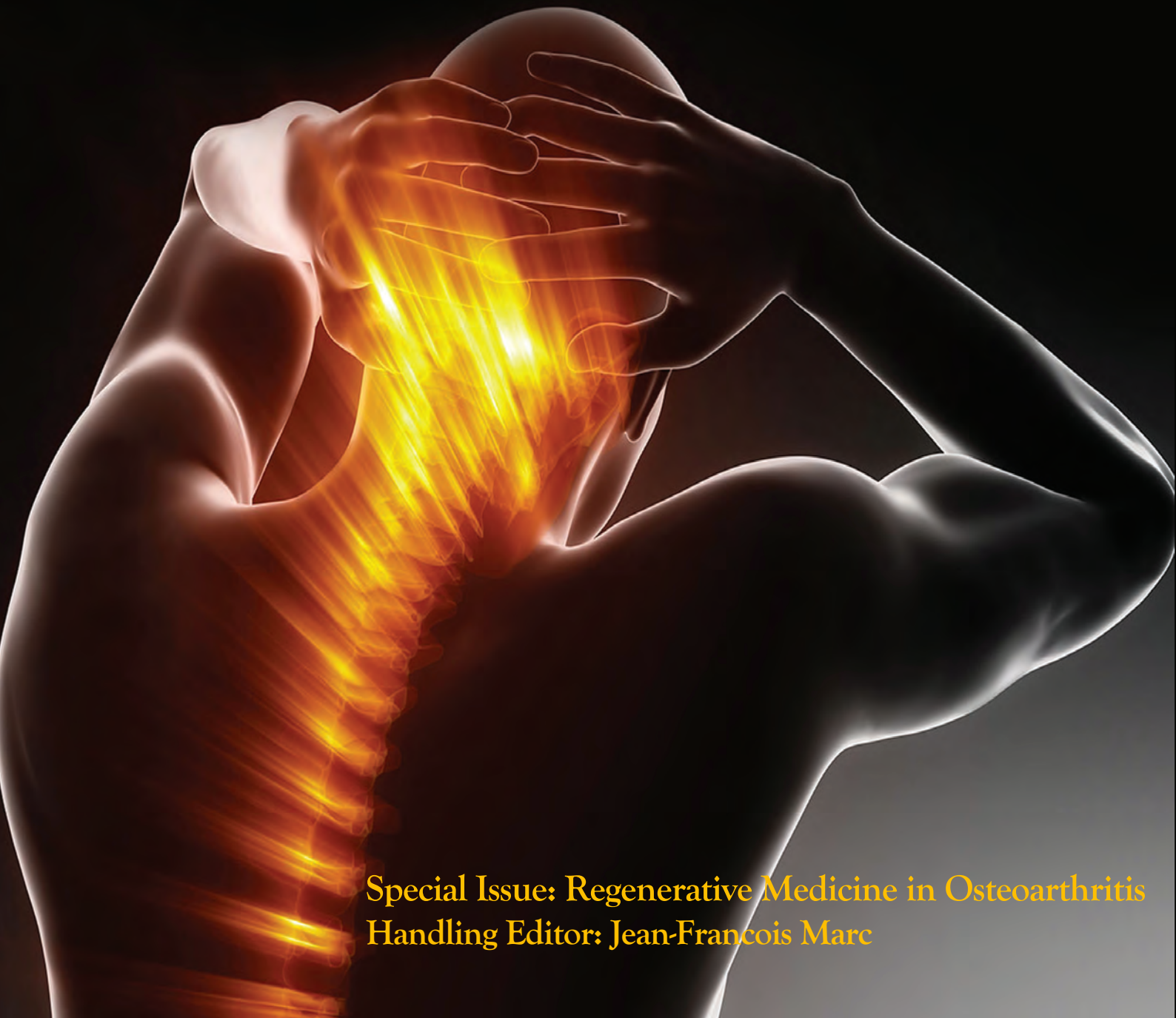


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Regenerative medicine in osteoarthritis

-A new chance for knee osteoarthritis patients

Editorial

There have been many exciting breakthroughs over the last years in medical research in terms of regenerative treatments for degenerative joint disorders in Humans, among which we can mention:

- **Mesenchymal stem cells (MSCs)**, whose benefits to date are essentially clinical and modest, for a high cost of treatment and without histological evidence of definite efficacy [1]. According to the FDA, manipulations prior to the ex vivo cell expansion may represent a significant risk in addition to the potential in vivo secondary transformation of these stem cells or even their differentiation towards an osteogenic lineage [2].
- **Three-dimensional biomaterial scaffolds** (Biomaterials for Tissue Engineering) supplemented with growth factors, more or less cellularized, or chemotactic biomaterials for MSCs represent promising directions for future research. Advanced biocompatible materials mimicking the three-dimensional organization of joint cartilage incorporate stem cells and growth factors either produced locally, or by biotechnological techniques. The future will tell us if this research axis of tissue bioengineering will be one of the therapeutic options for osteoarthritis. It seems however misleading to implant such systems in diffuse osteoarthritic lesions for which the preferred route remains intra-articular-injections [3].
- The local application of **Bone Marrow Cell concentrates** (BMC) harvested by bone puncture, often combined with a Hyaluronic Acid (HA)-based scaffold soaked in Platelet-Rich Plasma (PRP) or even associated with the microfracture technique, has only been described in case reports or small case series on localized cartilage defects so far. On one hand, it

is difficult to determine the specific effect of BMC in these multiple therapeutic combinations and, on the other hand, the second-look arthroscopy shows only dubious results with a neocartilage more fibrocartilaginous than natural hyaline. This histological result is similar to those observed with chondrocyte grafts for the treatment of osteochondritis dissecans [4].

- **Superfactors** such as Prostaglandin Factor 2 (PGF2), involved in regulating PPAR γ expression (protective effects of the peroxisome proliferator-activated receptor gamma) or Fibroblast Growth Factor 18 (FGF18), referred to as Sprifermin, are currently being studied [5]. The promising work conducted in 2014 on cartilage thickness following cycles of 3 injections per week administered every 6 to 12 months of Sprifermin seems to be supported by the post-hoc analysis performed in 2018 [6].

At this stage of our knowledge, we wanted to remain consistent in this special issue and focus on the **PRP/HA combination (CM-PRP-HA)** obtained using a dedicated medical device: Cellular Matrix A-CP-HA kit. Treatment with CM-PRP-HA has the advantage of being a validated simple, inexpensive and clinically effective procedure in terms of pain, stiffness and joint function, while remaining non-invasive and safe [7]. Interestingly, it was recently demonstrated that CM-PRP-HA was structurally effective using both ultrasound and high field MRI.

But, how does it work ?

The rationale underlying the use of a combination of PRP and HA is attractive, the patient being his «own treatment». CM-PRP-HA exerts its anti-inflammatory and regenerative effects through the compounds of the platelet secretome in a three-dimensional network of HA.

W-H Chen et al [8] investigated in vitro the dual biochemical mode of action of the

Jean-Francois Marc*

Rheumatologist, Health Consulting
Agency AGCOSS 12 rue Pierre Dépierré
42300 Roanne, France

*Author for correspondence:
contact.agcoss@gmail.com

combination of PRP and HA, with its anti-inflammatory and potentially regenerative effects on articular cartilage.

In this special issue, Dr. JL Renevier's publication demonstrates the long-lasting clinical efficacy of the CM-PRP-HA combination in patients who had unsuccessful response to previous treatment with HA alone (9). The HA present in the combination seems to improve the biological action of the PRP and to bring superior results than standard treatments with HA alone [10].

Dr. P Adam's publication demonstrates the interest of treating, with this product, degenerative meniscal lesions at an early stage, which otherwise are the precursors of the more advanced stages of knee osteoarthritis.

The clinical and structural effects of this new therapeutic approach for knee osteoarthritis treatment are proved in two studies. Dr B Barac and Dr JF Marc, demonstrate, following repeated injections of the CM-PRP-HA, the quantitative increase of cartilage thickness by ultrasound in 53 patients (90 knees) and the qualitative improvement in proteoglycan (PG) content in cartilage areas by 3 Tesla MRI in 6 patients, respectively.

S Vischer and al, explain the interest of the concept of combining PRP and HA treatment using a specific medical device. Cellular Matrix is the first medical device that has been designed and certified for the preparation of PRP combined with HA (CM-PRP-HA) in compliance with regulations and good practices for class III medical devices and for the therapeutic use of biological tissues for autologous therapy.

To conclude this special issue, the medico-economic study of Dr S Landi describe the significant savings to be expected from this type of treatment for the French national social organizations protecting the health of citizens. This data could be useful for a future consideration of reimbursement of this therapy for patients suffering from osteoarthritis.

However, in the context of evidence-based medicine, it is of key importance to confirm with a larger number of patients the results obtained in the structural proof-of-concept study. It is also crucial to standardize the injection protocol of CM-PRP-HA. A structural clinical study with various injection protocols is, therefore, already underway to clarify these two points.

In practice, and in the primary interest of patients, the place of Cellular Matrix, which is registered in Europe and many other countries, is to be part of the conservative

medical management of osteoarthritis as a first-line treatment and concomitantly with both non-pharmacological (weight loss, physical activity, orthoses, physical rehabilitation) and pharmacological treatment options (analgesics, short-term AINS for inflammatory flares, slow-acting anti-rheumatic drugs).

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“Cellular matrix™ PRP-HA”: A new treatment option with platelet-rich plasma and hyaluronic acid for patients with osteoarthritis having had an unsatisfactory clinical response to hyaluronic acid alone: Results of a pilot, multicenter French study with long-term follow-up

Objective: To evaluate the safety and efficacy of Cellular Matrix™, a new medical device designed for one-step preparation of platelet-rich plasma in presence of hyaluronic acid, for the management of tibiofemoral knee osteoarthritis in patients who had failed to respond adequately to previous treatment with hyaluronic acid alone.

Methods: Multicentre, open-label, uncontrolled, pilot study in 77 patients with grade II or III knee osteoarthritis and a pain at walking score between 3 and 8 on a Numeric Rating Scale. The treatment consisted of a series of 3 intra-articular injections scheduled at D0, D60 and D180 into the affected knee of a combination of platelet-rich plasma and hyaluronic acid prepared with the device Cellular Matrix. The primary efficacy criterion was the variation of pain at walking, as assessed with the Western Ontario and McMaster Universities Osteoarthritis Index (A1 score) between baseline and D270.

Results: Treatment with the combination of platelet-rich plasma and hyaluronic acid prepared with Cellular Matrix significantly reduced pain at walking between baseline and D270. The percentage of responders according to the criteria of the Outcome Measures in Rheumatology Clinical Trial and Osteoarthritis Research Society International was 94.4%. The treatment provided long-lasting benefits for half of the patients and allowed avoiding surgery for almost 80% of them at four years.

Conclusion: A 3-injection course of a combination of platelet-rich plasma and hyaluronic acid prepared with Cellular Matrix was well tolerated and effective in the long-term to relieve pain associated with symptomatic knee osteoarthritis.

Keywords: knee • osteoarthritis • platelet-rich plasma • hyaluronic acid • cellular matrix PRP-HA combination

Introduction

Osteoarthritis (OA) mostly affects people over 60 years of age, and more frequently women, with a sex ratio of 2 women for every man. Knee is a common localization for the disease and it is estimated that the number of subjects with knee OA in France is between 1.8 and 2.3 million [1,2].

Intra-articular injections of Hyaluronic Acid (HA) represent a treatment of choice for

knee OA since they can relieve symptoms for several months. They are designed to restore the concentration and molecular weight of HA in the synovial fluid, leading to a reduction in pain and improvement in physical function of the joint. Effectiveness of HA injections for improving synovial fluid viscoelasticity is widely documented. Indeed, many clinical trials testing different HA preparations have been carried out. Most of the placebo-controlled studies indicated a superiority of HA, whatever its molecular

Jean-Luc Renevier^{1*}, Jean-François Marc², Philippe Adam³, Nicolas Sans⁴, Jacques Le Coz⁵ & Ivan Prothoy⁶

¹Health Center du Lac, René Duguay Trouin Street, 78200 Mantes-la-Jolie, France

²Private practice, 2 Rue Elisée Reclus, 42300 Roanne, France

³Medipole Garonne Sports Clinic, 45 Gironis street, 31100 Toulouse, France

⁴CHU Toulouse, Pierre Riquet Hospital, Place du Docteur Baylac, 31059 Toulouse cedex, France

⁵Private practice, 94 Rue de l'Amiral Mouchez, 75014, Paris

⁶Polyclinic of the Southern Alps, 3-5 rue Antonin Coronat, 05010 Gap, France

*Author for correspondence:
jl.renevier@gmail.com

weight [3-10].

More recently, intra-articular injections of autologous Platelet-Rich Plasma (PRP) have proven to be an attractive alternative therapeutic option for OA. Indeed, the mechanism of action of PRP is based on its content of a range of biological mediators, some of which have anti-inflammatory activity, while others stimulate Mesenchymal Stem Cells (MSC's) and cartilage cells. *In vitro* studies have demonstrated the effects of individual growth factors on stimulation and chondrogenic differentiation of MSC's: MSC's cultured in the presence of Transforming Growth Factor-TGF- β produce significantly more proteoglycan and type II collagen [11]; Insulin-like growth factor 1 (IGF-1) has been shown to have a synergistic effect with TGF- β in inducing chondrogenic differentiation of MSC's [12]; basic fibroblastic growth factor (bFGF) induces proliferation and differentiation of chondrogenic MSC's [13]. The general clinical use of individual growth factors is currently prohibitive due to the complexity and cost of their methods of manufacture and potential adverse effects. Autologous point-of-care PRP is the easiest and safest solution to provide growth factors locally and to render this therapy quickly accessible in the clinical setting. The interest and potential efficacy of PRP in the treatment of cartilage lesions have been validated by *in vitro* studies: PRP increases the synthesis of proteoglycans and collagen in the extracellular matrix of cultivated intervertebral disc cells [14], stimulates proliferation and matrix biosynthesis of porcine articular chondrocytes [15] and shows superior efficacy than a standard culture medium on MSC's proliferation and differentiation into chondrocytes [16].

With respect to clinical data, initially many case series and a pilot study [17] showed improved symptoms following PRP therapy with no serious adverse side effects. A number of larger clinical trials have then been conducted, including one trial on 115 knees [18] which showed that autologous PRP injections improved, in a statistically significant and stable manner, the clinical scores of patients from the end of treatment to six months with respect to baseline scores ($p < 0.0005$). These beneficial effects decreased between 6 and 12 months ($p < 0.02$ with respect to baseline), although they remained better than the baseline scores. All these results suggest that PRP, due to its specific mechanism of action, is an effective and innovative tool in the therapeutic arsenal for the treatment of the

symptoms of knee OA.

Based on the above data, it is reasonable to assume that a combination of PRP and HA could provide added benefit in knee OA with respect to each of the products alone. HA would result in restoration of the rheological properties of the synovial fluid and would potentially favour the biological activities of PRP. Cellular Matrix is a Class III Medical Device which has recently become available and is the sole device which allows the combination of HA with PRP in conformity with regulations.

The objective of this study was to evaluate the effectiveness and safety profile of the Cellular Matrix PRP-HA mix in the management of tibiofemoral knee OA in patients who had failed to respond adequately to previous treatment with HA alone.

Methods

Study design and participants

This is an open-label, uncontrolled, pilot study conducted in 77 patients recruited in 6 French centres. Eligible patients were aged between 40 and 85 years, had radiographically ascertained grade II or III gonarthrosis according to Kellgren and Lawrence scale, had pain at walking between 3 and 8 on a 11-point Numeric Rating Scale and had previously been treated with HA with no satisfactory clinical response (defined as a Western Ontario and McMaster Universities Osteoarthritis Index [WOMAC] A1 score that did not show improvement of at least 3 points three months after the last injection).

Patients were excluded if they had acute inflammatory flare of OA in the affected knee, HA injection in the past 3 months, corticosteroid injection in the previous 2 months, any knee or hip surgery planned within the following 6 months, use of gluco-corticosteroids (except those that are inhaled) and level analgesics in the past 3 months, treatment with symptomatic slow acting drugs for osteoarthritis (diacerein, avocado and soy unsaponifiables, glucosamine sulfate, chondroitin sulfate) initiated in the previous 3 months, an history of allergy to HA, rheumatoid arthritis, surgery in the affected knee in the past 3 months, knee infection during the previous 6 months, a severe disease, and if pregnant or breastfeeding.

Routine laboratory tests (including a platelet count) were performed prior to study inclusion. All patients gave written informed consent.

Treatment protocol

The treatment consisted of a series of 3 intra-articular knee injections of around 4 ml of the combination of PRP and HA prepared with Cellular Matrix device (Cellular Matrix A-CP HA Kit, Regen Lab SA, Le Mont-sur-Lausanne, Switzerland) in accordance with operating instructions supplied with the kit. The device allows automated blood collection and blood component separation in closed circuit. After a five-minute-centrifugation, the resulting product, CM-PRP-HA, is a PRP with a platelet concentration 1.5-1.6 times higher than the baseline in blood, deprived of contamination with red and white blood cells, entrapped in a 3D network of HA.

After study inclusion, the patient was given the first intra-articular injection (D0) under strictly aseptic conditions while lying in the supine or semi-sitting position with the knee extended. Injection was performed using a classical external suprapatellar approach without local anaesthetic following aspiration of synovial fluid in case of intra-articular effusion. After treatment, patients were asked to limit the use of the affected leg for 10 hours; then, patients were allowed to gradually resume normal physical activity. Second and third injections were performed at D60 and D180, respectively, under the same conditions.

Efficacy and safety parameters

All patients were evaluated before the first injection (D0, baseline) and at D60, D180 and D270. The primary efficacy endpoint was the variation of pain at walking (WOMAC A1 score), as measured on an 11-point Numeric Rating Scale, between baseline and D270. Secondary efficacy endpoints consisted of

- The variation of the WOMAC A1 score between baseline and other timepoints and
- The variation of all other items of the WOMAC questionnaire between baseline and D270. Percentage of responders according to Outcome Measures in Rheumatology Clinical Trial and Osteoarthritis Research Society International (OMERACT-OARSI) criteria was calculated as recommended [19].

Briefly, strict responders were defined by a $\geq 50\%$ improvement in pain or function and reduction ≥ 20 mm on a 100 mm Visual Analogue Scale (VAS), whereas responders were defined by a \geq

20% improvement and reduction ≥ 10 mm on a 100 mm VAS in at least 2 of the 3 following areas: pain, function, global assessment of the patient. Safety was evaluated through the collection of information on adverse events at each follow-up visit or if the patients had complaints.

In addition, in order to evaluate the long-term performance of the treatment, a survey among study participants was conducted in December 2017. Questions were about the duration of the benefit of the CM-PRP-HA treatment in terms of pain and function, possible alternative treatments received by the patient such as viscosupplementation, and possible knee surgery undergone by the patient since the end of the study.

Statistical analysis

For statistical analysis, the averages for quantitative variables were compared between timepoints using the Student t test for paired data. In all statistical tests, the significance level

Table 1. Baseline demographic and clinical characteristics of patients.

Age (years)	
N	83 knees
Missing Data	0
Minimum/Maximum	40/84
Median	63
Mean (SD1)	62 (10.817)
Sex	
Male	48 (57.8%)
Female	35 (42.2%)
BMI	
N	83
Missing Data	0
Minimum/Maximum	20.32/39.06
Median	26.83
Mean (SD1)	27.406 (3.90)
Normal corpulence	23 (27.7%)
Overweight	42 (50.6%)
Moderate obesity	13 (15.7%)
Severe obesity	5 (6%)
Kellgren and Lawrence grades	
N	83
Missing data	0
Grade II	36 (43.4%)
Grade III	47 (56.6%)
Number of patients included by centre	
Centre 1	20 (24.1%)
Centre 2	10 (12%)
Center 3	16 (19.3%)
Center 4	8 (9.6%)
Center 5	17 (20.5%)
Center 6	12 (14.5%)
SD: standard deviation; BMI: Body Mass Index	

was set at 0.05.

Results

Demographic and clinical data

In total, 77 patients (83 knees) were recruited for this study between September 2013 and April 2014. Baseline demographic and clinical characteristics for these patients are summarized in Table 1. Out of these 77 patients, 10 withdrew for various reasons (6 dropout, 1 lost to follow-up, 1 for reasons independent of the study, 1 due to osteonecrosis of the lateral femoral condyle and 1 because of a worsening on X-ray of the arthritic disease from Kellgren & Lawrence grade II at baseline to III).

All patients reported having been treated previously with HA but did not respond satisfactorily to it. The most frequently reported HA were: Go-On (28%), Structovial (14%) and Durolane (12%). The percentage of other reported HAs was less than 10%.

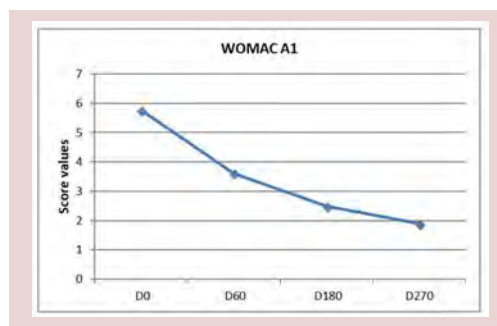
Primary outcome

Treatment with CM-PRP-HA decreased pain at walking between baseline and D270 by 65%, as measured by the WOMAC A1 score ($p < 0.05$). This decrease was constant throughout the 9 month follow-up period, although the reduction was less marked between D180 and D270 ($p = 0.079$) (Figure 1 and Table 2). This reduction was observed whatever the Body Mass Index (BMI) of patients (Figure 2) and regardless of the Kellgren and Lawrence grade II or III of knee OA (Figure 3).

There was no interaction between the variation of the WOMAC A1 score and the investigating centres.

Secondary outcomes

The studied treatment also significantly improved



WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index

Figure 1. Graphical representation Assessment of pain on walking (WOMAC A1) at D0, D60, D180 and D270.

the WOMAC pain subscore (taking into account all 5 items of the WOMAC A subscale) (Figure 4A), WOMAC stiffness subscore (Figure 4B), WOMAC physical function subscore (Figure 4C) and the overall WOMAC score between baseline and D270 in a statistically significant way ($p < 0.05$ for all assessed scores) (Figure 4D).

