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Quantitative evaluation of meniscal healing process of degenerative meniscus lesions treated with hyaluronic acid.

A clinical and MRI study

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SUMMARY

ABSTRACT

Background: The management of degenerative meniscus lesions (DMLs) deals with a contradiction between daily practice and scientific evidence. Arthroscopic partial meniscectomy (APM) is frequently used for DMLs, but randomized controlled trials failed to demonstrate any additional benefit compared to non-operative treatment or sham surgery. Moreover, APM is associated with an increased risk of osteoarthritis (OA). The European Society of Sports Traumatology, Knee Surgery & Arthroscopy (ESSKA) Meniscus Consensus stated that "surgery shouldn't be proposed as a first line of treatment of DMLs (Grade A)". Non-operative treatment can include rehabilitation, nonsteroidal anti-inflammatory drugs (NSAIDs), and intra-articular injections; however, there is "no evidence of which time/type of non-operative treatment should be proposed".

Purpose: The purpose of this study was to evaluate clinical efficacy and healing effects of conservative management of DMLs with a hyaluronic acid (HA) hydrogel. The primary aim was to objectively demonstrate meniscal healing by T2 measurements, providing a quantitative evaluation of qualitative changes in the meniscus. The long-term goal is to be able to treat DMLs conservatively, to avoid APM and prevent the onset of early OA.

Methods: Patients were subjected to two HA injections two weeks apart. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Patient's Global Assessment (PtGA) and Clinical Observer Global Assessment (CoGA) of the disease were assessed at baseline, 30, and 60 days after treatment. Short Form (36) Health Survey (SF-36) was assessed at baseline and 60 days after treatment. One year after treatment, patients were called to know whether any of them had undergone APM. All patients underwent magnetic resonance imaging using a 1.5-T Magnetic Resonance Imaging scanner, which included a T2 mapping pulse sequence with multiple echoes, at baseline and 60 days after treatment. The following meniscus compartments were analyzed according to International Society of Arthroscopy, Knee Surgery and Orthopedic Sports Medicine

(ISAKOS) classification: anterior horn lateral meniscus (AHLAT), anterior horn medial meniscus (AHMED), posterior horn lateral meniscus (PHLAT), and posterior horn medial meniscus (PHMED). As the vascularity and cell profiling are different between the inner and outer meniscus, each compartment was divided into three zones: red, red-white, white. Non-parametric tests (Fisher's and Wilcoxon test) were conducted to compare pre- and posttreatment data. *P*-values <0.05 were considered statistically significant. The relationship between clinical evaluation and meniscal healing was assessed using the Pearson's correlation test.

Results: 40 patients were enrolled. Patient compliance to treatment was good as all patients completed it. WOMAC score, physical function subscale, PtGA, CoGA, and SF-36 physical functioning and pain score showed a statistically significant difference between baseline and follow-ups (*p=0.024* WOMAC 30 days, *p=0.04* physical function subscale 30 days, *p=0.024* WOMAC 60 days, *p =0.02* physical function subscale 60 days, *p=0.008* PtGA 30 days, *p<0.001* CoGA 30 days, *p=0.001* PtGA 60 days, *p<0.001* CoGA 60 days, *p=0.01* SF-36 physical functioning, *p=0.03* SF-36 pain score). One year after treatment, only one patient had undergone APM. A decrease in the T2 measurement was detected in the PHMED in 39% of cases in both the red and red–white zone, and in 60% of cases in the white zone; in the PHLAT in 55% of cases in both the red and white zones, and in 65% of cases in the red–white zone. Only for the latter, there was a statistically significant difference between baseline and posttreatment T2 measurements (*p=0.03*). Correlation between clinical scores and T2 measurements was noted for some zones of the meniscus (pretreatment CoGA and AHMED red zone *p=0.030*, red and white zone *p=0.020*, white zone *p=0.054*, pretreatment PtGA and AHMED red and white zone *p=0.067*, pretreatment SF36 mental health score and PHMED red zone *p=0.036*, red and white zone *p=0.018*, pretreatment SF36 physical functioning score and PHMED white zone *p=0.048*, posttreatment SF36 emotional role functioning and AHLAT white zone *p=0.047*, posttreatment SF36 physical functioning score and PHLAT red zone *p=0.038*).

Conclusion: This is the first study to evaluate the healing of DMLs treated with hyaluronic acid injections in vivo with an objective method such as T2 mapping.

It is a first step in an ongoing investigation of conservative treatments for DMLs. This study supports the use of HA in the conservative management of DMLs as it is clinically effective and enhances meniscus healing as demonstrated by T2 measurements. Moreover, it reduces the need for APM at 1-year follow-up, representing a less invasive and cost-effective option compared to APM. Before this treatment is implemented clinically it needs to be further investigated to confirm collected results in a larger population.

