartilage and underlying subchondral bone to obtain a reproducible and durable repair.[28,30,36,37] In fact, the treatment goal of osteochondral cartilage defects should be to restore the physiological properties of the entire osteochondral unit. Thus, we applied this 2-step combined implant for the treatment of knee OCD. This surgical procedure, with the advantage of an

arthoscopic approach, enabled us to restore the articular surface by reconstructing both bone and cartilage, with good results at mid-term follow-up.

CONCLUSIONS

Arthroscopic MACT with bone grafting is a valid treatment option for knee OCD and can offer a good clinical outcome at 6 years. Better results can be obtained in men, sport-active patients, and smaller lesions. Further studies are needed to confirm the results over-time and determine whether this procedure may also reinstate correct knee biomechanics and homeostasis, thus preventing or delaying knee degeneration.

Filardo G, Kon E, Berruto M, Di Martino A, Patella S, Marcheggiani Muccioli GM, Zaffagnini S, Marcacci M. Arthroscopic second generation autologous chondrocytes implantation associated with bone grafting for the treatment of knee osteochondritis dissecans: Results at 6 years. Knee. 2012 Oct;19(5):658-63. The final publication is available at http://www.journals.elsevier.com/the-knee/ doi: 10.1016/j.knee.2011.08.007.

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Treatment of knee osteochondritis dissecans with a biomimetic osteochondral scaffold: clinical and imaging evaluation at 2 years

INTRODUCTION

Osteochondritis dissecans (OCD) is an acquired lesion of the subchondral bone that may result in separation and instability of the overlying cartilage.[1,2,3,4,5] Unstable lesions must be treated surgically. Left untreated, OCD can lead to the development of degenerative osteoarthritis.[6] Articular cartilage and its supporting bone functional conditions are closely related and the biomechanical perturbations caused by osteochondral alterations substantially alter pattern and magnitude of contact pressures and cartilage strains in the joint.[7] Thus, surgical goals should always try to re-establish the joint surface as anatomically as possible, aiming to achieve a more predictable repair tissue that closely resembles native articular structure at both cartilage and bone levels.

The purpose of this study was to evaluate the potential of a biomimetic osteochondral scaffold, by analyzing the results at 2 years. The hypothesis was that this 3-layer collagen-hydroxyapatite nanostructured scaffold developed to treat the entire osteochondral unit might restore the joint surface and improve symptoms and function in patients affected by OCD.

MATERIALS AND METHODS

Patient selection

Twenty-seven consecutive patients, affected by symptomatic OCD of the femoral knee condyles, grade III or IV on the ICRS (International Cartilage Repair Society) scale,[8] were enrolled and treated by osteochondral scaffold implantation. No patients were lost to follow-up. The patients included 19 men and 8 women, with a mean age of 25.5±7.7 years. Non-corrected axial deviations, evaluated clinically and via x-ray examination, were study exclusion criteria. Patients with infectious, neoplastic, metabolic and inflammatory pathologies were also excluded.

The defect site was the medial femoral condyle in 17 cases, whereas in 10 patients the lateral femoral condyle was involved. The average dimension of the defects was 3.4±2.2 cm^2. Thirteen patients were treated for the first time, and 14 patients had undergone previous surgeries. In 7 patients, other associated procedures were performed during the same operation.

Scaffold preparation

The osteochondral (OC) biomimetic scaffold (Fin-Ceramica Faenza S.p.A., Faenza - Italy) has a porous 3-D composite 3-layered structure, which mimics the whole osteochondral anatomy. The cartilaginous layer, consisting of type I collagen, has a smooth surface. The intermediate layer (tide-mark-like) consists of a combination of type I collagen (60%) and HA (40%), whereas the lower layer consists of a mineralized blend of type I collagen (30%) and HA (70%) reproducing the subchondral bone layer. The final construct was obtained by physically combining the layers on top of a Mylar sheet; the product was finally freeze-dried and gamma-sterilized at 25 KGray.[9]

Surgical technique

The surgical procedure was performed with the patient in the supine position and under general anesthesia. A pneumatic tourniquet was placed on the proximal thigh, and an arthrotomic medial or lateral parapatellar approach was used to expose the defect. The lesion was then prepared: the sclerotic subchondral bone was removed, and 8-mm deep lodgings with a stable shoulder were made to house the implant. The lesion was templated using aluminum foil to obtain the exact size of the graft needed. The template was then used to prepare the graft that was implanted using a press-fit technique (Fig. 1). The stability of the transplant was tested by cyclic bending of the knee while the graft was visualized, both before and after tourniquet removal.

Rehabilitation protocol

On the second postoperative day, self-assisted mobilization of the knee was started. Early isometric and isotonic exercises and controlled mechanical compression were performed. Muscular voluntary contraction and neuromuscular electrical stimulation were indicated and could be started at patient discharge. In the third or fourth week, weight touchdown with crutches was allowed, and the patient could then move progressively towards full weight bearing.

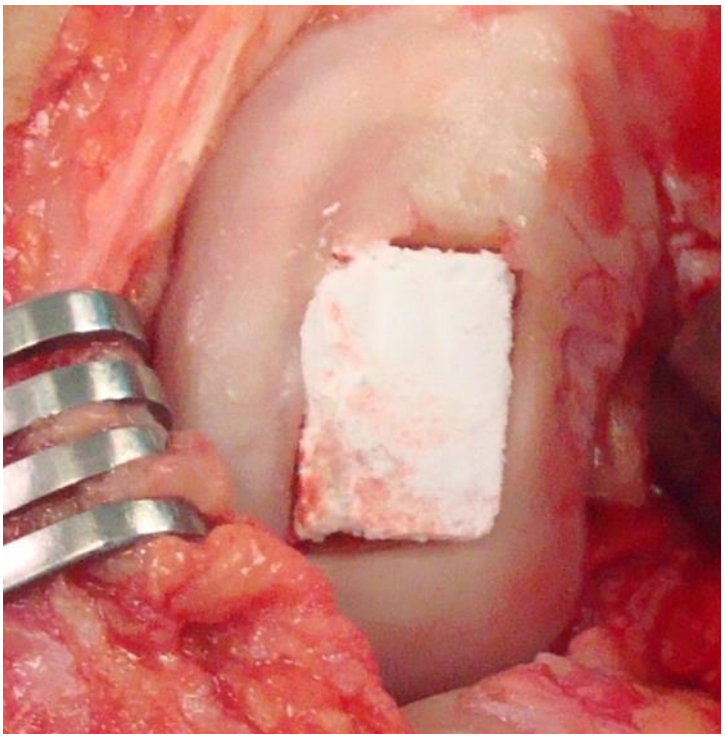

Fig. 1: Surgical view: osteochondral scaffold implantation at the medial femoral condyle.

Follow-up evaluation

Patients were prospectively evaluated preoperatively and postoperatively at 12 and 24 months of follow-up. The clinical outcome of all patients was analyzed using the cartilage standard evaluation form as proposed by the ICRS (International Cartilage Repair Society).[10] A knee functional test was performed according to the IKDC knee examination form. The lowest ratings in effusion, passive motion deficit and ligament examination were used to determine the final functional grade of the knee (normal, nearly normal, abnormal or severely abnormal). Returning back to sport was also evaluated with the Tegner score and compared with pre-operative and pre-injury levels.[11]

One patient presented a re-injury during the second year of follow-up, thus this score could not be considered for the final evaluation.

Twenty-three patients (85%) were available for the MRI evaluation at 12 months' follow-up, 2 of which could not be assessed because of the presence of artifacts due to hardware around

the joint. Therefore, 21 (out of 27 - 78%) MRIs were evaluated at 12 months, whereas 18 (out of 26 - 69%) were available for the analysis at 24 months.

Examinations were carried out using a GE MRI with a 1.5-Tesla super conducting magnet (General Electric Company, Fairfield, CN) with a dedicated quadrature detection knee coil (Quadknee, diameter 18 cm). For the description and evaluation of the graft maturation, an MRI scoring system (MOCART) was used.[12,13]

Statistical Analysis

Continuous data were expressed in terms of the mean and the standard deviation of the mean. One Way ANOVA was used to evaluate differences among groups when the Levene test for homogeneity of variances was not significant; otherwise, the Mann Whitney test was applied. For small groups the exact method as used. The GLM for repeated measures with Sidak correction for multiple comparisons was used to study score differences at different follow-up times. To evaluate the differences over time of ordinal scores the Friedmann Test was applied with the Wilcoxon post-hoc pairwise analysis with Bonferroni's correction. The Pearson Correlation was performed to study the relationship between normally distributed variables and the Spearman Correlation was performed to study the relationship between not normally distributed variables. $P<0.05$ was considered significant. Statistical Analysis was carried out by the SPSS software version 15.0 (SPSS Inc., Chicago, USA).

RESULTS

Adverse events: 2 patients presented fever during the first days and 3 patients had joint stiffness and required knee mobilization under narcosis.

IKDC subj score: marked improvement from baseline to 1 year (from 48.4±17.8 to 76.0±12.8, $p<0.0005$), and further improvement at 2 years (82.3±12.2; $p<0.0005$) (Fig. 2). IKDC obj score: from 40.1 % normal knees before the treatment to 59.3 % normal knees at 1 year ($p=0.027$), further improvement at 2 years with 84.6 % normal knees ($p=0.01$). Tegner score: 5.7±2.3 pre-injuries, 2.4±1.7 pre-operative, 3.6±1.2 at 1 year and 4.5±1.6 at 2 years, with a significant improvement ($p=0.01$) from the pre-operative level to 1 year and a further

improvement at 2 years (p=0.01): the sport activity final level was lower (p=0.06) than the pre-injury level (Fig. 3).

Parameters that influence the clinical outcome: gender, age, BMI, defect size, site, previous and combined surgery, preoperative activity level, and adverse events did not significantly influence the final outcome in this series.

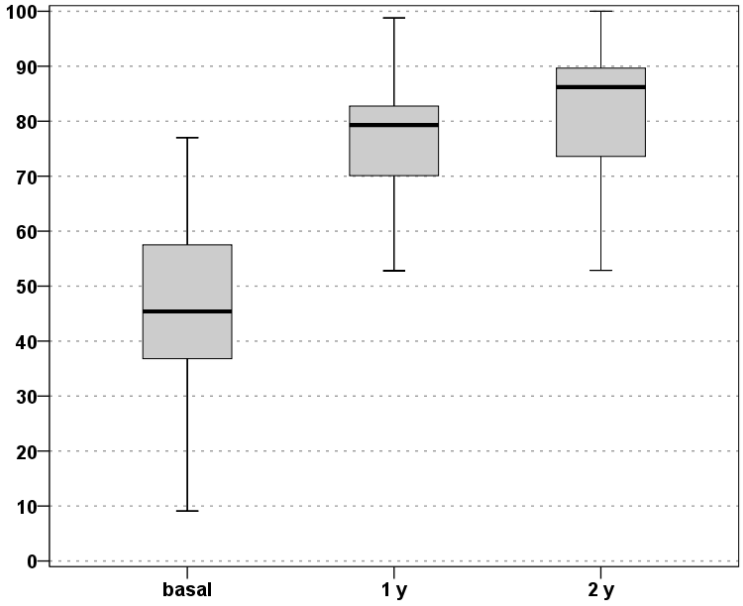

Fig. 2: IKDC subjective score: improvement from pre-operative level to 1 and 2 years' follow-up.