The proportion of responders according to the OMERACT-OARSI criteria at D270 was 94.4% (Figure 5), whereas the proportion of strict responders was 83.6% (Figure 5). However, due to missing data, the percentage calculation could only be performed on 54 patients for the responders, and on 60 patients for the strict responders.

Long-term evaluation

We were able to collect long-term data for 62 out of 77 study participants (80.5%). 59.7% of the them still perceived substantial clinical benefit 2 years after the treatment, while 50% were still satisfied with it at the time of the survey (4 years after the treatment). This effect was due to the initial 3-injection course for 61.2% of them, while 38.8% had to receive additional injections of the treatment to continue to perceive its positive effects on the long-term (1 to 3 injection(s)/year). For 79% of them, the treatment allowed avoiding surgery.

Safety

No serious adverse events were reported. Only 13.25% of patients experienced one or more adverse events (11 in all) related to the treatment. Most of them consisted of mild to moderate inflammatory reactions at the treated site; only one consisted of violent pain which lasted 6 hours.

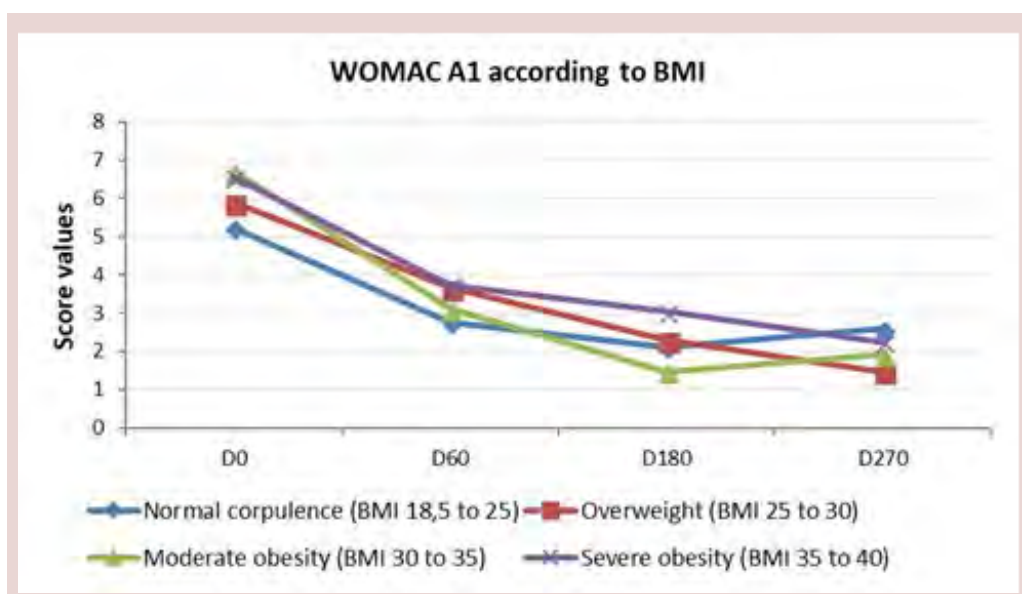
Discussion

In this French multicentre study, we show that a 3-injection course of the Cellular Matrix combination of PRP and HA can safely and drastically decrease pain and stiffness and improve function of the joint in patients with mild to moderate knee OA. In addition, we demonstrate the long-term efficacy of this treatment, as half of treated patients who answered our survey continued to experience improvement of their condition two to four years after the end of the initial 3-injection course treatment.

HA has long been recognized as a treatment option in the conservative management of knee OA, due to its lubrication, shock-absorption, anti-inflammatory and chondroprotective

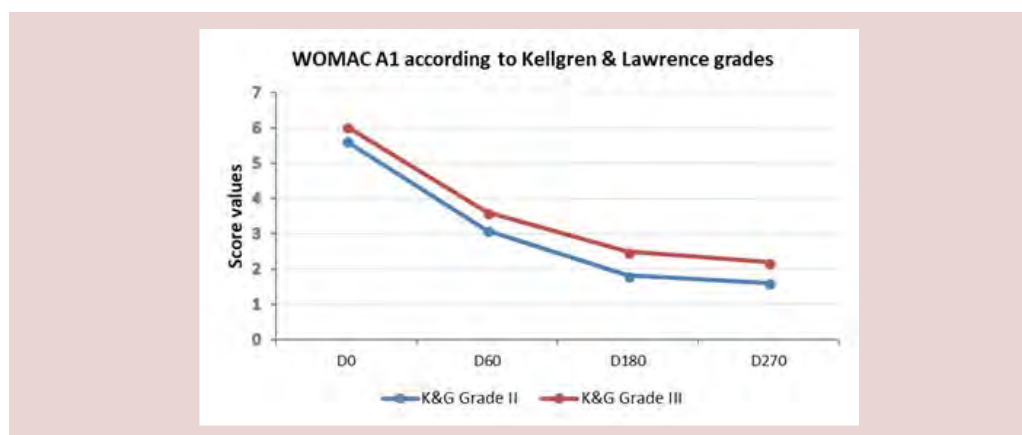
Table 2. Assessment of pain on walking (WOMAC A1) at D0, D60, D180 and D270.

	Score WOMAC A1 (SD)	P value versus baseline	Improvement from baseline
D0 (Baseline)	5.87 ± 1.53	N/A	N/A
D60	3.39 ± 1.88	0.000**	40.16%
D180	2.18 ± 2.03	0.000**	62.92%
D270	1.89 ± 1.76	0.000**	64.97%
P values			
D60 Versus D80	0.000**		0.000**
D60 Versus D270	0.000**		0.000**
D180 Versus D270	0.079 ns		0.208 ns
**highly significant; ns: non-significant WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index			



BMI: Body Mass Index; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index

Figure 2. Assessment of pain on walking (WOMAC A1) at D0, D60, D180 and D270, according to BMI categories.



K&G: Kellgren & Lawrence; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index

Figure 3. Assessment of pain on walking (WOMAC A1) at D0, D60, D180 and D270, according to Kellgren & Lawrence grades.

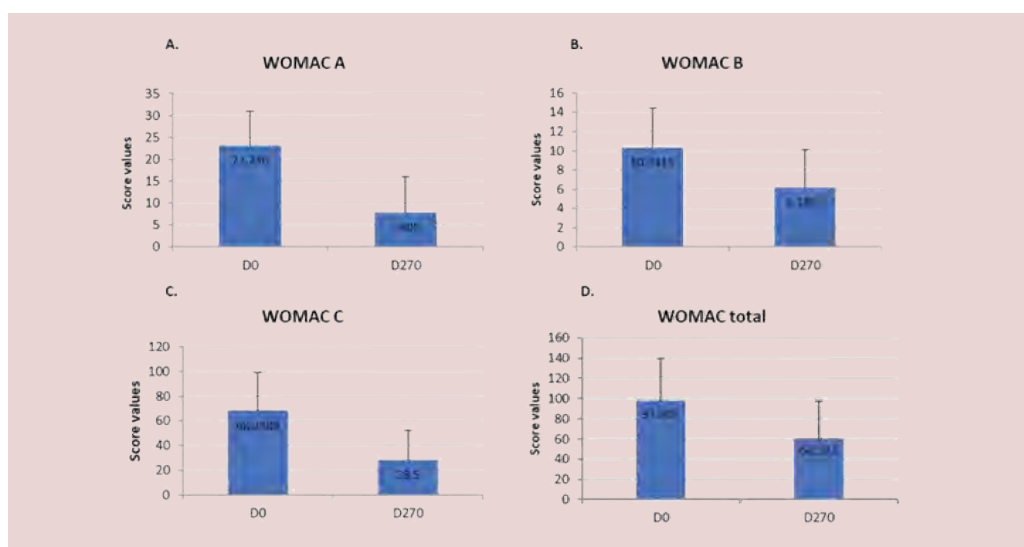
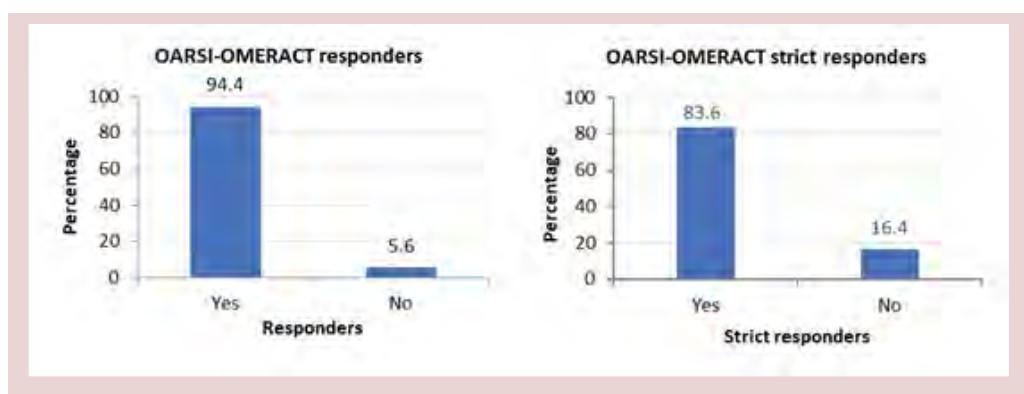


Figure 4. WOMAC Assessment- Assessment of WOMAC pain subscore (WOMAC A) (Figure 4A), WOMAC stiffness subscore (WOMAC B (Figure 4B), WOMAC function subscore (WOMAC C) (Figure 4C) and total WOMAC score at baseline and D270 (Figure 4D). WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.



OMERACT-OARSI: Outcome Measures in Rheumatology Clinical Trial and Osteoarthritis, Research Society International

Figure 5. Percentage of responders and strict responders according to OMERACT-OARSI criteria.

properties. Nevertheless, a significant proportion of patients doesn't respond positively to HA therapy and is therefore more likely to undergo surgery [20].

In this study, we focused on this well-defined population of OA patients whose symptoms could not be satisfactorily relieved by previous treatment with hyaluronic acid. Each patient aged 40 to 84 years received three intra-articular injections of CM-PRP-HA at D0, D60 and D180, and was followed-up for a period of 9 months. Results showed that treatment of Kellgren and Lawrence grade II or III OA with CM-PRP-HA decreased pain on walking by 65% at the end of the 9-month follow-up period and also improved WOMAC stiffness and physical

function scores. In addition, 94.4% of patients were considered responders to treatment based on OMERACT-OARSI criteria. This percentage has to be compared with that from Bowman et al. study who tried to identify patient and treatment factors related to successful HA treatment [20]. The authors report that only 57% of the patients met OMERACT-OARSI criteria for successful response to HA treatment following a 3-week regimen of HA, and that patients with grade I or II OA were 2.2 times more likely to respond to HA injections than those with grade III OA. In our study, we found no difference in treatment response between grade II and III OA patients. This implies that treatment with Cellular Matrix may be particularly relevant for patients with grade III OA who are more likely to have

unsuccessful outcomes with HA. As in Bowman's study, we found no statistical correlation with BMI, although we observed a trend to higher improvement in overweight patients.

On contrary, PRP is a relatively new option for OA treatment. It has been shown through several meta-analyses that PRP is superior to HA to relieve pain and improve function in patients suffering from knee OA [21-29]. More precisely, the Riboh et al. meta-analysis showed that only leukocyte-poor PRP, such as the PRP in CM-PRP-HA combination, was significantly superior to HA [30]. The rationale for PRP use is based on the biological stimulation of cartilage and mesenchymal stem cells through the active secretion of platelet growth factors during treatment. Additionally, PRP exerts its beneficial effects through the modulation of the inflammatory response by balancing pro- and anti-inflammatory factors [31]. This has been specifically demonstrated for the PRP prepared using the same technology as in the Cellular Matrix device in the Chen et al. study [32].

In recent years, based on a number of *in vitro* studies, it has become more and more obvious that the association of PRP with HA could provide added benefit for the treatment of joint degenerative diseases, due to their different mechanisms of actions to modulate the disease process [33]. Indeed, Sundman et al. [34] showed in their study measuring the effects of PRP and HA separately on synovioocyte and cartilage co-cultures that only HA-treated co-cultures resulted in a decrease in the pro-inflammatory cytokine IL-6, while only PRP-treated co-cultures resulted in a decreased gene expression of metalloproteinase-3 (MMP-3) and in an increased gene expression of hyaluronan synthase-2. This suggests that PRP and HA could have complementary beneficial anti-inflammatory and anabolic effects on joint cells and that a combination of them might produce better outcomes than either PRP or HA alone for the treatment of OA.

In support of this hypothesis, Chen et al. [35] demonstrated in their *in vitro* OA cell model cultured in presence of either PRP, or HA or a combination of both that PRP+HA can inhibit inflammation more efficiently than do PRP or HA alone. Chondrogenesis was also induced more strongly by PRP+HA than by PRP or HA alone. In addition, rescue of the decreased extracellular matrix synthesis by the PRP+HA combination was also higher than by PRP or HA

only. These findings were further supported by similar analyses conducted in their 3D arthritic neo-cartilage model, as well as in an OA mice model injected with either PRP, or HA, or both. Finally, Russo et al. [36] demonstrated that chondrocytes cultured in a PRP+HA-containing medium synthesize glycosaminoglycan at a significantly higher level than when cultured in the other culture conditions (PRP or HA only).

From a clinical point of view, the association of PRP and HA treatments also provided promising outcomes. Indeed, Lana et al. [37] who treated 105 patients suffering from Kellgren and Lawrence I to III knee OA with either HA, or PRP or both, found that the improvement in pain and physical function scores was significantly higher in patients treated with consecutive injections of HA and PRP, in comparison to each product administered separately. Interestingly, Saturveithan [38] and Chen [39] reported that the association of PRP and HA injections was also able to provide pain relief and functional improvement in patients with advanced knee OA, suggesting that combining these treatments could allow postponing the need for arthroplasty [39].

In these studies, however, the association of PRP and HA was obtained by sequential injections of PRP and HA. Cellular Matrix is the first dedicated medical device allowing to prepare PRP in presence of HA in a simple, safe and reproducible procedure. Abate et al. [40] conducted a retrospective comparative study on a patient group treated with PRP only compared to a patient group treated with the Cellular Matrix CM-PRP-HA combination. Interestingly, in this study, the device used to prepare PRP (RegenKit-BCT, Regen Lab, Switzerland) was based on the same technology as Cellular Matrix, except that it didn't contain HA and the PRP volume was almost twofold that of Cellular Matrix. As the authors observed that the CM-PRP-HA combination had the same efficacy as PRP prepared with RegenKit-BCT administered in higher volume, they concluded that the presence of HA could improve PRP properties, hypothesizing that this could be done by creating a bioactive scaffold around cells that would increase the residence time of growth factors.

Our study has some limitations. First, it doesn't include a control group with which to compare the effects. Patients' improvement is compared against their baseline values but it could have

been much more evidenced if compared with a placebo-treated group. Second, there is a high rate of missing data due to incomplete WOMAC questionnaire at the 9 month assessment that could have introduced bias in the estimates.

In conclusion, our study aimed at exploring the feasibility, safety and efficacy of using a combination of PRP and HA prepared with a dedicated medical device (Cellular Matrix) to treat patients suffering from mild to moderate knee OA who failed to respond adequately to a previous treatment with HA alone. Our results suggest that the association of both components using the Cellular Matrix technology is a safe and effective treatment for relieving symptoms associated with knee OA. Interestingly, long-term evaluation demonstrated that this treatment was still effective for at least 2 years for 50% of the patients that completed our survey and allowed avoiding surgery for almost 80% of them. Cellular Matrix technology may therefore represent a new medical alternative to knee surgery after failure of HA or, at least, a viable strategy allowing to delay the need for joint replacement surgery. Even though the exact cellular and molecular mechanisms underlying the association of PRP and HA still need to be elucidated, currently available clinical data with Cellular Matrix clearly makes it a promising and safe new player in the therapeutic arsenal for knee osteoarthritis.

Disclosure of Interest

JLR, JFM and PA received consulting fees from Regen Lab SA. The other authors declare that they have no competing interests.

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A novel treatment of knee degenerative disorders all-in-one intra-articular injection of platelet-rich plasma combined with hyaluronic acid

Purpose: Therapeutic evaluation of all-in-one intra-articular (IA) injections of autologous platelet rich plasma (PRP) combined with hyaluronic acid (HA) for knee degenerative disorders.

Material and methods: The protocol consisted in one or three IA injections, generally ultrasound guided, of a combination of PRP and HA prepared with the innovative Cellular Matrix device (CM-PRP-HA) on three cohorts of patients: A first cohort of 202 patients with meniscal lesions, comprising 93 patients with grade II or III stable meniscal tear, 59 patients after meniscal suture and 50 patients with meniscal cyst, a second cohort of 20 patients with Grade II or III osteoarthritis (OA) and a third cohort of 40 patients presenting post traumatic bone marrow edema (BME) comprising 20 patients with post-traumatic algodystrophy (PTA) and 20 patients with post-traumatic osteoarthritis (PTOA). The International Knee Documentation Committee (IKDC) subjective knee score and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain scale as well as magnetic resonance imaging (MRI) and ultrasounds were used to assess results.

Results: Patients with meniscal lesion were treated with one injection of CM-PRP-HA. The follow up evaluation was done after one year. Significant improvement in the IKDC score (79.6/100 vs. 42/100 before treatment) was observed for patients with meniscal tears. Patients with meniscal suture presented no failure, while the success rate for patients treated for meniscal cysts was 70%. Patients with degenerative OA received 3 IA injections of CM-PRP-HA at day 0, at 2 months and at 6 months. Significant difference in the WOMAC pain scale was observed during the final evaluation at 9 months compared with value at day 0 (2.45 vs. 5.65). Patients presenting post traumatic bone marrow edema were treated with one IA injection of CM-PRP-HA and evaluated after one month. The pain score decreases from 8 to 4 for PTA and PTOA patients. This result was correlated with a reduction of bone marrow edema observed with MRI.

Conclusions: The Cellular Matrix device has been designed to prepare intra-articular injections of a combination of PRP and HA for symptomatic treatment of articular pain and mobility improvement, essentially for patient suffering from knee OA. In this study, other indications for this innovative treatment are also proposed. CM-PRP-HA appears to be a more efficient alternative to visco-supplementation with HA for the symptomatic treatment of knee articular disorders, including pain reduction and increase of knee functionality for patients suffering of osteoarthritis, post traumatic bone marrow edema, meniscal tears, healing of meniscal suture and size reduction of meniscal cysts. Further investigations will determine the optimal frequency of IA injections with CM-PRP-HA, and whether this innovative product would represent not only a conservative treatment for various knee articular disorders but also a preventive treatment for OA, thus delaying the need for knee surgery.

Keywords: platelet-rich plasma • hyaluronic acid • degenerative osteoarthritis • bone marrow lesions • bone marrow edema • meniscal lesions • regenerative medicine

Introduction

Knee is the most common location for symptomatic degenerative OA and the risk of developing this pathology is almost 40% for men and 47% for women [1]. To treat this frequent and expensive pathology, recommendations of all Academic Societies relate to the use of non-pharmacological and pharmacological therapies [2-4]. Intra-articular injection of corticosteroids

only claims to be effective in the acute phases of OA, by reducing pain and articular effusion, thus allowing to dry up the joint. However, the frequency of these injections is limited due to the side effects of corticoids.

The interest of visco-supplementation with HA IA injections in managing osteoarthritic knee pain is well known [5-7]. HA is a major component of healthy synovial fluid. It acts

Philippe Adam¹, Jean Luc Renavier² & Jean-Francois Marc³

¹Head, Medical Imaging Department, Medipole Garonne Sports Clinic - 45 rue de Gironis, 31100 Toulouse, France

²Health Center du Lac, René Dugay Trouin Street, 78200 Mantes-la-Jolie, France

³Private Practice, AGCOSS, 2 Elisée Reclus Street, 42300 Roanne, France

*Author for correspondence:

adamphhj@yahoo.fr

as a lubricant and shock absorbing agent and contributes to joint homeostasis through various biomechanical and biological mechanisms, including cell-receptor interactions [5]. The loss of endogenous HA appears to contribute to joint pain and stiffness. The place of the visco-supplementation among current therapeutics agents seems to be a third alternative after failure of analgesic drugs and failure or intolerance to NSAIDs. However, the effectiveness of HA for OA is mainly on reducing pain and doesn't last more than a few months [6].

To improve the efficiency of IA medical treatment of OA, Sampson et al. [8] were the first to study the effect of IA injection of PRP in 14 patients with primary and secondary knee OA. These patients received 3 PRP injections at a four-week interval in the affected knee. There were no adverse events reported. Moreover, this study demonstrated significant and almost linear improvements in knee injury and OA outcome scores, including pain and symptom relief. The majority of the patients expressed a favorable outcome at 12 months after treatment.

Filardo et al. [9] treated 91 patients presenting with a chronic knee degenerative condition equally with three IA PRP injections with a follow up at 6, 12 and 24 months. Significant improvement was seen the first year however, all the evaluated parameters were significantly lower at 24 months with respect to the 12-month evaluation. The IKDC objective evaluation fell from 67 to 59% of normal and nearly normal knees while the IKDC subjective score fell from 60 to 51. The median duration of the clinical improvement was 9 months even though the results at 24 months remained significantly higher than the value before treatment. These findings confirmed that treatment with PRP injections can reduce pain and improve knee function and quality of life with a longer efficacy than HA.

Subsequently, Anitua et al. [10] showed that PRP stimulated the biological properties of HA, and Guler et al. [11] evoked a favorable action of IA PRP by comparing its use with that of HA in early-stage knee arthritis. The 2015 study of Gobbi et al. [12] also showed that IA PRP injections used for symptomatic early stages of knee OA induced significant reduction in pain and improved function after 12 months, which can be further improved at 18 months by annual repetition of the treatment. The same year, Marmotti et al. [13] reviewed the efficiency of

IA injections with PRP for cartilage lesions and evoked the potential negative impact of the presence of high neutrophil content in leukocyte rich PRP.