INTRODUCTION

DEGENERATIVE MENISCUS LESION

Degenerative meniscus lesion (DML) presents in adult patients (35 to 65 years of age) who have not had a trauma and consists in a progressive delamination and surface fibrillation. DMLs cause knee problems with years, as they have been clearly correlated with the development of tibiofemoral osteoarthritis (OA) (1-4).

Degenerative tears often are very complex tears. Patients present with chronic knee pain, swelling, and sometimes locking. The diagnosis is based on the physical examination supplemented with MRI. Palpation, joint line compression, rotation with axial loading, and the different meniscal tests are helpful in diagnosing a degenerative tear. Plain X-rays can be used for the evaluation of degenerative knee joint changes.

DMLs are completely different from traumatic meniscal lesions occurring in young patients. Traumatic meniscal lesions can be described as a true fracture produced by a twisting injury to the knee. Given the difference in morphological characteristics of DML and traumatic meniscal lesion, their management cannot be the same.

Due to its decisive role in load bearing, load transmission, load dissipation and in providing joint stability, congruity and lubrication (5), meniscus integrity in form and function is of utmost importance to the knee joint's long-term health (6). Meniscus functionality, which is the tissue's ability to function properly, i.e. to disperse loads and reduce friction, is heavily dependent on its extracellular matrix properties. Collagen fibers, primarily type-I, define the tissue's tensile strength and shock-absorbing properties, while proteoglycans contribute to compressive strength by upholding swelling pressure (5, 7, 8). Lately, therapeutic efforts have been aimed at preserving and restoring the damaged meniscus secondary to trauma or degeneration (9, 10). This is of particular relevance as meniscus and cartilage pathologies are closely interrelated. Consequently, meniscus damage and loss are key

features of and risk factors for developing OA (11). The discussion on whether meniscus pathologies are a cause or consequence of knee OA is ongoing (12, 13).

Yet common consensus prevails that morphological meniscus defects, i.e. surface breakdown and tissue tearing, are the consequence of degenerative changes of the extracellular matrix (14-16), and contribute to the evolution of OA by altering load distribution and transmission to the adjacent articular cartilage. Hence, detecting such degenerative changes early is necessary in therapeutic efforts to preserve the meniscus and prevent or delay the onset of early OA (2, 17).

Pathology

Macroscopic degenerative lesions of non-disrupted menisci present as yellow, opaque areas of meniscal tissue, which correspond to myxoid degeneration, perimeniscal cysts and meniscal calcifications.

Microscopically, the following findings can be noted:

(a) acellular eosinophilic hyaline degeneration often associated with fissural or horizontal lesions;

(b) myxoid degeneration, which is a collection of a normal mucoid substance. It can be located within the meniscus and is graded according to its severity. It has been found in more than half the cases in a study of Ferrer-Roca and Vilalta, who considered it to be a normal condition (18). Myxoid degeneration can also affect the perimeniscal zone and give rise to tears and pseudocysts, which can lead to the formation of perimeniscal cysts;

(c) regeneration zones: peripheral lesions situated within the vascularized zone, which can heal spontaneously forming a scar that is often surrounded by proliferating chondrocytes.

Frequency of Degenerative Meniscal Lesions

The medial meniscus seems to be frequently involved, independent of associated chondral damage. An analysis of 115 cadaveric or postamputation knee specimens, more than half of which were obtained from subjects aged 65 years or older and 61% from male subjects, documented a lesion of the medial meniscus in 38% of cases (19). These were predominantly horizontal tears, either confined to the posterior horn

or generalized, as well as unstable flap tears. Chondral lesions of the femoral condyles or the tibial plateau were a common finding, while there were no abnormalities of the menisci and vice versa. The vast majority of them were localized outside the area covered by the menisci, which seemed to act efficiently in terms of protecting the cartilage. The authors did not consider the menisci, whether torn or not, to be the cause of osteoarthritis.

The prevalence of intra-meniscal high signal intensity on MRI of asymptomatic subjects increases with age. It is estimated to occur in 5% of subjects under the age of 30 years, rising progressively to 13–15% of subjects between 30 and 45 years, 25–63% of subjects above 50 years and 65% of subjects above 65 years of age (20-22). In a histomagnetic study conducted by Raunest et al (23), who analyzed 480 MRI slices of 40 cadaveric knees derived equally from male and female subjects with a mean age of 71 years, meniscal lesions were detected in 80% of knees. In case of painful osteoarthritis of the knee, their prevalence was estimated to be 91% (24). In 1992 the French Arthroscopy Society carried out a survey of medial meniscus lesions which has now become a reference standard in France (25). The lesions detected in patients above 50 years of age accounted for one third of all 1,436 reported lesions. Moreover, they were markedly different from those in subjects under 50 years of age. Vertical lesions were traumatic in origin and predominantly occurred in young patients. Complex lesions and flap tears occurred in the absence of major trauma and affected older patients, whose mean age (54 years) was 17 years higher than that of patients with vertical tears (37 years). Flap tears were observed in patients with a mean age of 46 years, situating them in between the previous two age groups.

