Infiltrative treatment with Platelet Rich Plasma (PRP) in gonarthrosis

Giuseppe Mangone¹

Annalisa Orioli²

Angela Pinna²

Pietro Pasquetti¹

¹ Specialization in Physical Medicine and Rehabilitation, Recovery and Rehabilitation Agency, University Hospital of Careggi, Florence, Italy

² Postgraduate Specialization School in Physical Medicine and Rehabilitation, Recovery and Rehabilitation Agency, University Hospital of Careggi, Florence, Italy

Address for correspondence:

Angela Pinna, MD

Recovery and rehabilitation Agency

University Hospital of Careggi

Largo Palagi 1, 50134 Florence, Italy

E-mail: angelapinna983@gmail.com

Summary

The aim of the study is to evaluate and to quantify the effects on the quality of life and the decrease of pain in short and middle term in patients affected of gonarthrosis and treated with a series of 3 injections of Platelet Rich Plasma (PRP).

Gonarthrosis is one of the most frequent causes of disability on old ages and leads to difficulties in social, relational and daily activities (1). The most common therapeutic approach depends on the stage of the disease, it can be conservative (FKT), palliative (HA injections, chondro - protector) or reconstructive (TKR).

Between October 2010 and January 2013, 72 patients referring to the outpatient clinic of the Rehabilitation Department of the Trauma Center, University Hospital of Careggi, Florence, have been enrolled if a primary gonarthrosis was diagnosed. The patients, after a hematology visit in Immune-Hematology Department of Careggi, have been evaluated with the WOMAC scale for the knee, VAS at rest and VAS in movement before a series of 3 injections with PRP (T0), after 1 months (T1), after 3 months (T2) after 6 year (T3) and after 1 year (T4) from the last injection.

PRP injections can be considered a valid method in the control of pain, stiffness and joint function (24, 25) but it have to be considered as a second approach to the treatment of knee OA, due to the high cost and complexity of the procedure.

Most of our patients shown good clinical results after one year, were satisfied of the treatment and returned to their previous daily activities.

KEY WORDS: gonarthrosis; Platelet Rich Plasma; PRP; knee injections.

Introduction

One of the most interesting therapeutic choices in regenerative medicine for the gonarthrosis treatment is the use of Platelet Rich Plasma (PRP), a concentrate of autologous blood that contains a high number of platelets.

Gonarthrosis is one of the most frequent causes of disability in old ages, because it leads to difficulties not only for daily activities, social and relational aspects, but also hinders maintaining socio-economical level and a normal emotional life. This led to a decrease of quality of life both for patients and relatives [27:1].

Arthritis in Italy is the most frequent disease, it represents 72,6% of rheumatic diseases; according to the EULAR (European League Against Rheumatism) guidelines about 4 million people are affected of symptomatic osteoarthritis.

The ICARE study (heart failure for elderlies who live in Dicomano - 2003), analyzing 697 subjects who were more than 65 years old, demonstrates that the prevalence of gonarthrosis was 29,8%.

Pro.V.A. study (Progetto Veneto Anziani 2002) shows that, between 1854 females and 1245 males who are more than 65 years old, the prevalence of gonarthrosis is 26% for females and 12% for males.

Nowadays the osteoarthritis is considered as a dynamic pathologic process characterized by the imbalance between degeneration and autoriparative capacity of the articular cartilage (22).

The typical therapeutic approaches to gonarthrosis depend on the stage of the disease, it can be pharmacologic and not pharmacologic, conservative (FKT), palliative (hyaluronic acid injections), or reconstructive (OC graft, UKT, TKT). The local treatment is a very good therapeutic option, compared with systemic pharmacologic treatment that can have frequent adverse reactions and surgery option that is reserved to very advanced stage of gonarthrosis only (4, 19).

Before taking the surgery approach into account we propose the intrarticular injection of platelet rich plasma. From the '90s the interest in growth factors has increased to such an extent that it has become the "Holy Graal" of "wound healing". Thanks to the research on platelet growth (6, 14, 16, 17, 21) factors which led to the discovery of "Platelet Rich Plasma", platelets have been recognized as key cells in the healing of all tissues of the human body. In the bloodstream they are metabolically very active, they synthesize and release growth factors that are involved in the repair of damaged tissues. Recent researches showed the efficacy of growth factors in raising the chondrogenesis and preventing the degeneration of articular cartilage (7-9, 12, 13, 15, 18, 20-23).

