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UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
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ȘCOALA DOCTORALĂ DOMENIUL MEDICINĂ

INFLUENCE OF LUMBAR INTERVERTEBRAL DISC REGENERATION BY SURGICAL TREATMENT

ABSTRACT OF THE DOCTORAL THESIS

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List of abbreviations

AF	annulus fibrosus
ASA	American Society of Anesthesiology
BMI	body mass index
dGEMRIC	delayed gadolinium-enhanced magnetic resonance imaging of cartilage
DD	disc degeneration
DWI	diffusion-weighted imaging
EPs	endplates
ET	echo time
FEA	finite element analysis
GAGs	glycosaminoglycans
IVD	intervertebral disc
MRI	magnetic resonance imaging
NP	nucleus pulposus
ODI	Oswestry Disability Index
PCR	polymerase chain reaction
PGs	proteoglycans
PI	pelvic incidence
PV	pelvic version
RT	repetition time
SS	sacral slope

The fundamental problem

The etiology of degenerative disc disease is multifactorial, one of the most important factors involved in the degeneration process being vertical overload which causes intervertebral disc (IVD) compression.

This thesis attempts to answer the question of whether IVD degeneration can be stopped or reversed by reducing pressure on the discs during surgical treatment. Illes et al showed that posterior lumbar fusion after distraction on curved rods and using monoaxial transpedicular screws helps restore the anatomical height of the intervertebral disc and improves the quality of life in patients suffering from low back pain due to degenerative lumbar segmental instability. Postoperative imaging MRI results indicate that restoring the disc height allows a better rehydration and a better nutrient supply to the IVD [1].

Hypothesis

The hypothesis of this study was that the posterior distraction achieved during lumbar fusions decreases the pressure on the IVD and improves the content of GAGs in the nucleus pulposus, leading to the possibility of preserving or even regenerating the IVD, the purpose being to preserve the biomechanical properties of the lumbar spine.

Objectives

A clinical and an experimental study on animal model were designed to support the hypothesis formulated above.

The main objective of these studies was to assess the status of IVD, either by quantifying changes in GAGs in vivo, using imagistic techniques, during the clinical trial, or by histological analysis performed during the animal study.

Summarizing, we list below the objectives pursued:

- disappearance of nociceptive disc pain
- analysis of the functional outcome related to MRI signal changes before and after surgery - description of a new surgical method to improve IVD metabolism by restoring disc height and by reducing hydrostatic pressure
- analysis of IVD by determining quantitative and qualitative changes in the content of GAGs and water after surgery using specific dGEMRIC MRI sequences

- slowing or reversing the intradiscal catabolic metabolism, with partial or total restoration of the disc composition
- characterization of the lesions discovered at the histological examination of the IVD from the animal model

Research methodology

Intervertebral disc degeneration is characterized by a progressive loss of extracellular matrix molecules, especially GAGs, while an increase in GAGs is an indicator of regeneration [2]. To determine the quantitative changes in GAGs, MRI with the dGEMRIC protocol (delayed gadolinium-enhanced magnetic resonance imaging of cartilage) was used. It is a method of studying in vivo cartilage abnormalities (GAGs), noninvasive and generally accepted for IVD also [3].

The method is based on the ability of the gadolinium-based contrast agent to be evenly distributed in cartilage structures, including the intervertebral disc. Because the contrast agent is negatively charged, it cannot penetrate a healthy IVD, because GAGs which are also negatively charged are present in large amounts in normal discs and prevent it. With the deterioration of IVD, the amount of GAGs decreases, and the contrast agent will be distributed in the matrix of the cartilage or vertebral disc. The absorbed contrast concentration, which is inversely proportional to the amount of GAGs, can be calculated from the T1-weighted MRI sequence values before and after contrast administration. The same principle can be used to quantify GAGs from pre- and postoperative IVD, by comparing post-contrast T1 values calculated before and after treatment [4].