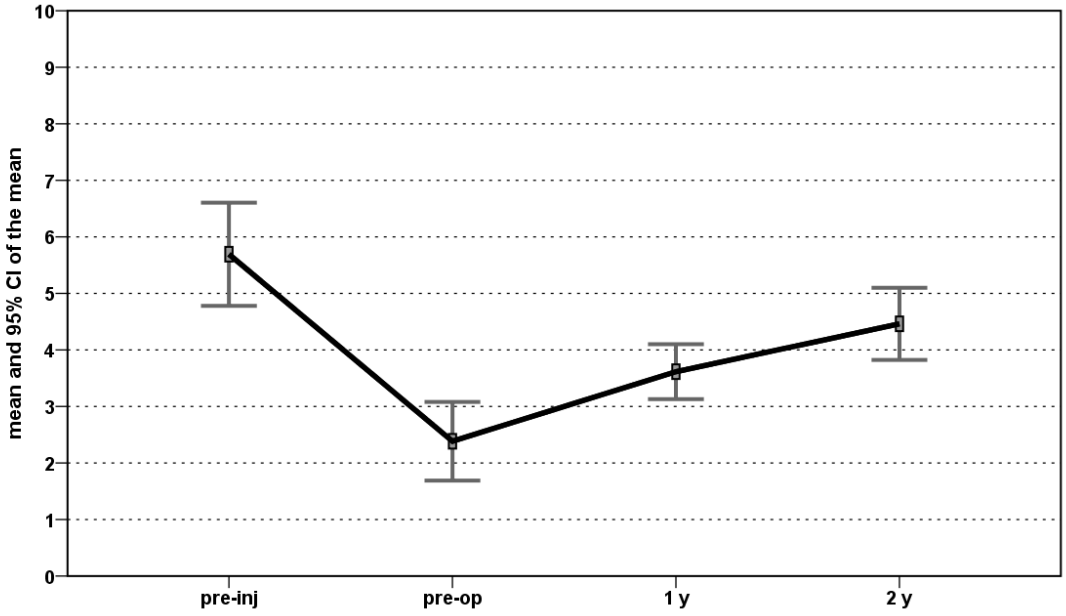

Fig. 3: Comparison of Tegner scores before injury, before treatment, at the 1 and 2-year f-ups.

Twenty-one patients were studied at 12 months and 18 at 24 months after surgery with high resolution MRI (Fig. 4).

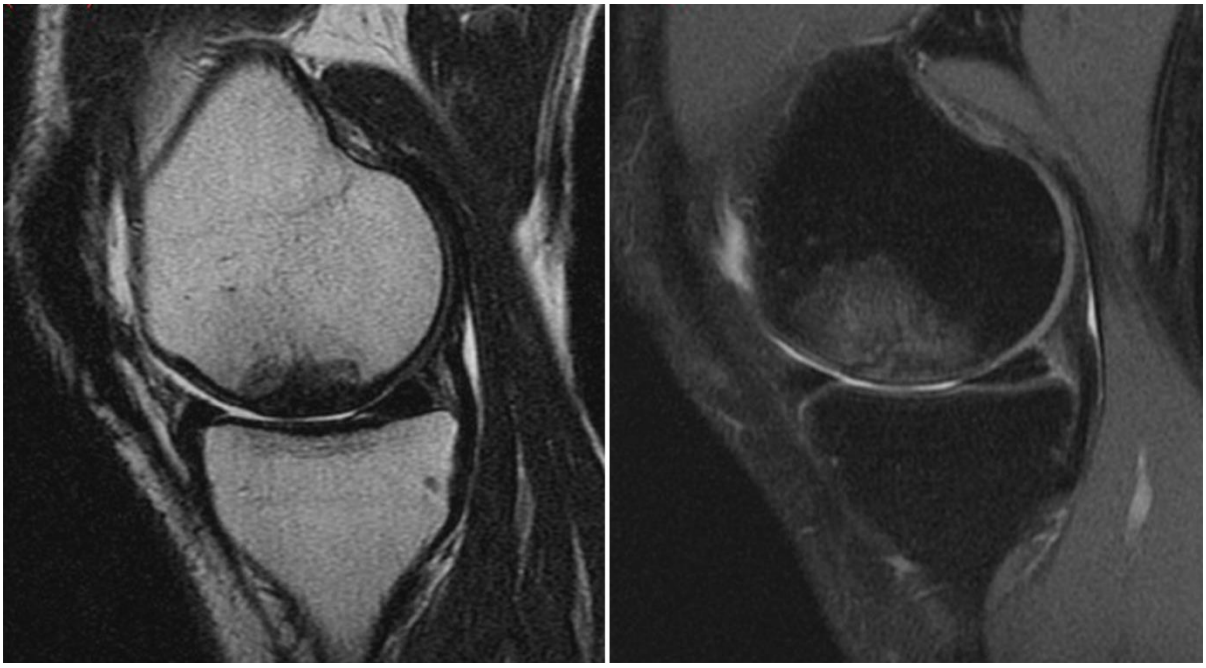

Fig. 4: MRI of an OCD lesion of the medial condyle treated with the osteochondral scaffold implantation: despite good scaffold integration and defect filling, subchondral bone changes with edema of the surrounding tissue remain at 2 years' follow-up.

At 1-year MRI showed complete filling of the cartilage in 76%, complete integration of the graft in 71%, the repair tissue surface was intact in 48%, the structure of the repair tissue was homogeneous in 33%, and the graft signal intensity score was iso-intense with the adjacent native cartilage in 43% and 38% of the cases in dual T2-FSE and 3D-GE-FS sequences, respectively. The subchondral lamina was never completely restored and subchondral bone changes were present in 62% of cases. Adhesion and effusion were found in 0% and 43% of cases, respectively. At 2 years MRI showed a complete filling of the cartilage was shown in 72%, complete integration of the graft in 83%, the repair tissue surface was intact in 56%, the structure of the repair tissue was homogeneous in 39%, and the graft signal intensity score was iso-intense with the adjacent native cartilage in 56% and 61% of cases in dual T2-FSE and 3D-GE-FS sequences, respectively. Subchondral lamina was never completely restored

and subchondral bone changes were observed in 61% of cases. Adhesion and effusion were found in 0% and 22%, respectively.

Effusion was correlated with a lower IKDC subj score at 1 year (p=0.04). No correlation was found between MOCART score and clinical results at both follow-up times.

DISCUSSION

The present study suggests that this osteochondral scaffold can be used to treat knee OCD with good clinical results at 2 years.

The best surgical strategy for the treatment of OCD is still controversial, and the available evidence is insufficient to provide recommendations for a specific technique.[14,15,16]

Regenerative procedures have been successfully proposed for restoring the damaged articular surface,[17,18,19,20,21,22] and a satisfactory outcome has also been reported for the treatment of OCD lesions.[23,24] Cole et al.[23] reported the analysis of the STAR study on the effectiveness of autologous chondrocyte implantation (ACI) in adult patients with OCD knee defects. The treatment was considered successful in 85% of the 32 patients at the 4-year follow-up, even if 35% of them had a subsequent surgical procedure and treatment failure percentage at the final follow-up was 19%. Teo et al.[24] focused on the treatment for patellar OCD, showing good short-term results with ACI as well as with the implantation of cultured bone marrow stem cells.

Most of the cell-based regenerative approaches reported in the literature are primarily focused on the reconstruction of the superficial layer of cartilage, and for osteochondral defects deeper than 8 - 10 mm they can present a delayed maturation and incomplete repair of subchondral bone.[25] Therefore, several authors have tried to address the subchondral bone as well. The "sandwich technique" developed by Peterson et al.[26] involves the use of cancellous bone to fill the defect, then the grafted chondrocytes are suspended between two periosteal flaps: the mid-term evaluation showed a higher success rate, with 93% of the patients reporting an improvement. Since the periosteal requires a second incision and causes a high rate of hyperthrophy,[22] and since a much lower complication rate has been shown by several authors using a type I/III collagen membrane instead of periosteum,[27,28] the use of a

porcine collagen membrane has also been used for OCD,[29,30] with good results at short-term follow-up. Krishnan et al.[30] confirmed the different outcome between juvenile-onset and adult-onset disease, and also showed that the main cause of the lower success rate of this technique for adult OCD lesions was the defect size. MACT[31] has also been modified to treat OCD. Ochs et al. used MACT on cancellous bone cylinders (diameter 8 mm) used to reconstruct the subchondral plate: the short-term evaluation of 22 patients showed encouraging results.[32] Vijayan et al.[33] confirmed these good results at a mean 5-year follow-up 14 patients treated with the implant of cancellous bone graft and two collagen-based membranes. Maus et al. treated 13 patients and reported 83% excellent or good results at 3 years.[34] Steinhagen et al.[35] treated 21 patients and the evaluation at 3 years confirmed the potential of this procedure, with 85% good or excellent results. They also showed poorer results in larger defects, and the influence of lesion size has been confirmed also at longer follow-up.[36,37]

To address the issue of treating challenging osteochondral lesions a biphasic scaffold has been developed. The structure of this biomimetic nanostructured scaffold, composed of type-I collagen and nano-structured hydroxyapatite, was conceived with the aim of confining bone formation to the deepest portion of construct without involving any superficial layer where the process of cartilaginous-like connective tissue formation should begin. Preclinical studies showed good results, in terms of both cartilage and bone tissue formation,[38,39,40] also with a cell free approach, suggesting osteochondral regeneration by harnessing and guiding the body's self-regenerative potential.[41,42] Thus, this osteochondral scaffold was introduced into clinical practice as a cell-free approach with promising preliminary results.[9,43,44,45]

OCD is a disease of the joint surface that primarily involves the subchondral bone and should therefore be one of the best indications for the implantation of this osteochondral scaffold. The prospective evaluation at 2 years confirmed the potential of this surgical approach, showing an improvement in all the parameters evaluated. All the questionnaires presented better scores 1 year after surgery, and a further improvement was found at the 2-year evaluation. Moreover, unlike what has been reported in the literature for other procedures,[30,35,36] no correlation with lesion size was observed.

More controversial findings were revealed by MRI. Whereas a complete filling of the defect and a complete integration of the graft was observed in most cases, the structure of the repair

tissue was often not homogeneous and subchondral bone changes (edema, granulation tissue, cysts, and sclerosis) were observed in most cases. However, none of these aspects was correlated with a poorer clinical outcome. A CT evaluation, more focused on the bone phase, might help to study better the subchondral regeneration process driven by this scaffold, and future studies are needed to determine the real significance of the alterations observed by MRI.

A different biological healing potential among patients can be hypothesized, but other aspects more related to the procedure itself might also be implicated. A rate of 13.3% of partial detachment was reported in an early stability analysis;[9] a possible reason for the early detachment of the implanted biomaterials might be weak mechanical fixation due to inadequate surgical technique. The press-fit implantation might not have been optimal in some cases, thus jeopardizing the integration. Moreover, the effusion reported might be caused by an inflammatory process triggered by this procedure, which, if not properly controlled, might also affect the scaffold and hinder the healing process. Other limitations of the present study are the lack of a control group and the short-term follow-up. Conversely, the strong points are the number of homogeneous patients prospectively evaluated both clinically and with imaging.

