Autologous platelet gel for tissue regeneration in degenerative disorders of the knee

Marcello Napolitano[1], Saverio Matera[1], Marcello Bossio[1], Antonio Crescibene[2], Enrico Costabile[2], Joan Almolla[3], Hesham Almolla[3], Francesco Togo[2], Casimiro Giannuzzi[3], Giampiero Guido[3]

[1]Immunohaematology and Transfusion Medicine Unit; [2]Orthopaedics and Traumatology Unit; [3]Radiology Unit, Cosenza Hospital, Cosenza, Italy

Background. The refinement of the use of platelet-derived growth factors that has occurred over the last decade has led to a broadening of the fields of use, in particular for new treatments in orthopaedics aimed at improving tissue regeneration.

Materials and methods. Twenty-seven patients, aged between 18 and 81 years, with a diagnosis of degenerative joint disease lasting for more than 1 year were treated. The patients were divided into two groups, one with arthritis of the knee, the other with degenerative cartilage disease of the knee. Both groups were treated with a therapeutic protocol consisting of a cycle of three infiltrations of platelet-rich plasma at weekly intervals.

The extemporaneous preparation was made from a sample of about 8 mL of venous whole blood collected into a specific Fibrin Polymer 2 test-tube from RegenLab® and centrifuged before addition of calcium gluconate.

During the initial pre-treatment evaluation, specific questionnaires were administered, the Numerical Rating Scale (NRS) for subjective measurement of pain and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC); these assessments were repeated 7 days after the end of the treatment and at 6 months during the follow-up.

Results. The parameters evaluated improved in both groups after treatment and there was a further improvement after 6 months of follow-up; furthermore, there was a substantial decrease in pain right after the first infiltration.

Discussion. The patients were treated on an out-patient basis by a specifically created multidisciplinary team comprising a transfusion specialist, an orthopaedist and a radiologist, who collaborate in a symbiotic manner. The out-patient protocol exploits the regenerative properties of platelet-rich plasma, which is a low cost treatment; in practice, a diagnostic-therapeutic programme of lower intensity, but of high technical and professional quality is created. The strategy also reduces both the number of hospital services and the pharmacological support required, thereby optimising the use of health care resources.

Keywords: knee, platelet-rich plasma, infiltration, growth factors, knee arthritis.

Introduction

The refinement of the use of platelet-derived growth factors that has occurred over the last decade has led to a broadening of the fields of use, in particular for new treatments in orthopaedics. These include the use of growth factors contained in platelet-rich plasma (PRP), obtained from centrifugation of autologous blood and applied to the site of tissue lesions in order to activate and accelerate physiological processes of healing. The tissues of orthopaedic interest that undergo cellular regeneration are cartilage, tendons, ligaments, muscle and bone. The fields of application, besides orthopaedic surgery, include plastic surgery, maxillofacial surgery and heart surgery[1-6].

PRP is a simple, economic and minimally invasive therapy that provides a concentrate of autologous

growth factors which can be used to activate and accelerate the physiological processes of healing.

Activated platelets release growth factors contained in their a-granules. In this way, the plasma becomes a vehicle of growth factors such as transforming growth factor beta (TGF-ß), platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF)[7-8], which it is thought play key roles in the process of healing of many tissues[9-10].

The first clinical use of PRP goes back to the early 1970s, when studies showed the different concentration of platelets in the plasma and, therefore, the highest concentrations of growth factors[11-12].

These growth factors are known to induce biological changes in cell proliferation, regulating bone cell metabolism, stimulating the replication of stem cells and bone progenitor cells and promoting angiogenesis, epithelialisation and formation of granulation tissue; furthermore, they influence the activity of collagen, promoting endothelial and fibroblast proliferation in various different connective tissues[7,8,13,14].

Arthritis is one of the most common chronic diseases in humans and the most frequent cause of disability. It has been calculated that at least 4,000,000 subjects in Italy suffer from symptoms of arthritis, for a total cost of about 6.5 milliard euros[15]; this cost is destined to increase given the ageing of the Italian population.

An important epidemiological study, carried out in the region of Veneto on more than 3,000 subjects over 65 years old living in the provinces of Padua and Rovigo, analysed the frequency of arthritis determined by radiography of the hips, hands and knees and photography of the hands in order to assess the degree of swelling and deformity as well. It was found that 19% of the participants had arthritis of the hands, 20% of the knees and 11% of the hips. Fourteen percent of the women and 10% of the males affected by lower limb arthritis had severe functional limitations and disability[16].

