



REZUMAT TEZĂ DE DOCTORAT

**COMPREHENSIVE STUDY OF THE EFFECT OF
REGENERATIVE THERAPIES IN TREATING
MENISCAL/CARTILAGE LESIONAL COMPLEX AND
TENDINOPATHIES**

DOCTORAND

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MOTIVATION

The motivation of the present study is to bring further information and insight into the present knowledge in the domain of regenerative therapies, but with elements of originality represented by the simulation of a common clinical situation: during surgical intervention for a meniscal tear (that was diagnosed beforehand), a cartilage lesion is observed (previously not diagnosed on imaging study), that needs to be addressed.

The main objectives of the study consist of:

- Demonstrating the regenerative potential of two types of regenerative therapies, PRP and BMAC respectively, in treating the cartilage/meniscus lesion complex (post-meniscectomy status) with experimental simulation of a relatively frequent situation in clinical context, and with poor treatment options in the present.
- Demonstrating the profilactic role of these therapies in the evolution and progression of chondropathy to arthrosis at a relatively young age.
- Comparative study of the results, using two regenerative therapies consisting of platelet rich plasma and bone marrow aspirate concentrate.
- Evaluating the effect of PRP therapy in the management of an extremely problematic pathology represented by recurrent Achilles Tendon tendinopathy that does not respond to conservative treatment measures.
- Proposing confocal microscopy as an efficient technique in evaluating articular surface with the possibility of 3D reconstruction offering a detailed picture of cartilage remodelling potential of studied therapies.

The present approach is an original one by conducting a comparative study between the two types of therapies. The main innovative aspect consists of the experimental simulation, *in vivo*, of a lesion complex frequently seen in clinical practice: complex knee injury that includes a meniscal tear, a cartilage lesion and +/- ligament injuries. Both therapies were administered after surgical treatment of the meniscal lesion by partial meniscectomy of the internal meniscus, recreating the clinical context in which during surgery for meniscal tear, a cartilage lesion is diagnosed, lesion that was previously not obvious on imaging studies. Follow-up time until the knees were harvested for further examination and studies was 6 months. The main method we used for studying osteochondral remodelling and healing

was confocal microscopy with 3D reconstruction of the articular surface in the cartilage defect area, and obtaining representative images for the effect of these therapies in the process of healing and remodelling of joint surface. Quality of the articular surface at the end of the healing process is strongly related biomechanically with the long term functional result on the affected joint.

GENERAL PART

The general part treats the current state of knowledge but also the clinical reality in the case of cartilage pathology, with the therapeutic options unanimously accepted in the present.

Regenerative medicine is developing at a fast pace in recent decades, being both promoted by the medical community and supported by the pharmaceutical industry. The definition of regenerative medicine is the treatment of pathologies by engaging the body's ability to heal and maximizing this physiological response by various methods of concentrating enzymes and biochemical factors involved in this process, administered at the site of injury or damaged structure, the ultimate goal being faster healing and preferably through regenerated healthy tissue and not scar tissue or fibrosis.

The ultimate goal of regenerative medicine is to improve the performance of the human body, with some scientists going as far as the idea of increasing life expectancy. The accelerated development of this field has led to a mismatch between the availability of these therapies for clinicians and patients and the lack of clear regulations regarding therapeutic protocols. The absence of regulations regarding the establishment of the indication, the administration protocol, the fixed harvesting protocol, the processing / centrifugation protocol, will lead to the delay in obtaining objective evidence confirming the effectiveness of these therapies as viable alternatives in the treatment of musculoskeletal disorders commonly encountered in orthopedics and traumatology. The standardization of the

conditions for the application of these therapies is currently an urgent need according to the concept of evidence-based medicine.

Although the use of these techniques is very popular in other surgical specialties, the most important being plastic surgery, or even dentistry, with various variants of use of biological products, orthopedics reluctantly adopts regenerative therapies due to lack of long-term evidence of articular side effects. Certainly in the next period, biological treatments will become very popular and the application will become more and more diverse as the orthopedic surgeon gains trust. Studies are beginning to report favorable results in the treatment of chronic tendon pathology, a classic example being the supraspinatus tendon in the shoulder, or as an adjunctive treatment in Achilles tendon reconstruction when at the end of surgery the tendon is infiltrated with platelet-enriched plasma (PRP) for accelerated healing, but also in other complex pathologies that pose major problems to the orthopedist such as pseudarthrosis or massive bone tissue defects.