A few clinical articles are concerned with the novel concept of combining PRP and HA injections for the treatment of degenerative disorders of the knee. This therapeutic association would take advantage of the synergistic anabolic actions of these two active substances introduced into the synovial environment. This is further supported by the fact that stimulation of collagen synthesis is correlated with growth factors and the regenerative effect of PRP on the chondrocyte [14-16].

Chen et al. [14] studied the effects of HA and PRP in an *in vitro* OA chondrocyte model and found that this association could rescue pro inflammatory cytokines-induced degeneration through chondrogenic signaling recovery. They strongly believed that PRP combined with HA could attenuate cartilage degeneration.

In the study by Sundman et al. [15], an *ex vivo* co-culture system of OA cartilage and synoviocytes, both PRP and HA enhanced metabolism (decreasing catabolism) and diminished markers of inflammation and nociception (TNF α). In synoviocytes, the use of PRP alone significantly decreased Matrix Metallo-Proteinase (MMP)-13 expression. MMP-13 is recognized as integral in cartilage matrix degradation during the development and continuation of OA.

These two studies were very helpful in understanding the mechanisms of this biological treatment which can produce a fundamental analgesic and anti-inflammatory effect by controlling the secretion of nociceptive and inflammatory mediators from cartilage and synoviocytes. Overall, an association of HA and PRP could act against the articular damage generated by trauma and degenerative OA, and improve patient-reported pain and functional scores and, if successful, could delay or avoid surgery [16-19].

The Cellular Matrix device is the sole medical device that has been designed and certified for the preparation of PRP combined with HA in a manner conform to regulations and good practices. Two recent papers highlighted the effects of intra-articular injections of the Cellular Matrix PRP-HA combination for the treatment of patients suffering from knee OA [20,21]. Renevier et al. [21] reported the good

results of CM-PRP-HA in a pilot, multicenter French study with a long-term follow-up, after a series of three I-A injections scheduled at D0, D60 and D180, providing long-lasting benefits for half of the patients and avoiding surgery for almost 80% of them at four years. The Medipole Garonne Sports Clinic was one of the centers participating in this study.

The purpose of this work, performed in Medipole Garonne is to assess the current situation of the CM-PRP-HA protocol for the treatment not only of degenerative knee OA but also of other knee disorders and to determine practical indications for clinical use.

Materials and methods

CM-PRP-HA protocol

The Cellular Matrix (CM) A-CP HA Kit (Regen Lab SA, Le Mont sur Lausanne, Switzerland) is a Class III medical device (European classification). CM has been specifically approved for the single step preparation, from a small sample of patient's blood, of autologous PRP in presence of HA in a sterile closed-circuit system. The CM device is an evacuated tube in which the patient blood sample (6 ml) is automatically collected. The blood-filled tube is centrifuged for 5 minutes at a relative centrifugal force of 1500 g (following the manufacturer's instructions). Thanks to the Regen Lab separating gel technology, the platelets and plasma are isolated from the other blood components and combined with the HA solution present in the device. The resulting CM-PRP-HA consists of around 3 ml of autologous PRP, with a platelet concentration 1.5 to 1.6 times higher than the baseline value in venous blood and with a low contamination in red and white blood cells (neutrophil poor PRP), entrapped in a 3D network of HA. The device contains 2 ml of natural, non-cross-linked HA at a concentration of 20 mg/ml (40 mg total). The HA is produced by bacterial fermentation, thus devoid of animal proteins. CM is approved for both orthopaedic and dermal applications in Europe, and clinical studies are ongoing in the US to obtain FDA pre-market approval.

CM-PRP-HA injection was always preceded by a medical consultation with analysis of clinical and imaging data, collection of patient consent and examination for infectious or haemorrhagic risks with temporary interruption of anticoagulants drugs if necessary. A blood count was also required. Systematic control MRI was done after the treatment. To reduce pain, premedication

(antalgic drug and anaesthetic patch one hour before injection) and inhalation of analgesic gas (Entonox® MEOPA, Linde Healthcare, during injection) were systematically used.

The I-A injection (generally US guided) of the CM-PRP-HA combination was performed just a few minutes after its preparation in the same room, with all aseptic precautions during the entire procedure. The overall procedure is simple, easy and relatively short, typically lasting less than 20 minutes. NSAIDs were prohibited one month before and after IA injection to avoid hampering the PRP effect on the healing process.

Selected pathologies

Meniscal cohort

The meniscal cohort included patients with stable meniscal tear and patients after meniscal suture or with meniscal cyst. The first group of patients suffered from grade II and III meniscal degenerative tears with an unsatisfactory clinical response to standard medical treatment (NSAIDs or HA alone). MRI classification of Stoller et al. [22] was used before and after the treatment: Grade I lesion is described as nodular intrameniscal hypersignal, Grade II as linear intrameniscal hypersignal without articular surface extension, and Grade III as linear intra-meniscal hypersignal with extension towards at least one articular surface (e.g. meniscal tear, unhooking sign). The main clinical criterion for patient inclusion was a stable meniscal lesion into a stable knee. The IKDC subjective knee score ("well-being" scale between 0 and 100) [23] evaluated reliability, validity, and responsiveness to the CM-PRP-HA treatment.

From August 2012 to June 2013, 93 patients (aged between 23 and 84 years, mean age 49, gender ratio: 24% female vs 75% male) suffering from Grade II or III (80% grade III) stable horizontal lesion (85% medial meniscus, 15% lateral meniscus, RR or RW meniscal area) were treated with only one IA injection of CM-PRP-HA. If effusion was present, an arthrocentesis was done before injecting the product into the joint. All injections were US-guided into the joint by sub-patellar way. The IKDC score for this group was evaluated at a final follow up in August 2015.

A second group of 59 patients (50 men, 9 women, mean age 25 years) benefited of one injection of CM-PRP-HA at one month after surgical meniscal suture for bucket handle lesion (40 cases) or unstable meniscal flap (19 cases), in

an attempt to strengthen meniscal healing and to reduce failures of surgery with recurrence of an instable tear [24].

US guided mechanical treatment of meniscal cysts was also performed for 50 patients with drilling and emptying of the cyst followed by a single injection of CM-PRP-HA into the joint by patellar way. Our purpose was to avoid the surgical ablation of the cyst and meniscus by stimulating the healing of the meniscal tear and the communicating channel.

Knee degenerative OA cohort

From September 2013 to April 2014, 20 patients (13 males and 7 females, aged between 40 and 77 years, mean age 59 years, mean BMI 25,83) suffering from knee OA of Kellgren and Lawrence [25] grade II (10 patients) and III (10 patients) were enrolled in Medipole Garonne as part of the multicenter French study of Renevier et al. [21]. Other selection criteria were failure to respond to HA treatment in the previous 3 months, and not taking analgesics or NSAIDs or anti-OA medication in the previous 3 months.

Therapeutic injections with CM-PRP-HA were performed by sub-patellar access at Day 0, Month 2 and Month 6 and evaluated at these three time-points using the WOMAC scale (Visual Analogue Scale (VAS) for pain, 0-10 cm) [26] WOMAC evaluation was also done at a final follow-up at Month 9. If effusion was present, an arthrocentesis was performed before injecting the product into the joint.

MRI was performed before the first injection in order to characterize the pathology, and at the end at 9 months. Only one MRI feature was noted: namely the presence or absence of Bone Marrow Edema (BME) before and after the treatment. BME was found in bone under areas where cartilage was damaged and was correlated with Bone Marrow Lesions (BML). MR Fat Sat PD-weighted sequences were always performed in the same order to detect the hyper-signal assimilated to edema femoral and/or tibial BME.

Bone marrow edema cohort

Twenty patients (13 men and 7 women) presenting condylar (N=15) or tibial (N=5) BME with PTA following surgery for cruciate ligament repair received one US-guided IA injection of CM-PRP-HA. Two criteria were noted at one month, namely VAS pain score and decrease or disappearance of BME *via* MRI.

Twenty patients with PTOA, 16 men and 4

women, presenting medial (N=15) or lateral (N=5) condylar BME, associated with an infra-centimeter superficial osteochondral defect, received one US-guided IA injection. Two criteria were noted at one month, namely VAS pain score and decrease or disappearance of BME around traumatic osteochondritis *via* MRI.

Results

Meniscal cohort

For patients with grade II and III degenerative meniscal tears there was a significant improvement in the IKDC subjective score one year after the beginning of CM-PRP-HA treatment, with a mean score of 79.6 (range 50 to 100/100) compared with 42 (range 0 to 60/100) before CM-PRP-HA. Improvement of meniscal tear (partial or total reduction of the tear with decrease of grade III towards grade II, reduction of peripheral cyst around the meniscal wall), and reduction of associated signs (synovial and collateral ligament hyper-signal, joint effusion) were always correlated with clinical improvement (Figure 1). It should be noted that 10 patients (10/93) failed to respond to the treatment and required surgery (1 suture and 9 partial meniscectomy) due to a poor evaluation of meniscal stability before the beginning of the study. A follow-up study at 2 years in August 2015 revealed that 52% of subjects exhibited long-term improvement with no severe adverse events reported.

For meniscal sutures injected after surgery, we found no failure of meniscal suture at the one year follow up, which indicates that CM-PRP-HA seems to enhance healing of meniscal sutures. For meniscal cyst, rate of success at one year was 70%. Fifteen patients (30%) had an unsatisfactory response to the treatment with pain and no reduction in size of the cyst, particularly when cyst emptying was not satisfactory at the beginning of the process.

Knee degenerative OA cohort

The results for the sub-cohort of patients from Medipole Garonne from the French multicenter study (21) were considered satisfactory with a reduction in pain at 9 months always correlated with a decrease or total resolution of BME observed by MRI (Figure 2). The hyper-signal assimilated to edema was always decreasing or disappearing. The variations for the WOMAC pain scale were also significant with a mean value of 5.65 at the beginning of the treatment, 3.8 at Month 2, 2.95 at Month 6 and 2.45 at Month

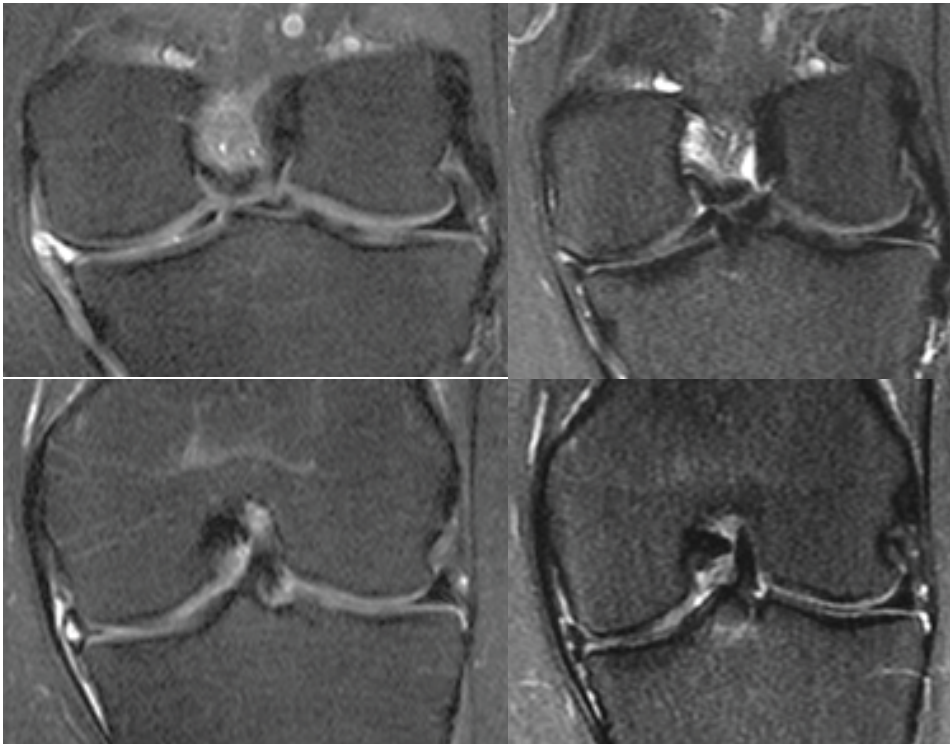


Figure 1. Grade III medial meniscus degenerative tear with peripheral cyst; Control MRI before (left side) and after CM-PRP-HA (five weeks) clearly demonstrate the decrease of meniscal wall edema, cyst and tear. Clinical improvement was correlated.

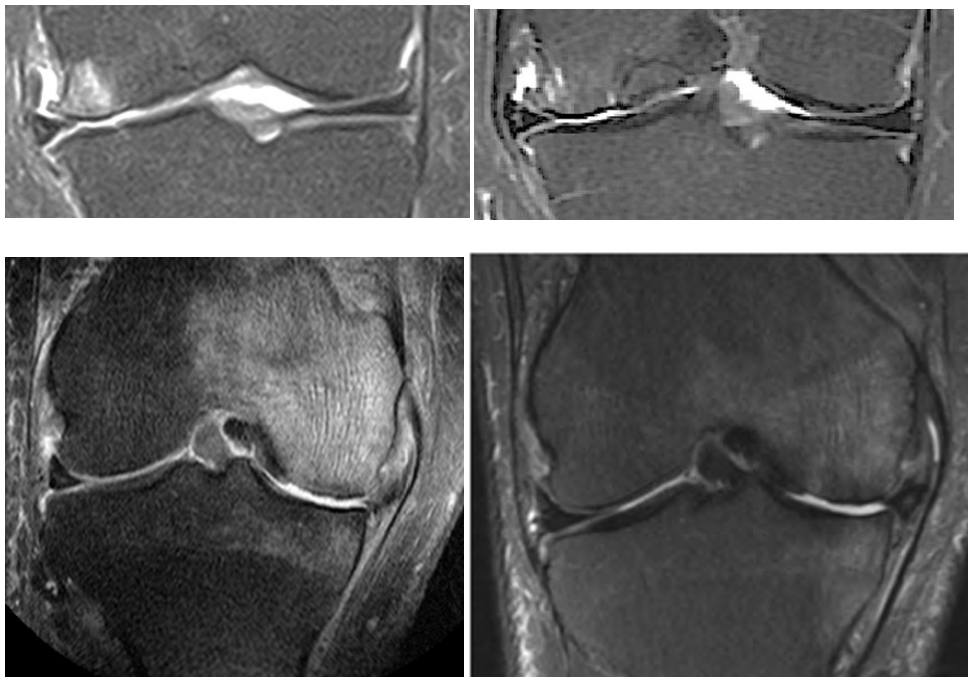


Figure 2. Two cases of Knee KL III OA; MRI was performed before (left side) and after CM-PRP-HA (right side). Condylar BME obviously decreased, and degenerative meniscal lesion was improved (meniscus arthritis) with reduced pain.

9 (Figure 3). Only two adverse events were recorded. They were inflammatory reactions which lasted 5 and 7 days respectively, after the first injection. They were treated with ice applications and analgesics.

In November 2017, approximately 4 years after the start of the OA study in Medipole Garonne, we saw in consultation half of patients (the other half was lost to follow up). Most of them had a satisfactory functionality, and had not undergone prosthetic device surgery. These patients were keen to repeat the treatment. Thus, we can confirm the long term efficiency of intra-articular injection with CM-PRP-HA in comparison with a standard visco-supplementation. Results in the sub-cohort from Medipole Garonne are parallel to that of global multicenter study [21] in which 94.4% of the treated patients for OA of the knee were considered responders to treatment based on the OMERACT-OARSI criteria [27].

Bone marrow edema cohort

For the 20 patients with PTA, the decrease of pain (mean VAS score reduced from 8 to 4) was always correlated with a reduction or disappearance of BME *via* MRI (Figure 4). For the 20 patients with PTOA, the results were similar, with a reduction of the hyper-signal ring around osteochondritis (target sign) (Figure 5). Reduction of BME was highly correlated with a reduction in pain. CM-PRP-HA was effective at one month, and the results remained good for a sample of 5 patients seen again at 6 months.

Since this study, CM-PRP-HA has been used on a regular basis in our clinic. A total of 2328 IA injection procedures with CM-PRP-HA were performed from August 2012 to June 2018 (Figure 6). The largest age group (62%) was between 41 and 60 years of age, with a relatively small group aged less than 21 years (1%). The anatomical distribution of IA injections is provided in Figure 7, with the majority for the

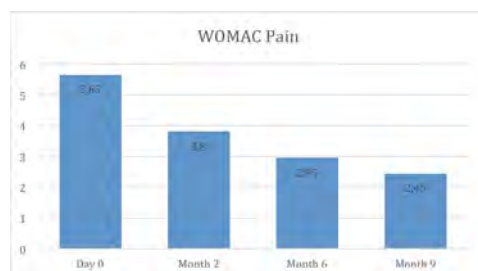


Figure 3. WOMAC Pain at Day 0, Month 2, Month 6 & Month 9 after 3 sub-patellar CM-PRP-HA injections (sub-cohort of 20 patients from “Medipole Garonne”).

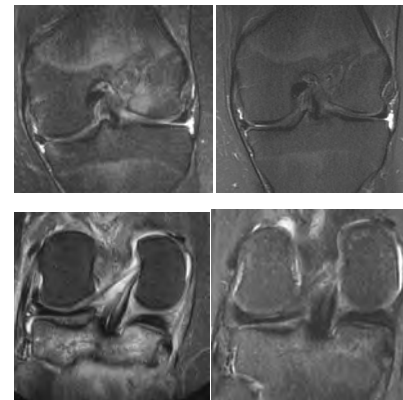


Figure 4. Knee PTA with BME. Lateral condylar edema along the tunnel of ligamentary plastic surgery (left top side). Tibial plate edema (left bottom side). Substantial improvement of the signal for cancellous bone after CM-PRP-HA at one month, correlated with decreased pain (right side).

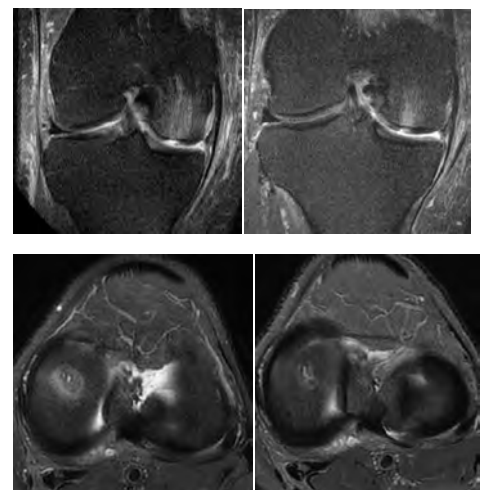


Figure 5. Knee PTOA with BME of medial femoral condyle and superficial cartilage defect (osteochondritis); Frontal plane (top) and axial plane (bottom) with a target sign. Edema highly decreased after CM-PRP-HA at one month.

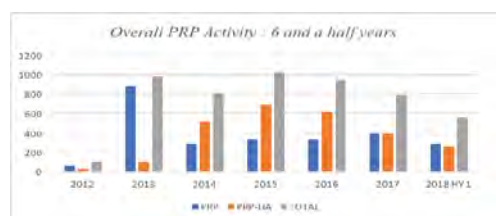


Figure 6. Global PRP therapy with Regen Lab devices; 2328 sessions of CM-PRP-HA from 2012/8 till 2018/6.

knee (84%). The gender ratio was 36% female vs 64% male.

Discussion

The literature supports the use of PRP IA

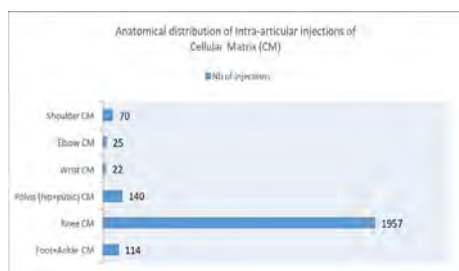


Figure 7. Anatomical distribution of CM-PRP-HA (84% for the knee, 6% for the hip, 4,9% for ankle and foot).

injections for the treatment of knee disorders [8-13]. In a recent review about the efficiency of I-A injection of PRP, Marmotti et al. [13] stated that PRP appears to have a potential for improving knee function and quality of life of patients suffering from chondropathy or initial OA, by reducing the inflammation and, in a lesser extent, the degenerative articular processes. Nevertheless, better results are seen in younger male patients, with low BMI and lower degree of cartilage degeneration with protocols implying a repeated course of injections.

Medipole Garonne is a Sports Clinic where meniscal injuries are frequently seen. Our aim is to conserve the meniscus and avoid early primary degenerative OA and other pathologies caused by repeated traumas such as PTOA and meniscus arthritis. For these reasons, and with the aim of bringing a more effective and sustainable service to our patients, we propose to complement the therapeutic arsenal of degenerative disorders of the knee by the use of IA injection of CM-PRP-HA. This proposal, particularly for young sportsmen, is in compliance with the statements of Marmotti et al. [13].

The question of whether CM-PRP-HA was more effective than classical visco-supplementation can also be addressed from this study. According to the type of HA (viscosity), either a single or three IA injections separated by an interval of one week is recommended. In the study of Trigkilidas and Anand [6], the HA IA injections had a modest effect on early to moderate knee OA with the effect peaking at around 6-8 weeks following administration, with a doubtful effect at 6 months. In our clinical experience, cases of failure are also numerous for the treatment of knee OA with classical visco-supplementation. A new treatment option such as CM-PRP-HA, is thus necessary before opting for surgical solution like of tibial osteotomy or replacement with prosthetic device.

The need for treatment for BME and BML was first addressed by Davies-Tuck et al. [28], who stated that resolution of BML was associated with reduced progression of cartilage pathology. The presence of BML is associated with progressive cartilage loss and pain, thus BME is an important bio-marker however observable only by iterative MRI examination. Secondary MRI signs included the decrease of joint effusion and of soft para-articular tissues swelling. Consequently, the pattern of BME is an important consideration in degenerative follow-up that can only be observed with help of MRI, as X-ray cannot show cartilage and ignores the edema. The correlation of a reduction of pain and BME noted in the current study after CM-PRP-HA treatment seems thus to be promising.