In the context of traumatology, cartilaginous disorders are increasing continuously, a fact which can be related to occupational activities and the population's increase in motor and recreational activities. In a multicentre study carried out by Hjelle *et al.* in 2002, the prevalence of cartilage defects in 1,000 arthroscopies was 61%[17]. In a trauma setting, Shelbourne *et al*. found a 23% prevalence of cartilage damage in patients with acute lesions to the anterior cruciate ligaments, while the prevalence in people with chronic laxity of the anterior cruciate ligaments was 54%[18].

Materials and methods

The subjects treated in this study were 27 patients aged between 18 and 81 years with a diagnosis of degenerative disease of the knee joint present for more than 1 year. The subjects were evaluated in a period of 6 months and were divided into two groups: one group had arthritis of the knee, the other had cartilage disease. The patients in this latter group had a first or second degree lesion, according to the classification of Outherbridge[19]. The patients in the former group were stratified according to the radiographic degree of joint degeneration using the classification of Kellgren and Lawrence[20]; seven patients had grade 1 disease, four had grade 2 and two had grade 3 disease.

We used a therapeutic protocol consisting of a cycle of three infiltrations of PRP at weekly intervals, for a total of about 15 mL. During the initial pre-treatment evaluation, specific questionnaires were administered, the Numerical Rating Scale (NRS)[21] for subjective measurement of pain and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score[22]; these assessments were repeated 7 and 180 days after the end of the treatment.

The mean age of the patients in the first group was 64±11 years; there were seven men and six women and six patients had bilateral disease so we treated nine left-sided joints and ten right-sided joints. The mean body mass index (BMI) of the patients in this group was 29.2±5.9 (range, 23-43).

The mean age of the patients in the second group was 26.2±2.0 years; all 14 patients in this group were male. We treated nine right-sided joints and six left-sided ones, since one patient had bilateral disease. The mean BMI of the patients in this group was 26.2±2.0 (range, 23-29).

During the first outpatient appointment, the patient's current and past medical history and family history were taken, and a blood count performed. Patients with a haemoglobin concentration <11g/dL, with a white blood cell count >$10x10^9$/L or a platelet count <$120x10^9$/L were excluded.

All the patients who underwent treatment had a normal blood count. Patients with systemic disorders, such as uncompensated diabetes mellitus, cardiovascular disease or immunodepression and patients receiving anticoagulant treatment were also excluded.

Each of the three infiltrations required an extemporaneous preparation of platelet gel, made from a sample of about 8 mL of venous whole blood, collected into a specific Fibrin Polymer 2 test-tube from RegenLab®, and subsequently centrifuged at 3,100 rpm for 8 minutes. In this way the red blood cells and plasma were separated physically, while the platelets were sedimented onto a specific separation surface.

Approximately 5 mL of PRP were obtained in this way, to which calcium gluconate 10% was added. They were prepared under a laminar flow hood and immediately applied by infiltration; the gelification process was completed within 2-7 minutes, exploiting contact with the body heat[23].

The results of the quality controls performed, on a sample of 15 of the 27 patients treated, are as follows:
- mean platelet count in the peripheral blood: $230x10^9$/L (minimum $183x10^9$/L, maximum $370x10^9$/L);
- platelet recovery (evaluated on 1 mL of PRP): approximately 30% (minimum 2.13%, maximum 65.44%) - this value was obtained using the following formula: volume of PRPxplatelet concentration in the PRPx100/8 mLxconcentration of platelets in the peripheral blood;
- platelet concentration factor: 2.3 (minimum 0.17, maximum 5.23); this datum derives from the ratio between the concentration of platelets in the PRP and that in the peripheral blood.

Infiltration technique

The intra-articular infiltration is conducted, following careful disinfection of the skin, with the patient in the supine position and the knee to be treated flexed at 90 °, following careful disinfection of the skin; subsequently, when the needle is removed, the skin is disinfected again and a dressing is applied. The patient is observed for a period of 15 minutes with the limb in a functionally resting position.

During the period of treatment the patient is forbidden to take non-steroidal anti-inflammatory drugs and is advised not to carry out heavy physical activity involving the lower limb for at least 2 weeks after the procedure.

Results

The use of PRP accelerated and improved the healing process, which could be evaluated in terms of:
- measurable efficacy, intended as an improvement of the psycho-physical state of the patient and his or her quality of life, related to a clear improvement in the range of joint movements and reduction in pain;
- cheapness and ease of use of the preparation in an out-patient setting;
- lack of side effects.