Numerous techniques have been proposed for the evaluation of the biochemical composition of cartilage, lesions and its repair, these studying the concentration of GAGs, collagen, sodium and water distribution (T2 mapping, dGEMRIC, T1rho mapping, sodium imaging MRI, diffusion-weighted imaging (DWI), etc.) [5]. In literature, dGEMRIC is mainly used for the study of knee cartilage and studies on the spine are rare and very heterogeneous [6]. The interest of dGEMRIC is to highlight cartilage damage in the preradiographic stage, before the appearance of joint space decrease. It is also used to evaluate the effectiveness of certain treatments for the cartilage [7]. In published research, the product used is $Gd\text{-}DTPA2^{-}$ whose trade name is Magnevist. However, since January 2018, marketing authorisations for Magnevist have been suspended by the European authorities. Therefore, we used another contrast agent, Dotarem (Gd-DOTA-), also negatively charged and used in particular by the

Schleich C et al team [8] for the exploration of the lumbar spine. Similar to Magnevist, the dose injected to each patient was 0.2 mmol/kg. As for the time between injecting the contrast agent and acquiring the images, there is no consensus for the spine. In studies involving dGEMRIC analysis of intervertebral discs, either the time between injection and imaging is not given or the time is given in a range of 40 minutes to 4 hours. For exploring the knee [9], the most studied region, the recommended time is 90 minutes. Therefore, we decided to wait 90 minutes between the gadolinium injection and the acquisition of the images, the chosen time representing a consensus between the good impregnation of the cartilage and the acceptance of the wait by the patients. The examination was performed on a 1.5 Tesla MRI before surgery and at a minimum of 6 months after surgery.

The dGEMRIC protocol includes a 3D ultra-fast gradient echo (VIBE) sequence with a repetition time (RT) of 15 ms, an echo time (ET) of 2 ms, a return angle of 5° - 26°, a FOV of 230 x 230 mm, a section thickness of 3 mm and a spatial resolution of 0.6 x 0.6 mm. Image analysis was performed with Syngo.via software (Siemens, Munich, Germany), which allowed the calculation of the dGEMRIC values/index in the T1-weighted MRI sequence. Pre- and postoperative examinations were compared, choosing the sections to evaluate the discs at the same level.

An animal study (rabbits) was designed and performed in order to analyze the imaging and histological characteristics of the intervertebral disc in order to highlight the signs of degeneration / regeneration after the application of a compression – distraction device at the level of the lumbar spine.

Summary of chapters

This work is structured in two parts, *general and special, respectively.* The general part includes information about the anatomo-physiology, biomechanics and the role of the intervertebral disc and the spine, as well as about the etiology and pathophysiology of the disc degeneration (DD), its classification from a morphological, histological, imaging point of view and about the classical therapeutic possibilities and the possibilities of disc regeneration in humans described in the specialized literature. The second part of the work consists of the clinical study itself followed by results, discussions and the experiment performed on an animal model, with results from the literature, the protocol of the experimental study, the development of the device used, its validation and finite element analysis, the results obtained and discussions.

The general part consists of four chapters.

The first chapter provides data on the normal vertebral disc and on disc degeneration.

Lumbar intervertebral disc degeneration is the most important cause of low back pain [10]. Low back pain is pain or discomfort in the lumbar region which may radiate to one or both lower limbs, being associated with various pathologies of the spine. Low back pain is the second reason for consultation for adults at the family doctor after upper respiratory tract infections. But since all age categories can be affected, the prevalence is increasing. Approximately 70 - 80% of the adult population will have at least one episode of low back pain in their lifetime, so important financial resources are allocated each year for diagnosis and treatment [11, 12, 13].

The intervertebral disc, which plays a role in the mobility and stability of the spine, has 3 parts that differ histologically, physiologically and biomechanically. It is avascular, so there is no possibility of healing and regenerating if degenerative lesions occur. It is made up of a central portion called the nucleus pulposus (NP) where cells (chondrocytes-like, notochordal cells, stem cells) are found in an extracellular matrix rich in proteoglycans (PGs), with the role of hydrating the disc. The NP is surrounded by a fibrous ring, made up of extracellular matrix, type I collagen to the outside and type II collagen and chondrocytes to the central part, called the annulus fibrosus (AF). Endplates (EPs) contain mainly type II collagen, but also proteoglycans. They separate NP and AF from the vertebral body, ensuring the diffusion of nutrients and oxygen.

The mechanical role of the intervertebral disc depends on its composition. Thus, the nucleus pulposus is stressed by compression due to vertical loading, having an intrinsic positive hydrostatic pressure, while the annulus fibrosus opposes the tension forces. EPs, in addition to the homeostatic role, also have a mechanical role, fixing the IVD to the upper and lower vertebral bodies and dampening the vertical load.

The mechanism of disc degeneration, from a biological point of view, begins with the number decrease and the disappearance of the notochordal cells in the NP, which coincides with the decrease of vascularization and thus of disc nutrition [14]. It is followed by decreased PGs production and degradation of PGs from NP associated with alteration of the extracellular matrix. NP dehydration and the degradation of the collagen fibers in AF lead to a decrease in the number of cells and a change in their phenotype, to the structural deformation of the disc, with a decrease in height and with the instability of the lumbar segment. Degeneration is a cascade of chemical reactions, with increased production of inflammatory cytokines and catabolic enzymes. This process leads to overloading of the structures adjacent to the intervertebral disc [15, 16].