The best frequency for administering CM-PRP-HA to maintain a good clinical result for pain beyond one, two, three or four years, and to avoid or postpone surgical intervention is still to be determined. In particular for sportsmen, should CM-PRP-HA injection be performed once each year, or a course of iterative IA injections with a two-month interval, as a preventive treatment for OA?

Conclusions

The first key element for effective treatment of degenerative OA is the early detection and stabilization of fibro-cartilaginous tears since meniscal destruction is highly predictive for OA [8]. Secondly, the reduction of BME [28] favors healing, as BME is correlated with loss of cartilage. Therefore, early detection and early preventive treatment of OA might avoid the destructive evolution that could lead to the need for a prosthetic device.

Many favorable studies to the use of PRP, are already present in the recent literature: PRP *vs* placebo [29-30], PRP *vs* HA [31-34], a systematic review with comparison of the efficiency of PRP *vs* corticosteroid injections or visco-supplementation or placebo injections in knee osteoarthritis [35], and also the potential for PRP to activate sub-chondral progenitor cells [36]. We assume that neither PRP nor CM-PRP-HA are placebos for the treatment of joint diseases.

The use of CM-PRP-HA has the potential to reduce pain more effectively than classical visco-supplementation, and to prevent, or at least to slow down, the progression of meniscal lesions and OA. Protection of fibro-cartilaginous

structures is clearly coupled with protection of articular cartilage and future satisfactory state of the joint. Therefore, preventive treatment is extremely important to reduce pain, functional limitation and cost of public health. However, we cannot ignore the fact that being overweight, or having traumatic instability or distortions of the skeleton disadvantages the therapeutic benefits of any treatment.

Based on results obtained in this study we are able to propose the CM-PRP-HA protocol for the following pathologies:

- Grade II and III stable meniscal degenerative lesion in a stable knee with no meniscal extrusion, and with functional cruciate ligaments. Preventive treatment of meniscal lesions appears good for possibly avoiding meniscal arthritis.
- Kellgren and Lawrence Grade II and III Knee OA, and possibly Grade IV if surgery is refused by the patient.
- Post-Traumatic Algodystrophy of the knee.
- Post-Traumatic OA with BME and edematous lesions of superficial cartilage (osteocondritis).

In the future, a comparative analysis of CM-PRP-HA vs HA visco-supplementation, as well as determining the most effective treatment regimen, will be probably useful.

Disclosure of interest

PA, JLR and JFM receive consulting fees from Regen Lab SA.

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Acknowledgement

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The new treatment approach in knee osteoarthritis: Efficacy of cellular matrix combination of platelet rich plasma with hyaluronic acid versus two different types of hyaluronic acid (HA)

Osteoarthritis pathogenesis is a complex process associated with decreased ability to regenerate cartilage mainly due to lack of physiological vascularization. One of the most commonly affected joints is the knee.

Purpose: The aim of this study was to compare the efficacy of intra-articular (IA) injections of platelet rich plasma (PRP) combined with hyaluronic acid (HA) prepared with the Cellular Matrix device versus IA injections with two different types of hyaluronic acid for treatment of knee osteoarthritis.

Material and methods: This is a prospective, randomized, double-blind, controlled study on 53 patients (90 knees) suffering from knee osteoarthritis, divided in 3 groups. The first group comprised 19 patients (30 knees) treated with 3 IA injections, one every second week, of Cellular Matrix (CM) PRP-HA combination. The second group of 19 patients (30 knees) was treated with 3 weekly IA injections of 2% non-cross-linked sodium hyaluronate (ArthroVisc[®], AV) and the third group of 15 patients (30 knees) treated with 3 weekly IA injections of 2% non-cross-linked sodium hyaluronate with mannitol (Ostenil[®] Plus, OP). All groups were homogeneous concerning gender, age and Kellgren Lawrence scale (I to III). For all patients visual analog pain scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Knee Injury and Osteoarthritis Outcome Score (KOOS), The International Knee Documentation Committee (IKDC) score ("well-being" scale for all 4 scores between 0 and 100) and ultrasound (US) cartilage thickness on lateral, trochlear, and medial compartments, with normal range values from 2 to 2.5 mm, were measured at the beginning of the treatment (baseline) and at each follow up visit, that is at 2, 6 and 12 months after the last injection.

Results: A statistically significant difference ($p < 0.05$) in the CM group was found compared to AV and OP group in the values of VAS, WOMAC, KOOS and IKDC after two months, although an improvement, compared to baseline values, was observed for the indicated parameters in all groups. A high statistically significant difference ($p < 0.01$) was obtained in the CM group compared to the AV and OP group for VAS, WOMAC, KOOS and IKDC after 6 and 12 months. In both groups of patients treated with hyaluronic acid, a deterioration of values for VAS, WOMAC, KOOS and IKDC score was seen at 12 months in relation to values at 6 months. The CM treated group showed statistically significant improvement ($p < 0.05$) of the cartilage thickness after 2, 6 and 12 months in the medial and highly statistically significant improvement ($p < 0.01$) in the lateral segments of knee cartilage in comparison to baseline values.

Conclusion: The Cellular Matrix PRP-HA combination (CM-PRP-HA) might be one of the most potent, safe, fast and novel therapeutic option for osteoarthritis of the knee (Kellgren–Lawrence grade I to III), as well as a useful tool for postponing arthroplasty surgery when it is necessary. For further investigations, we need larger prospective double-blind studies with MRI quantification of CM-PRP-HA effects on cartilage. Taking all this in consideration we are very close to believe that the future therapeutic option for osteoarthritis, will be combining therapeutic effects of Cellular Matrix CM-PRP-HA with bone marrow mesenchymal stem.

Keywords: cellular matrix • platelet rich plasma • hyaluronic acid • knee osteoarthritis • regenerative medicine

Introduction

Osteoarthritis (OA) pathogenesis is a complex process associated with decreased ability to regenerate cartilage mainly due to

lack of physiological vascularization. One of the most commonly affected joints is the knee [1,2]. Although OA is a disease of the entire joint (cartilage, ligaments, synovium, and bone), the initial lesion is usually in the articular cartilage.

Branko Barac*, Nemanja Damjanov & Ana Zekovic
Institute for Rheumatology, Belgrade, Serbia

*Author for correspondence:
baracbranko3@gmail.com

OA has a strong genetic component and, in most cases, has mechanical overload as an initiator of the process of cartilage damage, which evolves to a vicious inflammatory cycle, perpetuating joint degradation. This inflammatory pathway has as its primary agents, Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF), which induce increased expression of metalloproteinases and Nitric Oxide (NO), the main catabolic agents produced by chondrocytes in response to injury, in addition to more IL-1. Therefore, the treatment of osteoarthritis should target both the mechanical overload that leads to joint damage, for example with visco-supplementation with hyaluronic acid, and the inflammatory cycle that perpetuates the injury at one or more points in this chain, with treatments such as corticosteroid IA injections. In the treatment of knee osteoarthritis, many pharmacological and non-pharmacological therapeutic procedures have been used thus far [3,4]. Currently, the use of corticosteroids is still necessary in order to address secondary inflammation, as well as to prepare the knee joint for further therapy after arthrocentesis and evacuation of synovial fluid [5]. HA is one of the main components of synovial fluid. It ameliorates absorption during impact as well as lubrication of the joints. HA molecular weight and concentration, are reduced in synovial fluid from patients suffering from osteoarthritis (OA). Twenty-five years of clinical experience with numerous studies have shown pain reduction and functional improvement of knee OA following IA HA injections lasting up to 6 months on average. The mechanism of action is both biomechanical and biological, including anti-inflammatory effects. The network of HA chains forms a perfect matrix for cells [6]. Intra-articular injections of hyaluronic acid have had an effect on reducing the discomfort and slowing down the progression of the disease itself, along with the improvement of viscoelasticity, but have been unable to make possible regeneration of cartilage [7,8].

Biological, regenerative, minimally invasive therapy, such as the one with PRP, has been researched in many studies [9,10]. PRP, with its growth factors can stimulate cartilage repair, normalize viscoelasticity of synovial fluid, reduce pain, improve the joint function and improve the quality of life [10,11]. Activated and concentrated platelets release a large amount of different growth factors from their alpha granules, such as: PDGF (plated derived growth factor) which stimulates cell growth, generation and repair of blood vessels, and production of collagen, TGF-

beta (transforming growth factor beta) which stimulates cell proliferation, promotes production of extracellular matrix, stimulates angiogenesis and healing of wounds, VEGF (vascular endothelial growth factor) which stimulates proliferation and migration of endothelial cells, FGF (fibroblast growth factor) which stimulates proliferation, EGF (epidermal growth factor) which stimulates angiogenesis, regulates fluctuation of the extracellular matrix, stimulates proliferation and migration of fibroblasts, IGF (insulin like growth factor) which stimulates cell proliferation, accelerates synthesis of collagen, and stimulates the migration of fibroblasts. The active secretion of these growth factors is initiated by platelet contact with the extracellular matrix. Once secreted, growth factors induce different signal cascades in cells that activate cell proliferation, differentiation and synthesis of the new matrix for tissue regeneration. Numerous *in vitro* studies have demonstrated the influence of isolated growth factors on chondrogenic stimulation and differentiation of mesenchymal stem cells [12]. In addition, it has been shown that PRP has a significant role in the treatment of soft and hard tissues, with a key effect on cellular migration, proliferation and differentiation [13]. The idea of combining PRP with HA was based on their possible synergistic therapeutic effect in osteoarthritis. With that goal in mind, some *in vitro* studies have been carried out. Their synergism and positive metabolic balance have been proven in the work from 2014 of Wei-Hong Chen and associates, where the *in vitro* model has schematically shown the molecular mechanism of chondrogenesis, enhanced by PRP-HA treatment combination. The HA and PRP cooperatively activated surface receptors that triggered release of signaling molecules and finally enhanced chondrogenesis in human articular chondrocyte [14]. Taking into consideration all the aforementioned, it is reasonable to consider the use of a combination of PRP and HA in the treatment of osteoarthritis. Cellular Matrix is the first and the only device on the market allowing the combination of PRP with HA in conformity with regulations and good practice.

The aim of this study was to compare the efficacy of IA injections of PRP combined with HA, prepared with the Cellular Matrix device, versus two different types of HA IA injections in the treatment of knee osteoarthritis.

Methods

Study design and participants

This is a prospective, randomized, double-blind, controlled study on 53 patients (90 knees)

suffering from knee osteoarthritis divided in 3 groups. The first group (CM) comprised 19 patients (30 knees) treated with 3 IA injections, one every second week, of around 5 ml of CM-PRP-HA combination. The second group (AV) of 19 patients (30 knees) was treated with 3 weekly IA injections of 2 ml of 2% non-cross-linked sodium hyaluronate (ArthroVisc) and the third group (OP) of 15 patients (30 knees) was treated with 3 weekly IA injections of 2 ml of 2% non-cross-linked sodium hyaluronate combined with mannitol (Ostenil Plus). All groups were homogeneous concerning gender, age and Kellgren-Lawrence score. Clinical examination and recruitment of participants were conducted at the Institute of Rheumatology, Belgrade Serbia in the period from February 2016 to June 2017. All patients were informed concerning the method and methodology of the study and all of them voluntarily filled out the information consent.

For all patients visual analog pain scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Knee Injury and Osteoarthritis Outcome Score (KOOS) and The International Knee Documentation Committee (IKDC) score ("well-being" scale for all 4 scores between 0 and 100) and ultrasound cartilage thickness on lateral, trochlear, and medial compartments, with normal range values from 2 to 2.5 mm, were measured at each visit. Patients were evaluated before the first injection, and then, two, six and twelve months after the last injection. Routine laboratory tests, including blood platelet count, were performed before each injection.

Treatment protocol

The Cellular Matrix (CM) A-CP HA Kit (Regen Lab SA, Le Mont sur Lausanne, Switzerland) is a Class III medical device (European classification). CM has been specifically approved for the single step preparation, from a small sample of patient's blood, of autologous PRP in presence of HA in a sterile closed-circuit system. The CM device is an evacuated tube in which the patient blood sample (6 ml) is automatically collected. The blood-filled tube is centrifuged for 5 minutes at a relative centrifugal force of 1500 g (following the manufacturer's instructions), which corresponds to a speed of 3600 spin/minute in our centrifuge model. Thanks to the Regen Lab separating gel technology, the platelets and plasma are isolated from the other blood components and combined with the HA solution pre-

sent in the device. The resulting CM-PRP-HA consists of around 3 ml of autologous PRP, with a platelet concentration 1.5 to 1.6 times higher than the baseline value in venous blood and with a low contamination in red and white blood cells (neutrophil poor PRP), entrapped in a 3D network of HA. The device contains 2 ml of natural, non-cross-linked, HA at a concentration of 20 mg/ml (40 mg in total). The HA is produced by bacterial fermentation, thus devoid of animal proteins.

CM is approved for both orthopedic and dermal applications in Europe, and clinical studies are ongoing in the US to obtain FDA pre-market approval.

The CM Group was treated by a series of 3 CM-PRP-HA injections, one injection every two weeks. Each Injection consisted of around 5 ml of the combination of PRP (3 ml) and HA (2 ml, 2% non-cross-linked), prepared with the Cellular Matrix device.

The IA injection (US guided, lateral aspect of suprapatellar recess), while patient was laying on the back with legs in full extension) of the CM-PRP-HA combination was performed just a few minutes after its preparation in the same room, with all aseptic precautions during the entire procedure. The overall procedure is simple, easy and relatively short, typically lasting less than 20 minutes.

The second Group (AV) was treated by a series of 3 weekly injections of 2 ml of ArthroVisc (2% non-cross-linked HA, 40 mg/2 ml, Regen Lab SA, Le Mont sur Lausanne, Switzerland). The IA injection was US guided with the same patient position and needle application route, with all aseptic precautions during the entire procedure.

The third Group (OP) was treated by series of 3 weekly injections of 2 ml of OSTENIL PLUS (2% non-cross-linked HA with mannitol, 40 mg/2 ml, TRB Chemedica, Switzerland). The same IA injection procedure was applied as for the second group.

Blood sample was taken from each patient before the treatments. Each injection was administered under sonography control, by the same sonographer. Patient was blinded for the treatment option. An independent rheumatologist, blinded for the treatment option, examined the patients and measured the cartilage thickness by US at each visit.

Inclusion criteria were age above 30 years and below 80 years, history (at least 4 months) of chronic pain or swelling of the knee, imaging findings of degenerative changes of the joint (Kellgren-Lawrence Score up to 3 at X-ray evaluation, or US findings of degenerative changes (in patients with no OA signs visible with X-ray), and VAS score larger than 50. Exclusion criteria were age lower than 30 and above 80 years, Kellgren-Lawrence score higher than 3, systemic diseases such as rheumatoid arthritis, systemic lupus erythematosus etc., major axial deviation (varus >5°, valgus >5°), history of HA IA treatment, hematological diseases (coagulopathy), severe cardiovascular diseases, infections, immuno-depression, patients in therapy with anticoagulants or aggregation inhibitors, use of NSAIDs in the 5 days before blood donation. Patients with hemoglobin values < 11 g/dl and platelet values < 150,000/mm³, patients with corticoid treatment in past 2 months, symptomatic coxarthrosis on ipsi- or contra-lateral side, any hip or knee surgery planned within 6 months, patients showing past allergic reactions to one of the tested components, patients with serious cardiovascular pathologies, with active gastro duodenal ulcers, digestive hemorrhages, hepatic impairment and pregnant or breast feeding women were also excluded.

Statistical analyses

Results were reported as mean and range of values and presented in tables and charts. Differences between groups and in the same group were assessed by Student's t test and T par test as well as ANOVA. Interobserver correlation coefficient was also used. Differences were considered statistically significant at p<0.05. SPSS 20.0 software was used for the statistical analysis.

Results

In total 53 patients (90 knees) were examined. The follow up period was up to 12 months after the last injection. Visits were organized at 2, 6 and 12 months after the last IA injection. In Table 1, demographic data as well as body mass index and Kellgren-Lawrence score for all three groups are shown. In Table 2, all measured scores (VAS, WOMAC, IKDC and KOOS) for the Cellular Matrix Group are summarized with values at baseline and at 2, 6 and 12 months after the last injection. Tables 3 and 4 report the scores, at the same time points, for AV and OP groups, respectively.

Figure 1 shows that there were no statistically significant differences for: VAS, WOMAC,

Table 1. Gender, Age and Kellgren–Lawrence Score in all three groups.

	Cellular matrix (n=30)	ArthroVisc (n=30)	Ostenil Plus (n=30)
Gender, F:M (%)	16 (53) : 14(47)	17 (57): 13 (43)	17 (57): 13 (43)
Age, mean ± SD (range)	61.3 ± 10.9 (39-80)	61.3 ± 10.9 (39-80)	66.2 ± 6.8 (55-78)
Kellgren-Lawrence grade 1:2:3 (%)	10 (33) : 12 (40) : 8 (27)	12 (40): 10 (33): 8 (27)	11 (36) : 8 (27) : 11 (37)

Table 2. Group 1 (Cellular Matrix, CM), all scores.

Cellular matrix	Baseline	After 2 months	After 6 months	After 12 months
VAS	75.8 (50-90)	28.3 (0-60)	16.4 (0-50)	17.2 (0-50)
WOMAC	42.5 (14.4-82.6)	75 (43.9-100)	83.4 (66.7-94.7)	82.4 (64.2-100)
IKDC	28 (14.9-57.5)	57.1 (23-94.3)	67.7 (43.7-90.8)	66.2 (42.9-90.2)
KOOS	38.5 (9.5-70.8)	70.4 (46.4-100)	80.8 (62.5-94.6)	80.2 (61.9-93.6)

Table 3. Group 2 (ArthroVisc, AV), all Scores.

Arthrovisc	Baseline	After 2 months	After 6 months	After 12 months
VAS	70.2 (50-90)	46.9 (0-90)	56.2 (20-95)	62.4 (15-98)
WOMAC	53.7 (24.2-81.8)	69.1 (37.5-91.7)	67.1 (32.6-94.7)	59.1 (23.7-80.8)
IKDC	36.4 (13-65.5)	46.4 (18.4-71.3)	41.3 (14.9-86.2)	37.5 (12-78.2)
KOOS	49.7 (20.8-83.9)	65 (41.4-82.9)	58.8 (27.4-90)	51.1 (22-80.1)

Table 4. Group 3 (Ostenil Plus, OP), all scores.

Ostenil plus	Baseline	After 2 months	After 6 months	After 12 months
VAS	68.9 (50-90)	44.8 (0-100)	55.5 (10-100)	61.8 (17-100)
WOMAC	45.3 (12.9-73.4)	67.3 (24.2-99)	63.6 (25-94.7)	58.7 (22-89.1)
IKDC	25.3 (10.3-43.7)	40.2 (21.8-65.5)	40.7 (17.2-75.9)	35.8 (13.1-69.7)
KOOS	41 (13.1-62.5)	58.7 (19.6-86.6)	54.7 (27.4-90.5)	46.3 (17.3-82.4)

IKDC and KOOS scores between the three groups at baseline (p>0.05). Two months after the last injection (Figure 2), there were statistical significant differences in CM Group when compared to AV and OP groups in VAS, WOMAC, KOOS and IKDC scores p<0.05, even though we found improvement in all groups in all these parameters when compared to baseline values. There were high statistical significant differences (p<0.01) in CM Group

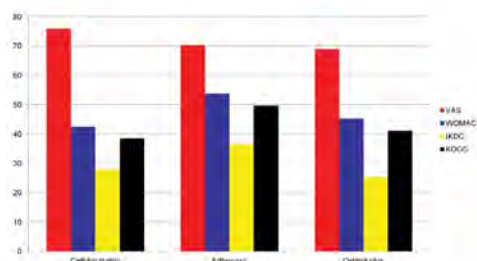


Figure 1. Baseline, all scores in all groups, $p>0.05$.

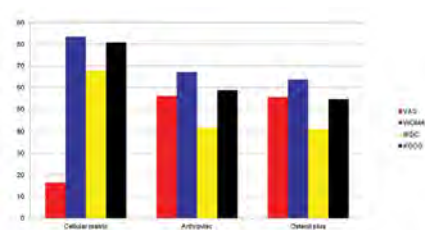


Figure 3. After 6 months, all scores in all groups, $p<0.01$.

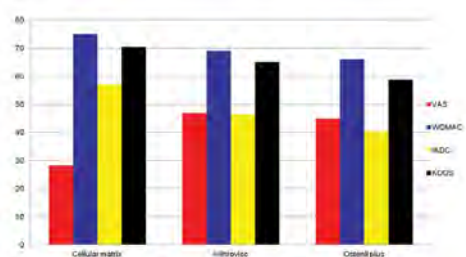


Figure 2. After 2 months all scores in all groups, $p<0.05$.

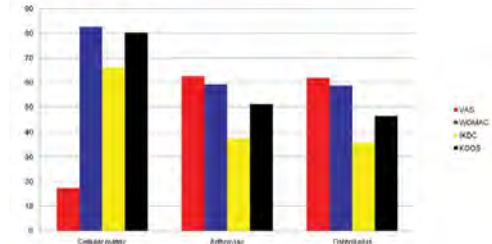


Figure 4. After 12 months, all scores in all groups, $p<0.01$.

when compared to AV and OP groups in VAS, WOMAC, KOOS and IKDC score 6 months after the last injection (Figure 3). On the other hand, in both groups treated with HA (AV and OP groups) we found deterioration in VAS, WOMAC, KOOS and IKDC score after 6 months. The effect of CM-PRP-HA therapy was persistent even 12 months after the last injection. After a period of one year follow up, high statistical significant differences ($p<0.01$) was observed in the CM group, when compared to AV and OP groups in VAS, WOMAC, KOOS and IKDC scores (Figure 4).

The special focus of this study was on the CM-PRP-HA therapy effects on cartilage thickness at 2, 6, and 12 months after the last IA injection as well as after HA IA injections. As foreseen, neither statistical significant differences, nor cartilage thickening, were seen in AV and OP groups 2, 6 and 12 months after the treatment ($p>0.05$, data not shown). On the other hand, for patients treated with CM-PRP-HA, we found statistically significant ($p<0.05$) improvement in cartilage thickness already after 2 months and also after 6 and 12 months in medial compartment, and high statistically significant improvement ($p<0.001$) in lateral compartments (Table 5).