The patients with knee arthritis obtained a clear improvement in the NRS score from a mean pre-treatment value of 8.1±1.7 to 3.4±2.5 at the post-treatment control. The mean WOMAC score, divided into its three components, was as follows: (i) *pain*, pre-treatment 10.4±3.9, post-treatment 17±2.5 and, for 7 patients, at the 6-month follow-up 17.9±2.8; (ii) *joint stiffness*, pre-treatment 4.9±2.2, post-treatment 7±0.9 and at the 6-month follow-up 7.4±0.9; (iii) *function*, pre-treatment 36.3±11.8, post-treatment 58.9±9.9 and at the 6-month follow-up 60.7±7.6 (Figure 1).

In the patients with cartilage disease, the mean NRS score changed from a pre-treatment value of 6.8±1.7 to 2.3±2.1 post-treatment. The mean WOMAC score, divided into its three components, was as follows: (i) *pain*, pre-treatment 13.0±4.8, post-treatment 18±2.5 and, for 6 patients, at the 6-month follow-up 18±2.8; (ii) *joint stiffness*, pre-treatment 5.1±2.2, post-treatment 6.8±1.0 and at the 6-month follow-up 6.8±1.3; (iii) *function*, pre-treatment 46.2±13.1, post-treatment 61.0±4.7 and at the 6-month follow-up 63.1±4.3.

None of the patients had any adverse effects or allergic reactions.

Our work was a pilot study on the use of PRP in joints affected by continuous and worsening degenerative diseases; the preliminary data provide an excellent starting point for further development of the technique with possible applications also in other anatomical districts.

Both groups studied had improvements in results in the long-term and pain decreased substantially from the time of the first infiltration.

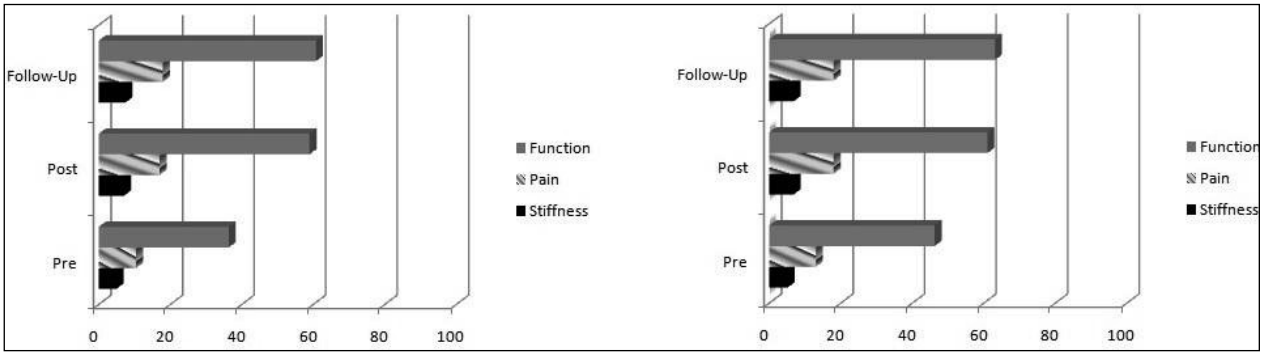

Figure 1 - WOMAC score in patients with knee arthritis (left) and cartilage disease (right).

Literature review and discussion

PRP is a concentrate of autologous growth factors used in different fields of medicine in order to improve and potentiate tissue regeneration. We reviewed the literature, finding and evaluating four studies in which patients with knee arthritis were treated similarly to ours with three infiltrations of PRP: the results of these studies were comparable to those in our study.

In a prospective study by Kon *et al*. in 2009, patients with a diagnosis of degenerative cartilage lesions were treated with injections of PRP[24]. The 100 patients were clinically evaluated before and at the end of treatment and during the follow-up using appropriate questionnaires and had improvements in all clinical scores, including an improvement in joint function and a decrease in pain[24].

In 2010, Filardo *et al*. carried out a study in which 91 patients were followed up for 12 months; 90 of these patients were also re-evaluated after 2 years. The results showed a decrease in pain and an improvement in joint function and, therefore, quality of life[25].

Also in 2010, Sampson *et al*. demonstrated significant and almost linear improvements in the scores on the evaluation scales used, a reduction in pain and relief from symptoms 12 months after treatment[26].

