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ORIGINAL STUDY

The impact of platelet-rich plasma on chronic synovitis in hemophilia

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Untreated chronic haemophiliac synovitis leads to the development of haemophilic arthropathy (HA) by affecting the metabolism of chondrocytes. Symptoms are progressive and often surgical intervention is required to prevent total loss of joint function. The focus of our study was to influence the chronic haemophiliac synovitis by means of autologous platelet-rich plasma (PRP) injection.

Six patients with hemophilia (PWH), aged between 9 and 45 and manifesting chronic synovitis of the ankle joint on one or on both sides (8 joints in total) were included into the PRP-study. The patients were classified depending on their joint status using the Hemophilia Joint Health Score (HJHS) prior to and again two months after treatment. Three to five ml of PRP was injected into the joint cavity within 30 seconds.

In all of the tested PWH pain relief has been reported subjectively by means of the HJHS and VAS scoring systems, comparing the pain intensity before PRP injection and two months after. The difference of pain perception has been found statistically significant for the VAS-scores. Considering the objective synovitis signs shown on MRI before and after PRP injection we recorded a decrease in the volume of free synovial fluid after PRP. All of the tested patients reported benefit of the PRP therapy.

Key words : haemophilia, arthropathy, platelet rich plasma, growth factor, synovitis

INTRODUCTION

Hemophilia is an inherited, gonosomal recessive illness. The most frequent is the A-type, in which factor VIII is deficient, in type B, there is a deficiency of factor IX. According to the amount of the factor present in blood there are three forms : mild, moderate and severe. About 50% of hemophiliacs have the severe form. People with hemophilia (PWH) suffer bleedings mostly in soft tissues or in joints, less frequently in mucose membranes.

After several repeated intraarticular bleedings in one joint a chronic synovitis is a common finding in PWH. These bleedings originate from the subsynovial venous plexus underlying the capsule, where a lack of thromboplastic activity has been demonstrated. The function of synovial cells (synoviocytes) is to absorb the blood from the intraarticular space and the presence of iron causes the

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synovial membrane to hypertrophy, so the synovium becomes richly vascularized and therefore vulnerable for repeated bleeding (*2*). The joint affected with chronic synovitis appears swollen, but is often painless, what is the most important finding in differentiation with haemarthrosis. Untreated chronic haemophilic synovitis leads to the development of haemophilic arthropathy (HA) by affecting the metabolism of chondrocytes.Hemophilic arthropathy is a most disabling problem in patients with hemophilia. Knees, ankles, elbows, hips and shoulders are the most common joints involved. Symptoms are progressive and often surgical intervention is required to prevent total loss of joint function. Management of HA has advanced with the development of purified clotting factor concentrates and procedures to prevent chronic synovitis.

Platelet-rich plasma (PRP) is a simple, low cost and minimally invasive way to obtain growth factors and is currently being widely experimented in different fields of medicine for its ability to aid the regeneration of tissue with a low healing potential (*19*). PRP is a concentrate of platelets in a small amount of plasma derived from peripheral blood, with a natural concentration of autologous growth factors. After platelet activation, several growth factors are released from the alpha-granules. Biological characteristics of PRP are based on the activity of different growth factors included in PRP (Table 1). The growth factors play a key role in regulation and stimulation of healing processes and regeneration of different tissues. The growth factors are able to stimulate cell proliferation, matrix remodelling and angiogenesis (*10*). The potential use of the patients' own growth factors to enhance reparative processes in tissues with low healing potential is evidenced by promising preliminary clinical applications (*16*).

The first published in vitro studies demonstrated a general tendency of PRP to stimulate the proliferation of several cell types, including osteoblasts (*8, 31*), fibroblasts (*21*), tendon cells (*5*), chondrocytes 1), periodontal ligament cells (*27*) and bone mesenchymal stem cells (*23*). PRP counteracts effects of an inflammatory environment on genes regulating matrix degradation and formation in human chondrocytes. Further, PRP decreases NFκB (Nuclear Factor Kappa Beta) activation, a major inflammatory pathway involved in the pathogenesis of ostheoarthrosis (*32*).