Besides the controversial imaging findings, the clinical evaluation gave good results, showing that this approach targeted to the damaged osteochondral unit may be successfully applied for the treatment of knee OCD with the advantages of one-step surgery, reduced costs and a simplified procedure. Thanks to the plasticity of the graft, large osteochondral defects can be treated through minor incisions, and the results showed that large lesions can also be treated with this technique and good clinical results can be obtained.

CONCLUSIONS

The results obtained in the present study showed that this biomimetic osteochondral scaffold is a valid treatment for knee OCD and might offer a good clinical outcome at 2 years of follow-up. The improvement is not correlated with the lesion dimension, and large lesions can also benefit from this implant. More controversial findings were obtained by the MRI

evaluation, with a not homogeneous structure of the regenerated tissue and subchondral bone changes observed in most cases at the short-term.

Filardo G, Kon E, Di Martino A, Busacca M, Altadonna G, Marcacci M. Treatment of knee osteochondritis dissecans with a cell-free biomimetic osteochondral scaffold: clinical and imaging evaluation at 2-year follow-up. Am J Sports Med. 2013 Aug;41(8):1786-93. The final publication is available at http://journal.ajsm.org/ doi: 10.1177/0363546513490658.

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Matrix-assisted autologous chondrocyte implantation for cartilage regeneration in osteoarthritic knees: results and failures at mid-term follow-up

INTRODUCTION

Young patients with osteoarthritic knees are a challenging population due to a combination of high functional demands and limited indication for joint replacement.[21] Whereas arthroplasty is a successful treatment for older patients, younger patients are less satisfied and in this patient population revision arthroplasty has increased morbidity and poorer results.[34] Conservative treatments such as injection and physical therapy can provide short-term pain relief but are often not a suitable solution for satisfactory durable results and postpone the need of this sacrificing and limiting prosthetic replacement.[7,18,19]

Therefore, whereas traditionally not indicated for the treatment of osteoarthritis (OA), cartilage regenerative procedures have become a focus of increased interest with the hope of delaying the need for joint replacement.[11,12,17,38,39] Good clinical results have been achieved with these techniques,[5,6,8,9,15,26,31,32,33,42] and several authors have also presented some promising preclinical findings in OA joints.[2,40] However, how OA processes may affect cartilage regeneration in the clinical setting is controversial and these procedures are currently not indicated for OA joints.[25]

The purpose of this study is to investigate the real potential of the cartilage regenerative approach MACT in a population of patients affected by knee OA lesions who had refused or were not indicated for a prosthetic replacement.

MATERIALS AND METHODS

Patient selection

Forty-four patients affected by knee OA lesions where previous conservative or surgical treatments had failed and had refused a prosthetic replacement underwent MACT as a salvage procedure by four senior surgeons at the authors' Institute from 2000. The indication

criteria were: full-thickness cartilage lesions in Kellgren 2-3 OA knees complaining of clinical symptoms (pain, swelling, locking, and giving way). Exclusion criteria were: kissing lesions, previous or combined complete meniscectomies (>50%), untreated tibio-femoral or patello-femoral malalignment or knee instability, infection, tumor pathology or other general medical conditions (diabetes, rheumatoid arthritis, etc).

Selected patients were enrolled, treated, and evaluated prospectively. The patients evaluated were 25 men and 19 women, with a mean age at surgery of 42 years (20 - 58) and a mean BMI of 26 (18 - 31). The Kellgren grade at X-ray was 2 in 31 patients and 3 in 13 patients and the average defect size of the treated lesions was 4 cm^2 (1.5 - 9). The most common treatment site was the medial femoral condyle (MFC) in 28 cases; 8 cases involved the lateral femoral condyle (LFC), whereas the patella and the tibial plateau were involved in 4 and 3 cases, respectively; finally 1 patient was treated for lesions at both condyles. Patient history showed a micro traumatic/degenerative etiology in 31 cases, trauma and OCD in 9 and 4 cases, respectively.

Thirty-seven patients (84%) had undergone previous knee surgery and in 24 patients (55%), associated procedures were performed.

Surgical technique and rehabilitation protocol were described before.[22,10]

Follow-up evaluation

All 44 patients underwent a prospective evaluation at 1, 2, 5 years, and at a minimum final follow-up of 7 years (mean 9 years and maximum 10 years). The clinical outcome was analyzed using the Cartilage standard Evaluation Form as proposed by International Cartilage Repair Society.[14] In particular, patients were evaluated with IKDC subjective score. Activity level was evaluated with the Tegner score relatively to pre-operative and pre-injury levels.[41] Patients were also asked if they thought their condition had improved or not, and if they would repeat the treatment based on the results obtained. Surgery was considered to have failed if the patient needed a re-operation because of symptoms related to the primary defect. For re-treated patients, the last clinical evaluation before re-operation was considered at every further follow-up.

Statistical Methods

Continuous data were expressed in terms of the mean and the standard deviation of the mean. One Way ANOVA with the Scheffé post-hoc pairwise analysis was used to study differences

among groups when the Levene test for homogeneity of variances was not significant; otherwise, the Mann Whitney test or the Kruskal-Wallis test with the non-parametric post-hoc pairwise LSD test were applied. The GLM for repeated measures with Sidak's correction for multiple comparisons was applied to evaluate score differences at different follow-ups. The Spearman rank correlation was applied to study the correlation between scores and continuous variables. The Kaplan-Meier survival analysis was performed to determine the failure rate. $P<0.05$ was considered significant. Statistical Analysis was carried out by the SPSS software version 15.0 (SPSS Inc., Chicago, USA).

RESULTS

No major adverse events were observed. Four patients presented a marked swelling that required arthrocentesis, and 3 presented fever postoperatively that resolved within one week. IKDC subj score: improvement from 38.0±15.8 to 63.5±17.4 at 1 year ($p<0.0005$), stable results at 2 years (67.0±18.3), and then gradual deterioration with 62.9±21.3 at 5 years and 57.8±20.6 at 9 years (lower than at 2 yeas, $p=0.012$) (Fig. 1).

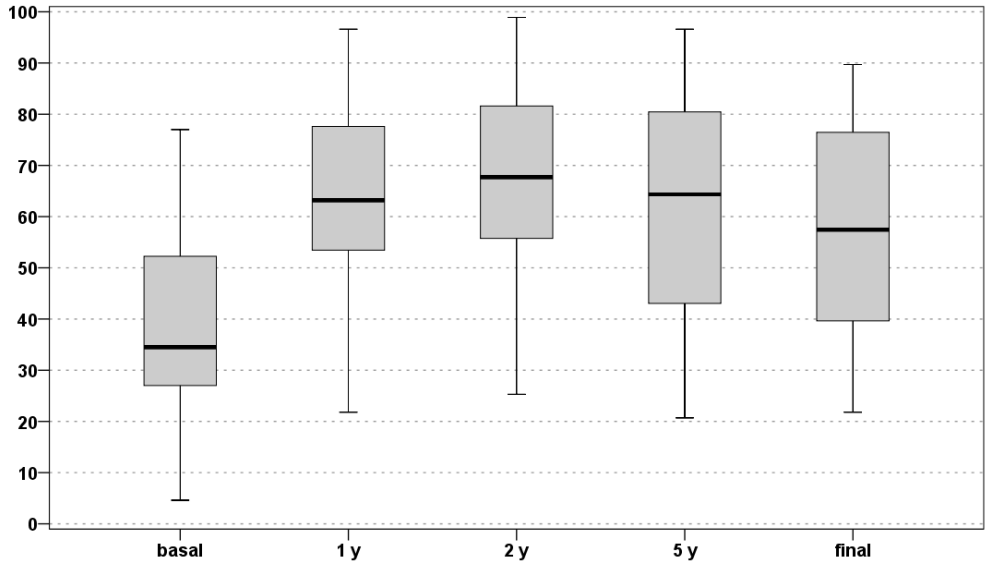

Fig. 1: IKDC subjective score at basal level, 1, 2, 5, and final mean 9-year follow-up.

Tegner score: significant improvement ($p<0.0005$) from pre-surgery (1.8±1.4) to all follow-ups (3.1±1.3 at 1 year, 3.2±1.3 at 2 years, 3.1±1.3 at 5 years, and 2.8±1.2 at the final follow-up) and stable results. Despite the improvement, the score remained lower than before the onset of symptoms (4.6±2.0) ($p<0.0005$) (Fig. 2).

Failures: 12 patients failed, for a cumulative failure rate of 27.3% (Fig. 3). At the last follow-up 47.7% evaluated their condition not better than before surgery and 39% would not repeat it considering the results achiewed.

Parameters that influenced the clinical outcome: age, gender, BMI, defect size, previous or combined surgery, etiology, site, and Kellgren level did not significantly influence the final outcome in this series. The only factor that was found to influence the results was the meniscus condition: knees without history of meniscectomy reached a score of 74.5±17.4 and 71.6±18.8 at 2 and 5 years, respectively, higher than the meniscectomized knees ($p=0.011$ and $p=0.012$, respectively). However, these scores were not stable over time, with a final result of 64.2±19.0 (Fig. 4).

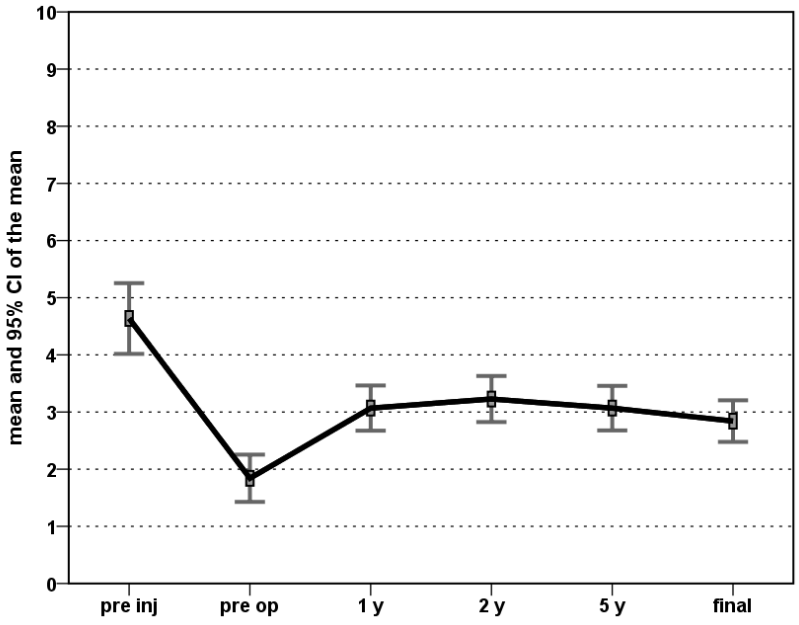

Fig. 2: Tegner score at pre-injury and pre-operative level, and at 1, 2, 5, and final mean 9-year f-up.