There are four categories of PRP, depending on their leuco-

cytes and fibrin content: pure platelet-rich-plasma (P-PRP), leucocyte and platelet-rich-plasma (L-PRP), such as cell separator Regen, the kit we use, pure platelet-rich fibrin (P-PRF) and leukocyte- and platelet-rich-plasma (L-PRF) (11). The reparative process is the same of the other tissues and it consists of 3 steps: inflammation, proliferation, and re-make. In the first step the platelets conduct their physiological role of starter and modulator of the healing process. In the lesion region the degranulation of platelets leads to the release of a lot of growth factors, as TGF (Transforming Growth Factor), PDGF (Platelet Derived Growth Factor), EGF (epidermal growth factor), VEGF (Vascular Endothelial Growth Factor), IGF (Insulin-like Growth Factor). These promote the chondrocytes proliferation, the cells migration and the extracellular matrix proteins synthesis. HGF and bHGF cytokines are chemotactic and encourage mitosis of endothelial cells, angiogenesis and neovascularization. In Platelet Rich Plasma these functions are enhanced as the number of platelets is greatly increased (22, 24, 25).

Platelet Rich Plasma is obtained from the whole blood of the patients through the centrifugation of a test tube or a blood bag or through autologous aphaeresis using cell separator. It is a concentrate of autologous blood that contains a high number of platelets and cannot give hypersensitivity reactions (9, 10, 22).

The use of growth factors is an innovative therapy that provide the possibility of healing to thousands of people suffering from orthopaedic problems (22, 23).

It deals with a series of three intrarticular injections of a concentrate of autologous blood containing a high number of platelet, 4-7 times the physiological amount of platelet in circulation.

The healing of cartilage tissue is quite slow, compared with that of the other connective tissues; this peculiarity is probably due to its poor vascularity. This, together with the load of the body weight, damages the reparative process and stimulates the cartilage degeneration and the loss of articular function.

Platelet Rich Plasma gives the possibility of repairing the damages of knee cartilage, prevents the degeneration, reduces pain and accelerates the joint functionality. PRP induces the proliferation of several cell types, inhibits the release of interleukin 1 (IL-1) from macrophages and reduces the proliferation of the macrophages themselves by limiting the initial inflammatory process, and stimulates quiescent stem cells in order to differentiate them into the type of the damaged tissue. Recent researches show that platelets have the possibility of reducing pain, probably due to the proteases which activate the receptor 4-peptide platelet having antinociceptive properties. Further activated PRP in chondrocytes reduces the transactivating activity of NF- κ B, critical regulator of the inflammatory process, and decreases the expression of COX-2 and CXCR4 target genes. Increasing in hepatocyte growth factor (HGF), interleukin-4 and tumor necrosis factor- α (TNF- α). HGF and TNF- α , by disrupting NF- κ B-transactivating activity, are important for the anti-inflammatory function of activated PRP. Therefore, activated PRP in U937-monocytic cells reduced chemotaxis by inhibiting chemokine transactivation and CXCR4-receptor expression, thus possibly controlling local inflammation in cartilage (4).

PRP technique is safe, it has only few contraindications, it cannot give adverse effects and gives good outcomes. It has been developed to promote the long-term healing of tissues.

Therefore, these new approaches related to arthritis have to be considered in the biological arthroplasty field as well (5). The main aims of intrarticular injections with platelets growth factors in gonarthrosis are:

- to improve the quality of life
- to control pain
- to maintain and/or increase joint function
- to contain the progression of the disease
- to restore viscoelasticity of the synovial fluid
- to stimulate the growth and reparation of articular cartilage.

Materials and methods

Between October 2010 and January 2013 72 patients referring to the outpatient clinic of the Rehabilitation Department of the Trauma Center, University Hospital of Careggi, Florence, have been enrolled if a primary gonarthrosis was diagnosed. Before the treatment all patients were evaluated by an Immune-hematologist in Immune Hematology Department of University of Careggi, Firenze. There were 39 males and 33 females, average age was 63 with a minimum of 52 and a maximum of 82 years. All patients have been suffering from pain for more than 4-6 months at the moment of enrollment.