Table 1. — Short summary of the growth factors active in Platelet- rich plasma concentrate.

Growth factor	Function
PDGF : Platelet-derived growth factor	Promotes mitogen Activity of mesenchymal cells (smooth muscle cells, glioblasts, fibroblasts, osteoblasts), regulates colagen synthesis
TGF-β : Transforming growth factor beta	Controls proliferation, Cellular differentiation and other functions in most cells, regulates activity of other growth factors, stimulates angiogenesis, inhibits macrophages and lymphocytes proliferation
FGFs : Fibroblast growth factors	Promotes angiogenesis and fibroblasts proliferation
VEGF : Vascular endothelial growth factor	Stimulates migration and mitogen activity of endothelial cells
EGF : Epidermal growth factor	Stimulates cell growth, proliferation and differentiation
CTGF : Connective tissue growth factor	Stimulates cell adhesion, migration, proliferation and angiogenesis

Multiple studies proved that mature mesenchymal cells active in the synovium healing proces have specific membrane receptors for platelet growth factors and thus the growth factors can activate these cells in the healing process of soft and bone tissues (*19,10*).

The use in orthopaedic surgery and traumatology has been wide spread. The most common indications are patellar tendinosis (14), tennis elbow (*15*), rotator cuff repair (*9*), anterior cruciate ligamentoplasty (*30*), tendon ruptures (*29*), plantar fasciitis (*24*), other tendinopathies (*28*) as well as bone cysts in children (*16*). It can also be used in treatment of non-unions and pseudoarthroses (*25*) and in cartilage repair (*7*).

Using PRP in other medical disciplines has been tested as well, such as in general (*26*), maxillofacial (*12*) and plastic (*22*) surgery, gynecology and cardiosurgery (*13*) and in laparoscopic operations for gastric bypass, hysterectomy or sternotomy.

However, despite wide clinical application and many trials ongoing worldwide, currently there is only scarce scientific evidence of the efficacy of PRP (*11*). Some studies on blood-derived growth factors have shown interesting preclinical results and a promising clinical outcome after the treatment of cartilage lesions in humans. But high-quality studies are required to confirm the preliminary results of PRP- administration and provide scientific evidence to support its use (*19*).

The aim of this study was to decribe the effect of PRP in PWH, who manifested chronic synovits of the ankle joints.

MATERIAL AND METHODS

Study design and patient characteristics

From July to September 2012 we treated 6 patients with severe hemophilia type A (aged 9-45). They manifested chronic synovitis of the ankle joint on one or on both sides ; in total we treated 8 joints. The study followed the principles of the Declaration of Helsinki (*34*).

Joint status

The patients were classified depending on their joint status prior to treatment and again two months after, applying the Hemophilia Joint Health Score (HJHS) (*18*). In every affected joint the following 8 signs or symptoms were evaluated : swelling, duration of swelling, muscle atrophy, crepitus on motion, flexion loss, extension loss, joint pain and muscle strength. All patients were also asked to rate their pain on the Visual Analogue Scale (VAS) (*33*).
The joint status in 5 patients was examined by MRI before the application of PRP and then 2 months after. One patient has been examined by ultrasound, because of claustrophobia (Table 2).