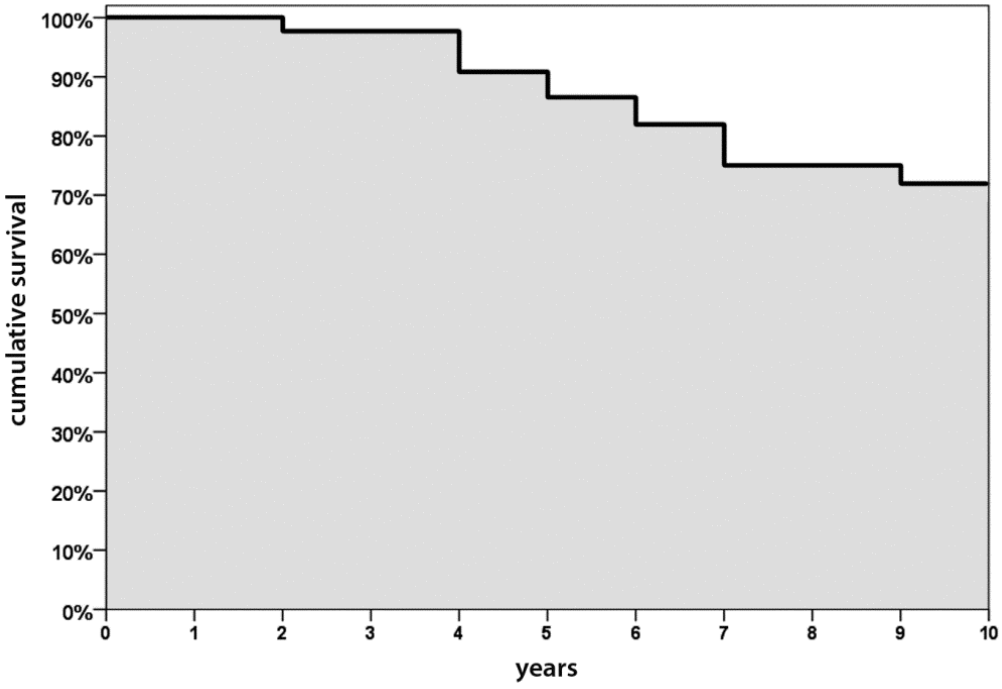

Fig. 3: Survival curve: 27.3 % of the patients failed during the study period.

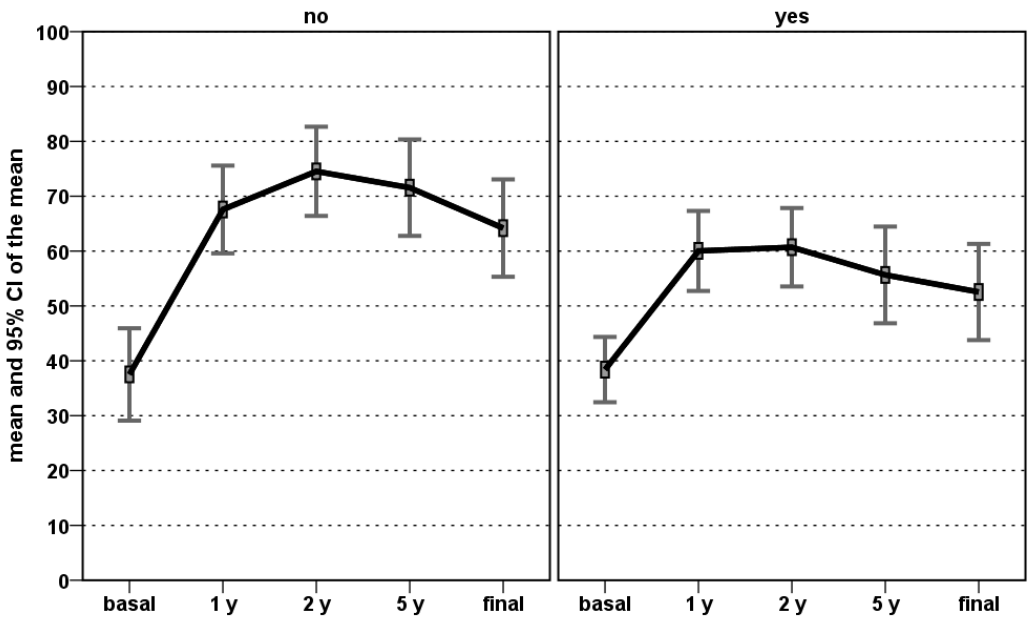

Fig. 4: IKDC subjective score at the different follow-ups for knees with or without previous/associated partial meniscectomies.

DISCUSSION

Regenerative procedures in OA lesions presents some additional problems: in a degenerative process the adjacent areas can be involved, leading to excessive mechanical forces and risk of graft loosening, and the inflammatory cytokines might cause apoptosis of the implanted bioengineered cell-based scaffold.[23] In OA processes several intra-articular changes, such as inflammatory, cellular or molecular factors, come into play, all unfavourable conditions for tissue regeneration as supported by some preclinical findings.[30,35,37]

However, some other authors suggested the potential of regenerative procedures[2,40] and, more important, even some promising clinical findings have been reported. Hollander et al.[13] showed that tissue engineering is not inhibited by the degenerative articular environment and Minas et al.[24] reported that at 5 years 92% of patients were able to delay the need for joint replacement. Ossendorf et al.[28] treated mild degenerative cartilage lesions and focal OA knee defects and reported an improvement in quality of life, as confirmed by. Kreuz et al.[20] results at 4 years.[29] Bauer et al.[1] treated patients with MACT and tibial osteotomy, showing satisfactory results up to 60 months but with a worsening over time and with overall unsatisfactory MRI at the final evaluation. Rosenberger et al.[36] reported good results if all comorbidities were addressed concomitantly with cartilage treatment.

However, the literature also reports less positive findings. Niemeyer et al. showed a high failure rate. 26.7% at 2 years.[27] Nehrer also concluded that MACT for the treatment of OA and for salvage treatment has to be considered with caution because the LOW chance of success.[26]

Finally, in a study[9] on cartilage degenerative lesions results were inferior with respect to the outcome reported in different study populations, and the number of failures was also markedly higher, suggesting the limits of an unfavorable environment.

In this study we focused on the potential of MACT for the most challenging clinical condition: knee OA lesions. No severe adverse events were observed and a significant improvement was observed, but the outcome was inferior with respect to the one reported for different study populations,[3,4,5,15,16] and results presented a worsening over time with a total failure rate of 27.3%. These results seriously question the indication of this regenerative surgical approach as a salvage procedure for such a challenging knee condition.

Interestingly, patients with untouched meniscus presented a satisfactory clinical condition at short/mid-term follow-up. However, results deteriorated over time, and results were compromised regardless of the severity of the joint condition.

The high number of patients prospectively evaluated at mid-long term follow-up allowed us to demonstrate the potential of MACT. In fact, conversely to the available studies that show promising findings at short-term follow-up, this study shows that even the most responsive OA patient categories achieved a low outcome at mid/long-term follow-up. Therefore, the indication as salvage procedure is questionable, and the high failure rate should be considered before applying MACT in OA joints.

CONCLUSIONS

The clinical outcome of patients affected by knee OA lesions who underwent MACT as a salvage procedure was poor. A higher improvement could be obtained in patients who had not undergone previous or associated meniscectomies, but this was limited over time. The failure rate was high, regardless of the OA degree. Limits of MACT have to be remembered if considered for young patients affected by knee OA.

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PART 3

Platelet Rich Plasma knee injections for the treatment of degenerative cartilage lesions and osteoarthritis

INTRODUCTION

The management of chondral pathology is challenging because the regeneration capacity of cartilage is limited.[6,7,9,14,21,31]

Numerous strategies have been proposed as non-invasive solutions for pain treatment, improving function and reducing disability, and ultimately modifying the course of degenerative processes with variable success rates.[16] However, these therapies may offer only short-term benefit and present side-effects or even deleterious consequences on knee structures.[4,19,20,23,34] Finally, they have not been clearly shown to be able to alter the natural history of the disease, and none of the currently available treatments can be considered an ideal procedure for the treatment of chondropathy or OA.[8,25]

The most recent knowledge regarding tissue biology highlights the complex regulation of growth factors (GFs) for the normal tissue structure, and the influence of these GFs on cartilage repair has been widely investigated.[10,13,15,19,30] Platelet-Rich Plasma (PRP) therapy is a simple, low cost and minimally invasive method to obtain a natural concentrate of autologous GFs from the blood.[1,29] In a previous analysis, we evaluated at a 12-month follow-up 91 patients (115 knees) affected by a chronic degenerative condition of the knee and treated with 3 PRP intra-articular injections. The preliminary results indicated that this procedure is safe and has the potential to reduce pain and improve knee function in younger patients with a low degree of joint degeneration.[17]

The main purpose of this study is to evaluate the results of PRP to treat degenerative knee articular cartilage lesions at a 2-year follow-up and to determine the persistence of the beneficial effects obtained over time. A secondary aim of the study is to determine the best indication criteria and application modalities.

MATERIALS AND METHODS

Patient selection

The following criteria were used for patient selection: history of chronic knee symptoms and imaging findings of joint degeneration. Exclusion criteria were: systemic disorders such as diabetes, rheumatoid arthritis, major axial deviation, haematological diseases, severe cardiovascular diseases, infections, immunodepression, patients in therapy with anticoagulants or antiaggregants, use of NSAIDs in the 5 days before blood harvesting, and patients with Hb values of < 11 and platelet values of < 150,000/mmc.

Of the 91 patients evaluated in the previous 2-, 6- and 12-month follow-up study, 90 were available for the 24-month follow-up, whereas 1 patient was lost at the time of the follow-up study. The analysed patients consisted of 57 men and 33 women, with a mean age of 49.9 ± 13.6 years (range 24-82). There were 66 patients who were affected by a monolateral lesion, whereas 24 patients presented a bilateral lesion, for a total of 114 knees treated. The mean BMI was 25 ± 3 (range 18-32) and 27 patients underwent a previous knee surgery. All of the patients presented a chronic degenerative condition; 58 knees presented a degenerative chondral lesion (Kellgren 0), 32 knees presented early osteoarthritis (Kellgren I-III), and 24 knees were affected by advanced osteoarthritis (Kellgren IV).

Platelet-Rich Plasma preparation

The procedure used a 150-ml venous blood sample (in a bag with 21 sodium citrate) for each lesion treated. The samples were centrifuged twice to separate erythrocytes and to concentrate platelets, producing a unit of 20 ml of PRP. The collected PRP was divided into 4 units of 5 ml each: 1 unit for platelet count and bacteriological test, 1 unit for the first injection, and the other 2 units were stored at -30° C. An average of 6.8 billion platelets were administered to the lesion site for each injection.

Injections were administered every 3 weeks; for the second and third treatments, the samples were thawed in a dry–thermostat at 37° C for 30 min before application; 10% calcium chloride was added to the PRP unit to activate the platelets before the injection.

Treatment procedure and follow-up evaluation

The infiltration was performed using a classic lateral approach with a 22-g needle. At the end of the procedure, the patient was asked to bend and extend the knee a few times to allow the PRP to distribute throughout the joint (Fig. 1).