Changes in the disc also occur with age and are generally irreversible. The distinction between these lesions and those caused by degeneration still remains unclear due to their multifactorial origin. Studies carried out by Conventry et al as early as 1945 show the dynamic changes that take place at the level of the disc during lifetime. Observations related to morphology and age include: disappearance of blood vessels at the level of the vertebral plates, densification of the fibers that constitute AF, progressive loss of distinction between NP and AF, replacement of NP with fibrocartilage, appearance of chondrocyte conglomerates in the deep part of the disc, cartilaginous hyalinization of the vertebral plates, vascularization of the posterior portion of the AF, appearance of fissures at the level of the disc, extrusion in small quantities of NP at the level of the plates (Schmorl nodules). Changes that occur in pathological conditions, for example in degenerative disc disease, include: the appearance of osteophytes, anterior and posterior protrusion due to total or partial rupture of AF fibers, the presence of Schmorl nodules and NP calcifications, flattening of the disc [17, 18, 19].

The etiology of disc degeneration is multifactorial. There are genetic, biomechanical and local factors involved.

Thus, as the intervertebral disc is a dynamic structure similar to the cartilage, being avascular, after degeneration there is no intrinsic possibility of NP cell restoration. Another important factor is the limited nutrition of the disc, because the vessels that cross the vertebral plateau become obliterated in childhood, so that the main mechanism for feeding becomes diffusion.

Among the biomechanical factors, the vertical load that determines compressive forces at the level of the IVD is a primary factor in the occurrence and accentuation of disc degeneration (DD). The size, repetition, duration of these forces influence the production of PGs, collagen types I and II and degrade EPs by calcification [20, 21].

The genetic factor [22] has been intensively studied and DD heredity can reach up to 74% in the case of the lumbar spine.

The action of inflammation is well described by Rannou, who shows the involvement of certain metalloproteinases, cytokines and growth factors in DD. There is a direct link between mechanical stress and the activity of metalloproteinases [23].

Smith et al show the involvement of infection with cutibacterium acnes, the bacterium most frequently detected in culture, by microscopy and conventional PCR (Polymerase Chain Reaction) in the pathogenesis of the degenerative disc disease [24].

Segmental instability of the spine can favor the development of the degenerative disease of IVD. It was described by Kirkaldy-Willis who classified it into three phases: temporal dysfunction, unstable phase, secondary stabilization [25]. In the first phase, the dysfunctions can only be monitored by magnetic resonance imaging and we speak of "dehydrated disc" or "black disc". In the second phase, the changes lead to a decrease in the height of the disc with the alteration of the function of the elements that stabilize the column, the result being instability. The decrease in the height of the interdiscal space is influenced by the decrease in the level of water content and the decrease in the content of GAGs. In the third phase, the stabilization of the affected segment occurs due to the lesions that appear and that decrease intervertebral mobility.

In the second chapter, notions about the biomechanics of the spine and about the reciprocal influence of sagittal balance and the intervertebral disc on each other are presented. The main parameters of the spinopelvic balance are presented, namely the pelvic incidence (PI), the sacral slope (SS) and the pelvic version (PV), the pelvic incidence being equal to the sum of the sacral slope and the pelvic version [26]. Disc degeneration causes the loss of lumbar lordosis with the alteration of the global sagittal balance and the appearance of compensatory mechanisms intended to restore this balance: at the level of the thoracic spine – hypokyphosis is the main compensatory mechanism if the muscles are still efficient; at the pelvic level – pelvic retroversion, which increases stress in the sacroiliac joints, tension in the iliosacral ligaments, contraction of the piriformis muscle, contraction of the posterior spinal muscles, contraction of the rectus abdominis muscles, any of these elements being able to cause pain. The PI value has an influence in the process of lumbar degeneration, the transmission of the load, as we have seen, being different. A large PI causes canal stenosis more frequently than a small PI due to facet arthrosis that causes a decrease in the diameter of the vertebral canal. A low PI can cause ductal stenosis by thickening of the yellow ligament and by disc protrusion.

As the social and economic consequences are important, a diagnosis in the early stages is desirable. Medical imaging is basic for this objective, the MRI exam being the gold standard in the detection of DD. Quantitative MRI can detect intradiscal changes at the molecular level. For our study we used the delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) exam, which helps determine the status of GAGs in IVD. All of this is presented in chapter three.