PRP-administration

Peripheral blood was drawn after the patients were preloaded with clotting factors according to their weight. Gravitational Platelet Separation (GPS) System by CFT Cell Factor Technologies, Inc., Biomet Europe was used for PRP-preparation. The GPS© II System separates the blood components, on the basis of density, by centrifugation. Whole blood is collected with anticoagulants and briefly centrifuged with low forces (softspin). Three layers are obtained : red blood cells (RBCs), 'buffy coat' (BC) layer and platelet-poor plasma (PPP). BC is typically of whitish colour and contains the major proportion of the platelets and leucocytes (*10*). Arthrocentesis was than done under sterile conditions, aspiration of the fluid was done and PRP (3-5 ml) was injected into the joint cavity. The joint was stabilised in a soft bandage for 24 hours and sport activities were prohibited for four weeks. Patients were allowed to bear full weight. Pain relief and volume of free synovial fluid was evaluated and compared pre- and post-treatment.

Statistics

The variance of the paired values HJHS and VAS was evaluated by Fisher exact test. The concrete numbers of HJHS and VAS were compared pre- and post- treatment by means of the t-test. A p-value less than 0.05 was considered statistically significant.

RESULTS

The common features of all patients, before PRP injection, were : chronic synovitis, recurrent ankle swelling on one or both sides, associated with loading , without recurrent intrarticular bleeding recently and with relatively high HJHS (average 6.75) and VAS- score (average 2.85). All patients were on preventive treatment with factor VIII and had no bleeding for at least one year before PRP injection.

Two months after PRP injection, the HJHS score decreased (average 4.85), but not significantly. All patients reported pain relief, shown also by the VAS score values, which decreased significantly (average 0.75, $p \leq 0{,}0001$) Objectively, we may conclude, that the pain perception was lowered by PRP injection (Table 2).

Case studies (Table 2)

The first patient is a 9 year old boy, the youngest patient, who suffered from left ankle problems. Soft tissue swelling was reported and shown by MRI with an apparent swelling of the synovial lining and the presence of joint fluid. After treatment with PRP the ankle swelling disappeared and the patient described a minimal post-workout pain. The MRI image showed a regression of synovial hyper-

Table 2. — Characteristics of PWH treated with PRP- injection before and after therapy (difference 2 months)

No.	age (years)	localization	PRP injeciton (ml)	HJHS pre	HJHS post	VAS pre	VAS post	MRI pre	MRI post
1	9	left ankle	5,0	5	1	3	0	hypertrophic synovium, free fluid	significant free fluid regression, mild synovial hypertrophy regression
2	45	left ankle	3,0	10	8	4	2	mild hypertrophic synovium	no change
3	45	right ankle	3,0	12	10	3	1	moderate hypertrophic synovium	no change
4	24	right ankle	6,0	7	5	3	0	hypertrophic synovium, free fluid	significant free fluid regression, mild synovial hypertrophy regression
5	20	right ankle	6,0	11	8	3	1	moderate hypertrophic synovium	mild synovial hypertrophy regression
6	13	right ankle	3,5	3	0	3	0	US : mild hypertrophic synovitis, no free fluid	US : no change
7	21	left ankle	3,5	3	1	2	1	synovial fibrosis	no change
8	21	right ankle	3,5	3	1	2	1	synovial fibrosis	no change

throphy. This was accompanied by the disappearance of free joint fluid (Fig. 1A-D).

The second and oldest patient (45 years), complained of both his ankle joints showing signs of severe HA with synovial lining irritation. After application of PRP, he was able to walk without pain. Moderate and mild synovial hypertrophy was seen on the MRI of both ankles, which remained unchanged after the PRP treatment, but his subjective evaluation was improved bilaterally as shown by the VAS- score.

The third patient was a very active young man of 24 years. His main problem was the morning stiffness in his right ankle. Clinically the joint presented mild swelling and symptoms of chronic synovitis. After application of PRP the time needed to move the joint in the morning was shortened and the usual morning stiffness was reduced. This subjective and clinical improvement corresponded with the MRI findings, where a significant regression of free fluid and a slight decrease of synovial hypertrophy was seen. The VAS score was reported as 0.