Platelet-rich plasma or PRP has been used for decades to facilitate tissue healing during surgery. Currently, percutaneous injection for various pathologies has become a relatively common practice, being the next natural step after the results obtained in various surgical specialties. The diseases in which it is used are osteoarthritis, tendinopathy, chondropathy, ligament ruptures, muscle, etc. although scientific evidence is limited on the actual biological effect of PRP. Also, variations in the preparation and administration protocol may affect the results, so a standardization of this therapy is necessary with the enrichment of scientific evidence of PRP effects. In the field of orthopedics and traumatology, PRP therapy seems to be becoming a viable option in certain pathologies in which current therapeutic measures are not satisfactory, such as various forms of tendinopathy, such as chronic supraspinatus tendinopathy, chronic Achilles tendonopathy, but perhaps the most interestingly, it is the

pathology of the cartilage or the field of chondropathies. Cartilage pathology has always made treatment difficult and clear solutions are not yet foreseen in the near future.

The contribution of the study in defining a specific place of regenerative therapies in the arsenal of the orthopedic surgeon consists in taking a post-traumatic clinical context familiar to arthroscopic knee surgery and comparative evaluation of the results obtained using the two techniques. The clinical context with a potential indication for the application of regenerative therapies is the association of partial post-menisectomy status with focal osteochondritis.

Therapeutic behavior in the case of osteochondral injury associated with a meniscus injury and / or knee ligament injury, usually in the context of sports trauma, varies depending on many factors ranging from the experience of the orthopedic surgeon and the degree of the hospital unit to which the patient is referred. to economic factors. One thing is certain, so far no consensus or standard protocol has been established in the management of this lesion complex, due in large part to the lack of an option to cure the osteochondral lesion without long-term effects on the joint.

SPECIAL PART

The special part includes three studies carried out in order to achieve the above-mentioned objectives, as follows:

First study:

It consisted in the standardization of the confocal microscopy technique as an extremely valuable method in the detailed description and study of the joint surface but also in

establishing a clear protocol for the study of osteocartilaginous pieces. Thus, a study of 'mapping' the femoral head in secondary hip osteoarthritis was performed demonstrating regions with quasi-normal joint surface at the level of the non-bearing lower pole and at the level of the posterior region of the partially supporting femoral head, using images obtained by confocal microscopy. The results obtained contribute to the knowledge base that suggests the importance of additional studies in the field of physiological bone salvage procedures in favor of replacement by arthroplasty.

The aim of the present study was to "map" the joint surface of osteochondral fragments in the human femoral head, in the context of coxarthrosis with surgical indication. The main objective of our study was to demonstrate the presence of quasi-normal cartilage over a significant area of the femoral head surface in patients with indication for hip endoprosthesis. The secondary objective was to support research in osteotomy procedures for the preservation of the natural femoral head and the postponement of replacement with total hip stents.

In this study, the methodology of scanning the joint surface at the level of the portant and non-bearing areas of the femoral head was standardized using confocal microscopy.

The working hypothesis is that, in carefully selected cases, the femoral head has regions with articular surface and quasi-normal cartilage in non-load-bearing or partially load-bearing areas. For these cases, total hip arthroplasty seems an aggressive therapeutic option, especially at a young age. As such, innovative studies are needed in the field of bone / joint rescue procedures, so that these patients can benefit, even for a limited period of time, from the advantages of the biological joint.

Confocal microscopy has the advantage of studying the joint surface with a detailed description of cartilage defects, and respectively, obtaining extremely accurate 3D reconstructions.

The results obtained from the scanning of osteochondral fragments but also the reconstruction of 3D images claim without a doubt that this technique of analysis of the contact surface is extremely valuable and can provide additional explanations in the comprehensive understanding of the biomechanics of the arthritic joint.

Based on these preliminary results, a new surgical technique could be imagined in which the osteotomy is performed at the basicervical level, without the involvement of the trochanteric mass and obligatorily without dislocation of the femoral head during the intervention to preserve the artery of the round ligament. Although many voices would challenge the viability of the femoral head following basicervical osteotomy that would disrupt blood supply through the cervical epiphyseal arteries, we identified two solid arguments that theoretically support bone consolidation and viability of the rotated femoral head, as follows:

1. preservation of the artery of the round ligament which contributes significantly to the vascularization of the femoral head and could ensure the minimum blood supply necessary to maintain the viability of the femoral head.
2. very high rate of consolidation of the subcapital fracture following open reduction and osteosynthesis with interposition of the graft harvested from the iliac, with the absence of consolidation in only 3% of cases. In the case of osteotomy, the time elapsed from trauma to reduction is excluded and the reduction will be anatomical considering that the osteotomy tranche creates a perfectly flat "fracture" line (elements that decrease even more in theory, the percentage of consolidation complications).

An animal study can confirm these theoretical assumptions and is a future project to propose an original technique of osteotomy of rotation of the femoral head, designed to allow even temporary preservation of the natural joint, until the patient's age is "compatible" with the proposal. total hip arthroplasty surgery.