The criteria for inclusion in this study were: failure of conservative therapies, primary gonarthrosis diagnosis following ACR criteria, presence of pain lasting for at least 4 months, a radiographic picture of II or III grade of Kellgren and Lawrence Scale (26) of gonarthrosis, and age between 50 and 85 years old, of the patient (26).

Patients with oral anticoagulant therapy or coagulopathies, not controlled systemic disease, neoplastic disease, or anticoagulant therapy, thrombosis, traumatic cartilage lesions and uncooperative patients were excluded from the study.

The evaluation of the presence of inclusion criteria and the absence of exclusion criteria was carried out during the clinical examination by medical personnel specialized in Physical and Rehabilitation Medicine and after an Immune-Hematology visit at the Hematology Department of Careggi Hospital. Our patients have been evaluated with the WOMAC scale for knee (Western Ontario and Mc Master Universities Osteoarthritis Index) and VAS (Visual Analogue Scale) at rest and in movement. All the patients have been evaluated before the treatment (T0), 1 month after the last session, (T1), 3 months after the last session (T2), 6 months after the last session (T3) and then the last evaluation (T4) took place 1 year after the last session.

To prepare the PRP we used the "Crossover 2" REGEN Kit ATHENA (Florence).

Our infiltration protocol consists of 3 sessions, one session every 21 days; the infiltrative technique is with patient supine on the bed, flexed knee and injections access ways are antero-medial or antero-lateral.

Patients should not take ASA and NSAIDs five days before and after each injection of PRP because it can interact with the activation of platelets; after the injection they have to spend 2 days of relative rest, assume paracetamol 1000 mg in case of pain or use cryotherapy.

The technique used to prepare the injections starts with taking of 14 cc of peripheral blood, that is immediately centrifuged in 2 dedicated tubes at 3200 rpm for 12 minutes. We obtain then the separation of the corpuscular part from the solid one, with the platelets placed on a filter. After the re-

ACR (American College of Rheumatology) clinical criteria for primary gonarthrosis diagnosis

. Knee pain
At least 3 of the following 6 criteria age > 50 less than 30 mins of morning stiffness cracking joints pain at bone pressure knee bone enlargement No increase of local temperature
SENSIBILITY: 95%
SPECIFICITY: 69%

Kellgren and Lawrence Scale

0 LEVEL NO SERIOUSNESS	No finding of arthritis
1 LEVEL UNCERTAIN SERIOUSNESS	Little Osteophytes uncertain meaning
2 LEVEL MINIMAL SERIOUSNESS	Definite osteophytes, regular articular space
3 LEVEL MODERATE SERIOUSNESS	Moderate reduction of articular space, osteophytes
4 LEVEL HIGH SERIOUSNESS	Severe reduction of articular space with subcondral bone sclerosis

moval of excess of the supernatant, platelets are put back into suspension through delicate balancing movements in 2 cc of supernatant. The leukocytes filter as well and this is the reason why we speak about L-PRP (Plasma rich in platelet and leukocytes). In this preparation the number of platelets is 3-5 times higher than that of the blood (1 mln PLT). About 2 to 2.5 cc of L-PRP are obtained with this process. In a second moment calcium gluconate is added in a percentage compared to the volume (0.5-1%), and the syringe is gently shaken so that it starts the platelet degranulation (2, 3).

After a careful disinfection made with iodopovidone we can infiltrate the articulation.

The whole procedure is conducted at the presence of the physiatrists, who takes care of the injections, a Immune-Hematologist, who stands over the manipulation and the autologous transfusion of the blood, and a biologist, who prepares the Crossover 2 kit.