The fourth patient was a 20 year old athlete, who complained of chronic post-workout pain and swelling of the right ankle that limited him in his sports activities. The MRI images showed synovial hypertrophy but no free fluid.After application of PRP the MRi examination showed a slight decline of synovial hypertrophy. Clinically, decrease in swelling and subjective improvement of performance wa reported.While the joint pain still lasted, the duration of it was shorter.

The fifth patient was a 13 year old boy with symptoms of chronic synovitis of the right ankle. He was unable to undergo an MRI examination because of claustrophobia, he was consecutively examined by ultrasound. A mild synovial hypertrophy without presence of free fluid was reported before PRP administration. The same image was reported after PRP treatment, but subjective complaints improved (VAS score 0) with regression of soft tissues swelling.

The last patient was a 21 year old man with a chronic synovitis in both ankles. He described them as painful and stiff, with mild soft tissue swelling. The MRI image showed a synovial fibrosis in both ankles. This picture remained unchanged even after treatment with PRP, but the swelling was reduced

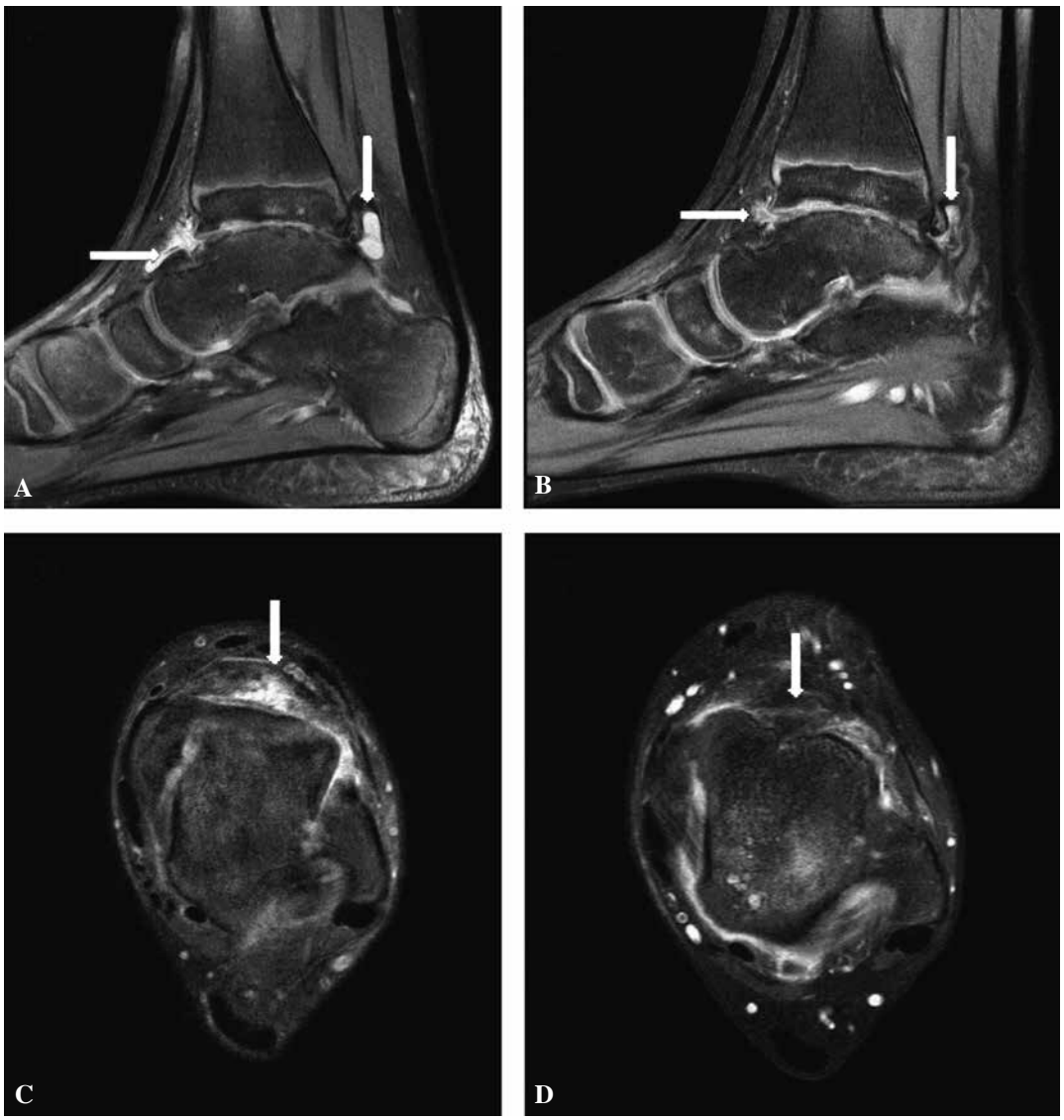

Fig. 1. — MRI of the left ankle joint.
Side view : Tumefied synovium and free fluid in both the ventral and the dorsal recesses are seen before treatment (A). Mild synovial hypertrophy and significant free fluid regression is seen after treatment (B).
Transversal view : Free fluid in the ventral recessus is seen before treatment (C). Regression of this fluid is seen after treatment (D).

and the patient described the joints as less rigid and painless.

DISCUSSION

The positive effect of PRP therapy reported in the literature is evident, but there are still too few studies reporting the PRP action in HA. Many aspects still need to be clarified, such as the dosage, the timing and the way of application. Intra-lesional injections appear to be the most reasonable approach, in order to directly stimulate the affected site (*19*). This approach has been used in our PWH study, too. The proper timing of application is still unclear. We may hypothesize that the count and frequency of bleeding episodes, could be a determining factor, but this must be tested more precisely in the future.

Nevertheless, several PRP studies showed an effect in degenerated or damaged joints if compared to the standard therapy (*11*). Generally, analysing PRP effect in patients with OA, we may assume, that the PRP therapy works better in young people and is more effective if the joint shows milder cartilage degeneration (*20*).