The last chapter of the general part is dedicated to the treatment of degenerative disc disease. The current treatment of IVD degeneration is represented by conservative therapies (rest, non-steroidal anti-inflammatory drugs, muscle relaxants, physical therapy, acupuncture, lumbar orthoses) and by invasive therapies (epidural infiltrations, ablation techniques, surgical techniques, such as fusion or arthroplasty). All these treatments address the clinical symptoms and not the causes that led to the damage of the disc, they do not restore or preserve the disc.

Regenerative techniques would have curative intent by repairing and regenerating the disc. Regenerative therapies hold great promise because they target the degeneration cascade that fuels disc pain. These treatments may propose exogenous repair based on cell therapy and tissue engineering or endogenous repair based on molecular biotherapy and gene therapy. Although clinical, histological and imaging improvement trends can be observed in the short term, large-scale and long-term studies are needed to see the true potential of these new therapies. Thus, growth factors could be useful for early stages of degeneration, gene and cell therapy for moderate stages, and tissue techniques for late stages [27, 28, 29]. Certainly, in order to improve the results of these treatments, especially in the medium and long term, modern techniques that act directly on the disc should be applied only after the metabolic problems and the problems of the mechanical factor have been solved.

An effective treatment that stops or regenerates the disc has not yet been established. Ideally, 3 objectives should be achieved: the disappearance of nociceptive disc pain, the partial or total restoration of disc composition, the slowing down or reversal of the intradiscal catabolic metabolism.

It is important to start treatment early, in the initial phases, before the loss of mechanical function, because that is the moment when segment instability appears.

The special part / the personal contributions begins with the description of the hypothesis and the objectives, followed by the general methodology of the research (presented at the beginning of the abstract). Afterwards the actual clinical study and the experiment on the animal model are described. The last chapter is that of conclusions and personal contributions.

Our clinical study proposes an improvement of intradiscal metabolic conditions, by influencing the mechanical factor represented by the vertical loading through surgery. In the subchapter "material and method" the selection of patients, the surgical treatment, the clinical (evaluation) and radiological evaluation, the type of statistical analysis were described.

Thus, in this study we included 27 patients (38 intervertebral discs) who underwent the same type of surgical treatment performed by the same surgical team specialized in spine surgery.

The indication for surgery was lumbar segmental instability with marked pain, in all cases due to mono or multilevel lumbar disc disease. Instability was diagnosed both functionally – wearing a lumbar corset – and by preoperative imaging techniques such as standard X-rays and MRI images. Surgery was indicated only if the low back pain was resistant to correct drug and preservation treatment for at least 6 months and if patients did not described a significant reduction in low back pain after continuous wearing of a lumbar corset for 3 months.

Our exclusion criteria included clinical or radiological signs of neurological compression, spinal stenosis or other specific radiological signs, such as spondylolisthesis (greater than grade I), fracture, infection or neoplasm. Exclusion criteria also included contraindications to the use of the contrast agent (gadolinium), as well as uncertainty of the possibility of postoperative follow-up.

From the patients' files, data related to age, sex, body mass index (BMI), ASA score (American Society of Anesthesiologists – assesses the patient's anesthetic risk), the operated level, the duration of follow-up were recorded.

All patients in this study underwent posterior lumbar spine fusion surgery, mono or plurisegmental, using a system of monoaxial transpedicular rods and screws (Clariance, France) for fixation of the affected segment(s). In each case, a posterior distraction force was applied between the monoaxial screws on curved rods according to the desired postoperative lordosis. The degree of posterior distraction was determined by the average height of healthy discs above and below the pathological segment. After the correction maneuver, posterolateral osteosynthesis was associated with a lyophilized allograft from a bone bank to obtain segmental fusion.

Clinical evaluation of patients was performed using the visual analog scale and the Oswestry disability index.

The radiological evaluation followed the same protocol for all patients, before and after surgery. To measure the lumbar lordosis and the height of the intervertebral space, the pre- and postoperative magnetic resonance examination (the dGEMRIC examinations) was used, using the Cobb [30] and Dabss [31] methods, respectively. The use of mid-sagittal MRI images to compare lumbar lordosis and disc height values before and after surgery has been studied and accepted as a reliable method [32, 33].