Safety

No single serious adverse events were reported in patient treated with CM-PRP-HA combination. In 5 patients treated with HA, 2 in AV group and 3 in OP group, mild

Table 5. US measured cartilage thickness in CM group, lateral, medial and trochlear compartment.

	Baseline	After 2 months	After 6 months	After 12 months	ICC 95% CI
LAT**	1.5 (0.5-3)	1.9 (0.8-3.1)	2.1 (1-3.1)	2.2 (1.3-3.5)	0.93 (0.84-0.97)
MED*	1.6 (0.7-2.5)	1.9 (0.5-3)	2 (1-2.8)	2.1 (1.3-2.9)	0.87 (0.72-0.94)
TRO	2.2 (0.9-3.5)	2.4 (1.2-3.6)	2.4 (1.4-3.5)	2.3 (1.5-3.4)	0.9 (0.8-0.96)

* $p<0.05$; ** $p<0.01$

inflammatory reactions, with redness on treated spot, were recorded which lasted for a maximum of 12 hours.

Discussion

PRP, as one of the new therapeutic options for knee osteoarthritis, was compared to conservative HA treatment in several studies. One of the studies [15] dealt with comparison of the effect of PRP with two different types of HA. It was a prospective comparative study testing PRP against low molecular weight HA (LW-HA) and high molecular weight HA (HW-HA). There were 3 homogeneous groups of 50 patients each. After a follow-up period of 6 months better performance for VAS and WOMAC scores were found in the PRP group [15]. In another study [16], efficacy of single-spinning leukocyte-free PRP injection was compared to HA in 153 patients evaluated up to 6 months of follow-up. Contrary to the previous study the only parameter where a clear superiority of PRP was found, was

the percentage of responders (patients with at least 50 % of pain reduction) [16]. On the other hand, in several meta-analyses, it has been shown that PRP is superior to HA in pain management and improving mobility for patients suffering from knee osteoarthritis [17-24].

In our study, we show that therapeutic application of series of 3 CM-PRP-HA injections were superior to traditional HA therapy in all measured scores (WOMAC, IKDC and KOOS) as well as for Visual Analog Pain Scale. One of the contribution of our study was the long-term efficacy of CM-PRP-HA with a 12-month follow-up period, in comparison to both HA treated groups. Although improvement was recorded in all three groups after two months of follow-up, significant deterioration was detected in both HA treated groups after 6 and especially after 12 months of follow-up.

Synergistic effect of PRP and HA was demonstrated in many *in vitro* studies. Their different mechanism of action could modulate different aspects of osteoarthritis, affecting both sides of disease, visco-supplementation and treatment of secondary inflammation and degenerative changes [25]. An *in vitro* and OA animal model study from Chen, et al. (14) supports this hypothesis. This study also suggested that chondrogenesis was induced more strongly by the PRP+HA combination than by PRP or HA only. Postponing the surgical procedure was the goal for many studies. Treatments with consecutive PRP and HA injections was able to reduce the pain and improve functional ability in patients with advanced knee OA, and in some studies it helped to postpone knee surgery and arthroplasty [26,27]. Renevier, et al. reported positive results with CM-PRP-HA treatment in a pilot multicenter French study with a long-term follow-up, after a series of three IA injections scheduled at day 0, day 60 and day 180, that provided long-lasting benefits for half of the patients and avoiding surgery for almost 80% of them at four years [28]. According to all results CM-PRP-HA treatment maintain long lasting good clinical outcome for pain management and functional improvement of the knee. It could be used as well for traumatic cartilage pathologies as for patients with degenerative OA.

Conclusion

For the first time in literature, to our knowledge, we quantified in our study therapeutic effect of PRP and HA combination (prepared with the Cellular Matrix A-CP-HA device)

on cartilage. With a very strong interobserver correlation rate coefficient, we show statistical significant thickening of the cartilage 2, 6 and 12 months after the end of CM-PRP-HA treatment on both lateral ($p < 0.05$), and medial ($p < 0.01$) compartments. With statistical significant improvement in all measured scores (WOMAC, IKDC, KOOS and VAS) at all follow-up visits in CM group when compared to both HA groups and statistical significant cartilage thickening, we can conclude that CM-PRP-HA might be one of the most potent and safe new therapeutic option for the treatment of knee osteoarthritis with Kellgren–Lawrence grade I to III, as well as a useful tool in postponing arthroplasty surgery, when it is necessary. For further investigations, we need larger prospective double-blind studies, with MRI quantification of CM-PRP-HA effects on cartilage. Taking all this in consideration, we are very close to believe that the future therapeutic option for osteoarthritis pathology will be combining therapeutic effects of Cellular Matrix CM-PRP-HA with bone marrow mesenchymal stem cells.

Disclosure of Interest

BB received consulting fees from Regen lab SA. The other authors declare that they have no competing interest.

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The new treatment approach in knee osteoarthritis: Efficacy of cellular matrix combination of platelet rich plasma with hyaluronic acid versus two different types of hyaluronic acid (HA) Research Article

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High-field MRI exploration of the structural effects of cellular matrix™ on articular cartilage in knee osteoarthritis: A pilot study in 6 patients

Objective: To analyze the potential modulatory effect of Cellular Matrix, a new medical device designed for the one-step preparation of platelet-rich plasma in presence of hyaluronic acid, on the structure of articular cartilage in patients suffering from knee osteoarthritis using high-field Magnetic Resonance Imaging measurements of longitudinal relaxation time after gadolinium injection.

Methods: The treatment consisted of a series of 3 intra-articular injections scheduled at D0, D60 and D180 into the affected knee of six patients with Kellgren-Lawrence grades of 1.5 to 3. Magnetic Resonance Imaging acquisitions were performed before the first injection at D0 (baseline), at D180 (just after the third injection) and at D270 (3 months after the third injection). The efficacy criterion was the variation of T1 relaxation time in different selected cartilage regions.

Results: Our study reveals a positive "time-dependent" structural effect of the combination of PRP and HA obtained with Cellular Matrix on the proteoglycan content of the knee joint cartilage. At D180, the weight-bearing areas were involved in two patients with Kellgren-Lawrence grades equal to or greater than 2. At D270, 5 patients showed an initial improvement in the weight-bearing area; only one patient with early external femoropatellar osteoarthritis (with a Kellgren-Lawrence grade of 1.5) had no improvement.

Conclusion: This pilot study demonstrates for the first time the modulatory effect on the structure of the knee joint cartilage of a combination of platelet-rich plasma and hyaluronic acid prepared with a specially dedicated medical device (Cellular Matrix) during the course treatment. Cellular Matrix could therefore be considered a Disease Modifying Osteoarthritis Device.

Keywords: cellular matrix • osteoarthritis • pilot

Introduction

While osteoarthritis (OA) is the most common cause of pain and disability among people over 50 years of age [1], knee OA is becoming a real public health issue as populations age. Knee OA is an underestimated condition. Its increasing prevalence [2-4] has been causally linked with obesity [5]. In the United States, surgeons performed 686,000 knee replacements in 2009, and projections predict the implantation of 1,520,000 prostheses in 2020 and 3,480,000 in 2030. Prosthetic revision rate (unicompartmental or total) continues to progress. A 600% increase is expected by 2030 [6].

Intra-articular injection of Hyaluronic Acid (HA), referred to as viscosupplementation, represents a recognized treatment for knee

OA. Many clinical trials testing different HA preparations have been performed in humans, some of which report results versus saline placebo. Most of these studies conclude that HA is superior to a saline placebo, whatever its molecular weight [7-13].

More recently, Platelet-Rich Plasma (PRP) injections have proven to be an interesting treatment option [14-23]. The potential efficacy of PRP in the treatment of cartilage lesions has already been evaluated *in vitro*; particularly, PRP has been shown to increase the synthesis of proteoglycans and collagen in the extracellular matrix of cultured intervertebral disc cells [24]. However, very few studies have documented a possible modulatory effect of PRP on cartilage structure in Humans to date.

Jean-François Marc*¹ & Jean-Luc Renevier²

¹Rheumatologist, Health Consulting Agency AGCOSS 12 rue Pierre Dépierré 42300 Roanne, France

²Rheumatologist, Centre Médico Chirurgical du Mantois, 78200 Mantes-la-Jolie, France

*Author for correspondence:
contact.agcoss@gmail.com

In recent years, it has become more and more obvious that the association of PRP with HA could provide added benefit for the treatment of joint degenerative diseases, due to their different mechanisms of actions to modulate the disease process [25-32].

Joint cartilage is made of water (60%-80%) and chondrocytes surrounded by an extracellular matrix [33]. This matrix is composed of type II collagen (5%-10%) and proteoglycans (10%-20%) (PG) [34]. Cartilage damage in osteoarthritis is accompanied by biochemical changes in the collagen network and proteoglycans. The loss of proteoglycans has been associated with the early phases of osteoarthritis based on studies conducted in animal models [35,36] and anatomical parts [37,38]. These biochemical alterations, which escape conventional radiology techniques, can be detected by Magnetic Resonance Imaging (MRI), which represents therefore a tool of choice as a non-invasive approach to osteoarthritis.

Different functional approaches by MRI based essentially on relaxation time measurements coupled or not with the injection of a contrast agent have been developed. The T1 relaxation time measurement after injection of a gadolinium (Gd)-based contrast agent is the most commonly used technique [39] with measurements made about 90 minutes after the injection phase. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) is based on the demonstration that Gd distributes in inverse relationship to cartilage PG content, leading to a reduction of T1 relaxation time.

Van Tiel described and used a promising reproducible methodology based on this technique to explore the potentially structural effect of HA in early stage knee OA, unsuccessfully [40].

Our study aimed at demonstrating with the same validated methodology that PRP combined with HA can be structurally effective on articular cartilage in knee OA. Using an innovative medical device allowing the preparation of autologous PRP in presence of HA in a one-step procedure and in close circuit (Cellular Matrix™), this collaborative work between rheumatologists and the Centre National de Recherche Scientifique (CNRS) has made it possible to study the effect of a combination of PRP and HA on the cartilage of the knee using high-field 3 Tesla (3T) MRI measurements. The safety and efficacy of Cellular Matrix has already

been assessed in several clinical studies, including a recent one still showing a clinical benefit on pain and function 4 years after a 3-injection course treatment [41].

Objectives of the study

To analyze the potential modulatory effect of the combination of PRP and HA prepared with Cellular Matrix (CM-PRP-HA) on the structure of the articular cartilage in patients suffering from knee OA using high-field MRI measurements of longitudinal relaxation time after gadolinium injection (dGEMRIC), a scientifically recognized indirect index of proteoglycan (PG) content.

Patients and methods

Patients

Six patients were included after they provided their written informed consent. Inclusion criteria were as follows: participants older than 18 years, knee pain duration longer than 3 months and radiographic knee OA with Kellgren-Lawrence (KL) grades of 1 to 3 [24,42]. Exclusion criteria were: contraindications to MRI, renal insufficiency (glomerular filtration rate < 60 ml/min), knee surgery within the last year, recent viscosupplementation or glucocorticoid injection. The study protocol was authorized by the French National Authority for Health (ANSM) and approved by local Ethics Committee (CPP Sud-Est I). The study was conducted according to Good Clinical Practice and guidelines of the Declaration of Helsinki.

Treatment

The combination of PRP and HA was obtained using the Cellular Matrix device, as per instructions for use supplied with the kit. Cellular Matrix, manufactured by Regen Lab SA, Le Mont-sur-Lausanne, Switzerland, is a class III medical device. It allows for the extemporaneous preparation of a combination of autologous PRP and non-crosslinked HA gel 2% (CM-PRP-HA) intended to be used for intra-articular injection (Figure 1). The HA used (2 ml) has a molecular weight of 1550 kDa. Each patient received a series of three intra-articular injections of CM-PRP-HA at D0 (baseline), D60 and D180, as described by Renevier et al. [41].

MRI acquisition

MRI acquisitions were performed before the injection of CM-PRP-HA at D0 (baseline), D180 (just after the third injection) and D270 (3 months after the third injection). Before each

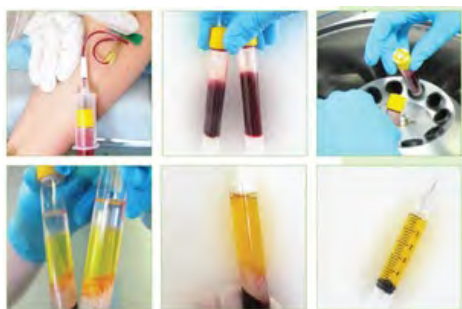


Figure 1. One step procedure for the preparation of a combination of PRP and HA with cellular matrix device.

Following blood withdrawal, the tube is centrifuged for 5 minutes at 1500 g. During this centrifugation, the gel migrates to form a barrier that mechanically separates the plasma and platelets from the other blood components (red and white blood cells). This supernatant containing plasma and concentrated platelets is PRP. The HA gel, which was located at the bottom of the tube before centrifugation, migrates to the top of the tube and sits above the PRP fraction. This allows for an easy mixing of HA and PRP after centrifugation by gentle and repetitive inversions and rotations of the tube. The final PRP/HA combination is collected using a blood transfer device and is ready to be used by the physician. The full procedure is done in close-circuit.

MRI session, a double dose (0.2 mmol/kg) of gadoteric acid (Dotarem®, Guerbet, France) was injected intravenously approximately 95 min before the MRI session. Patients were then asked to exercise for 15 minutes on a cyclo-ergometer at a comfortable rate and pedaling frequency in order to promote the contrast agent distribution within the knee articular cartilage as previously described [39,40]. MRI measurements were started after an additional 80 min resting period. MR imaging was performed on a 3.0 Tesla MRI scanner (Verio, Siemens Germany) using a set of phase array surface coils positioned above and below the knee (Figure 2). After a localization procedure using scout images, quantitative sagittal T1 mapping was performed using a dual-flip angle 3D GRE sequence with the following parameters as previously described [39,40,43] flip angles: 6 and 33°, TR: 15 ms, TE: 2.58 ms, Field-of-view (FOV): 144 mm; slice thickness: 3 mm, slice oversampling: 28.6%, matrix size: 384 * 384, bandwidth: 380 Hz/pixel, in plane resolution 0.4 * 0.4 mm. The resulting scan time was 4.5 min for a slab with 28 slices. In addition to the quantitative map, morphological evaluation was performed using a sagittal T1-W turbo spin echo sequence (TR-TE: 700-18 ms,



Figure 2. 3.0 tesla MRI scanner used at the Center for metabolic exploration by magnetic resonance (CEMEREM, Marseille).

MRI : Magnetic Resonance Imaging; CEMEREM: Centre d'exploration métabolique par résonance magnétique

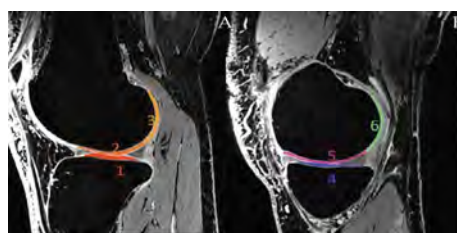


Figure 3. Six masks manually segmented. 1 & 2 and 4 & 5: Weight-bearing anterofemoral (2 & 5) and tibial (1 & 4) areas, lateral (A) and medial (B); 3 & 6: Non-weight-bearing posterofemoral areas, lateral (A) and medial (B).

voxel size: 0.5 * 0.4 mm), and both sagittal and coronal proton-density turbo spin echo sequences including a fat saturation scheme (TR-TE: 4000-37 ms, voxel size 0.5 * 0.4 mm and TR-TE: 3800-37 ms, voxel size: 0.4 * 0.4 mm). The FOV was 130 mm. the total scan time for the morphological evaluation was 7.1 min.

Data analysis

From the T1W-MRI baseline dataset, a central slice was selected in the external and internal tibiofemoral areas. For each area, three cartilage regions of interest (masks) were manually drawn by an expert surgeon using FSL View, the 3D viewer included in the FSL toolbox [39,40]. These 3 regions consisted of the weight-bearing cartilage of the femoral condyles (Areas #2 and #5), the posterior non-weight-bearing cartilage of the femoral condyles (Areas #3 and #6) and the weight-bearing cartilage of the tibial plateaus (Areas #1 and #4) (Figure 3). Using a non-linear registration method, these manual masks were propagated to the superior and inferior slices and then to the MRI datasets recorded at D180 and D270. For this purpose, T1W-MRI obtained at D180 and D270 have been registered into the baseline T1W-MRI dataset. The corresponding T1 maps have also been resampled using the

baseline T1W-MRI dataset as a target.

All registration and resampling have been performed using the ANTS library (<http://stnava.github.io/ANTs/>) tools [44]. This registration process eliminated subjective visual slice matching and additional manual segmentations. As previously reported, cartilage regions with long T1 relaxation time have relatively high glycosaminoglycan (GAG) content compared to cartilage regions with short T1 relaxation time which indicates reduced GAG content (refs). In order to avoid all possible partial volume effects from the cortical bone, a very conservative segmentation procedure was used so that cartilage pixels in the close vicinity of bone pixels were systematically excluded. It has been previously suggested that a 95 ms difference in T1 relaxation time corresponding to a 19% change could be considered as clinically relevant and indicative of an improved cartilage GAG content as measured by 3D dGEMRIC at 3.0 Tesla [39]. We used a similar approach in order to compare the post-contrast T1 values at different times.

Results

Radiological and WORMS score

The radiological scores determined by two clinicians in charge of recruiting patients on the Kellgren-Lawrence (KL) scale (42) was between 1.5 and 3 (Table 1). The WORMS score (Figure 4) for cartilage ranged from 0 (patient, P1) to 32.5 for the patient 6 (P6) (Table 2) [45]. As shown in Figure 5, both scores were highly correlated (R2=0.8).

Quantitative MRI analyses

Analysis #1: D0 vs D180

External compartment: At D0, the mean T1 values (\pm SD) were 358 ± 109 , 373 ± 82 et 415 ± 123 for the tibial, anterofemoral and posterofemoral zones, respectively (Table 3 and Figure 6). The

Table 1. Radiological scores of the patients included in the study, according to the Kellgren-Lawrence grading system.

	KL score	Type of osteoarthritis
P1	1.5	Early external femoropatellar
P2	3	Global with effusion
P3	2	Internal femorotibial
P4	2	External femorotibial and patellofemoral
P5	3	Internal femorotibial
P6	3	Internal femorotibial

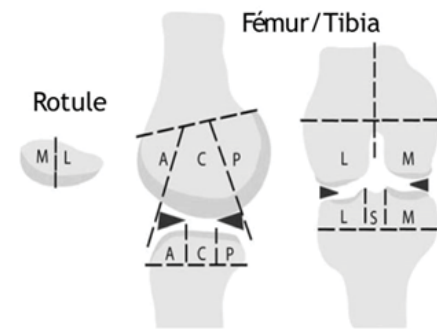


Figure 4. WORMS: Whole organ magnetic resonance imaging score.

At time D0, the WORMS score was established for each patient according to the following items. Cartilage (signal and morphology), Sub-articular compartment (bone marrow, cysts, osteophytes, bone wear), meniscus (integrity) cruciate ligaments (integrity). Analysis area: medial, lateral, tibial spine, anterior, posterior, central.

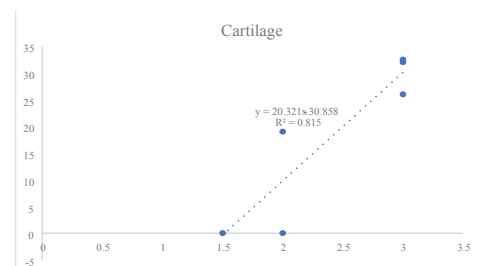


Figure 5. Correlation between Kellgren-Lawrence grade (x-axis) and WORMS (y-axis).

Table 2. WORMS scores of the patients included in the study.

Score WORMS Cartilage	Score			rank
	WORMS Total	rank	rank	
P1	0	1	6	6
P2	32.5	209	1	1
P3	21	62	5	5
P4	19	75	4	3
P5	26	85	3	2
P6	32	64	2	4

coefficients of variation (CV) were comparable between zones (22 to 31%). As shown in Table 3, the T1 values measured at D180 were not different from those measured at D0.

Internal compartment: At D0, the mean T1 values (\pm SD) were 284 ± 63 , 293 ± 41 et 387 ± 45 respectively for the tibial, anterofemoral and posterofemoral zones, respectively (Table 4 and Figure 7). The coefficients of variation (CV) were comparable between zones (12 to 22%). As shown in Table 4, the T1 values measured at D180 were not different from those measured at D0. For comparative purposes, we

Table 3. External compartment: T1 values measured at D0 and D180.

Compartment externe						
	Tibia		Fem ANT		Fem POST	
	J0	J180	J0	J180	J0	J180
P1	340	309	362	332	344	327
P2	214	211	262	240	339	220
P3	489	695	487	717	588	682
P4	436	356	422	263	549	324
P5	250	239	304	253	291	197
P6	418	366	403	472	379	479
moyenne	358	363	373	379	415	371
SD	100	159	75	170	112	166
CV (%)	28	44	20	45	27	45

SD: Standard deviation; CV: Variation coefficient

Table 4. Internal compartment: T1 values measured at D0 and D180.

Compartment interne						
	Tibia		Fem ANT		Fem POST	
	J0	J180	J0	J180	J0	J180
P1	335	194	353	209	421	264
P2	208	160	289	240	358	365
P3	305	286	327	329	389	497
P4	239	154	257	182	361	253
P5	248	278	246	159	335	297
P6	371	265	286	405	458	465
moyenne	284	223	293	254	387	357
SD	57	55	38	86	41	95
CV (%)	20	25	13	34	11	27

SD: Standard deviation; CV: Variation coefficient

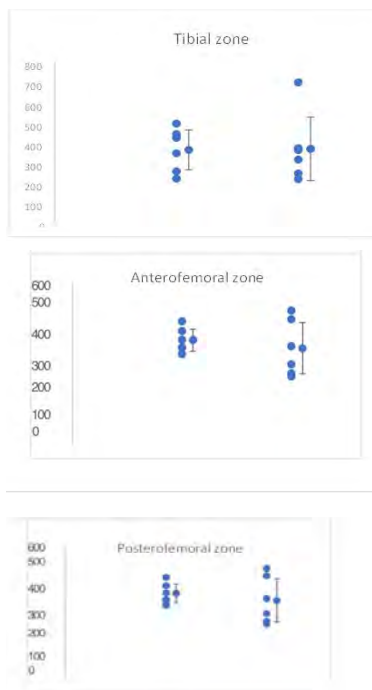


Figure 6. T1 values (ms), as measured before (left-hand side) and after (right-hand side) treatment in the three zones of the external compartment.