Arthroscopic classification

An arthroscopic DML classification system was first proposed in 1983 (26). It was further developed on the basis of a retrospective study of 2,100 arthroscopies, during which 310 degenerative lesions were detected (27).

Type I represents an alteration of the meniscus without interruption of its continuity. The meniscus is homogeneous but has lost its normal appearance: it is flat, looks drab and frosted, and its colour sometimes resembles that of chamois leather. Its surface is irregular and its inner edge is often ragged and frayed. On

palpation, it has lost its firm consistency and elasticity, and is sometimes soft to the feel. There are, however, no tears or instability. Only this type corresponds to the so-called meniscosis. Type II is characterized by the presence of calcium deposits on the surface of the meniscus as well as within its body (meniscocalcinosis). Type III indicates the presence of a horizontal cleavage tear.

Type IV refers to the likely presence of a radial tear (IVa), which is slightly oblique and originates from the inner edge of the medial meniscus at the junction of the middle and posterior one thirds of its body, extending towards the peripheral zone anteriorly or posteriorly. In case of such a tear, it is possible to mobilize a large pedunculated fragment of the meniscus with an arthroscopic probe. A tear continuing along the inner border of the meniscus, detaching a mobile and palpable flap, is called a type IVb tear. Type V is characterized by the presence of a complex lesion which cannot be precisely described. It is rarely encountered, but when it occurs, it is mostly in an osteoarthritic knee.

Initially, in this classification, the definition of a DML excluded a past trauma to the knee and radiologically documented osteoarthritis. Only minimal pinching of the edge of the medial meniscus was accepted. The aim of this definition was to differentiate these lesions from classic traumatic lesions and from meniscal lesions associated with osteoarthritis. At that time, these three entities were often confused in the Anglo-Saxon literature. Currently, it is thought that DML may occur before, at the same time, or after the onset of chondral lesions.

MRI classification

The classification system of Crues et al serves as a reference standard for MRI (28). It refers to meniscal lesions regardless of whether they are degenerative or traumatic in origin. A healthy meniscus is triangular and prismatic in shape, producing a low-intensity signal in all sequences, with a homogeneous and weaker signal than that of cartilage. Meniscal tears appear as linear areas of high signal intensity located within the normal low-intensity zones on both T1 and T2-weighted images. Degenerative changes related to the presence of local mucoid degeneration are seen as areas of high signal intensity on T1 and particularly T2-weighted scans (Figure 1).

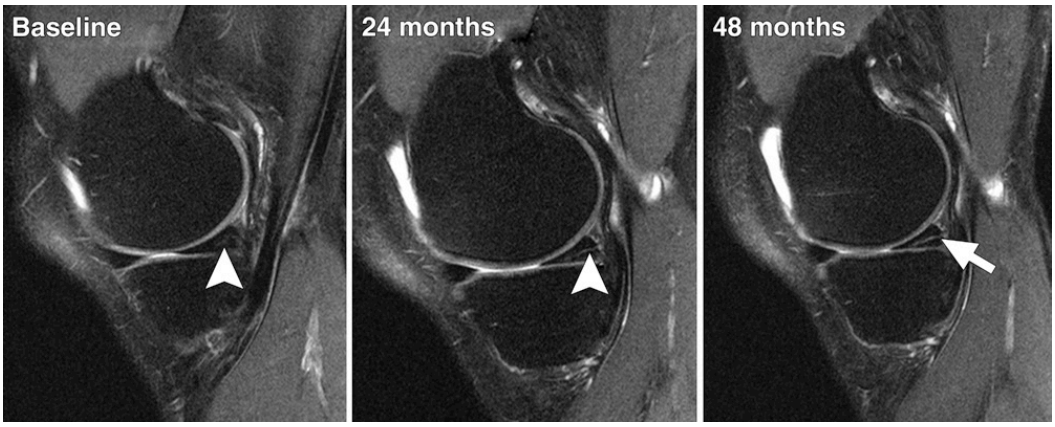

Figure 1: Sagittal intermediate-weighted fat-saturated MR images of the medial meniscus from four study participants demonstrate different grades of intrameniscal signal intensity in the posterior horn. A, Normal morphology of the medial meniscus. B, Focal grade 1 signal intensity (arrow) in the posterior horn of medial meniscus. C, Linear grade 2 signal intensity (arrow) not extending to the surface of the meniscus. D, Linear grade 3 signal intensity (arrow) involving the inferior surface of the posterior horn on only one image section. *From Kumm et al Natural History of Intrameniscal Signal Intensity on Knee MR Images: Six Years of Data from the Osteoarthritis Initiative. Radiology. 2016.*