Up to 6 weeks before surgery and between 6 and 12 months after surgery, T1weighted images were taken in sagittal planes with an MRI of 1.5 Tesla (Magnetom Avanto Fit 1.5 Tesla, Siemens, Munich, Germany) with a ET/RT value of 10/500 ms and T2 intensity images with a ET/RT value of 100/2800 ms, with a trench thickness of 3 mm. After initial MRI, a dGEMRIC examination was also performed according to the following protocol: a gadolinium-based contrast agent (gadoteric acid-gadoterate meglumine-Gd-DOTA D-Dotarem®) was injected in a dose of 0.2 mmol/kg at a rate of 2 ml/second. At 90 minutes after contrast injection, an ultra-fast MRI gradient (3D VIBE) was performed (repetition time - RT: 15 ms, echo time - ET: 2 ms, rotation angle: 5º-26°, FOV 230 x 230 mm, section thickness 3 mm, spatial resolution: 0.6 x 0.6 mm) using the same 1.5 Tesla MRI. The concentration of the absorbed contrast agent was determined from the T1 values on the preoperative dGEMRIC images. The procedure was the same for postoperative dGEMRIC MRI to determine the postoperative concentration of T1 contrast agent. All image and value analyses were obtained with the Syngo.via software (Siemens, Munich, Germany). We calculated the dGEMRIC T1 index, which is an indicator of the molecular state of the IVD and which shows an inverse relationship with the content of the GAGs in the disc [33]. During the dGEMRIC tests, the L1 - L2 intervertebral disc was used as a control disc to determine the content of GAGs at the operated levels. We assumed that there were no changes or only minimal changes in the fluid content of the disc at this level because the first lumbar disc and its surroundings are not touched during the operation and because of the short period between pre- and postoperative evaluations. Thus, normally there are no substantial differences at this level before and after surgery, but if they do occur, it means that they are caused by the MRI machine. An MRI coefficient has been developed to correct the postoperative dGEMRIC value in case of a significant difference. We also investigated the height of the L1 - L2 disc.

For all operated discs, as well as for the control discs, for the dGEMRIC calculation there was an identification of the sections for the evaluation of the discs at the same level and then a drawing of the outline of the respective discs. For each disc, 7 sagittal sections were selected and analyzed (1 mediosagittal, 3 parasagittal on the right and 3 parasagittal on the left), the final calculations representing the mean obtained. The mean concentration of gadolinium absorbed per unit area, an indicator of the condition of the intervertebral disc, was calculated using the pre- and postoperative T1-dGEMRIC values divided at the disc surface. We also calculated the ΔT1- dGEMRIC/cm2 which is an indicator of the molecular state of the intervertebral disc [4].

Among the results of the study we mention:

- out of the total of 39 patients included in the study, only 35 were operated on between 2017 and 2022. Of these, 4 had incomplete files, 2 did not perform the dGEMRIC postoperative control exam (1 patient lost from follow-up, 1 patient with important comorbidities) and 2 presented postoperative complications (1 case of infection, 1 case with degradation of the device caused by significant osteoporosis). Thus, we analyzed 38 intervertebral discs of the remaining 27 patients, of which 17 had a monosegmental disease and 10 had a multisegmental disease (9 patients with two affected levels, 1 patient with three affected levels)
- by gender the study included 16 women and 11 men
- the median age was 50.77 years
- the average follow-up time was 28.74 months
- overweight and class I obesity predominate (77.8% of patients)
- predominance of ASA II and III scores in 77.8% of patients
- the mean preoperative low back pain score was 8.33 and decreased to 1.7 after surgery; this improvement was considered significant ($p < 0.01$)
- the ODI score before surgery was 53.48%, and postoperatively it decreased to 18.44%; the improvement was statistically significant ($p < 0.01$)
- lumbar lordosis did not change significantly postoperatively (preoperatively, mean 46.81°; postoperatively 47.38º) ($p = 0.174$)
- IVD height increased by an average of 1.71 mm due to posterior distraction (from 7.9 mm to 9.6 mm); this change was considered statistically significant ($p < 0.01$)
- dGEMRIC examination: mean accumulation of 410.08 units/cm2 of preoperative gadolinium, while significantly less, 272.45 units/cm2, gadolinium was detected postoperatively; the mean value of $\Delta T1$ was 137.63 units/cm2 of gadolinium, a statistically significant difference
- for IVD L1 - L2, the mean value of dGEMRIC was 417.19 units/cm2 before and 403.90 units/cm2 (SD :153.8) after surgery ($p = 0.701$), with a mean index of 1.03; the height of this disc remained unchanged

Discussion

Most lumbar spine specialists agree that lumbar instability is based on the degeneration of IVD and is considered one of the major factors that trigger low back pain. After the lack of success of conservative treatment, spinal arthrodesis surgery by posterior transpedicular fixation supplemented by ventral fusion of vertebral bodies is the most frequently performed operation. Ventral fusion invariably involves the sacrifice of the intervertebral disc.