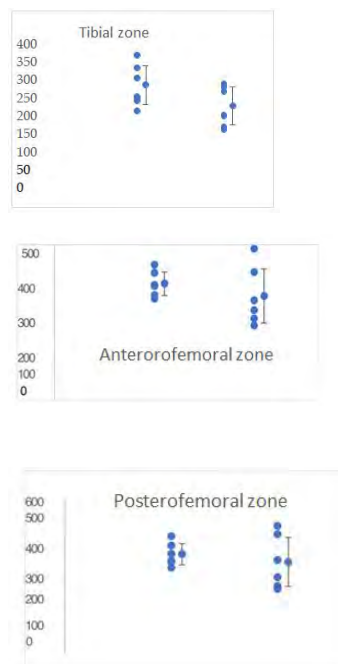


Figure 7. T1 values (ms), as measured before (left-hand side) and after (right-hand side) treatment in the three zones of the internal compartment.

calculated the ratio of T1 values between the external and internal compartments. For the tibial and anterofemoral compartments, the values were generally higher for the external compartment. This difference was not found for the posterofemoral compartment (Table 5).

Zone score D180: Four patients had no improvement in the T1 values, while two patients had localized improvements. More specifically, P3 showed improvements in the tibial and

anterofemoral zones of the external compartment and in the posterior femoral zones of the inner compartment. For P6, the increases were localized at the level of the posterofemoral zone (external compartment) and the anterofemoral zone of the internal compartment (Table 6).

Total score at D180: Table 7 summarizes all this data and presents the total score calculated on this basis. In this context, Patient P3 had the best outcomes with improvements on both the

Table 5. Ratio of T1 values between external and internal compartments.

C Ext / C int						
	Tibial		Anterofemoral		Posterofemoral	
	D0	D180	D0	D180	D0	D180
P1	1.02	1.6	1.02	1.59	0.82	1.24
P2	1.03	1.32	0.91	1	0.94	0.6
P3	1.6	2.43	1.49	2.18	1.51	1.37
P4	1.83	2.31	1.64	1.45	1.52	1.28
P5	1.01	0.86	1.24	1.59	0.87	0.66
P6	1.13	1.38	1.41	1.17	0.83	1.03
Mean value	1.27	1.65	1.28	1.49	1.08	1.03
SD	0	1	0	0	0	0
CV (%)	28	37	22	27	31	32

C Ext: External compartment; C int: Internal compartment; SD: Standard deviation; CV: Coefficient of variation

Table 6. Zone score at D180.

	External compartment			Internal compartment		
	Tibial	Antero-femoral	Postero-femoral	Tibial	Antero-femoral	Postero-femoral
P1	0	0	0	0	0	0
P2	0	0	0	0	0	0
P3	1	1	0	0	0	1
P4	0	0	0	0	0	0
P5	0	0	0	0	0	0
P6	0	0	1	0	1	0

Table 7. Total score at D180.

	Cpt EXT	Cpt INT	Total	Rank	Weight-bearing areas	Rank	Non-weight bearing areas	Rank
P1	0	0	0	3	0	3	0	3
P2	0	0	0	3	0	3	0	3
P3	2	1	3	1	2	1	1	1
P4	0	0	0	3	0	3	0	3
P5	0	0	0	3	0	3	0	3
P6	1	1	2	2	1	2	1	1

Cpt ext: External compartment; Cpt int: Internal compartment

weight-bearing and non-weight-bearing zones. Patient P6 also showed signs of improvement in both areas.

Analysis #2: D0 vs D270

External compartment: At D0, the mean T1 values (\pm SD) were 358 ± 109 , 373 ± 82 et 415 ± 123 , respectively for the tibial, anterofemoral and posterofemoral zones, respectively (Table 8). The coefficients of variation (CV) were comparable between zones (22 to 31%). As shown in Table 8, the T1 values measured at D270 were not different from those measured at D0.

Internal compartment: At D0, the mean T1 values (\pm SD) were 284 ± 63 , 293 ± 41 et 387 ± 45 , respectively for the tibial, anterofemoral and posterofemoral zones, respectively (Table 9). The coefficients of variation (CV) were

comparable between zones (12 to 22%). As shown in Table 9, the T1 values measured at D270 were not different from those measured at D0. For comparative purposes, we calculated the ratio of T1 values between the external and internal compartments (Table 10). For the tibial and anterofemoral compartments, the values were generally higher for the external compartment. This difference was not found for the posterofemoral compartment.

Zone score at D270: At this stage and given the small number of values, we opted for an individual analysis strategy by adapting the results of a previous study [40]. We chose 19% as a significant threshold of increase. This threshold was calculated on the basis of the T1 (500 ms) values reported by van Tiel et al and the 95 ms value reported as significant [39, 40]. In other

Table 8. External compartment: T1 values measured at D0 and D270.

External compartment						
	Tibial		Anterofemoral		Posterofemoral	
	J0	J270	J0	J270	J0	J270
P1	340	346	362	388	344	408
P2	214	288	262	324	339	313
P3	489	637	487	693	588	739
P4	436	295	422	310	549	259
P5	250	440	304	348	291	284
P6	418	441	403	349	379	445
Mean values	358	408	373	402	415	408
SD	100	120	75	133	112	162
CV (%)	28	29	20	33	27	40

SD: Standard deviation; CV: Variation coefficient

Table 9: Internal compartment: T1 values measured at D0 and D270

Internal compartment						
	Tibial		Anterofemoral		Posterofemoral	
	J0	J270	J0	J270	J0	J270
P1	335	261	353	302	421	339
P2	208	147	289	217	358	357
P3	305	409	327	450	389	616
P4	239	287	257	287	361	330
P5	248	238	246	299	335	369
P6	371	776	286	247	458	408
Mean values	284	353	293	300	387	403
SD	57	204	38	73	41	98
CV (%)	20	58	13	24	11	24

SD: Standard deviation; CV: Variation coefficient

Table 10. Ratio of T1 values between external and internal compartments.

C Ext / C in						
	Tibial		Anterofemoral		Posterofemoral	
	J0	J270	J0	J270	J0	J270
P1	1.02	1.33	1.02	1.28	0.82	1.2
P2	1.03	1.96	0.91	1.49	0.94	0.88
P3	1.6	1.56	1.49	1.54	1.51	1.2
P4	1.83	1.03	1.64	1.08	1.52	0.79
P5	1.01	1.85	1.24	1.16	0.87	0.77
P6	1.13	0.57	1.41	1.41	0.83	1.09
Mean values	1.27	1.38	1.28	1.33	1.08	0.99
SD	0	1	0	0	0	0
CV (%)	28	38	22	14	31	20

SD: Standard deviation; CV: Variation coefficient

words, a 19% increase in the T1 value was considered as a sign of improvement (score = 1) while an increase of less than 19% was assigned a score of 0. Five patients had localized T1 values improvements. More specifically, P3 showed improvements in all areas (external and internal compartments). For P6, an increase was localized in the tibial zone of the internal compartment. The other three patients P2, P4 and P5 have each shown an initial improvement each time at the weight-bearing areas. On the other hand, patient

P1, who only suffered from an early stage of knee osteoarthritis, showed no change. Table 11 summarizes these zone scores.

Total score at D270: Table 12 summarizes all this data and presents the total score calculated on this basis. In this context, patient P3 had the best outcomes with improvements on both the weight-bearing and non-weight-bearing zones. Patient P6 showed signs of improvement only in the weight-bearing area.

Table 11. Zone score at D270.

	External compartment			Internal compartment		
	Tibial	Antero-femoral	Postero-femoral	Tibial	Antero- femoral	Postero-femoral
P1	0	0	0	0	0	0
P2	1	1	0	0	0	0
P3	1	1	1	1	1	1
P4	0	0	0	1	0	0
P5	1	0	0	0	1	0
P6	0	0	0	1	0	0

SD: Standard deviation; CV: Variation coefficient

Table 12. Total score at D270.

	Cpt EXT	Cpt INT	Total	Weight-bearing areas	Non-weight-bearing areas
P1	0	0	0	0	0
P2	2	0	2	2	0
P3	3	3	6	4	2
P4	0	1	1	1	0
P5	1	1	2	2	0
P6	1	0	1	1	0

Discussion

Our study is based on the well documented inverse relationship between Gadolinium penetration into the cartilage and T1 relaxation time, and the relationship between T1 relaxation time and the proteoglycan content in cartilage [39,40]. In general, the T1 values reported in this study are lower than the values reported in the literature for healthy subjects but also for subjects with osteoarthritis [39,40]. In accordance with the results reported in the literature [43,46-49], this decrease clearly indicates a significant loss of proteoglycans. This loss seems to be less pronounced in the external compartment than in the internal compartment, in line with previous work [39,40].

In addition, T1 values were correlated with WOMBS radiological score values, strengthening the adequacy of T1 measurements as a quantitative tool for cartilage monitoring. Such a conclusion has also been proposed on the basis of a comparative analysis between T1 measurements and T1rho measurements [50].

It should be noted that the measurement method we chose for the T1 relaxation time was different from the one used in the work of Van Tiel et al. [39,40]. This could explain the differences in values between the two studies without impacting our comparative analysis. On the basis of this difference, we chose to adapt our analysis method to the 95 ms threshold previously reported [39] for T1 values close to 500 ms. Consequently, we considered a 19% increase in the T1 value as an indication of improvement in the proteoglycan content.

Statistically, (at mean values, Mann Whitney paired series tests were performed with a statistical threshold $p < 0.05$), there was no difference between the measurements at D0 and D180, nor between D0 and D270, which probably reflects the small number of subjects. However, based on an individualized analysis and a 19% threshold increase in the T1 value, five patients showed localized improvements.

At D180, these improvements were localized in the tibial and anterofemoral areas of the outer compartment and in the posterofemoral area of the inner compartment for Patient P3. For patient P6, these improvements were localized in the posterofemoral area (outer compartment) and the anterofemoral area of the inner compartment. In both cases, the weight-bearing areas were involved in these two patients who had a Kellgren-Lawrence score equal to or greater than 2.

At D270, we observed a consolidation for Patient P3 with a score that tripled. In this case, all cartilage areas showed improvement. For Patient P6, the score was reduced from 2 to 1 with an improvement in the tibial area of the internal compartment. While Patients P2, P4 and P5 showed an initial improvement in the weight-bearing area, only Patient P1 had no improvement.

Our study therefore reveals a positive "time-dependent" structural effect of the combination of PRP and HA obtained with Cellular Matrix on the proteoglycan content of the knee joint cartilage. Patients responded relatively quickly

given the avascular and paucicellular nature of the cartilage tissue which is characterized by a very slow turn-over in physiological situations and even more so in the hostile inflammatory context of knee osteoarthritis.

Beside growth and regeneration factors, the platelet secretome contains anti-inflammatory cytokines; PRP probably acts through this dual effect [51]. Hyaluronic acid, on the other hand, is expected to act as a support potentiating PRP activity [32] and have a facilitating role that may potentiate or maximize tissue response to growth factors [52].

Clinically, all treated patients experienced improvement in pain and stiffness in accordance with the study of Renevier et al. [41]. This confirms the clinical relevance of the variations in T1 values defined by Van Tiel et al. [39,40] (95 ms for values close to 500 ms or a 19% difference).

Conclusion

This is a pilot, proof of concept study, aiming at demonstrating for the first time the modulatory effect on the structure of knee joint cartilage of a combination of PRP and HA prepared with a specially dedicated medical device (Cellular Matrix).

The small number of patients did not allow for a relevant statistical study; however, the individual analysis strategy adapted from Van Tiel et al. [39,40] was appropriate. Thus, the individual comparative analysis considering a 19% increase in the T1 value as significant clearly indicated that 2 out of 6 patients had positive outcomes at D180, while 5 out of 6 patients had improvements at D270. One patient aggregated positive outcomes over the 6 zones studied. Only Patient 1 did not show any improvement.

A modulatory time-dependent effect on cartilage structure is thus demonstrated. The clinical confrontation is unequivocal with improvement of all treated patients for both pain and stiffness scores, in line with the Renevier's study [41]. In the end, this research work demonstrates that the combination of PRP and HA (Cellular Matrix) is structurally effective at D270. Cellular Matrix can therefore be considered as a true anti-osteoarthritis treatment with a proven structural effect on knee joint cartilage in Humans. A large-scale multicenter European study with the same methodology and with the same parameters but with a long-term MRI analysis scheduled at D360 is therefore required in order to confirm our positive preliminary data.

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Knee OA management: A cost-effectiveness analysis of platelet-rich-plasma versus hyaluronic acid for the intra-articular treatment of knee OA in France

Objectives: The aim of this work is to carry out an economic evaluation of the intra-articular (i.a.) use of the platelet-rich plasma (PRP) therapy in the short period treatment for knee osteoarthritis (OA). Recently the scientific literature has shown the effectiveness of this treatment. The comparator adopted is the Hyaluronic acid (HA) which represents the standard i.a. therapy.

Methods: A cost-effectiveness analysis was performed using a decision tree model. The effectiveness outcomes are reported in terms of Quality Adjusted Life Year (QALY). The costs are reported in Euro (€) currency evaluated in 2016. Deterministic and probabilistic sensibility analyses are reported in order to evaluate the robustness of the results and account for the different sources of uncertainty.

Results: The PRP therapy results more costly but also more effective than HA. Using a Willingness to pay thresholds of € 10,000/QALY, the PRP is cost-effective with respect to HA, for patient with moderate to severe knee OA, presenting an Incremental Cost Effectiveness Ratio (ICER) of €760 per QALY.

Keywords: Platelet-rich plasma • hyaluronic acid • cost effectiveness • cost-utility • knee osteoarthritis • knee osteoarthritis management

Introduction

Osteoarthritis (OA) is a chronic and degenerative pathology that affects joints in particular hands, knee, hip and lower back resulting in joint inflammation with associated pain, stiffness and loss of movement. Its incidence turns out to be higher in the population older than 60-years-old, for whom prevalence is estimated 10% worldwide [1-5]. As shown in Global Health Observatory data repository musculoskeletal diseases are the 8th in the whole world and the 4th in western countries for disability adjusted life years (DALYs) [6,7]. OA results to be the 11th out of 291 pathologies for burden of disability (YLDs) and 38th per DALYs [8,9].

In France Knee OA prevalence was estimated in a range from 2.1% to 10.1% for men and 1.6 and 14.9% for women (in a population of 40-75 years old). The knee standardized prevalence was 4.7% for men and 6.6% for women, respectively [10]. According to data on Hospital discharges by diagnosis, provided by Eurostat, OA has an important impact on health care activity.

In France hospital discharges for OA were around 206,000 (103,236 hip and 103,334

knee), that were the 2% of the discharges with an average hospital stay of 7.5 days in 2014. These numbers are increasing steadily every year (Figure 1 and Table 1). In France hospital discharges for OA were around 156,000 (84,583 hip and 71,703 knee) in 2006 and 190,000 (97,654 hip and 92,902 knee) in 2012.

In western countries the rise of OA prevalence is leading to an increase in the number of total joint arthroplasty, which can be considered as the final stage of OA (knee and hip OA) [11,12]. In a study on Total Knee Replacement (TKR) incidence, the annual growth varies by country, from the 17% and 14% of Portugal and Switzerland to the 7% of Germany. In France the compound annual growth of TKR was estimated around 5.3% [13]. The burden of OA is correlated to a high economic impact in terms of both direct health-related costs and indirect [14-21]. A systematic review concludes that the social cost of OA could be between 0.25% and 0.50% of a country's GDP [15,16]. In France, the study by Le Pen et al. estimated that healthcare costs (doctor visits, medicines, and hospitalizations) for patients with osteoarthritis account for around 1.7% of France's total healthcare expenditure in 2002 [20].

Stefano Landi*¹, Paolo Landa² & Salvatore Russo¹

¹Department of Management, University "Ca' Foscari" Venice, Venice, Italy

²Medical School, University of Exeter, Exeter, UK

*Author for correspondence:
stefano.land@unive.it

Number of hospital discharge for OA - France

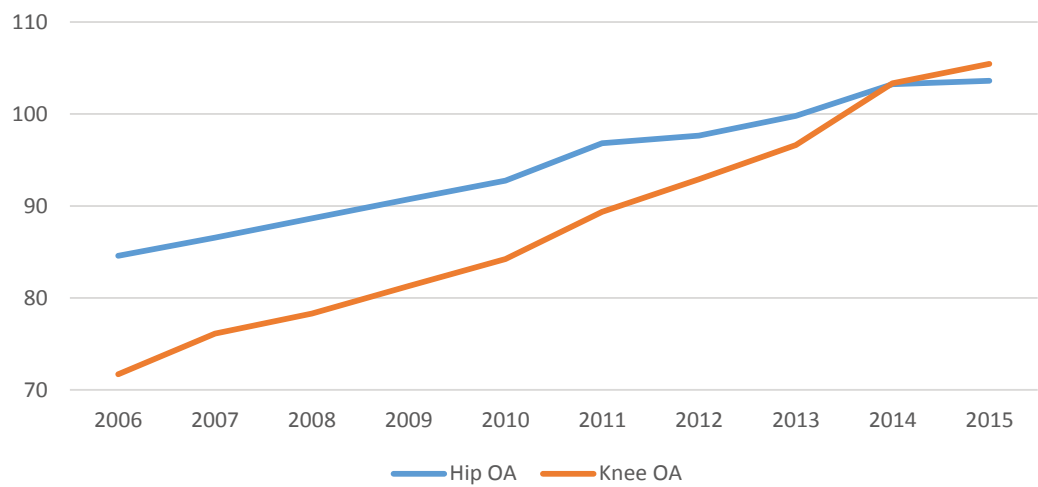


Figure 1. The increasing number of hospital discharges for OA in France (Source Eurostat).

	Hip OA	Knee OA	Total Hospital discharges for OA
2006	84,583	71,703	156,286
2007	86,562	76,127	162,689
2008	88,656	78,304	166,960
2009	90,735	81,288	172,023
2010	92,759	84,222	176,979
2011	96,831	89,354	186,185
2012	97,654	92,902	190,556
2013	99,791	96,619	196,410
2014	103,236	103,334	206,570

Bertin et al. [21] provide a health economic update of the patient costs associated with hip or knee OA treated in the community and in Medical, Surgical and Obstetric care (MSO) and Post-Acute Care and rehabilitation (PAC) hospitals in France in 2010. The annual costs per community patient were € 715 and € 764 for hip and knee OA, respectively, including a cost to the healthcare system of € 425 and € 454, that is, an estimated € 3.5 billion (€ 2 billion to the healthcare system) for 4.6 million patients. Hospitalization engendered annual costs of € 9,797 per patient with hip OA and € 11,644 per patient with knee OA, that is, a total cost of € 1.955 billion for patients hospitalized for hip or knee OA in 2010 [21].

The main direct health-related cost driver is the total joint arthroplasty [22]. The steady increasing, year after year, of surgery incidence leads costs to grow [22,23] in the future [11,12]. A possible strategy to adopt consists in slowing down the progression of the pathology in order to delay (or to avoid) the surgery [24-28].

Intra-articular therapies: Platelet-rich-plasma and hyaluronic acid

Intra-articular (IA) infiltration therapies are used after the failure of conservative treatments, between the pharmacological therapies and the surgery, in order to delay or to avoid the surgery [24-28]. Recently the scientific literature has shown the effectiveness of two IA therapies as Hyaluronic Acid (HA) and PRP.

It has been shown that an infiltration of HA in the joint can restore temporarily the patient's health, giving him relief. Several clinical studies show the HA efficacy in knee OA treatment [29-32]. HA, compared to corticosteroids infiltrations, has a longer effect on pain, rigidity and movement in patient with knee OA [33].

HA has been used for several years and it is considered a standard intra-articular therapy in the treatment of knee OA. Hatoum et al. showed the cost-effectiveness of HA compared to oral NSAIDs (non-steroidal anti-inflammatory drugs), physical therapy and assistive device [34].

On the other side PRP is a non-transfusion use blood component, collected from the patient's blood and used on the same patient [35,36]. The PRP treatment efficacy is shown in several studies [36-42]. PRP injections seem more effective in the treatment of knee OA over HA, in terms of pain relief and self-reported function improvement at 3, 6 and 12 months [36,43-50]. The results are confirmed by several meta-analyses [51-58].

The method to produce PRP needs a medical device leading the therapy to be more costly than the comparator (HA). It is a typical situation where a technology has better effectiveness but on the other hand higher costs. In these cases it is important to run an economic evaluation to understand the relative cost-effectiveness. Health economic evaluation can be defined as a comparative analysis of alternative courses of action in terms of both their costs and consequences in order to assist policy decisions. The goal of economic evaluation is to inform decision makers to choose activities where benefits outweigh opportunity costs.

The objective of the present study is to assess the cost-effectiveness of the i.a. PRP therapy with respect to HA, in France, for patients with mild-moderate to severe knee pain due to OA and who failed to respond to conventional therapy, usually represented by corticosteroids.

Materials and methods

Study design

A decision tree model that evaluates the choice between PRP and HA for OA knee disease has been developed, considering costs and clinical benefits of both therapies. The decision tree is one of the models used in decisional analysis to perform economic evaluations. In the model are represented two or more strategic choices, defined by the initial branches which depart from the main node. Each branch represents one

of the possible paths that could be undertaken while following a certain strategy. Each branch leads to a node, in which you find another event from which other branches depart up to a final outcome, represented by the leaf or terminal node. For each leaf node are associated the costs to bear during the path from root to leaf nodes and its outcome.