Second study:

It is on an animal model (rabbit), which included 21 animals divided into three groups, in which the complex knee trauma was surgically reproduced and later we used the two

regenerative techniques to observe the differences in the healing and remodeling process at the surface. joint. At the end of the 6-month observation period, the joints were harvested and analyzed using confocal microscopy. The obtained results showed for the first time nationally , the effectiveness of PRP therapy in the immediate post-traumatic context.

The experimental model was built in order to reproduce in experimental context a situation encountered relatively frequently in the usual practice, namely, the lesional association at the knee consisting in the duo meniscus lesion - osteochondral lesion. At present, the osteochondral defect in such a lesion complex is treated incompletely or insufficiently for a number of reasons, including the experience of the orthopedic surgeon and the level of the hospital unit where the patient is cared for. Thus, in our experiment, the first knee surgery consisted of osteochondral defect and excision of the anterior horn of the meniscus, an intervention to which all experimental animals were subjected, at the right knee, followed by random division into study groups for avoiding the influence of the results through possible differences between the created lesions. The study included 21 adult rabbits purchased from the Cantacuzino Institute and acclimatized in the biobase of the University of Agricultural Sciences and Veterinary Medicine of Banat Timisoara where the experimental part was performed with the approval of the University Bioethics Commission and the Sanitary Veterinary Directorate. Rabbits belong to the New Zealand white breed and adult animals > 3 years old have been selected to replicate the biological context of the adult and avoid false results due to the biological reserve of the youth. The rabbit was chosen because it has the biomechanical advantage of total knee loading during normal gait by replicating human joint stress where loading also occurs with full body weight. The place of choice for the osteochondral lesion was the internal femoral condyle, the motivation being also the most accurate simulation of the situations encountered in medical practice in which the internal compartment of the knee is most often affected, especially the internal femoral condyle. Also, the internal condyle is a region of maximum mechanical stress subjecting the lesion but also the techniques studied by us, to the test. After initial surgery, all animals were marked with a single code subcutaneous CIP to avoid errors after distribution in study groups and for ease of recognition at the end of the follow-up period.

The post-operative follow-up period ('post-traumatic') was 6 months after the initial intervention to ensure the onset and evolution of degenerative changes in the three

experimental groups. Given that the biology and lifespan of the rabbit extends to 8-12 years, the six months is a considerable amount of time, transposed into human biology would be about 4 years although such an equivalence is not scientific. At the end of the follow-up period, the knee was harvested for examination. The study of the knee consisted of 3 distinct stages:

- macroscopic examination of the entire joint, cartilage defect and remaining meniscus
- study in confocal microscopy with 3D reconstruction of the joint surface and multiple measurements on images to objectify the results
- histological study for control and confirmation of the results obtained in confocal microscopy

Experimental results suggest accelerating the healing process and increasing the quality of joint surface remodeling in the presence of PRP therapy but also improving the final result in the entire joint after injuries resulting in the lesion complex: cartilage defect / post meniscectomy status with relatively immediate administration in the postoperative or posttraumatic stage. The three-injection protocol was used at 7-10 day intervals and post-procedure joint mobilization was not limited in any way. In the animal model experiment it is practically impossible to limit the load of the treated joint but we do not exclude the possibility that mobilization without loading for a period of 6-10 weeks to further improve the final result, we will start a new experiment in this direction.

The results of hematogenous marrow concentrate therapy are not as scientifically sound as we would have initially liked for two major reasons: the first was the small amount of hematogenous marrow that can be harvested in the case of rabbits, which is impossible to predict in the design phase of the study, and the second is the single-procedure protocol with a single administration that may be insufficient in starting the regenerative process. Although animal studies using rabbits to study the effects of BMAC therapy are found in the literature, the exact hematogenous bone marrow harvesting protocol is not described and we cannot explain why in the present study the harvesting was so laborious and the maximum amount

harvested so reduced. The small amount of hematogenous marrow made it extremely difficult to process, leading to a final product of questionable quality, although the presence of mesenchymal cells was checked and confirmed microscopically. Recent data, however, suggest that not only mesenchymal cells are responsible for the success of therapy but the entire enzyme complex that may not have been obtained in the optimal parameters in our experiment. Another aspect is also the impossibility of following some recommendations after performing the procedure such as: limiting the load, limiting certain activities, etc. . Both therapies in the family of regenerative therapies improved the final result but significant results were obtained in the case of PRP therapy, which validates this procedure as an option in the arsenal of the orthopedic surgeon as an adjunct procedure in the treatment of complex knee injuries, even in postoperative context. operator, situation reproduced in the present experiment. However, PRP therapy must be included in a complex post-traumatic management from which it certainly does not replace either reconstructive surgical procedures or stages in the patient's postoperative recovery.