In our study, after surgery, all patients showed a clinically important improvement, with both pain and disability scores supporting this. The surgical technique includes posterior distraction on curved rods using transpedicular monoaxial screws. However, posterior distraction is not embraced in surgical practice, as it is considered the main cause of decreased lumbar lordosis and sagittal imbalance. This surgical "axiom" was based on the use of Harrington instrumentation, in which posterior distraction on a straight rod led to the flat back syndrome [34]. However, 3D instrumentation with curved rods and monoaxial screws fundamentally changed the chance of lordosis maintenance. As the axis of the monaxial screws is constant, the distraction between the screws on the lordotic rod is also transmitted to the vertebral bodies. We achieved this result in our cases, the lumbar lordosis was maintained despite the posterior distraction, the difference was only 0.57º. Due to the rear distraction, a significant increase in IVD height was achieved, averaging 1.71 mm.

However, the height of the L1 - L2 disc remained unchanged and the absorption of gadolinium did not change significantly, so we believe that this segment may be considered as control during our study. Our conclusion is that the pre- and postoperative changes in the operated segment during the dGEMRIC MRI examination are related to the quantitative change in GAGs. In our opinion, interbody fusion after sacrifice of IVDs is an intervention that significantly alters the normal biomechanics of the spine. We believe that despite the degenerative changes, IVD plays an essential role in maintaining the biomechanics of the spine. For this reason, we have always tried to preserve the IVD, because despite posterior fusion, there are always some degrees of residual flexion-extension motion in the fused segment [35]. Few human tissues are functionally related to the composition of their extracellular matrix, such as IVDs. The content of GAGs in the extracellular matrix is responsible for the water content of the nucleus pulposum which determines the thickness of IVD. Continuous hypertension increases catabolism, causing loss of GAGs accompanied by NP dehydration and IVD degeneration. In our preliminary study, a follow-up MRI performed after posterior distraction during lumbar fusions revealed an increase in the water content of IVD as a consequence of the increase in the amount of GAGs, as supported by dGEMRIC studies. Thus, the decrease in the concentration of gadolinium is a direct effect of the appreciable increase in the content of GAGs. This occurs through posterior intervertebral distraction that decreases pressure on the disc.

Based on these results, we believe that the loss of GAGs of IVDs can be stopped by reducing the pressure on them. Also, the accumulation of gadolinium in the pre- and post-operative L1

- L2 discs did not change significantly (accumulation coefficient close to 1.03), which justifies our theory that this disc can be used as a control for the operated levels. In our opinion, the increase in the amount of GAGs and water content in NP is a sign of the regenerative capacity of IVD. Our surgical technique has improved the environment and the metabolism of the disc. Our good results at least make us hope to reverse the catabolic metabolism into an anabolic one, with an increased production of GAGs, which is part of a possible regeneration process. We believe this is beneficial for new therapies to achieve successful results. The combination of these treatments can have a synergistic effect on the regeneration of the disc [36].

The current trend in the surgical treatment of lumbar instability is arthrodesis. Our approach is different and consists of preserving the intervertebral disc and promoting its regeneration. By performing a posterior intervertebral distraction, we reduce the overload and the hydrostatic pressure in the disc.

In the penultimate chapter, an experimental study on an animal model is presented in order to analyze the changes of the lumbar intervertebral disc after compression.

The objective of this experimental study aims to evaluate the appearance of changes specific to lumbar IVD degeneration by surgical implantation of an intervertebral compression device. The degenerated disc is then subjected for a certain period of time to a process of distraction by using a specific device. The disc changes that occur at the end of each period are analyzed histologically.

An important step is the choice of a relevant animal model, which varies according to the objectives of the research. However, even by this method we can only get closer to the characteristic human elements. In our case, an animal model must be found in which the intervertebral disc is histopathologically and pathophysiologically similar to the human one and in which the degeneration process generally follows the same stages. An important difference in the comparison of the pathophysiology and the process of human disc degeneration with that of the animal model is the lack of vertical stress on IVDs, the human being a bipedal being. From this point of view, the IVD of no animal model is similar in all respects to the human one, the differences being accepted and, if necessary, taken into account depending on the purpose of the experiment. Last but not least, the costs related to buying, feeding, caring for the animal are not negligible.