Results

In the study group the mean score of WOMAC (possible range 0-96) for all patients before treatment (T0) was 26.95 points (extremes 3 – 59). The score at the end of the treatment (T1) after 1 month was 11.1 points (extremes 0 – 45). After 3 months from the end of treatment (T2) the score was 9.3 points (extremes 0 – 36). After 6 months from the end of treatment (T3) the score was 8.8 points (extremes 0 – 39) and 1 year after the end of the treatment (T4) it went up to 7.5 points (extremes 0.– 25) (Tables 1- 6). Pain at rest (possible range 0 – 10) moved from a mean

score of 2.3 points in T0 (extremes 0 – 6) to 1.1 points in T1 (extremes 0 – 6) at 1 month. The value in T2 (3 months) was 0.6 points (extremes 0 – 4), in T3 the value was 0.7 (extremes 0 – 5) and 0.2 points (extremes 0 – 3.) 1 year after the end of the treatment (T4) (Tables 1-4).

Pain in movement (possible range 0 – 10) moved from a mean score of 6.1 points in T0 (extremes 4 – 10) to 3.5 points in T1 (extremes 0 – 10) at 1 month. The value in T2 (3 months) was 2.8 points (extremes 0 – 8), in T3 the value was 2.1 (extremes 0 – 8) and 2.4 points (extremes 0 – 8.) 1 year after the end of the treatment (T4) (Tables 1-5).

The results have been statistically evaluated using the Test T (T Student test). Patients showed a very significant improvement both in functional and pain environment ($p<0.005$). The improvement, compared with the pre-treatment records, lasted for almost one year (Table 2).

VAS at rest decreases constantly in each control and after 1 year from the end of the treatment is near to 0 (Table 4).

VAS at movement decreases constantly till the follow up at 6 months, but slightly grows up after 1 year (Table 5). This probably happens because the benefits of the treatment decrease, but also because most patients try to play new activities since they feel less pain.

We also analyzed with Test T the differences between the 3 items of WOMAC scale: pain, stiffness and function.

Pain and stiffness improve in a statistically significant way ($p<0.05$) till the third month, and after they maintain the result but the score don't decrease any more.

Stiffness decrease in a very statistically significant way ($p<0.005$) during the first month, but then there aren't sizable improvement (Table 3).

Table 1 - Results.

	T0	T1	T2	T3	T4
VAS AT REST	2,33	1,14	0,67	0,71	0,16
VAS IN MOVEMENT	6,11	3,53	2,79	2,10	2,44
WOMAC	26,95	11,32	9,30	8,84	7,5

Table 2 - Results.

	T0-T1	T0-T2	T0-T3	T0-T4
TEST T VAS AT REST	0,002	3,5928E-06	4,5953E-05	4,0808E-09
TEST T VAS IN MOVEMENT	5E-07	2,5E-13	9E-16	4,4E-05
TEST T WOMAC	9,2224E-09	2,41006E-11	4,62115E-10	2,71418E-08

Table 3 - Results (statistic evaluation using T student test).

WOMAC	T0-T1	T1-T2	T2-T3	T3-T4
T TEST PAIN	3.58991E-08	0.036	0.368	0.126
T TEST STIFFNESS	1.43082E-05	0.920	0.345	0.288
T TEST FUNCTION	1.28628E-08	0.010	0.531	0.736

Table 4 - VAS at rest.

Table 5 - VAS at movement.

Table 6 - WOMAC.

Conclusions

On the basis of the analysis of the results it is evident that patients treated with PRP have shown good clinical results immediately after the treatment and 1 year after the end of the treatment as well.

It is worth highlighting that this methodology gives both good clinical results and rapid improvements of joint function with a few number of therapy sessions.

However, the cartilage lesions remain a challenge: traditional techniques show no satisfactory and long-lasting results. Nevertheless, the PRP injections treatment has to be considered as a second approach to the treatment of knee osteoarthritis, because of the high cost (more expensive than HA injection) and complexity of the implementation of the procedure.

The usage of PRP for the treatment of cartilage lesions is a state-of-the-art technique compared with the traditional methods of cartilage grafts. Our procedure is less invasive, requires only three knee injections and it is less expensive. The novelty of the methodology consists of the injection of platelets growth factors that work actively inside the joint

with few contraindications, without the risk of adverse effects such as infections (the blood is autologous), and with a low level of invasivity (ambulatory treatment).

Therefore, based on all the above mentioned reasons, associated to the recovery outlook, it may be concluded that the infiltrative treatment with emocomponents in gonarthrosis provides for a better recovery outlook for the patients.

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