Meniscal lesions have been classified to fall within one of the following three grades (Figure 2): Grade 1 is defined as a high signal intensity area which is round or oval in shape, of variable size, and occupies a variable amount of the meniscal triangle, but does not extend to the surfaces. Grade 2 is defined as a high-intensity signal which is roughly linear, almost always horizontal and of variable size. It does not involve the surfaces of the meniscus, but can extend to the meniscosynovial junction. Its frequency is 24% according to LaPrade et al and 41% according to Jerosh et al (29, 30). Grade 3 is defined as an area of high signal intensity extending to the surface of the meniscus or to its free edge. It indicates a meniscal tear.

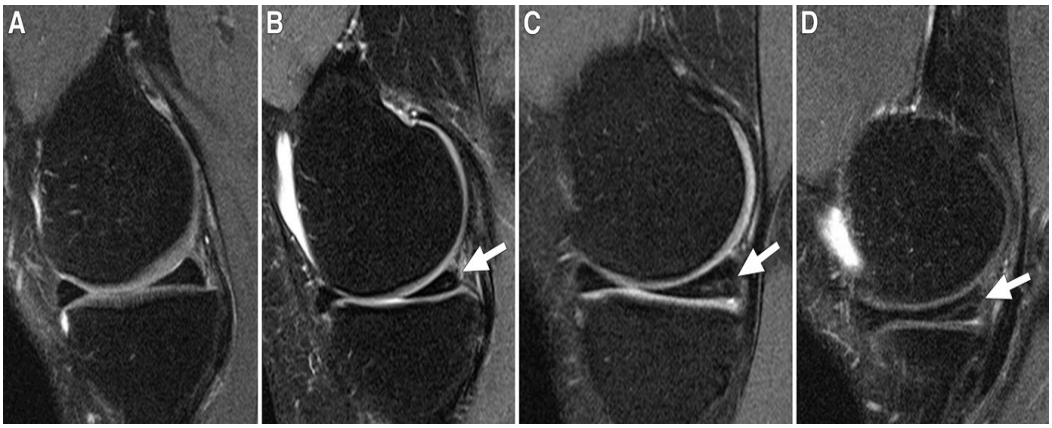

Figure 2: Sagittal intermediate-weighted fat-saturated MR images of the medial meniscus from four study participants demonstrate different grades of intrameniscal signal intensity in the posterior horn. A, Normal morphology of the medial meniscus. B, Focal grade 1 signal intensity (arrow) in the posterior horn of medial meniscus. C, Linear grade 2 signal intensity (arrow) not extending to the surface of the meniscus. D, Linear grade 3 signal intensity (arrow) involving the inferior surface of the posterior horn on only one image section. *From Kumm et al. Natural History of Intrameniscal Signal Intensity on Knee MR Images: Six Years of Data from the Osteoarthritis Initiative. Radiology. 2016.*

Correlation between MRI and Histology

The correlation between the MRI and histological findings has been the focus of much research. In 1992 Hodler et al after having analyzed 179 MRI scans and histological specimens of 20 cadaveric menisci, found the MRI results to have a relatively moderate sensibility of 72% and a specificity of 80% for the detection of tears (31). In fact, some fibrous and mucoid areas mimicked meniscal tears, which was the reason why the accuracy or the efficiency was only 76%. This number rose to 93% in a histomagnetic study of Raunest et al, which included all types of lesions, from degenerative ones to tears (23) .

Correlation between MRI and Arthroscopy

Arthroscopy of the knee remains the gold standard, but it does not allow detecting lesions which do not extend to the surface of the meniscus. It is of little use in case of grade 1 and 2 MRI abnormalities, which represent intrameniscal degeneration. However, in grade 1 lesions, a soft, compressible area can be sensed on the superior surface during meniscal probing. Tiny openings in the meniscal wall, through which intrameniscal lesions communicate with the articular cavity, are not always detected. The correlation between MRI and arthroscopic findings has been studied by many authors. According to Fischer et al the sensibility and specificity of MRI were 89 and 84%, respectively, for the medial meniscus and 69 and 94% for the lateral meniscus (32). We have no knowledge of any studies specifically dealing with DML. Bin et al demonstrated the superiority of arthroscopy over MRI for radial tear detection (32). Briole conducted a prospective study on the value of MRI in determining the instability of DML (33). The specificity was good for flap tears but weak for radial tears (type IVa).

Biomechanical and cellular segmental characterization of human meniscus

A deep knowledge on meniscus biology is essential to understand meniscus pathology and to develop effective treatments.