After the injection, the patients were sent home with instructions to limit the use of the leg for at least 24 hours, to use cold therapy/icing to treat pain, and to avoid the use of steroidal medications. During the treatment cycle, rest or mild activities (exercise bike or mild exercise in a pool) were permitted, and subsequently a gradual resume of normal sport or recreational activities was allowed, as tolerated.

Fig. 1: The blood sample is processed, obtaining 5 ml PRP samples for the intra-articular injections.

Patients were prospectively evaluated before and after the treatment, and at follow-up studies 6, 12 and 24 months. All results are presented as the number of knees.

IKDC, objective and subjective, were used for clinical evaluation. Complications, adverse events and patient satisfaction were also recorded.

For patients who decided to repeat the treatment between the 12- and 24-month follow-up because the beneficial effect ended, or for those who were operated or treated because of knee degeneration, the final available follow-up before the new treatment was used for the final evaluation.

Statistical analysis

Statistical analyses were carried out with the SPSS (Chicago, IL, USA) for Windows software program version 13.0. A P value lower than 0.05 was considered significant.

Results were expressed as mean ± SD. The Paired T test or The Wilcoxon test for non parametric data were applied to identify significant differences between baseline follow-ups. One Way ANOVA test or the Mann–Whitney test and the Kruskal–Wallis test for non parametric data were used to identify significant differences between and among groups. The

Spearman's and the Pearson's statistical correlations were applied to study the parameters that influenced the final outcome.

RESULTS

One patient presented a marked pain response and swelling for 2 weeks after the injection. No other complications were observed.

IKDC obj score: from 46.5% of normal and nearly normal knees before the treatment to 78.1% at the end of the therapy, then to 72.8% and 66.7% at the 6- and 12-month follow-ups, respectively, with a significant improvement ($p<0.0005$) at all these follow-ups with respect to the basal level. The evaluation after 2 years confirmed the worsening observed at 1 year ($p=0.018$) with a further decrease ($p=0.004$) for a 58.8% of normal and nearly normal knees. IKDC subj score: marked improvement from the basal evaluation to the end of therapy and the follow-up at 6 and 12 months ($p<0.0005$). The worsening at the 12-month follow-up ($p=0.02$) was further increased at 2 years ($p<0.0005$) (Fig. 2). The good level of satisfaction at the 12-month follow-up was confirmed at 2 years, with 80.2% (73/91) and 80.0% (72/90) of satisied patients, respectively. A significant worsening was observed in all the subgroups; however, better results were found in lower degrees of articular cartilage degeneration ($p<0.0005$, fig. 3), and in younger patients ($r=-0.421$, $p=0.0001$, fig. 4). Further analysis showed worst results in women at 2 years ($p=0.0002$).

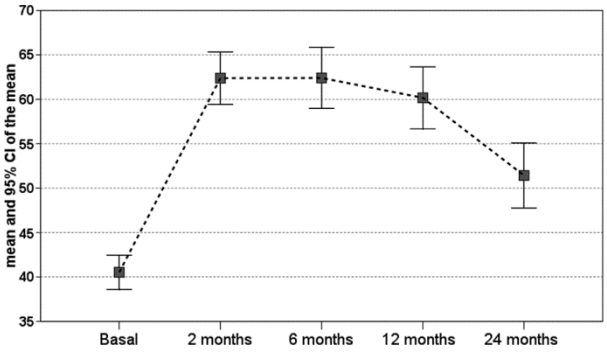

Fig. 2: Health status evaluated with IKDC Subjective score (0-100).

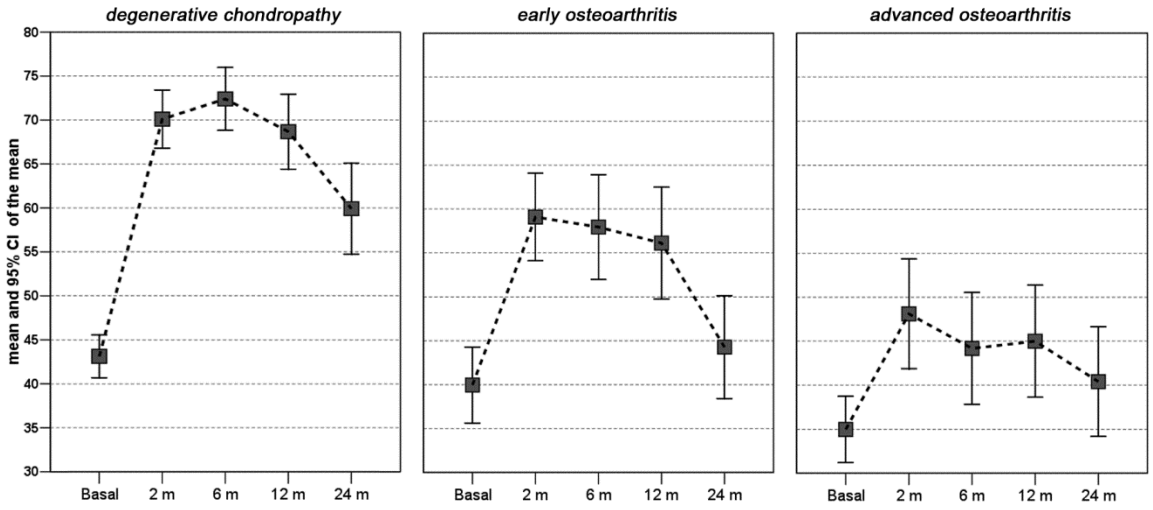

Fig. 3: Patients with degenerative chondropathy achieved better IKDC subj results with respect to patients affected by early OA, who presented a higher improvement compared to patients with advanced OA. All subgroups presented a marked worsening from the 12- to the 24-month f-up.

The mean beneficial effect duration was 10.9 ± 8.1 months. (Fig. 5). The effect duration was correlated with age, sex, and degree of degeneration: younger patients had longer lasting results (r=-0.343, p<0.0005), as well as men (12.6 ± 7.9 vs 7.8 ± 7.6; p=0.002) and cases with lower chondral degeneration (degenerative chondropathy: 13.7 ± 7.7, early osteoarthritis: 9.2 ± 6.9, advanced osteoarthritis: 6.1 ± 7.8, p<0.0005).

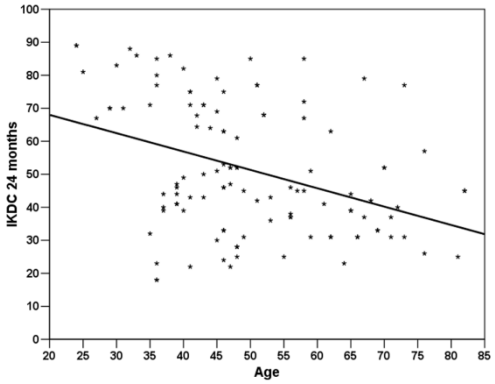

Fig. 4: Correlation between age and clinical outcome.

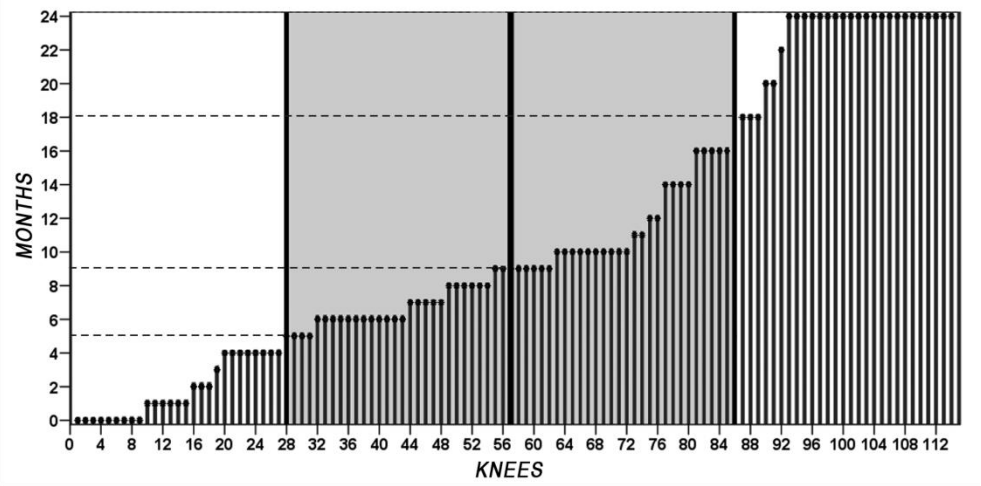

Fig. 5: PRP beneficial effect duration documented with the IKDC subjective evaluation in the 114 knees treated and evaluated for 24 months after the injection cycle.

DISCUSSION

Chondrocytes are affected by numerous extracellular stimuli influencing the regulation of biosynthetic and catabolic activity, including mechanical stress and soluble factors.[5] An imbalance of regulatory factors, which may result from aging, disease, or injury, may hinder tissue maintenance and repair, ultimately resulting in accelerated loss of the articular surface and leading to end-stage OA.[32]

Current pharmacological interventions may only temporarily reduce pain.[2] Thus, laboratory investigations are focusing on the possibility of preserving normal homeostasis to avoid or at least delay more invasive surgical procedures.

Several studies have described a complex regulation of GFs involved in normal tissue structure and the reaction to tissue damage.[1] The most studied GFs include the transforming TGF-β, PDGF, IGF, FGF, HGF, and many others. They modulate the expression of the chondrocyte phenotype, chondrogenic differentiation of mesenchymal stem cells, matrix deposition, and chondrocyte metabolism and proliferation.[10,15,18,24] They are also important for chemotaxis and regulation of regeneration and catabolism.[11,30] They have independent chondroinductive actions, but also additive effects and synergistic interactions.[12,18,22]

Blood-derived GFs have been documented in the literature in both preclinical and clinical studies on cartilage repair.[3,26,33] In fact, recently there has been increasing interest in the use of PRP, because of the activity of blood GFs carried in platelets, many of which have been shown to take part in the regulation of articular cartilage.[1,10,13,15,19,29,30]

PRP is derived from the centrifugation of autologous whole blood and contains 4 to 5 times more platelets than normal blood, offering a high concentration of GFs in physiologic proportions. Some researchers suggest a possible use of PRP for cartilage lesions.[26,27,28,33]

These studies suggest that these potent biological regulators of chondrocytes have an important role in cartilage repair. However, for the time being, the evidence for the clinical use of PRP injections is still in its infancy. We performed a study to explore this novel approach for the treatment of articular cartilage degenerative lesions and documented that this procedure is safe and has the potential to reduce pain and improve knee function and quality of life in younger patients with a low degree of cartilage degeneration. The positive results achieved at the end of the therapy were maintained at the 6-month follow-up, then a tendency for the results to worsen was observed over time. At 2 years we observed an overall worsening in all of the evaluated parameters, even though these parameters remained above the baseline values. Despite the overall worsening in all of the subgroups, younger male patients and those with a lower degree of cartilage degeneration still presented better results. The median duration of clinical improvement was 9 months. It should be emphasized that we observed a high range of PRP effect duration. In fact, young men with degenerative chondropathy presented not only higher clinical benefit, but also more durable results.