Among the available animal models, the rabbit model is widely accepted for the study of the intervertebral disc, but it also serves as a model for the study of human immunology, the pathology of various diseases and the response to various infections [37]. There are similarities but also differences in IVD between this model and humans. It is used in the analysis of pathophysiology and morphological, radiological, histological and biochemical changes of degenerated lumbar intervertebral discs, but also in the finding and validation of new treatments, such as new regeneration therapies.

After searching the recognized databases, an analysis of the studies in the literature was made that showed the frequent use of the rabbit model in the pathology of the spine and of the intervertebral disc, respectively. Disc degeneration can be achieved by different methods, but frequently a compression of the IVD has been achieved through various external devices, has caused an increase in pressure inside the NP and in the AF tension, affecting cell function and phenotype. An analysis of the degenerated disc subjected to intervertebral distraction was performed only on animals. There are already experimental studies that have demonstrated through biochemical analyses that posterior distraction can promote disc regeneration. The basic mechanism would be related to a decrease in hydrostatic pressure determined by decreasing the load. In the same study, magnetic resonance imaging showed a change in the MRI signal in the T2 sequence [38, 39].

The inclusion criteria in the sub-chapter "Material and method" were: healthy rabbit, aged 5-6 months, New Zealand breed, weighing approximately 3-3.5 kg. This breed is widely used in experimental studies in literature.

A study protocol was developed, an anesthetic, surgical and postoperative care protocol. The surgical protocol consisted of performing a posterior surgical procedure at the level of the lumbar spine under intramuscular or general anesthesia. Preoperative radiographs are performed in two incidences, face and profile, to identify and mark the L4 - L5 level. An external device is used to stimulate disc degeneration by 200 N compression for 28 days and, after this period, is used to stimulate regeneration of the disc by 120 N distraction for another 28 days. An external device is used, which is fixed by titanium pins (length 70 mm, diameter 5.5 mm) attached to two 1.5 mm Kirschner pins with a horizontal direction passed through the L4 and L5 vertebral bodies, respectively. After the operation, the animals are cared for at the biobase of the Faculty of Veterinary Medicine, without any restrictions. All complications are recorded, their occurrence excluding the animal from the study.

Before starting the actual experiment on the rabbit model, we carried out a theoretical and practical anatomical study on a rabbit carcass in order to choose the type of surgical approach. We highlighted the muscle planes, the characteristics of the type vertebra (by dissection), the main vascular-nervous bundles in the lumbar region (theoretically).

One stage of this study was the construction of the devices necessary for compression and distraction. The development of the compression and distraction device was done in several stages, starting from a study published by Kroeber et al [38]. We chose to manufacture the devices using a 3D printer, the material used being the eSUN PLA+ filament. This brought two great benefits:a reduced cost and a lower weight of the device. Next, a numerical calculation of the resistance of the compression and distraction devices was performed using the finite element method (FEM). To determine the stress and strain state in these devices, FEA (Finite Element Analysis) programs were used. The result of this calculation confirmed that the stress and strain state obtained in all variants does not exceed the permissible strengths of the materials used in the construction of the devices. The finite element method has helped us to improve the devices, making them more competitive from a functional point of view. The two devices were validated with the help of two different devices that use power cells. Thus, we calculated the force loss that occurs between the one applied to the device and the one transmitted to the IVD, which was about 65%. This force remains constant during the application period.

The results obtained from the histological analysis of normal IVD (2 rabbits that did not wake up after anesthesia) with IVD from 3 rabbits operated and to which the compression device was fitted, one not being put under tension, being considered as a control (2 died on the 9th day and 1 on the 10th day). The disc from the last control rabbit did not show differences from normal IVDs, the conclusion being that the presence of the device does not cause changes at this level. On the contrary, in the other 2 rabbits, disc degeneration lesions were highlighted, such as the distorted collagen lamellae of the annulus fibrosus, with loss of parallelism, fragmentation and bifurcation. NP became inhomogeneous, with groups of chondrocytes.

The conclusions obtained for this 10-day single-center descriptive longitudinal study carried out on rabbits in order to cause disc degeneration are as follows:

- the rabbit animal model is a relevant medium model in the study of the pathophysiology of the spine, and implicitly of the intervertebral disc
- it is a very sensitive model, in order to improve the results, clear, improved anesthetic, operative and postoperative protocols are needed
- through a process of compression of the intervertebral disc with the help of an external device, we were able to highlight degeneration using histological analysis.