The meniscus tissue is heterogeneous thus biomechanics, cellularity and architecture present segmental variation. Biomechanics of meniscus tissues is correlated with architectural and cellular characterization. A biomechanical study on fresh human menisci described different meniscus segments concerning the storage modulus (E'- which relates to the stiffness of the material), the loss factor (tan δ – that represent the ratio of amount of energy dissipated by viscous mechanisms relative to energy stored in the elastic component and proved information about the damping properties of the material) and the loss modulus (E'' = E' x tan δ) (34). Results have shown that anterior segments present significantly higher damping properties. Moreover, the mid body of medial meniscus is significantly stiffer than the lateral.

These profiles might be implicated in the specific role of the lateral and medial meniscus. Lateral meniscus is known to be more mobile and having a higher role in

load transmission within the less congruent lateral knee compartment comparing to medial meniscus (35). The lower mobility of medial meniscus can also influence conversion of axial load into meniscal hoop stresses (35).

Dynamic compressive moduli are region-specific and dependent on the loading frequency. This information has high relevance as loading condition of the joint should be considered when evaluating the in vivo behavior of this tissue.

Age also greatly influences dynamic stiffness as increased stiffness is associated with increasing age.

Increased BMI is associated with a decrease in stiffness but not in damping properties. Such combination could eventually be explained by greater difficulty of the structure to recover when a higher load (correspondent to higher BMI) is systematically applied. Influence of gene expression might also be implicated (36).

The cell-associated matrix (CAM) of one of the populations of meniscus cells is known to be composed of high amounts of type I and II collagen and low amounts of aggrecan48. On the other hand, a second population synthesizes a CAM containing high amounts of type I collagen, low amounts of type II collagen and high amounts of aggrecan. This population is known to be CD44+CD105+CD34-CD31 (37). A third population, CD34+ (a stem cell marker), has also been described but not associated to significant CAM production (37). The zone 1 of the meniscus contains more stem cells than zone 3 and these cells are known to play a role in meniscal regeneration (38). CD45 (marker for hematopoietic stem cells) was only present in an even smaller percentage of cells. This small number of CD45+ hematopoietic cells might play a role on chondrogenic differentiation of mesenchymal stem cells (MSCs) (39). It has been realized that meniscus-resident MSCs are efficient colony formers, possess strong chondrogenic activity, and share the same set of typical cell-surface markers as bone-marrow-derived MSCs (40). In addition, it has also been recognized that paracrine signaling by MSCs might play a decisive role in stimulating repair responses (38, 40).

Cells are the same regardless of age or despite admitting possible different activity levels in cell adhesion molecules (CAMs) production or some specific gene expression (36).

It has been recognized that cell density and distribution might have decisive role in further differentiation and maturation of constructs (41). Recently, it has been described despite the known circumferential variation from vascular part to the central and avascular parts there is also a radial variation in cells' distribution (34). The anterior segments of both lateral and medial meniscus have relatively lower cellularity (number of cells per area) as compared to mid body and posterior ones.

ARTHROSCOPIC PARTIAL MENISCECTOMY

The management of DMLs deals with a contradiction between daily practice and scientific evidence (42-44). Arthroscopic partial meniscectomy (APM) is frequently used for DMLs, which aims to relieve symptoms by removing torn meniscal fragments. The incidence of APM in the middle-aged has largely increased in several countries (45). In the U.S., almost 700,000 APMs are carried out each year, with annual direct medical costs around $4 billion (46). Numbers from Sweden confirm that meniscal procedures are the most common arthroscopic knee procedures (45). The Danish media have reported an increased frequency of meniscal procedures over recent years in Denmark. Thorlund et al analyzed the Danish National Patient Register to evaluate the precise numbers, age, sex, and diagnosis for patients who underwent meniscal procedures in the period 2000 – 2011 (45) (Figure 3). The incidence of arthroscopic meniscal procedures almost doubled. The largest relative increase in meniscal procedures (i.e. a 3-fold increase in incidence rate) was observed in patients older than 55, whereas the largest absolute increase (i.e. 4,481 procedures) occurred in the middle-aged population between 35 and 55 years of age. In contrast, the incidence rate of meniscal procedures in young patients under 35 was stable. These data suggest that the increased incidence of arthroscopic meniscal surgery mainly involved patients with degenerative meniscal tears. Meniscal procedures were carried out in men more frequently than in women but the proportion of women increased from 2000 to 2011.