The short-term effect documented makes it difficult to support disease-modifying properties for this approach. PRP probably influences the overall joint homeostasis, reducing synovial membrane hyperplasia and modulating the cytokine level, thus leading to an improvement in the clinical outcome, even if only temporarily and without affecting the cartilage tissue structure or the progression of joint degeneration.[11,26]

This study documents the results of PRP as a treatment of articular cartilage degeneration in humans, with a significant improvement in the clinical outcome at short follow-up. The findings obtained regarding the safety, feasibility and short-term efficacy of this treatment option confirm that it may represent a low-invasive and safe procedure that could be

cyclically repeated to improve knee function and quality of life, and to delay the need for more invasive surgical procedures.

CONCLUSIONS

PRP injections have the potential to reduce pain and improve both knee function and quality of life with short-term efficacy, especially in younger patients with chondral degenerative lesions or early OA. It still remains to be determined if there is only a temporary improvement in symptoms or if PRP therapy also presents disease modifying properties, and if different platelet concentrations or application modalities could further increase the clinical benefits.

Filardo G, Kon E, Buda R, Timoncini A, Di Martino A, Cenacchi A, Fornasari PM, Giannini S, Marcacci M. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. Knee Surg Sports Traumatol Arthrosc. 2011 Apr;19(4):528-35. The final publication is available at Springer via http://dx.doi.org/ doi: 10.1007/s00167-010-1238-6.

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Platelet Rich Plasma injections for cartilage degeneration and osteoarthritis: single- versus double-spinning procedures

INTRODUCTION

Societal impact of degenerative diseases such as articular cartilage pathology and osteoarthritis (OA) are increasing, due to the continued rise in the mean age population and greater emphasis on physical activity in all age groups.[1,2,3,4] Numerous approaches have been proposed as non-invasive treatment with variable success rates, but none has clearly shown to be able to alter the natural history of the disease.[5]

PRP has gained increasing attention as innovative procedure to stimulate repair or replace damaged cartilage, due to the pools of growth factors (GFs) stored in platelets' α-granules, that have been identified to take part in the regulation of articular cartilage.[6,7,8,9,10,11] PRP is a simple and minimally-invasive method to obtain a high concentration of autologous GFs that can be easily placed directly into the lesion site.[12] Due to the autologous nature of the platelet extract, the risk of allergy or infection is negligible.[13]

Despite some interesting findings,[14] results are still preliminary and controversial. The difficulty in this field of research is increased by the numerous products used. PRP concentrations have been reported to range widely, and the numerous preparation methods present many other different variables, such as presence of other cells, activation and storage methods, and many other aspects that could be important to determine PRP properties and clinical efficacy.[15] In particular, the presence of leukocytes is controversial, since some authors attribute better results to leucocyte depletion, because of the deleterious effects of proteases and reactive oxygen; others consider them as a source of cytokines and enzymes, and they could help preventioning infections.[16]

Aim of this study was to explore this novel biological therapy for degenerative lesions of articular cartilage and OA comparing two products, already applied in the clinical practice, that are based on different preparation approaches: single- vs double-spinning procedures.

We investigated if the difference in platelet concentration, cellularity, and storage modality may lead to different clinical results.

MATERIALS AND METHODS

Patient selection

The following selection criteria were used: patients affected by chronic pain or swelling of the knee and imaging findings of degenerative changes. Exclusion criteria included systemic disorders such as diabetes, rheumatic diseases, hematological diseases, severe cardiovascular diseases, infections, immunodepression, patients in therapy with anticoagulants-antiaggregants, use of NSAIDs in the 5 days before blood donation, patients with Hb values of < 11 and platelet values of < 150.000/mmc.

For this study, 144 patients affected by knee cartilage degenerative lesions and OA were treated with intra-articular injections: 72 were treated with 3 autologous PRGF, 72 with 3 PRP intra-articular injections. Each group of patients received a different treatment depending on a sort of "geographic" randomization; every center performed only one treatment, and so the patient treatment allocation was due to the center the patients went to. All 2 centers enrolled consecutive patients following the same inclusion criteria. All the patients were prospectively evaluated at 2, 6, and 12 months. In case of bilateral lesions, the worse knee was chosen for the clinical evaluation. PRP and PRGF groups were comparable in terms of age, sex, number of bilateral lesions, BMI, degeneration level, and previous surgery.

Platelet concentrate preparation and injection.

PRGF: The procedure consisted of a 36-ml venous blood sample for each injection. Four tubes of 9 ml of blood were centrifuged at 1800 rpm for 8 minutes, obtaining a concentration suspended in plasma that was extracted pipetting carefully in order to avoid leukocytes aspiration. Before the injection, 10% of Ca-chloride was added to the PRP unit to activate platelets.

PRP: The procedure consisted of a 150-ml venous blood sample for every knee treated. Two centrifugations (the first at 1800 rpm for 15 minutes to separate erythrocytes, and a second at 3500 rpm for 10 minutes to concentrate platelets) produced 20 ml of PRP. The unit of PRP

was divided into 4 small units of 5 ml each. All the open procedures were performed in an A-class sterile hood. One unit was sent to the laboratory for quality test, 1 unit was used for the first injection within 2 hours, and the other 2 units were stored at -30° C. For the second and third treatments, the samples were thawed in a dry–thermostat at 37° C for 30' just before application. Before the injection, 10% of Ca-chloride was added to activate platelets.

In both procedures injections were administered every 3 weeks through a classic lateral approach with a 22-g needle. At the end of the procedure, the patient was invited encouraged to bend and extend the knee a few times to allow the PRP to distribute itself all over the joint (Fig. 1).

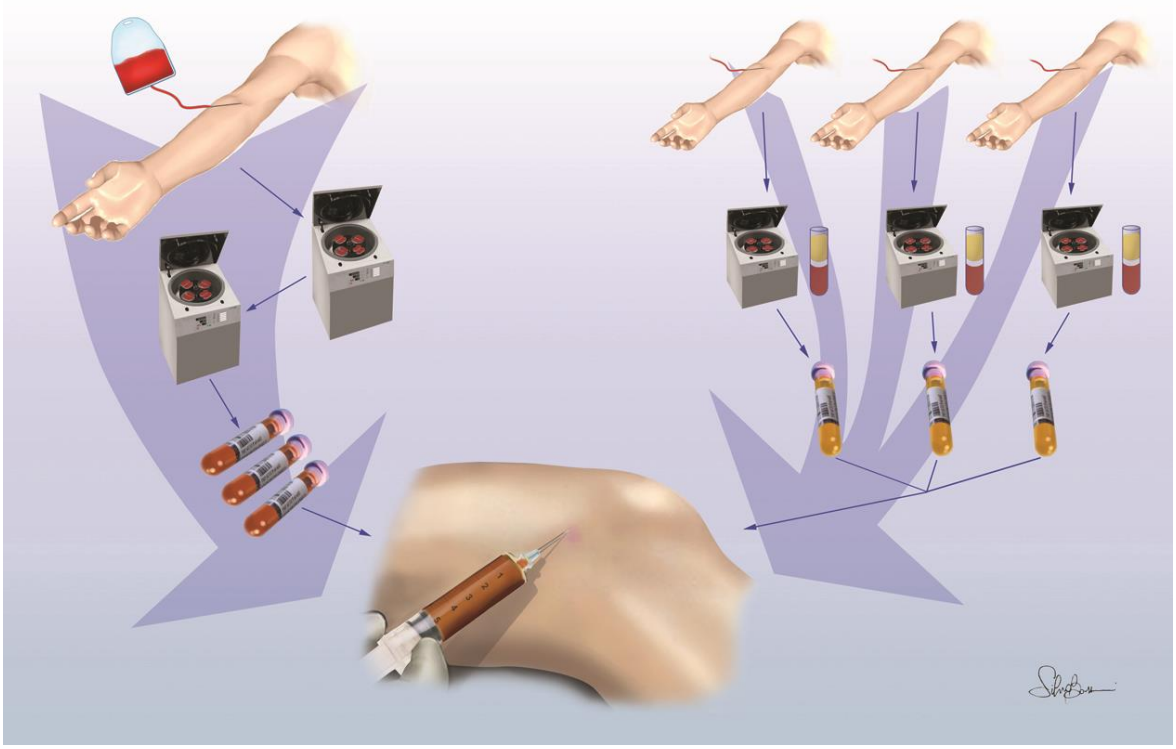

Fig. 1: PRP and PRGF preparation procedures.

Platelet and cell count

In order to analyze the different concentrates obtained, 7 volunteers underwent blood harvesting, and both PRGF and PRP were prepared from the same blood.

The mean final number of platelet concentrated was 350000/µl in the PRGF group and 1022000/µl in the PRP group, with a concentration factor of 1.7x with the single-spinning procedure and 5.0x with the double-spinning procedure.

The mean final number of leukocytes was 800/µl in the PRGF group and 9525/µl in the PRP group, with a concentration factor of 0.1x with the single-spinning procedure and 1.6x with the double-spinning procedure.

Post-procedure protocol and follow-up evaluation

Patients were sent home after the injection with instructions on limiting the use of the leg and to not use non-steroidal medication but cold therapy for pain for at least 24 hours. During the cycle of injections rest or mild activities were indicated. Subsequently, a gradual resumption of normal sport or recreational activities was allowed as tolerated in both the treatment groups.

Patients were prospectively, clinically evaluated before the treatment, at 2, 6, and 12 months of follow-up. Subjective IKDC and Tegner scores were used for clinical evaluation. Adverse events and patient satisfaction were also recorded.

Statistical analysis

A power analysis was performed for the primary endpoint (IKDC-S, 6-month follow-up). From a pilot study, a standard deviation of 15.8 points was identified. With an alpha error of 0.05, a beta error of 0.2 and a minimal clinically significant difference of 7,4 points corresponding at 1/3 of the documented mean improvement, the minimum sample size was identified as 72 for each group.

Continuous data were expressed in terms of the mean and the standard deviation of the mean. One Way ANOVA was used to assess differences among groups when the Levene test for homogeneity of variances was not significant; otherwise, the Mann Whitney test or the Kruskal Wallis test were performed. The Least Significant Difference test was used as post-hoc pair-wise analysis of the Kruskal Wallis test. GLM for repeated measures with Bonferroni correction for multiple comparisons was used to test score differences scores at different follow-ups. The influence of grouping variables on scores at different follow-ups were investigated by GLM for repeated measures with the grouping variable as fixed effect. The non parametric Pearson's Chi square test evaluated by Exact methods was applied to

study the relationships between grouping variables. Spearman rank correlation was applied to evaluate the correlation between continuous variables.

$P<0.05$ was considered significant. Statistical Analysis was carried out by means of the SPSS software version 15.0 (SPSS Inc., Chicago, USA).

RESULTS

No serious adverse events were observed. The two procedures presented a significant difference in the minor adverse events: both pain and swelling reaction after the injections were higher in the PRP group ($p=0.0005$ and $p=0.03$, respectively).