According to our results we may claim, that there is a significant benefit of pain relief in PWH reported by the VAS-score and that the HJHS score has improved as well, but not significantly. Our study included only 6 patients, which is not enough to make general conclusions, but after our experience, we would like to encourage orthopaedists, to choose a PRP injection as a possible alternative in treatment of hemophilic arthropathy. There is no recent socio-economic study monitoring the efficiency of the PRP treatment, but we would like to present it as an alternative with very satisfactory results. To our knowledge, there is at the moment no study reporting the PRP effect in HA.

A recent study explores the effect of PRP on cartilage in comparison to hyaluronic acid as an alternative to PRP, in patients with osteoarthritis. The authors found that autologous PRP injections showed more and longer efficacy than hyaluronic acid injections when pain reduction and articular function were evaluated. Better results were achieved in younger and more active patients with a low degree of cartilage degeneration, whereas a worse outcome was obtained in more degenerated joints and in older patients, in whom results similar to those of visco-supplementation have been observed (*20*). Another similar study reported comparable results. PRP injections showed a significant clinical improvement up to one year of follow-up. However for middle-aged patients with moderate signs of osteoarthritis the results were not better for PRP than those obtained with hyaluronic acid injections (*11*).

PRP therapies can delay joint cartilage deterioraton by interfering with the early catabolic and inflammatory events and by subsequently promoting anabolic responses (*3*). On the other hand, PRP can initiate an inflammatory response in the absence of an inciting injury in normal soft tissue in rabbits (*6,17*).

No simple pathway can be determined how PRP acts in the treatment of joint pathologies. PRP therapies are much more complex than previously thought (*3*) and their exploration poses more questions than answers.

CONCLUSION

Autologous PRP administered into the joint cavity reduces the symptoms of chronic hemophilic synovitis in the ankle joint. Patients treated with this method state a better quality of life, especially thanks to the pain relief. Their clinical status also improves.

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