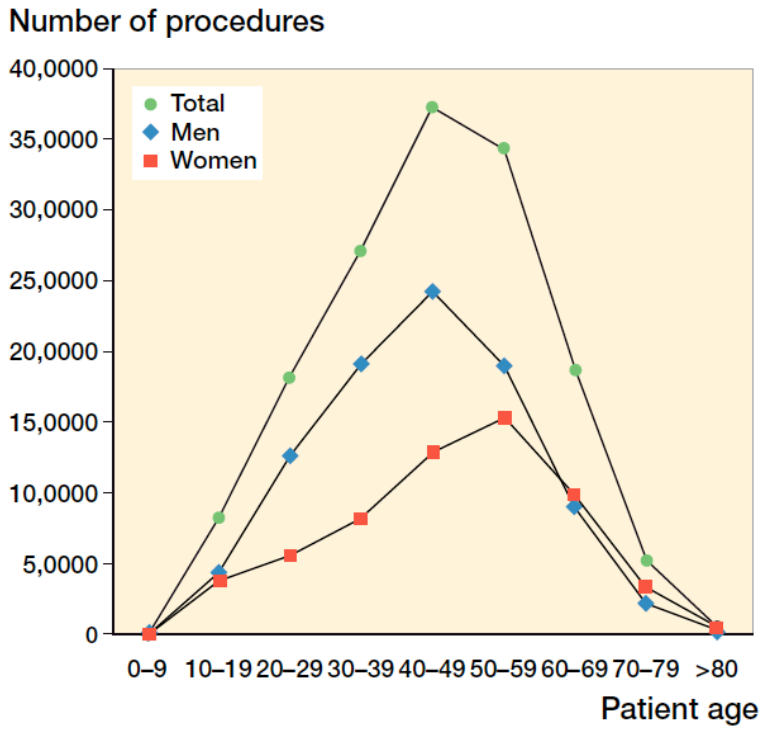

Figure 3: Number of meniscal procedures in Denmark (all codes) divided into age groups for the years 2000–2011. Blue: men; red: women; and green: men + women. *From Thorlund et al. Large increase in arthroscopic meniscus surgery in the middleaged and older population in Denmark from 2000 to 2011. Acta Orthopaedica. 2014.*

However, randomized controlled trials failed to demonstrate any additional benefit of APM for degenerative meniscal tears compared to non-operative treatment (47-51) or sham surgery (52, 53). All these studies except two (47, 53) excluded patients with OA. In a multicenter, randomized, double-blind, sham-controlled trial, 146 patients with symptomatic DML and no knee OA were assigned to APM or sham surgery (52). At 12 months after the procedure, there were no significant differences between groups in the Lysholm and Western Ontario Meniscal Evaluation Tool (WOMET) scores and in knee pain after exercise (52). In a meta-analysis of nine randomized trials, the advantage of arthroscopic surgery compared with sham surgery, lavage, exercise, and medical treatment was small and limited in time (54). Although well conducted, all the studies had biases and limitations. On the other hand, the cross-over from nonoperative treatment to arthroscopy ranged from 0 to 35%.

It should be taken into account that APM is associated with an increased risk of OA, as demonstrated by several studies (55) (Figure 4).

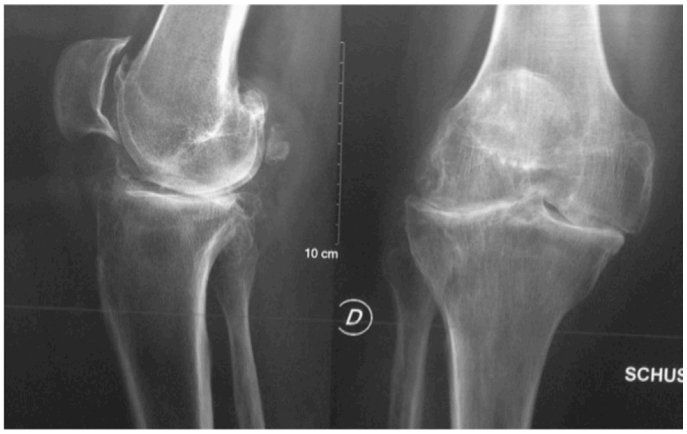

Figure 4: Generalized osteoarthritis 24 years after medial + lateral meniscectomy onstable knee. *From Beaufils et al. Management of traumatic meniscal tear and degenerative meniscal lesions. Save the meniscus. Orthopaedics & Traumatology: Surgery & Research. 2017.*

In a long-term follow-up study (mean 8.1 years), 57 patients who had undergone APM were clinically and radiographically evaluated for prevalence and progression of knee OA. Tibiofemoral

OA was evident in 62.69%, and it progressed from 17.2% preoperatively to 65.95% postoperatively (p = 0.001) in the medial compartment and from 17.64% preoperatively to 58.82% postoperatively (p = 0.0324) in the lateral compartment.