Conclusions and personal contributions

The distinction between age-related disc disease and degeneration still remains unclear due to the multifactorial origin of the lesions. Disc degeneration is clinically closely related to the presence of low back pain and biomechanically to the presence of vertical compression force which causes an increase in hydrostatic pressure associated with altered disc nutrition and increased production of inflammatory cytokines and catabolic enzymes. These reactions cause a decrease in PGs, alteration of the extracellular matrix and AF, IVD dehydration, a decrease in the number of cells, followed by morphological alterations with progressive loss of disc height and with disc deformation, thus producing instability of the vertebral segment and overload of the structures adjacent to the intervertebral disc. The development of vertebral segmental instability subsequently maintains a vicious circle.

The treatment is pathogenic and not etiological, it addresses the clinical symptoms and not the causes that led to the damage of the disc, it does not restore or preserve the disc.

Thus, contemporary treatments for discogenic back pain remain largely suboptimal. The current treatment of intervertebral disc degeneration involves conservative therapies and invasive therapies, fusion or arthroplasty, techniques that sacrifice the intervertebral disc.

The purpose of regenerative techniques is to repair and regenerate the disc, the intention being curative. However, it is necessary to select degenerated IVDs in which reparative therapy would be indicated. Among the conditions would be that they are the cause of low back pain and that they have a low degree of Pfirrmann degeneration. It is very likely that this therapy will not achieve its goal in the case of advanced degeneration. Thus, the type of regenerative therapy used would depend on the stage of degeneration: in the early stage, a therapy with growth factors can be proposed, in the intermediate stage, a cell or gene therapy, and in the advanced stage, a tissue engineering. For example, in the case of an alteration of NP, but with a normal AF and EPs, the replacement of NP with a hybrid substitute combining biomaterials and regenerative treatment could be indicated. There are already clinical trials evaluating qualitative outcomes, namely pain and disability scores, as well as imaging results, with encouraging results. Reporting of adverse reactions is necessary in view of their subsequent use in the treatment of human pathology.

In conclusion, an effective treatment that can stop or even reverse the process of disc degeneration that would imply the disappearance of pain, partial or total restoration of disc composition by reversing intradiscal catabolic metabolism, is still being studied.

The possibility of preserving / regenerating the intervertebral disc, thus improving the receptor environment, while preserving the biomechanical properties of the lumbar spine through posterior distraction is an important method for obtaining a favorable result in terms of new therapies.

In literature, distraction is not a treatment for intervertebral disc degeneration, the classic treatment being arthrodesis or arthroplasty. We have not found any specialized studies that analyze this aspect. There are animal studies presented in this work, which, after obtaining the degeneration of the intervertebral disc, analyze its behavior after the application of an intervertebral distraction. Qualitative and quantitative MRI, histological and histochemical analyses show an improvement in cellular metabolism, with an increase in the amount of GAGs which proves disc regeneration.

Therefore, our study showed that reducing vertical pressure on the intervertebral discs positively influences their metabolism. The increase in disc height by posterior distraction, without altering lordosis, causes an increase in the amount of glycosaminoglycans, indicated by the decrease in contrast agent binding in the dGEMRIC analysis. The increase in the amount of glycosaminoglycans in the nucleus pulposum, as well as the increase in water content are signs of the ability of the intervertebral discs to regenerate. The results suggest that by reducing/eliminating the axial load, the pressure on the discs by posterior fusion combined with a slight distraction, a hydration of the IVD is obtained, but also an increase in the concentration of proteoglycans due to the reactivation of cellular metabolism. So not only can the degeneration be stopped, but the process of disc regeneration can be initiated, thus obtaining an optimization of the local environment. The long-term goal would be to achieve temporary posterior distraction and fixation, if possible by a minimally invasive approach, until improvement in intervertebral disc metabolism is achieved, after which the posterior instrumentation could be removed. We believe that achieving good results in the treatment of disc degeneration through innovative cell, gene or tissue therapies is also related to a responsive disc environment that could be achieved with the technique described.

Another benefit of this study is the use of dGEMRIC, which is a non-invasive method, to assess the condition of the intervertebral discs. It can be proposed for the early diagnosis of disc degeneration and also for the follow-up of the results of the treatments applied.

Among the limitations of the clinical trial, we can note the small number of cases, the short follow-up period and the absence of a non-invasive analysis of the condition of the disc (for example, dGEMRIC) after at least two years after the surgical treatment. Of course, a multicenter study with a satisfactory number of cases and a longer follow-up is desirable. As far as the animal experiment is concerned, it is clear that an improvement in all stages related to surgery and anesthesia, as well as post-surgical care, would certainly lead to conclusive results that would further support the importance of posterior distraction.

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