In this model the root node of the tree represents the choice between the two therapies (PRP and HA) for the knee OA treatment. From this choice depart several paths and treatments that the patient could be submitted at. For both the therapies the first node splits the path in two, according to the positive or negative response to the therapy. The development of the tree is represented in Figure 2.

The results are reported in terms of Incremental Cost-Effectiveness Ratio (ICER). The incremental cost-effectiveness ratio (ICER) is used to summarize the cost-effectiveness of a health care intervention. It is defined by the difference in cost between two possible interventions, divided by the difference in their effect. It represents the average incremental cost associated with one additional unit of the measure of effect. The outcomes values express the additional costs implied by the adoption of PRP to gain an additional life year in perfect health status. The French Health system perspective was adopted to evaluate resources consumption. Only direct costs of the therapies were included. The time horizon considered in the model is one year. There are no robust clinic evidences on a longer period.

Cost-effectiveness and willingness to pay threshold

The ICER is a value that reports the incremental cost for a gained point of a QALY gained, that it represents one year in perfect health. In Figure 3 are represented in a Cartesian

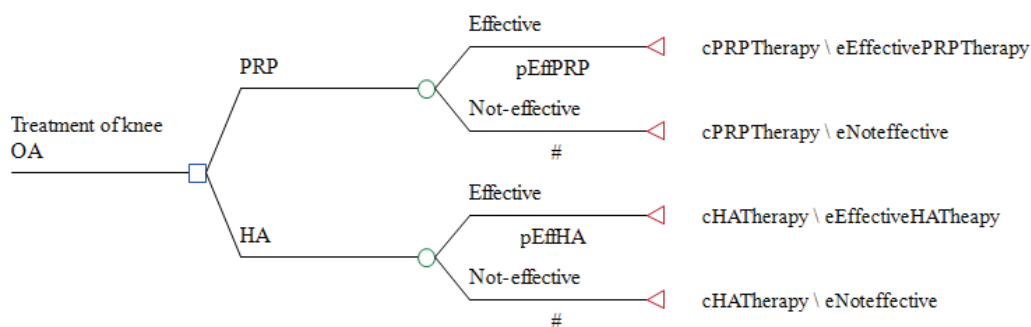


Figure 2. Cost-effectiveness model of PRP vs HA.

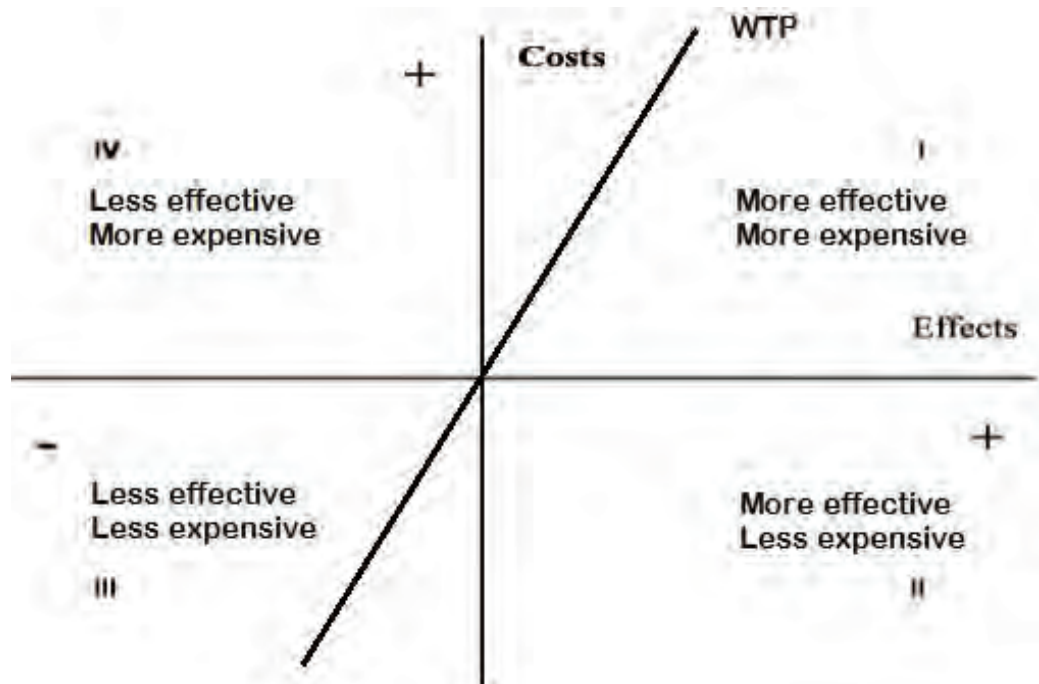


Figure 3. ICER plane with different possible scenarios.

plane all the possible results. In quadrant II the new technology dominates the standard of care, meaning that the new technology is more effective and more expensive. In quadrant III the new technology is dominated and not adopted. The quadrant I is the most common situation, where new technology is more effective and more costly, in this situation the adoption of the Incremental Cost Effectiveness Ratio and the use of WTP threshold will help to choose the best strategy. In France does not exist an official cost-effectiveness threshold to evaluate the cost-effectiveness of a therapy. In this study we refer to two WTP levels, the standard one (30,000 €/QALY) and the conservative (10,000€/QALY), but at the same time we report sensitivity analysis on different WTP thresholds.

Parameters of the model

Resources use and costs

The cost for the two i.a. therapies is affected both by the cost of the product/device used and by cost of the process with the medical professionals involved. In the analyses we inserted the cost of the product/device, the cost to perform the injections and the number of injections applied (Table 2).

In particular PRP has a longer process (and higher costs) with respect to HA. Considering HA costs, the market price of Hyalgan product has been adopted to represent the cost of HA.

In addition, given the possible fluctuation of product market prices and also the use of different products a sensitive analyses has been carried out on this parameter.

As said PRP needs a medical device to be ‘produced’; the medical device taken as reference for this work is the Regen kit BCT-1©, manufactured by Regenlab (CH), is a simplified, sterile kit for PRP preparation that permits to separate plasma and platelets from other blood components obtaining a PRP ready to use. The cost of the PRP is calculated using the the market price of the Regenkit BCT-1. costs € 65 per i.a.

Besides the i.a. injection, the production of PRP implies several preparation steps (as previously described) and need longer times for elaboration, 11 minutes for PRP against 2 minutes for HA. These important differences in the delivery of the two therapies has been included in the model. The duration of the PRP preparation process was taken by interview to clinicians from different health structure in different countries. (Local Health Authorities (Unità Locale Socio Sanitaria, ULSS) of transfusional centres in Veneto region, Italy, Rhumatologie, Hopital de Meulan-Les Mureaux, France, and Klinik für Orthopädie und Unfallchirurgie UKSH Lübeck, University, Germany) (Table 2).

PRP preparation starts taking a blood sample from a patient’s vein (8 ml). Than patient’s blood is put in the BCT-1 kit tube that contains

Table 2. Costs and effectiveness: Input parameters for the base case and ranges of the parameters for sensitivity analysis. Min and max values are the ones used in the deterministic sensitivity analyses. In the table are also reported the distribution used in probabilistic sensitivity analyses.

Direct costs (€)					
Parameters	Base case value (€)	Range		Distrib.	Data source
		Min	Max		
Medical Doctor/minute (€)	0.74	0.5	1.3	Log Normal	30 juin 2016 JOURNAL OFFICIEL DE LA RÉPUBLIQUE FRANÇAISE (Texte 26 sur 196) - (Data refer to the 12ème échelon of hospital doctor) Min: (4ème echelon of hospital doctor)) Max: MD extra hospital with 80 €/hour tariff
Knee OA i.a. injection cost	30.82	30.82	75.71	Fractile	CCAM (classification commune des actes médicaux) CODE: NZJB001 Evacuation of the articular collection of the lower limb, by transcutaneous route without guidance o CODE: NZLB001: Therapeutic injection of pharmacological agent into a serous joint or bursa of the lower limb, transcutaneous without guidance Max and Fractile distribution: model the case where both the codes need to be applied.
PRP process additional costs for single injection (about 9 minutes)	6.66	5.18 (7 minutes)	9.62 (13minutes)	-	Medical doctors opinion in France, Germany and Italy.
RegenKit BCT-1 Cost (Market cost)	65	52	78	Gamma	Market price
Base PRP therapeutic cycle cost (3 i.a. RegenKit BCT-1)	307			-	
HA Hyalgan	29.59	23	35	Gamma	Market price
Base HA Hyalgan therapeutic cycle cost (3 3 i.a.)	181			-	
Effectiveness (QALY-Scenario 2, severe symptoms)					
Parameters	Base case (QALY)	Range		Distrib.	References
		Min	Max		
HA Therapy	0.158	0.097	0.23	Uniform	Duymus et al. 2016 Wailoo et al, 2014
PRP Therapy	0.365	0.187	0.535	Uniform	Duymus et al. 2016 Wailoo et al, 2014
Therapies not effective	0.07	0.02	0.183	Uniform	Duymus et al. 2016 Wailoo et al, 2014
Effectiveness (Womac)					
Parameters	Base case (WOMAC)	Range		Distrib.	References
		Min	Max		
HA Therapy	32	26	38	Uniform	Duymus et al. 2016
PRP Therapy	46	36	56	Uniform	Duymus et al. 2016
Therapies not effective	24	14	34	Uniform	Duymus et al. 2016

an anticoagulant able to prevent the activation of the platelets and a cell selector gel that permits separation of red cells from other blood components. The BCT-1 tube is then submitted to a centrifugation at a speed of 1500 g-force (3400 RPM), which enables to obtain three components: red blood cells trapped under the gel, Platelet-Poor-Plasma (PPP) and PRP settled on the surface of the gel. By gently inverting the BCT-1 tube several times, it is possible to suspend cellular deposit in the supernatant and obtain PRP (about 4 ml). Then PRP is ready for use, collected by a sterile syringe it is injected into the patient's joint [39,59].

The HA treatment needs only the IA injection time, while the PRP treatment needs two minutes for the plasma withdrawal from the patient, around 7 minutes for the centrifugation and the other operations previously cited and finally 2 minutes for the injection that are in common for both therapies. Finally, the additional time to produce PRP with respect to HA is 9 minutes.

In order to include in the model the extra time due to produce PRP we multiply the cost per minute of a hospital doctor for the additional time. In this way we modeled the higher resource consumed for the PRP production process.

PRP production process costs count around 10% of the total therapeutic cycle cost. The data on the hospital doctor cost per minute was reported on the Journal Officiel de la Republique Francaise [60]. General costs were not inserted in the analysis because there is no significant difference and they do not change the results. The number of injections included for a PRP therapeutic cycle is 3, as reported in the most recent meta-analysis [51-54].

Clinical data sources and derivation of utility values

Studies on both therapies report effectiveness in terms of WOMAC scale (Western Ontario & Mc Master University Arthritis Index) that represents an illness specific measure of outcome widely adopted for lower extremity symptoms and function. Patients have to answer to a questionnaire assessing their status for three disease related domains: Pain, stiffness and functionality.

The scores are summed for items in each subscale, with possible ranges as follows: pain=0-20, stiffness=0-8, physical function=0-68. Total scores can range from 0 to 96, with higher scores

indicating increased pain and stiffness, and decreased physical function.

Illness specific scales are very sensitive to changes in patients' conditions and they can be really accurate to evaluate improvement related to a certain treatment. On the other hand they are not useful to make comparison among treatments out of the context of a certain pathology and lacks of standard willingness to pay useful to compare the results of cost effectiveness analyses. The general health status profiles are less sensitive, but allow to analyze and compare results also out of the context of a certain disease. A variety of generic preference-based measures have been developed, the most commonly used questionnaires include the EuroQol (EQ)-5D5L, the Short Form 6D (SF-6D) and the Health Utilities Index (HUI). Once completed, the questionnaires generate a score using an algorithm based on values that have been obtained from a sample of the general public [61]. The values are the health-related quality of life (HRQoL) and measure the utility from living in a specific health state. Health states assume HRQoL values between 0 (dead) and 1 (perfect health), negative value are possible when the health status is considered worse than death. QALYs are assessed by combining the weights calculated for health states alongside the time spent in those health states. QALYs represent the number of years lived in perfect health. The advantage of its use is the possibility to compare results among pathologies and among willingness to pay thresholds for health outcome. Illness specific scales can be transformed in QALYs using mapping techniques. In this work WOMAC scores were transformed in QALYs using the conversion procedure of Wailoo [62] which it has been showed to be the best performer mapping algorithm in literature [63] and uses a mixture model derived from a study where patients states are both expressed in terms of WOMAC and EQ-5D5L. The model predict HRQoL using demographic variable, WOMAC pain, stiffness and function subscales.

In regard to the probability to respond at the two therapies, several studies show a response rate that ranges between 70% and 90% for both treatments [29,30,40,41]. For the base case scenario we chose to set the probability of clinical effectiveness at 80% for both of the therapies.

Sensitivity analysis

Deterministic and probabilistic sensitivity analysis have been conducted to assess the impact

of the uncertainty of the parameters used in the model on the results. Deterministic Sensitivity Analysis (DSA) has been run for every min-max scenario of any parameters. In detail one way DSA have been run for every parameter where a min and max scenario is reported. Probabilistic Sensitivity Analysis (PSA) was performed through a Monte Carlo simulation, performing 10,000 scenarios, to assess the uncertainty around the ICER and the probability of the PRP therapy to be cost-effective at different willingness to pay thresholds. At each model input parameter was assigned a probability distribution (Table 2), that describe the different value the parameter can have with different probabilities [64]. For the parameters cited in literature where it was not estimated standard error, it was assumed a general standard error of 25% of the mean value [65].

Results

QALY

The base case of the scenario with more severe WOMAC scores confirms that none of these therapies dominates the other, but PRP results the cost-effective therapy. The average cost per QALY is respectively around € 761.5 for HA and € 761.7 for PRP. The incremental effectiveness of PRP is 0.166 QALY with an incremental cost of 126.21 €. The ICER of PRP introduction is €760/ QALY. In the tornado diagram (Figure 4) the deterministic sensitivity analysis was summarized. The most sensitive parameter is the effectiveness of the two therapies. In this case

the PRP have a greater effectiveness (in fact in the base case the ICER is lower) but also have a greater variability because the clinic study used 49 report a higher standard deviation. However, no sensitivity analysis of the parameters changes the base case scenario result, that looks quite robust (considering the best-worst of the one way sensitivity analysis the ICER range from 500 to 5,500 €/QALY). The PRP does not become a cost saving therapy (dominant), even in the best case scenario, but in every scenarios it is the cost effective therapy (according to a € 10,000 WTP).

The Probabilistic Sensitivity Analysis (PSA) was performed through a Monte Carlo simulation considering 10,000 scenarios. All the parameters and variables of the model vary according to the assigned distribution. Establishing a WTP of €10,000 per QALY, the PRP is cost-effective in the 99% of the scenarios (Figure 5), while considering a WTP of €30,000 the PRP is cost-effective in 100% of the iterations. In Figure 6 is reported the corresponding acceptability curve with the WTP threshold. For every WTP thresholds is indicated the percentage of cases in favor of PRP or HA.

Discussion

HA and PRP are two IA therapies used after the failure of conservative treatments and pharmacological therapies in order to delay or to avoid the surgery [24-26]. Recently the scientific literature has shown the effectiveness of two IA therapies as Hyaluronic Acid (HA) and PRP.

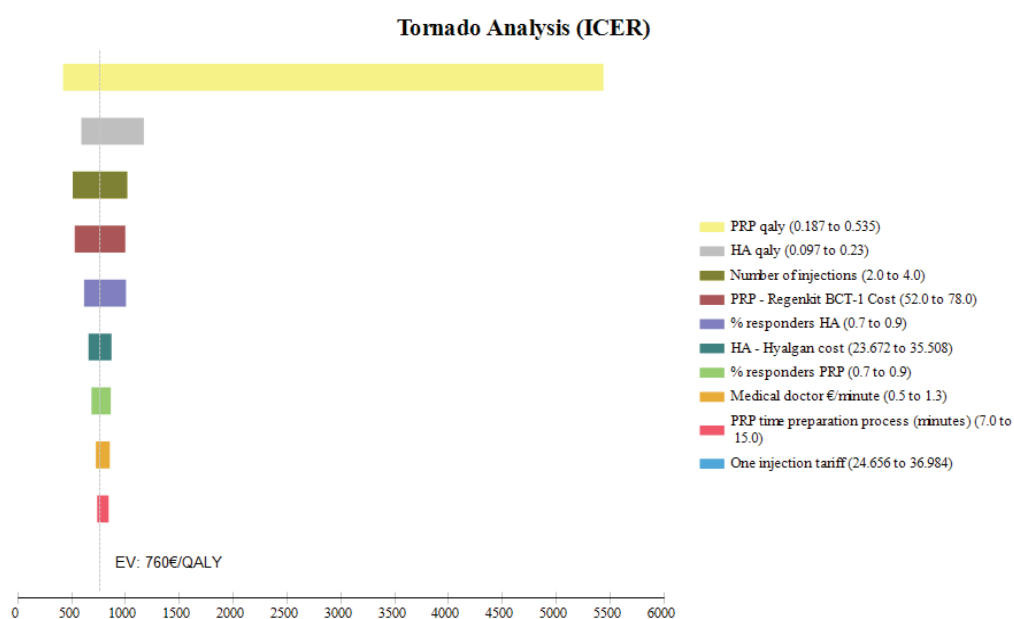


Figure 4. Results of one-way sensitive analysis (Tornado Analysis), PRP, Incremental cost-effectiveness €/QALY.

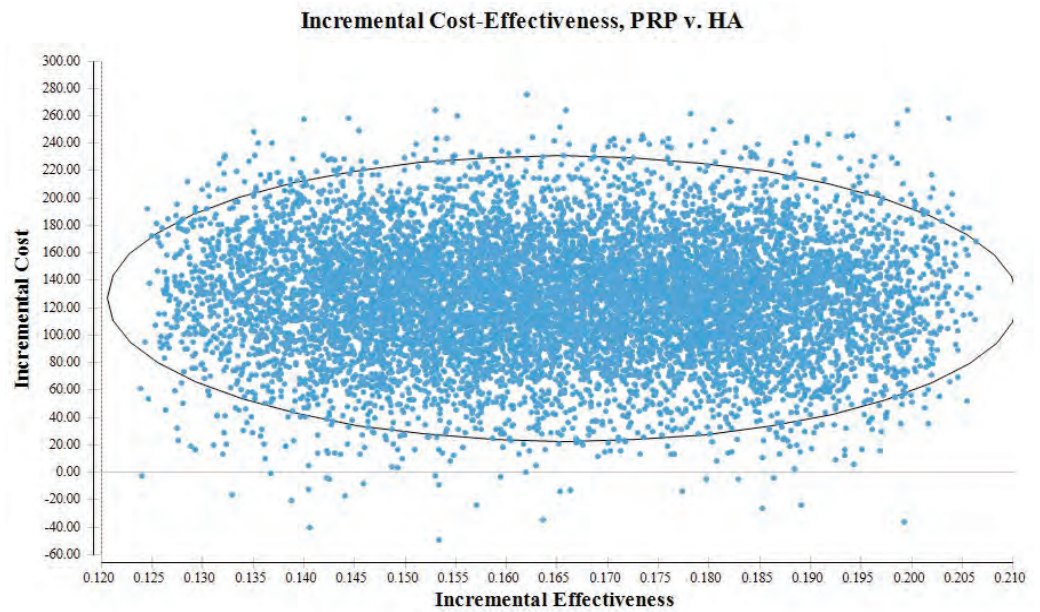


Figure 5. Cost-effectiveness plane of joint distribution of incremental cost and effectiveness for PRP. In the plane are reported 10,000 different cases according to the joint distribution of the variables. The diagonal line represents the WTP threshold of € 10,000/QALYs.

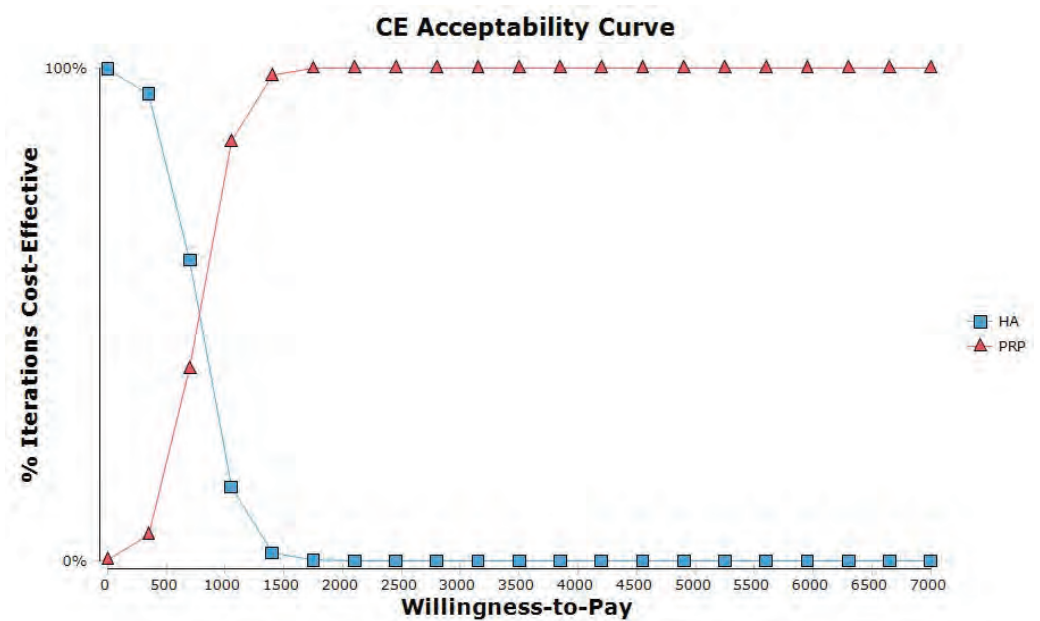


Figure 6. Cost-effectiveness acceptability curve of PRP vs HA under various WTP thresholds.

Several studies show that PRP is more effective than HA [36,43-58].

The rapid innovation in medicine leads to an increase in health outcomes, nowadays many incurable diseases are cured and the life expectancy is longer than few years ago. On the other hand new technologies in medicine are costly, impacting the health system sustainability. When deciding, all health care sectors and decision makers (public or private) are constrained by budgets.