The essential role of the meniscus in the lifespan of the knee has been clearly demonstrated in basic science and biomechanical studies (56). The meniscus has multiple and complex functions, such as load-bearing, load transmission, shock absorption, stability of the knee, as well as lubrication and nutrition of articular cartilage (57).

The capacity to resist forces imposed on the knee is related to the biomechanical properties of the meniscal tissue. The meniscus is highly hydrated (72% water). The remaining content consists of extracellular matrix and cells. The organic matter is mainly constituted by collagen (75%), glycosaminoglycans (GAGs) (17%), DNA (2%), adhesion glycoproteins (<1%), and elastin (<1%) (57). Age, injuries, and pathological conditions affect the proportion of biochemical contents (58, 59). The extracellular matrix and cells phenotype differ in the outer and inner portion. The

outer portion is similar to fibrocartilage, while the inner portion has some characteristics similar to the articular cartilage. Cells of the outer portion are fibroblast-like cells, with an oval and fusiform shape. They are surrounded by type I collagen and small percentages of glycoproteins and type III and V collagen (60). Cells of the inner portion can be considered as fibrochondrocytes or chondrocyte-like cells, with a round shape. They are surrounded mainly by type II collagen, a small amount of type I collagen, and a higher concentration of GAGs. Potential progenitor cells have been identified in the superficial zone. These cells are flattened and fusiform and have cell extensions (57).

The biomechanics of APM have been extensively studied. Several cadaver studies of meniscectomies observed increased contact pressures of up to 80% to 90%, which increased with progressively larger amounts of resection (61-64). Interestingly, horizontal tears, vertical (longitudinal), and radial tears alone had only a marginal increase in contact pressures with the greatest increase occurring after meniscectomy (61-64). The increased pressures experienced by the articular cartilage may lead to earlier cartilage wear. The key findings from these studies were that horizontal cleavage tears should be managed with no more than resection of a single leaflet and that high-grade radial tears should be repaired to minimize changes to Tibiofemoral contact pressures (61, 62).

The kinematics of the knee is also affected by APM. Joint moments represent an important indicator of joint loading. The knee adduction moment (KAM) determines the mediolateral distribution of load across the tibial plateau, such that large adduction moments concentrate load on the medial tibiofemoral compartment. Many studies have shown an association of increased KAM with the incidence and progression of tibiofemoral osteoarthritis (65, 66). After APM, there is increased KAM in the operative leg up to a year after surgery (65, 66). There are also changes in the knee extension and flexion moments, which affect the loads experienced in the patellofemoral joint (65-67). APM also has an effect on gait. A study found that 105 patients that recovered from APM (pain-free postmeniscectomy; mean age 39.7) had a reduced range of motion and lower peak moments in the sagittal plane compared to 47 healthy controls (mean age 38.2) at 11 weeks postoperatively (66). The

postmeniscectomy patients also had markedly larger peak KAM during all stages of stance compared with controls (66).

MANAGEMENT OF DEGENERATIVE MENISCUS LESION

In 2016, the European Society of Sports Traumatology, Knee Surgery & Arthroscopy (ESSKA) Meniscus Consensus analyzed literature and expert opinions to produce a guide for DMLs (42). Recent publications stated that nonoperative treatments have similar results to arthroscopic surgery. Several editorials and letters have been published either by medical physicians or orthopaedic surgeons in the defence of their own practice. These exchanges were confusing and have not been useful to the clinician in making treatment decisions. Therefore, there was a need for a more uniform and clearer message and the treatment of DML should be related to both scientific evidence and clinical expertise. Finding a consensus in such a diverse continent like Europe where medical culture and healthcare systems vary from country to country was not easy. A strict methodology was therefore applied and numerous European experts were involved in the process.

First of all, it was stated that there is very limited evidence that pain in the degenerative knee is directly attributable to a DML even if the lesion is considered to be unstable. Great caution must be taken before arriving at the conclusion that the DML is the direct cause of the patients' knee symptoms. It is still plausible that, in some patients, torn meniscus parts from the degenerative lesion (by its displacement) may cause knee joint symptoms.

Loss of meniscus function may negatively affect the knee in the long term. Therefore, in many people, the DML (which may impair the force transmission and load distribution capabilities of the meniscus) is a feature indicative of a knee joint with (or at increased risk of) developing osteoarthritis.

If DMLs are a cause or consequence of knee osteoarthritis is still unclear. However, one causal pathway does not necessarily exclude the other, i.e. one phenotype of knee osteoarthritis may start with meniscus degradation and degenerative lesion leading to loss of meniscus function and osteoarthritis development. In turn, osteoarthritis and its general degradation of the knee joint,

involving multiple structures, may also cause DMLs and extrusion that further accelerate structural progression of the disease (Figure 5).