PRGF group: the IKDC subj score passed from 45.0±10.1 preoperatively to 59.0±16.2, 61.3±16.3, and 61.6±16.2 at 2, 6, and 12 months of follow-up, respectively, with a significant improvement at 2 months ($p<0.0005$) maintained at 6 and 12 months ($p<0.0005$) (Fig. 2). Tegner score improved from 2.5±1.6 to 3.4±1.9 at 2 months ($p<0.0005$); a further improvement was seen at 6 months (3.6±2.0; $p=0.03$), then results remained stable (Fig. 3).

PRP group: the IKDC subj score passed from 42.1±13.5 to 60.8±16.6, 62.5±19.9, and 59.9±20.0 at 2, 6, and 12 months of follow-up, respectively, with a significant improvement at 2 months ($p<0.0005$) maintained at 6 and 12 months ($p<0.0005$) (Fig. 2). Tegner score improved from 2.9±1.7 to 3.6±1.7 at 2 months ($p<0.0005$); a further improvement was seen at 6 months (3.9±1.9; $p=0.03$), then results remained stable (Fig. 3).

When comparing the 2 groups, no differences were found in the subjective IKDC and Tegner scores at 2, 6, and 12 months. The satisfaction level was also similar: 76.4% in the PRGF group and 80.6% in the PRP group.

Parameters that influenced the clinical outcome. Worst IKDC subj results were observed in older patients at 1 year in both groups (rho=-0.217, $p=0.009$ in the PRGF group and rho=-0.296, $p=0.012$ in the PRP group) (Fig. 4). Better results for earlier degrees of knee degeneration were seen in both groups (Fig. 5). BMI, sex, bilateral lesions, and previous surgery, did not significantly influence the final results in this series.

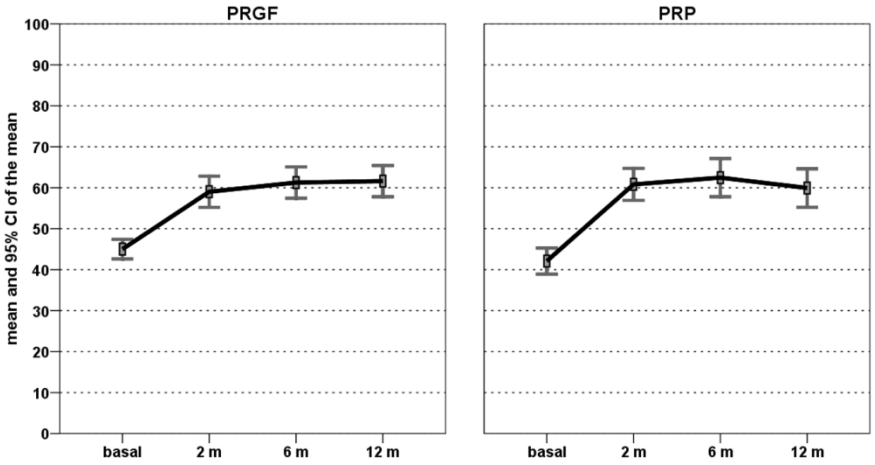

Fig. 2: Health status evaluated with IKDC score (0-100) in the 2 treatment groups.

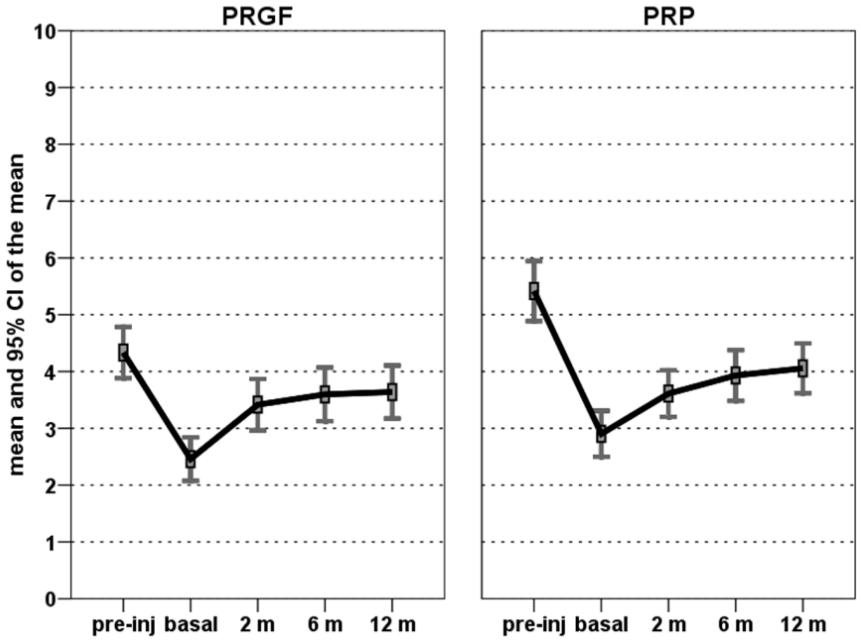

Fig. 3: Activity level evaluated with Tegner score (0-10) in the 2 treatment groups.

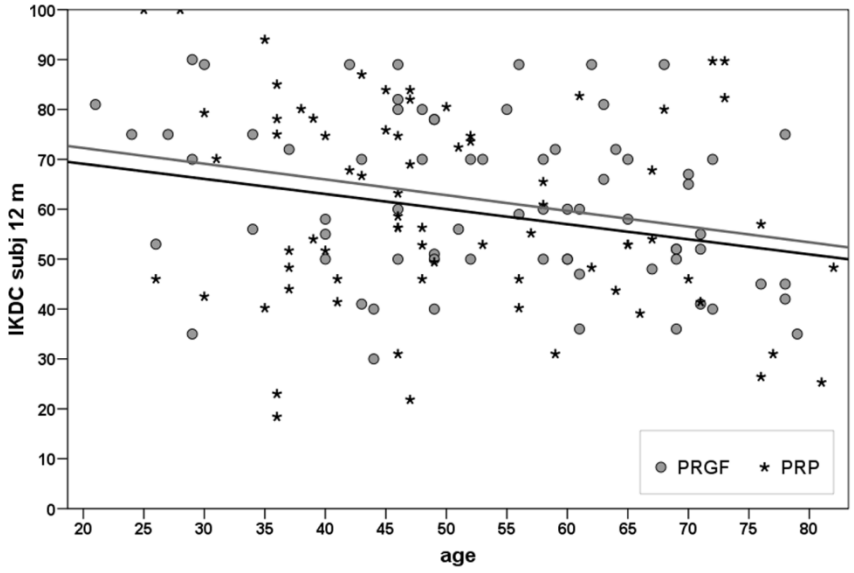

Fig. 4: In both treatment groups age was correlated with the clinical outcome: at 12 months of follow-up older patients obtained worst IKDC results.

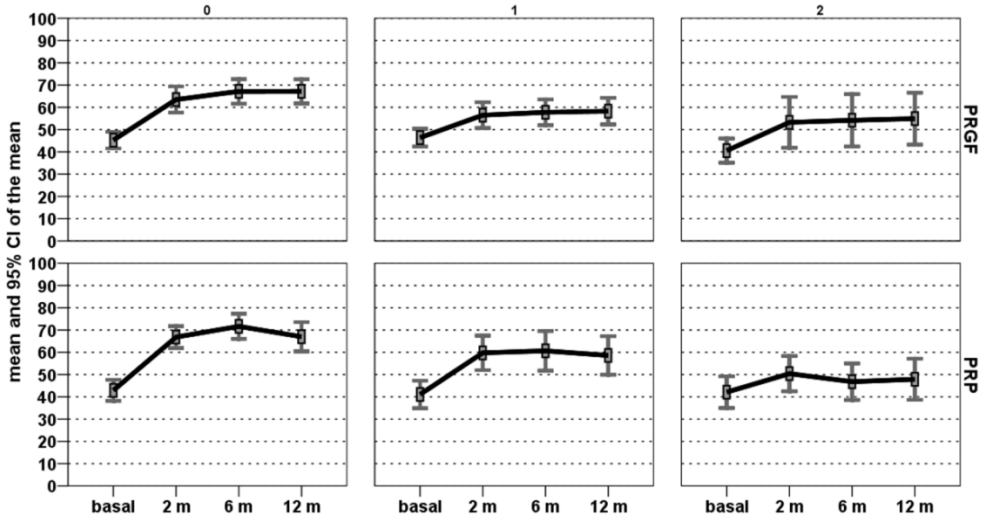

Fig. 5: In both treatment groups better IKDC subjective results have been achieved in patients with lower degrees of knee degeneration at 2months (tau=-0.207, p=0.029 and tau=-0.295, p=0.001 in the PRGF and PRP groups, respectively), 6months (tau=-0.272, p=0.004 and tau=-0.362, p<0.0005 in the PRGF and PRP groups, respectively), and 12 months of follow-up(tau=-0.265, p=0.005 and tau=-0.282, p=0.002 in the PRGF and PRP groups, respectively).

DISCUSSION

Recently, there has been an increasing use of autologous blood products that might provide cellular and humoral mediators to favor tissue healing in tissues with low healing potential.[14-19] Blood-derived products have already been studied as adjuvant for cartilage lesion or OA treatment.[20-26]

Clinical studies also support the role of PRP for the treatment of cartilage lesions. Sánchez et al.[27] treated a soccer player with PRGF for an articular cartilage avulsion, obtaining an accelerated and complete healing. They also reported[28] suggested safety and usefulness of this treatment approach in an observational retrospective cohort study on 30 patient. Wang-Saegusa et al.[29] evaluated 261 OA patients with 73.4% improvement at 6 months. Sampson et al.[30] reported a favorable outcome in a small group of patients who benefited from the injection results for at least 12 months. Kon et al.[31] published a pilot study of 100 patients, with evidence of safety, pain reduction and improved function. The evaluation at 2 years[32] showed an overall worsening and documented a median duration of the beneficial effect of 9 months.

Multiple systems have been developed to offer an easy, cost-effective strategy to obtain high concentrations of GFs. Protocols for producing PRP can be summarized into 3 methods: selective blood filtration, single-spinning methods, and double-spinning procedures.[33] Lower costs and higher feasibility explain the clinical application of the latter two approaches. The single spinning approach can concentrate platelets 1- to 3-fold baseline levels, while 5- to 8-fold baseline levels are achieved by double spinning. However, double spinning also concentrates leukocytes,[14] that could exert deleterious effects because of the proteases and reactive oxygen released, other than a premature platelet degranulation with consequently less GFs available.[34]

The aim of this study was to compare two products for the treatment of cartilage degenerative lesions and OA, already used in the clinical practice, based on different preparation approaches: single- vs double-spinning. Both groups showed a significant improvement of all clinical scores, with better outcome in younger patients with lower degrees of joint degeneration. The comparative analysis failed to show any difference in all the subjective evaluations at 2, 6, and 12 months. Satisfaction level and improvement were

also similar. On the contrary, the two procedures presented a significant difference in the minor adverse events: both pain and swelling reaction were higher in the PRP group. It is possible that the presence of leukocytes caused a local inflammation, thus explaining the higher symptomatology observed. However, the higher post-injection reaction didn't affect the final clinical outcome. It is possible that white cells can play a complex role, with immunomodulatory capability and influence on GFs concentration through their own release of GFs or by stimulating platelet release of GFs.[35,36]

Finally, despite the well known alterations after storing platelets in freezing conditions,[16] we documented good results also in the PRP group, thus suggesting that freeze-thawing doesn't adversely affects platelets' properties to the point of impairing their clinical efficacy.