The only retrospective, observational cohort study, using injections of hyaluronic acid as the control treatment, was performed by Sanchez *et al*. in 2008. Each group consisted of 30 patients with joint arthritis. The clinical results were evaluated using the WOMAC questionnaire before treatment and 5 weeks after treatment (Table I). The success rate at 5 weeks with regards to the pain scale had reached 33.4% for the group receiving treatment with plasma rich in growth factors and 10% for the group treated with hyaluronic acid[27].

The aim of our study was explore the use of this new approach to the treatment of degenerative lesions of cartilage of the knee joint.

Joint cartilage is often exposed to multiple macro- and micro-trauma which can lead to the loss of tissue and consequent loss of the cartilage layer and evolution towards arthritis. Cartilage has an intrinsically limited capacity to regenerate making degeneration of this tissue difficult to treat[28,29]. Growth factors play a crucial role in modulating chondrogenic expression. TGF-β binds to the layer of cartilage activating its regeneration through greater

Table I - Comparison of the clinical results in the two groups studied.

	Cartilage disease			Arthritis		
WOMAC score	**Stiffness**	**Pain**	**Function**	**Stiffness**	**Pain**	**Function**
Pre-treatment	5.1±2.2	13.0±4.8	46.2±13.1	4.9±2.2	10.4±3.9	36.3±11.8
Post-treatment	6.8±1.0	18±2.5	61.0±4.7	7±0.9	17±2.5	58.9±9.9
Follow-up	6.8±1.3	18±2.8	63.1±4.3	7.4±0.9	17.9±2.8	60.7±7.6
NRS score						
Pre-treatment	6.8±1.7			8.1±1.7		
Post-treatment	2.3±2.1			3.4±2.5		

phenotypic expression of chondrocytes and activating matrix synthesis, through the differentiation of mesenchymal stem cells towards the chondrogenic lineage and through the interaction with interleukin-1 which activates the synthesis of proteoglycans[30,31]. PDGF increases the proliferation of chondrocytes and stimulates the synthesis of proteoglycans.[32] Insulin-like growth factor-I (IGF-I) has been demonstrated to stimulate the synthesis of proteoglycans and suppress their catabolism[33,34]. Basic fibroblast growth factor (**b**FGF) and VEGF are involved in chondrocyte induction. These growth factors are all present in the α-granules of platelets, with the exception of IGF-I[34], and can be instilled into the joint at high concentrations[35].

Conclusions

The patients were treated as out-patients by a multidisciplinary team consisting of a transfusion specialist, an orthopaedist and a radiologist who collaborate in order to optimise the methods of selecting, diagnosing and treating patients.

The activity of this out-patient clinic, in line with Legislative Decree 502/92[36], is based on the union between technological innovations and reduction of health care costs by:

- less care-intensive diagnostic-therapeutic processes, which nevertheless remain of high technical and professional quality;
- continuous management and monitoring of the patient;
- the possibility of moderating/limiting the use of hospital services and pharmacological demands.

In accordance with Law n. 219 of October 21, 2005[37] and Legislative Decree n. 261 of December 20, 2007[38], PRP is prepared by a specialist in immunohaematology, who is flanked by an orthopaedist and radiologist for the performance of the infiltration. The patient is enrolled, following clinical and instrumental investigations in order to determine the correct diagnosis and appropriate therapeutic strategy, and undergoes serial evaluations over time.

This experience has led to the development of an organisation whose central role is played by the transfusion specialist and the other professional figures who cooperate in order to optimise the use of the organisational and economic resources.

The preliminary results indicate that treatment with injections of PRP is safe and can reduce pain and improve joint function and, therefore, the quality of life of patients. In line with other published studies, this treatment gave better results in younger patients with less severe joint degeneration[24-25]; this induces us to believe that a multicentre, prospective study is needed in order to uniform the diagnostic and therapeutic criteria, stratifying the patients on the basis of age, sex, BMI and the radiographically determined severity of the disease.

Additional efforts should be made to try to standardise both the techniques for preparing the PRP and the optimal therapeutic dose. This would add to the scientific knowledge on PRP and improve and broaden its use.

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Correspondence: Marcello Napolitano
U.O.C. di Immunoematologia e Medicina Trasfusionale
Azienda Ospedaliera di Cosenza
Via F. Migliori
87100 Cosenza, Italy
e-mail: manap@libero.it