Third study:

Comes to explore a separate field of application of PRP therapy, namely chronic non-compliant Achilles tendonopathy, addressing the effects of this regenerative therapy in the management of tendon pathology, a topic of great interest in the literature. Musculoskeletal ultrasound was used as a technique to evaluate the results, both compared to the healthy contralateral tendon before therapeutic interventions, and after these procedures at one month and 6 months. In the vast majority of cases, the administration of PRP therapy has led to a functional recovery superior to the therapeutic alternatives previously used through stability over time.

Tendon pathology in general and Achilles tendon pathology in particular pose problems in daily practice due to the fairly high incidence but also the limitation of activities and poor tolerance, being a condition that influences and limits gait. De Jonge et.al. publishes a paper in which he follows from the presentations to the general practitioner how many patients complain of gait disorders due to an Achilles tendon pathology and reaches the number of 2.35 out of 1000 patients who go to the family doctor with this problem. Of course, in the

case of following the orthopedic presentations, the number would be approximately 3-5% of the total number of patients who present with painful accusations, especially chronic ones. Precisely for this reason, the interest for therapeutic solutions in the case of chronic tendon pathology is constant and the practitioner is always looking for new options for the patient with chronic tendinopathy.

Achilles tendon pathology, especially chronic degenerative tendinopathies are relatively common especially after the age of 40 and often very difficult to treat. Although we would expect most tendinopathies to come from sports, this problem has been reported in sedentary people, the explanation being the degenerative changes that occur in the context of age and affect the vascularization of the tendon. Changes most often occur in the middle portion of the tendon, but can also occur in the bone-tendon junction. The high incidence of this pathology but also the high incidence of the failure of conservative treatment up to a quarter of cases, makes this subject to arouse interest when we think about the field of regenerative therapies. Although there are currently several techniques or strategies for treating this disease, PRP therapy is becoming increasingly popular due to several factors including: the biological nature of the therapy by which the injected product results from processing the patient's blood, cost / effectiveness , the degree of invasiveness and so on. The aim of the study was to document the medium and short term results obtained after the treatment of recalcitrant Achilles tendonopathy using local infiltrations with high concentration of PRP, rich in leukocytes.

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

Following the research activity carried out within the present doctoral study, we drew the main conclusions:

- PRP therapy accelerates the physiological healing process and improves the final result of the remodeling of the joint surface by limiting the global degeneration of the joint after traumas resulting in the lesion complex: cartilage defect / post meniscectomy status, on animal model. The protocol of three injections at a distance of 7-10 days was used, the first dose administered relatively close to the moment of “trauma” (14-28 days) and the mobilization of the post-procedure joint was not limited in any way.
- The results of hematogenous marrow concentrate therapy are not scientifically sound for two major reasons: the first was the small amount of hematogenous marrow that can be harvested in the case of rabbits, which is impossible to predict in the design phase of the study, and the second is single-procedure mono-procedural protocol that may be insufficient in starting the regenerative process. The small amount of hematogenous marrow made it too difficult to process, leading to a lower quality final product.
- Both regenerative therapies improve the end result at the joint level but due to the technical difficulties encountered in the preparation and study of BMAC, we consider that our results regarding PRP therapy are really valuable.
- Confocal microscopy can be an extraordinarily valuable tool along with 3D reconstruction using specific software to analyze joint contact surface and comprehensive understanding the sequence of events in the pathophysiology of osteoarthritis.
- - PRP therapy can bring a major benefit in chronic tendon pathology that does not respond to other conservative treatment options, taking into account a multitude of factors that may influence in one way or another the end result, such as: dose administered, quality final preparation, administration technique, number of doses and interval between administrations, type of PRP preparation according to the classifications and so on.

- PRP therapy should be considered not only as a stand-alone therapeutic procedure but also as an adjuvant form, in association with reconstruction surgery.
- Highlighting of the quasi-abnormal cartilage at the level of the femoral head with confocal microscopy reveals additional studies in the field of surgical techniques for preserving physiological bone, a good starting point in this direction being the rotational osteotomy proposed by Sugioka since 1992 .

ORIGINAL CONTRIBUTIONS

- Replication in experimental conditions on an animal model of a relatively common clinical situation in the knee, with a lesion complex consisting of post-traumatic cartilage defect diagnosed at the time of surgery for partial meniscectomy.
- Use of confocal microscopy to describe in detail the joint surface defect and the biomechanical involvement in the arthritic process of contact surface disorders.
- Standardization of the osteochondral fragment analysis technique using confocal microscopy.
- Proposing a surgical variant of rotational osteotomy at the basicervical level in order to preserve the joint in favor of replacement by arthroplasty in young adults.