Limitations of this study are the lack of randomization and placebo control group, but the high number of homogeneous patients analyzed, other than the similarity of the injection protocol, allowed to draw some conclusions.

Both treatments offered a significant improvement, especially in younger patients with lower degrees of joint degeneration, confirming some previously reported findings.[31,32]

The 2 procedures differ for volume of blood harvested, number of blood extractions, and final concentrate, with more platelets but also leukocytes in the PRP group, and less platelets but absence of leukocyte in the PRGF group. However, despite all these differences and the initial higher pain and swelling reaction in the PRP group, PRP and PRGF treatment offer same results at 12 months follow-up. Future studies are needed to clarify the role of platelet concentration and white cells presence, the influence of freezing, and if different PRP preparation and application modalities could further improve its clinical efficacy.

CONCLUSIONS

The comparative analysis documented a higher pain and swelling reaction after the injective treatment in the double-spinning PRP group, but failed to show any significant difference between single- and double- spinning procedures in the clinical improvement obtained up to 12 months of follow-up. Better results were achieved in younger patients with a low degree of cartilage degeneration.

Filardo G, Kon E, Pereira Ruiz MT, Vaccaro F, Guitaldi R, Di Martino A, Cenacchi A, Fornasari PM, Marcacci M. Platelet-rich plasma intra-articular injections for cartilage degeneration and osteoarthritis: single- versus double-spinning approach. Knee Surg Sports Traumatol Arthrosc. 2012 Oct;20(10):2082-91. The final publication is available at Springer via http://dx.doi.org/ doi: 10.1007/s00167-011-1837-x.

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Platelet Rich Plasma vs hyaluronic acid injections for knee degenerative pathology: a randomized controlled trial

INTRODUCTION

The influence of GFs on cartilage repair is now widely investigated in vitro and in vivo.[1,2,3] Platelet Rich Plasma (PRP) is a simple, low- cost and minimally- invasive method which allows one to obtain a natural concentrate of autologous GFs from the blood,[4] and it is increasingly applied in the clinical practice to treat knee degenerative pathology, such as chondropathy and early osteoarthritis (OA).[5,6,7,8,9,10] Although its widespread application, there are only a few high level studies in the literature to demonstrate the real efficacy of PRP. We believe that it is necessary to clearly prove the real potential of this biological approach in order to guide its clinical use and avoid an indiscriminate clinical application, and we therefore designed a high level study. We report the results of a prospective controlled double-blinded randomized trial on 109 patients treated and evaluated up to 12 months of follow-up after PRP or Hyaluronic Acid (HA) injections.

MATERIALS AND METHODS

The following selection criteria were used: patients affected by a monolateral lesion with history of chronic (for at least 4 months) pain or swelling of the knee and imaging findings of degenerative changes of the joint (Kellgren Lawrence 0 to III at X-ray evaluation). Exclusion criteria were: age > 80 years; Kellgren-Lawrence score > 3; systemic disorders such as diabetes, rheumatoid arthritis, major axial deviation (varus >5 , valgus > 5), haematological diseases (coagulopathy), severe cardiovascular diseases, infections, immunodepression, patients in therapy with anticoagulants or antiaggregants, use of NSAIDs

in the 5 days before blood donation and patients with Hb values < 11 g/dl and platelet values < 150,000/mmc.

Patients were prospectively evaluated basally and at 2, 6, and 12 months of follow-up using IKDC subjective, IKDC objective, KOOS, and Tegner scores. At basal evaluation and at every follow-up we also measured the ROM and the transpatellar circumference of both the index knee and the contralateral one to check if any changes occurred over time. Patient satisfaction and adverse events were also reported. All the clinical evaluations were performed by a medical staff which was not involved in the injective procedure, in order to maintain the study double blinded. At the end of the study the nature of the injected substance was revealed to the patients.

The groups were homogeneous for sex, age, BMI, symptoms duration, and previous treatments.

To guarantee the blinding of the patients, all of them underwent blood harvesting to obtain autologous PRP which was used only in half of them, according to a randomization list. The procedure consisted of a 150-ml venous blood sample for every knee treated. Then, 2 centrifugations (the first at 1480 rpm for 6 minutes to separate erythrocytes, and a second at 3400 rpm for 15 minutes to concentrate platelets) produced a unit (20 ml) of PRP. The unit of PRP was divided into 4 small units of 5 ml each. One unit was sent to the laboratory for analysis of platelet concentration and for a quality test, 3 units were stored at -30° C. One week later the injection cycle was started, with 3 weekly injections of PRP or HA. At the moment of the injection the syringe was properly covered to prevent the patient to discover the substance he was receiving. In case of PRP administration, the total number of platelets per milliliter in the PRP represented a mean increase of about 5 times compared with whole blood values.

The post-injective protocol has been previously described.

Statistical analysis

Continuous data were expressed in terms of the mean and the standard deviation of the mean. One Way ANOVA was applied to determine differences among groups when the Levene test for homogeneity of variances was not significant; otherwise, the Mann Whitney test or the

Kruskal Wallis test were performed. The Least Significant Difference test was used as post-hoc pair-wise analysis of the Kruskal Wallis test. GLM for repeated measures with Sidak correction for multiple comparisons was used to test score differences at different follow-ups. The non parametric Pearson's Chi square test evaluated by Exact methods was applied to investigate the relationships between grouping variables. $P<0.05$ was considered significant. Statistical Analysis was carried out by means of the SPSS software version 15.0 (SPSS Inc., Chicago, USA).

RESULTS

No major complications related to the injections were observed. A significantly higher post-injective pain reaction was observed in the PRP group ($P=0.039$). However, this reaction was self-limiting within a few days without compromising the overall outcome.

The analysis showed a significant improvement of all clinical scores from basal evaluation to the 2-, 6-, and 12-month follow-ups in both groups. No differences could be seen between groups in the clinical scores. ROM and knee circumference measurements were also comparable at the different follow-ups.

In the PRP group the IKDC subjective score passed from 50.2 ± 15.7 at the basal evaluation to 62.8 ± 17.6 at 2 months, 64.3 ±16.4 at 6 months, and 64.9 ± 16.8 at 12 months. In the HA group the IKDC score passed from 47.4 ± 15.7 at the basal evaluation to 61.4 ± 16.2 at 2 months, 61.0 ± 18.2 at 6 months, and 61.7 ± 19.0 at 12 months. The other scores used (KOOS, Tegner) presented the same trend with significant improvements in both groups.

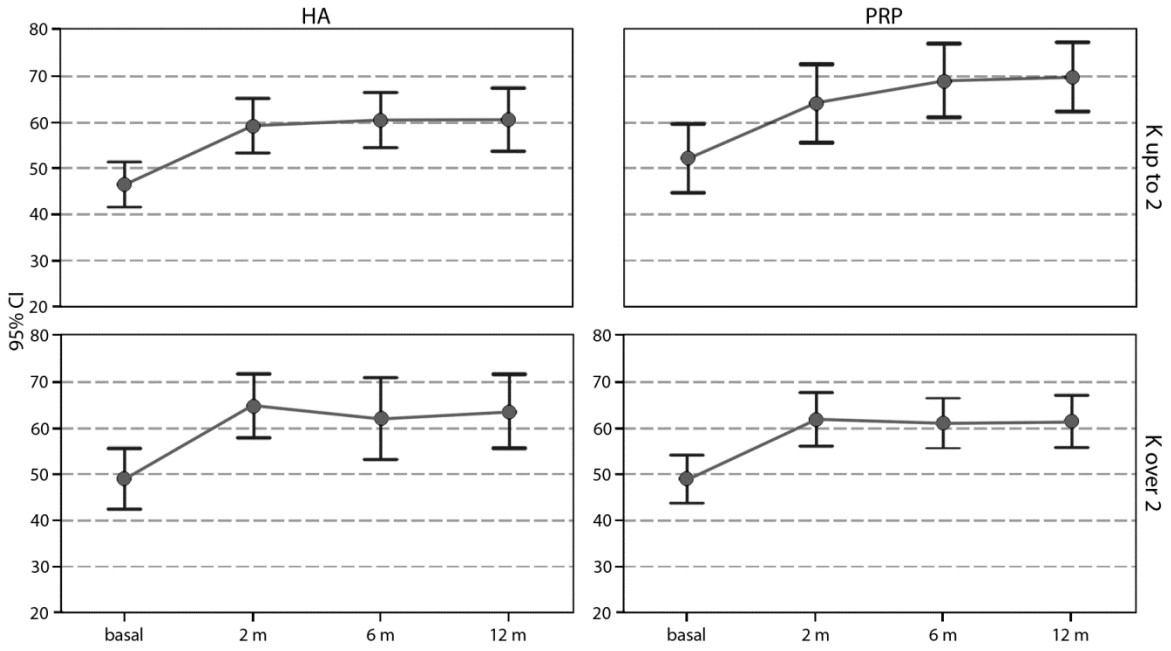

Fig. 1: IKDC subjective results obtained with PRP and HA treatments in two patient subgroups: patient affected by Kellgren Lawrence grade 0-II lesions and patients presenting grade III level of knee degeneration.

The analysis of the results in patients affected by different degrees of cartilage degeneration showed no demonstration of PRP benefit with respect to HA even in the less degenerated cases (Fig. 1).

We registered 3 failures, all in the HA group: in two cases we suspected an intolerance to some components of HA and we stopped the treatment after the first injection. In the third case the patient was still complaining intense pain and functional deficit and he seeked further treatment in another medical center.

DISCUSSION

Despite its wide clinical application, only a few reports have documented PRP results for the treatment of knee degenerative lesions in the literature.[5,6,7,8,9,10] We also previously

performed studies to evaluate this clinical application of PRP, documenting safety and interesting findings,[11,12,13,14] but high level studies are required to confirm these preliminary results. This double-blinded randomized clinical trial presents a number of patients who reached the final 12-month evaluation markedly higher than the majority of the studies published up to the present day. In fact, this currently represents the highest available evidence on PRP use for knee degenerative pathology and gives us some indications on the potential of this biological approach and on its most appropriate clinical use. The analysis of the results obtained with this randomized trial already underlined important aspects. The safety and the clinical improvement of this procedure was confirmed. We observed a higher pain reaction after PRP injection, but without jeopardizing subjective and objective results up to 1 year follow-up. Oppositely to what shown by the current literature, PRP didn't offer better results compared to HA in this series. However, it has to be emphasized that the average age of the selected patients was higher than in other studies.[11,12] Further studies on different patient populations and different PRP products are needed to clarify the potential of this biological approach for the treatment of cartilage lesions and joint degeneration.

CONCLUSIONS

The results of our trial suggest a clinical improvement offered by a PRP injection cycle but no benefit with respect to HA. Unless other studies on different platelet preparations will prove different results, PRP cannot be considered as first line treatment for knee OA, and should be reserved to patients who didn't benefit from other conservative or injective treatments such as viscosupplementation.

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