Economic evaluation facilitates comparisons between health care programs. Economic evaluation is an important part of the health technology assessment (HTA). Usually a new technology has a better effectiveness but higher cost with respect to the standard of care. The goal of health economic evaluation, through the use of modelling, is to maximize the benefits from health care spending.

The method to produce PRP needs a medical device leading the therapy to be more costly than

the comparator (HA). It is a typical situation where a technology has better effectiveness but on the other hand higher costs.

The goal of health economic evaluation, through the use of modelling, is to maximize the benefits from health care spending.

Up to date No economic analysis of PRP in the treatment of knee OA has previously been reported. The present study carried out a first economic evaluation to establish the economic value of this therapy for knee OA, in addition to evidence of safety and effectiveness. The specific objective of the study was to assess the cost-effectiveness of the i.a. PRP therapy with respect to HA, in France, for patients with mild-moderate to severe knee pain due to OA.

Results show that for patient with a mild to severe knee OA, considering a time horizon of 1 year in the French system context, PRP therapy is cost-effective with respect to HA. PRP production process implies additional costs that are outweighed by additional benefits in terms of pain relief. In French does not exist an official cost-effectiveness threshold for evaluating the cost-effectiveness of the therapy. In this study we refer to a conservative 10,000€/QALY WTP but at the same time we developed sensitivity analyses on the WTP thresholds. In fact we showed the cost effectiveness acceptability curves for every scenarios. In this way the decision makers can have a complete view. However even considering a conservative WTP threshold (€ 10,000.00/QALY) PRP is cost-effective.

Some limitations of the present study should be taken into account. First the reported cost-effectiveness ratio may be influenced in relation with the method used to convert WOMAC scores in QALYs and for this reason we mapped the QALY from two different studies. Second a chronic disease should be evaluated in a longer period of time. The lack of clinical evidences on longer-term follow up, than one year, does not allow to use a a long term model able to take into account the surgical intervention in the analysis. The economic impact of TKR in NHS is an important variable to take into account for the economic evaluation for the introduction of new therapies for knee OA. A more effective therapy can delay of some years TKR and this delay could lead to reduce the total OA economic impact on Healthcare Systems. Yet the definition of the structure of this study will be useful to extent the evaluation when data on longer follow ups will be collected.

Conclusion

In conclusion, despite the limits explained above, it is possible to state that the IA PRP-based therapy is cost-effective with regard to the IA HA considering a one year horizon. Future research should evaluates PRP effectiveness for a longer period, in particular with reference to the delay of TKR. The major effectiveness of PRP, in addition to quality-of-life improvement, could delay TKR and therefore reduce the total costs of the knee OA and the economic burden on Healthcare Systems.

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Technology rationale for the development of CellularMatrix[®] A-CP-HA Kit, certified medical device allowing the combination of platelet rich plasma and hyaluronic acid for the treatment of osteoarthritis

Solange Vischer*, Valerie de Fourmestraux & Antoine Turzi

Regen Lab SA, En Budron B, Le Mont-sur-Lausanne, Switzerland

*Author for correspondence:
svischer@regenlab.com

The composition of synovial fluid of patients suffering from osteoarthritis (OA) is altered with reduced concentration and molecular weight of Hyaluronic Acid (HA) and augmentation of catabolic enzymes and inflammatory markers. Intra-Articular (IA) injections of exogenous HA aim to restore the rheological properties of the synovial fluid in the osteoarthritic joints. However, clinical studies have shown that the benefit of HA injections lasts only for around 6 months and that many patients don't respond well to repeated course of HA treatment. On the other side, platelet rich plasma (PRP) IA injections have been shown to reduce pain and improve joint mobility, probably by modulating the expression of catabolic enzymes and inflammatory markers. As injections of HA and PRP use different pathway to alleviate symptoms in OA patients, the concept of combining these two treatments has emerged recently. *In vitro* evaluations and preliminary studies have demonstrated the therapeutic potential of this new therapeutic approach for OA treatment. To meet the needs of medical practitioners that want to treat their patients with this new treatment option, an innovative medical device, the CellularMatrix A-CP-HA Kit, has been specifically designed. This device is the first certified medical device that allows the combination of PRP and HA in manner compliant with medical devices regulation and good clinical practice.

Keywords: CellularMatrix • A-CP-HA Kit • platelet rich plasma • hyaluronic acid • knee osteoarthritis • regenerative medicine

Introduction

Osteoarthritis is a degenerative disease and represents by far the most common cause of articular pain [1]. It is associated with degeneration of the joint, subchondral sclerosis and inflammation of the synovial membrane [2]. The evolution of this condition is characterized by the following symptoms: pain, joint cracking/popping, stiffness, deformity, loss of mobility and especially in the case of knee OA, swelling and synovial effusion. The disease is related to aging and generally affects the joints that are subject to stress, such as the knee, hips, small joints of the hand, and the cervical and lumbar spine [2]. OA is the most common joint disorder in the United States. Among adults 60 years of age or older, the prevalence of symptomatic knee OA is approximately 10% in men and 13% in women. The number of people affected with symptomatic OA is likely to increase due to the aging of the population and the obesity epidemic [3].

In a healthy joint, cartilage and synovial fluid

allow for shock absorption and for the bones to slide over one another with ease, thus ensuring the joint mobility. Synovial fluid, that fills the intra-articular joint space, lubricates the joint and is a source of nutrients for the articular cartilage. It is mainly composed of HA, a highly hydrophilic molecule that gives to the synovial fluid its shock-absorbing properties. It has been shown that the HA concentration and molecular weight decrease in synovial fluid with age and in patients suffering from OA, which may cause symptoms of pain and physical loss of function. Treatment with IA injections of exogenous HA aims to restore the shock absorbing and lubrication properties of the synovial fluid in the osteoarthritic joints [4].

The synovial fluid of patients suffering from OA also contain increased catabolic and inflammatory markers [5]. PRP IA injections have been shown to decrease pain and improve joint mobility [6], but also to regulate the expression of catabolic and inflammatory markers in synovial fluid [7,8].

As injections of HA and PRP use different pathway to alleviate symptoms in OA patients, the concept of combining these two treatments has emerged recently. *In vitro* evaluations and preliminary studies have demonstrated the therapeutic potential of this promising new therapeutic approach for OA treatment. The CellularMatrix A-CP-HA Kit has been specifically designed, and certified, for allowing the combination of PRP and HA in a manner compliant with medical device regulations and good clinical practice. The device is a closed-circuit system in which autologous PRP is prepared from a small volume of the patient's blood in presence of a HA solution that is preloaded inside the device. The resulting product is a leukocyte poor PRP resuspended in a three-dimensional matrix of HA. It combines the mechanical properties of HA with the biological properties of PRP that act synergistically to alleviate the OA symptoms.

Hyaluronic acid in the treatment of osteoarthritis

HA is a naturally occurring linear polysaccharide, widely distributed in human tissues, where it constitutes the major part of the extracellular matrix. HA is a major component of synovial fluid and it is known that its concentration and its molecular weight are lower in synovial fluids from patients suffering from OA. Visco-supplementation treatment by intra-articular injections of exogenous hyaluronic acid solution is currently one of the possible treatment for OA, especially of the knee. HA IA injections are designed to improve HA content in synovial fluid and restore its rheological properties and its mechanical action on the cartilaginous structures of the joints, leading to a reduction of pain and an improvement of joint function. It has been demonstrated that locally injected HA is rapidly degraded, so it is thought that the long-term clinical benefits of HA may be due to its ability to promote the *de novo* synthesis of a high molecular weight HA, rather than just replacing the degraded endogenous HA.

In vitro, *in vivo* and clinical studies demonstrate that exogenous HA may also mediate therapeutic effects in OA by many other biochemical actions within the joint, including induction of proteoglycan aggregation and proteoglycan synthesis, inhibition of inflammatory mediators, and analgesic activity [9].

For almost two decades, chemically

modified HA derivatives have been developed to increase their molecular weight and thus their residence time within the joint. The resulting so-called hylans, made of chemically cross-linked HA molecules, exhibit a higher viscosity and consequently higher half-life in the joint, suggesting a potentially better effectiveness. Whether hylans do really show a greater efficacy is still controversial, as available data is not sufficient to be able to draw reliable conclusions [10,11].

The therapeutic effects of IA HA injections may last for a relatively long period, ranging from several weeks to several months [12-23]. In 2011, a systematic review showed evidence of a modest but significant efficacy of intra-articular HA for knee OA compared to a placebo four weeks post-injection with a moderate clinical significance after eight weeks and continued residual benefit until 6 months post injection [24].

Nevertheless, some meta-analyses [25,26] highlighted the fact that the extent of these positive effects appears to be only modest from the clinical point of view. Indeed, the authors showed that even though the outcome parameters exhibited statistically significant differences in favor of HA over the saline placebo, pain relief and functional improvement do not seem to represent an important clinical benefit. One reason may be that there was a generally high placebo effect. Possible explanations for it include synovium aspiration (arthrocentesis), as well as the injections per se, which are known to provide beneficial effects by themselves, making it difficult to distinguish them from the beneficial effects of the HA. In addition, HA has been shown to have a high rate of non-responding patients, 50% to 20% of the patients, depending on the studies and the HA used [27-31].

IA injections of HA represent an efficient and safe approach for the treatment of OA, particularly if it is considered within the framework of a global treatment, which also includes medication-based and non-medication-based therapeutic interventions, such as physical exercise and weight loss. Furthermore, because HA injections have no known medication interactions, it is a good option for patients on multiple medications [32].

A recently conducted literature search on a HA (Ostenil®, TRB Chemedica, Switzerland), with similar characteristics to the one contained in CellularMatrix A-CP-HA Kit, identified 12 studies [33-44]. In total, 694 patients suffering

from various degenerative joints conditions, including knee OA (62%), hip OA (17.5%), hallux rigidus (5.5%), temporomandibular joint disorders (7%) and rhizarthrosis (8%) were treated with Ostenil® or Ostenil®Mini according to different treatment courses (one to five intra-articular injections). All studies, except two case reports on acute pseudoseptic arthritis, showed that pain relief could be obtained after the first intra-articular injection, and was continuously improved with an increased number of injections. Additionally, functional improvement could also be obtained. Generally, HA effects could last for up to 6 months. In all studies, the product proved to be safe and well-tolerated, as no serious adverse reactions were observed.

Platelet rich plasma in the treatment of osteoarthritis

PRP is a volume of autologous plasma that has a platelet concentration above the baseline value in whole blood, [45]. As such, PRP contains not only a high concentration of platelets but also the full content of plasma clotting factors, the latter of which typically remain at their physiologic levels [46]. The rationale of PRP use for therapeutic applications is to mimic the biological healing process that normally occurs in the human body after injury [47]. PRP preparation consists in removing red and white blood cells, which delay the healing and concentrating platelets, thereby increasing factors that are useful in healing [48]. Because there are numerous PRP preparation protocols, differing by preparation devices, centrifugation conditions and operator dexterity, PRP is used to qualify biological products that greatly vary in their platelet concentration, quality and content in growth factors, and level of contamination with red blood cells and pro-inflammatory white blood cells [49].

This large variability in PRP preparations creates a challenge when trying to accurately draw conclusions from the literature to guide PRP production and determine indications for use. To fulfill the need of a standardized PRP preparation, Regen Lab SA, Switzerland, has developed a PRP preparation technology that works in closed-circuit, using polymer separating gels in evacuated tubes. Blood components are separated according to their specific density thanks to centrifugal force and the separating gel inserts itself precisely between the platelets and the white blood cells. At the end of the centrifugation, the separating gel forms a physical

barrier that efficiently isolates the platelets and the plasma in the upper part of the device while red and white blood cells are entrapped below the separating gel, in the lower part of the device. The resulting PRP is a plasma type PRP, that is a PRP with virtually no red blood cells and a very low level of pro-inflammatory white blood cells. The platelet concentration factor is low (1.6 times higher than baseline in whole blood) as the full volume of plasma is recovered, however it has been demonstrated that plasma type PRPs, also called leukocyte poor PRPs, are therapeutically efficient with a platelet concentration factor between 1 and 3 times over the baseline. The so-called therapeutic platelet concentration of 1 billion per ml (4 to 5 times over the baseline values) [45] concerns only leukocyte-rich PRP, as a higher platelet concentration is needed to compensate for the negative effects of pro-inflammatory white blood cells (neutrophils).

IA injections of autologous PRP, and more specifically of leukocyte poor PRP [50], has been found to be an attractive treatment option for the treatment of OA, due to the biological mechanism of action and the autologous nature of the product. A number of *in vitro* studies have already shown the impact of isolated growth factors on the chondrogenic stimulation and differentiation of Mesenchymal Stromal Cells (MSCs). For example, it was shown that MSCs produce significantly more proteoglycans and type II collagen when cultured in presence of TGF- β [51], while bFGF induces chondrogenic proliferation and differentiation of MSCs [52]. PRP represents a safe and cheaper alternative to recombinant growth factors, releasing an autologous and appropriate cocktail of growth factors within a natural and physiological range at the site of injection. *In vitro*, it has been shown that PRP has a proliferative effect on autologous chondrocytes and mesenchymal stem cells [53,54] and attenuates their pro-inflammatory chemokine and metalloproteinase expression [55]. Clinical studies evaluating the efficacy of PRP, prepared with Regen Lab technology, for the treatment of knee osteoarthritis demonstrated significant effects on pain relief and functional improvement, especially for those patients with lower degrees of OA, with safety of use and no serious adverse events attributable to the treatment [8,56-62].

Combination of PRP and HA prepared with the CellularMatrix A-CP-HA Kit

The combination of PRP and HA injections has been suggested as a promising treatment option for osteoarthritis. *In vitro*, it has been

demonstrated that the combination of HA and PRP can synergistically promote cartilage regeneration and inhibit OA inflammation markers [63]. Studies with consecutive injections of PRP and HA on patients suffering from moderate OA suggested that the combination of the two treatments provided an added benefit compared to each product administered individually [64-66]. The results of a case study showed for each patient an increase in the joint space width through objective X-ray measurements [66]. This suggests that the combination of PRP and HA injections may induce local cartilage regeneration and that this therapy may be an effective ultimate chance for patients whose last option might be a total arthroplasty. However, it is not known how PRP interact with HA when the two products are injected consecutively. In addition, there is no safety assessments for this type of procedure.

HA products intended for IA injections are class III medical devices, according to European classification. This imply that they should be used as indicated in their instructions for use and thus not be modified in any way or combined with any other products by the user. To meet the needs of medical practitioners that want to treat their OA patients with PRP combined with HA, Regen Lab has designed the CellularMatrix A-CP-HA Kit. This kit is a certified class III medical device specifically intended for the preparation of PRP in presence of HA and the injection of this combination in a single procedure. The CellularMatrix technology is the first available on the market that allows this combination and is protected by numerous worldwide patents of Antoine Turzi (US8945537, US9517255, EP2544697B1, EP3184114B, JP6076091, JP6321119, CN103079577B, IL221133, CA2789533C, AU2011225828B, RU2614722, KR20130067247, HK1179507, US2015151858, EP2771241, WO2016083549, WO2013061309, WO2011110948).

The European certification procedure for medical devices guarantees that they meet all regulatory requirements. Pre-clinical tests must be performed to demonstrate, among others, biocompatibility of the device components, stability of the device during its shelf life, maintenance of its sterility. Clinical evaluation must be performed for initial conformity assessment and on an ongoing basis to insure post-market surveillance and clinical follow-up. These requirements ensure the safety and

the performance of medical devices for their intended use.

Principle of operations of the CellularMatrix A-CP-HA Kit

The A-CP-HA device is a sterile evacuated tube in which 6 ml of the patient's blood are automatically collected by connecting the tube to a blood collection set. The tube contains 2 ml of hyaluronic acid solution at 20 mg/ml, a biologically inert separating gel and 0.6 ml of anticoagulant (sodium citrate 4% (w/v) solution). This anticoagulant is aimed to anticoagulate only the volume of blood that is collected in the device. It has no ancillary effect on the patient and its action is fully reversible. As for Regen Lab PRP devices, platelets and plasma are isolated from the other blood components by the separating gel through a simple centrifugation (Figure 1). During the centrifugation, the separating gel migrates in the device and intercalates itself precisely between blood components, while the HA gel migrates from the bottom of the device to the top of the plasma, thanks to its low density. At the end of centrifugation, platelets, plasma and the HA gel are isolated in the upper part of the device, while red and white blood cells are entrapped below the separating gel, that forms a physical barrier in the middle of the device. Upon gentle inversions of the device, platelets are put back in suspension in the plasma and, by using a transfer device, the resulting PRP combined with the HA gel is collected in the syringe that will serve for its injection (Figure 2). The preparation procedure is easy, rapid and done in closed-circuit. Provided that proper aseptic techniques are used during the procedure, this technology allows to avoid microbial contamination of the biological sample and operator exposure to the blood of the patient.

The PRP-HA combination prepared with the CellularMatrix A-CP-HA Kit (CM-PRP-

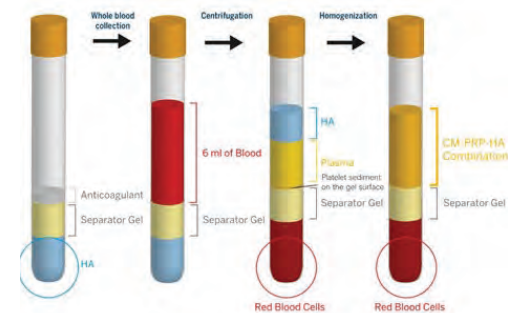


Figure 1. Schematic illustration of the preparation process.

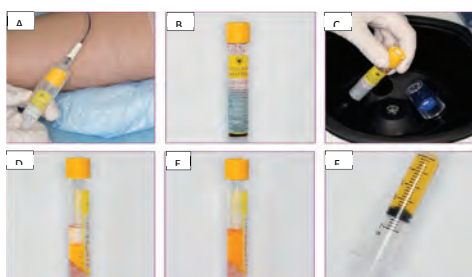


Figure 2. CM-PRP-HA mix preparation process: A: Automatic blood collection inside the A-CP-HA tube; B: Blood filled A-CP-HA tube; C: Centrifugation; D: At the end of the centrifugation the plasma and the platelets are recovered in the upper part of the device. The HA float over the plasma. E: After repeated tube inversion a homogeneous preparation is obtained. F: The resulting CM-PRP-HA mix is ready for injection.

HA) consists of around 3 ml of leukocyte poor PRP, whose composition is known to be therapeutically efficient for the treatment of OA [8,56-62], in suspension in 2 ml of natural HA that form a three-dimensional network. The HA is produced by bacterial fermentation, thus free of animal molecules and not chemically modified. It has similar characteristics to Ostenil® Plus (TRB Chemedica, Switzerland) however without the adjunction of mannitol. When injected into a joint, this HA network not only brings visco-supplementation in the intra-articular space, but also entraps the platelets and the plasma molecules, which probably optimize the biological action of the PRP [67].

Clinical Results

A pilot multicenter study was conducted in France to evaluate the safety and efficacy of the CM-PRP-HA combination obtained with CellularMatrix A-CP-HA Kit in a total of 77 patients suffering from mild to moderate knee OA, who had failed to respond adequately to previous treatment with HA alone [68]. The treatment with CM-PRP-HA (3 injections at day 0, day 60 and day 180) significantly reduced pain at walking between baseline and D270. The percentage of responders according to the criteria of the Outcome Measures in Rheumatology Clinical Trial and Osteoarthritis Research Society International was 94.4%. CM-PRP-HA provided long-lasting benefits for half of the patients and allowed avoiding surgery for almost 80% of them at four years. Another study retrospectively compared patients treated with CM-PRP-HA (40 patients) to a control group treated with PRP only [67]. The treatment was based on 3 intra-articular injections administered

at weekly intervals. Clinical results showed a statistically significant improvement compared to baseline over a six-month follow-up period for both groups. This study was not able to show a significant difference between the two treatments probably because the injection interval of one-week for both PRP and CM-PRP-HA was not optimized and too short. Indeed, it has been shown that optimal injection protocol for Regen Lab PRP is with one-month interval [60,61]. A third study compared patients treated with CM-PRP-HA (50 patients) to a control group treated with HA only [69]. The treatment was based on 3 intra-articular injections administered at a three-week interval. Patients were prospectively, clinically evaluated before the treatment and at 2, 6 and 12 months follow-up visits. At each follow up visit, both groups showed a highly significant improvement when compared with the basal assessment. The infra-group comparison showed, at each follow up evaluation, a significantly higher improvement for the group treated with CM-PRP-HA in respect to HA alone.

The side effects observed with CM-PRP-HA were similar to those observed with PRP IA injections. These adverse reactions appeared at the injection site and were mild inflammatory reactions, which were solved in a few days.

Conclusion

The CellularMatrix device, allows to combine PRP with HA in a safe and efficient manner, in respect with medical device regulations and good clinical practice. The clinical results show that the resulting CM-PRP-HA combination brings long term symptomatic amelioration in patients with mild to moderate knee OA (II-III OA grade, according to Kellgren and Lawrence grading scale), even on patients who had failed to respond to previous visco-supplementation with HA alone, and postpone the need for arthroplasty. The HA present in the combination seems to improve the biological action of PRP and thus to bring superior and longer lasting results than standard treatments with PRP or HA alone. Further clinical studies are needed to establish the most efficient treatment protocol (number of injections, interval between injections) for large joints (knee, hip, shoulder) but also for small joints (elbow, wrist, hand, ankle, foot) and for the different grade of OA at these various anatomical locations. The CM-PRP-HA combination seems to be a promising new treatment option and might be an answer for unmet therapeutic needs for patients suffering from OA.

Disclosure of Interest

Solange Vischer, MSc. and Valérie de Fourmestraux, PhD are employees from Regen Lab SA. Antoine Turzi is the CEO of Regen Lab SA.

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40 Bloomsbury Way

Lower Ground Floor

London, United Kingdom

WC1A 2SE

Ph: +44-203-769-1765

Email: rheumatology@clinicalinsight.org