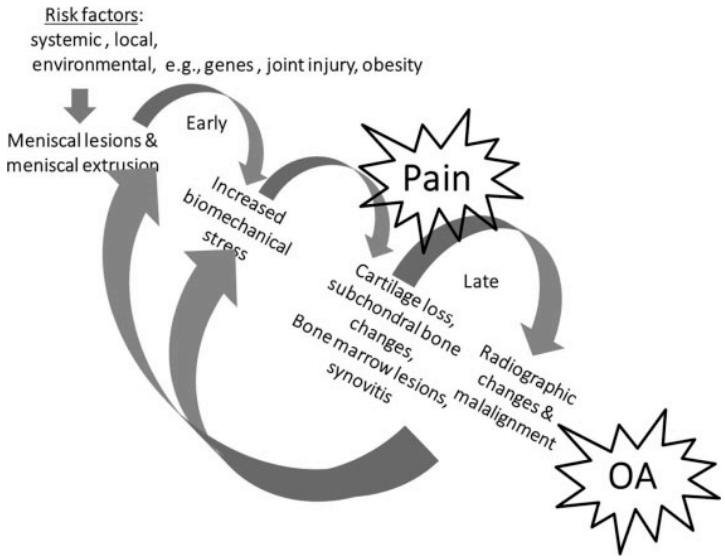

Figure 5. Meniscus pathway to knee osteoarthritis. *From Beaufils et al. Surgical Management of Degenerative Meniscus Lesions: The 2016 ESSKA Meniscus Consensus.*

The management of DMLs should take into account that meniscal lesions have a significant potential to become asymptomatic.

Rathleff et al performed a prospective cohort study on 291 consecutive patients with knee pain and MRI-verified meniscal lesion treated according to an arthroscopy restrictive strategy (68). Patients were treated conservatively with NSAID, referral to physiotherapy and reduced activity recommendation. Arthroscopy was offered patients who did not obtain an acceptable result from conservative treatment. Good results were observed for both operative and conservative treatment. The success rate for conservative treatment was 58 %. Arthroscopy was performed in 78 patients following failed conservative treatment after a median of 233 days.

In a randomized trial, Herrlin et al compared arthroscopic and conservative treatment for degenerative medial meniscal tears (69). Ninety patients (mean age 56 years) were evaluated using the Knee Injury and Osteoarthritis Outcome Score, the Lysholm Knee Scoring Scale, the Tegner Activity Scale and a Visual Analogue Scale for knee pain prior to the intervention, after 8 weeks of exercise and after 6 months. months. According to the outcome scores arthroscopic partial medial

meniscectomy combined with exercise did not lead to greater improvement than exercise alone. In agreement with other authors, they also found a reduction or cessation of sports after arthroscopic meniscectomy especially in patients with degenerative knee changes (70, 71). Fifty-nine per cent of the patients did not reach their pre-injury activity level after 6 months. This conclusion was confirmed in a recent study where they did a 5-year follow-up on the same study population (48). This indicates no additive effect of arthroscopy compared to conservative treatment in middle-aged patients with non-traumatic medial meniscal tears. Patients with non-degenerative meniscal tears are more satisfied with their knee function after arthroscopy than those patients with degenerative meniscal tears (70, 72-74). Since there is an increased risk of developing radiographic tibiofemoral osteoarthritis for individuals older than 40 years of age after arthroscopic meniscectomy, exercise treatment during several weeks is recommended as the first choice (75-77).

In the current literature, there is no evidence of which time/type of non-operative treatment should be proposed. It is important to note that no study has focused on functional outcomes of non-operative treatment versus placebo (or nothing). Randomized controlled trials have proposed various rehabilitation protocols, however, non-operative treatment could also consist of NSAID (if no contraindications), intra-articular injection, a physiotherapy and/or home exercises for 3–6 months.

Non-operative treatment is converted to surgery (crossover) in 0–35% of the patients. This cross-over rate has to be compared to the rate of arthroscopic treatment failure. When surgical treatment is proposed after a non-operative treatment failure, APM will result in similar but not superior results than successful non-operative treatment.

There are controversies regarding the definition and role of mechanical symptoms as an indication for APM. The definition of “mechanical symptoms” remains unclear and further investigations are needed, as it may cover a wide range of symptoms with different severity and frequency. In the randomized controlled trial by Gauffin et al patients’ history of symptoms (i.e. mechanical symptoms or acute onset of symptoms) did not affect outcomes (but patients with a joint locking lasting longer than 2s more than once a week were excluded) (78). Pooled results of all randomized

controlled trials reveal very limited added benefit of APM for degenerative meniscus regardless of pre-operative symptoms (fixed locking knee or knee with recurrent catching symptoms excluded). Sihvonen et al did not find any benefits over sham APM to relieve knee catching or occasional locking (79). Indication for early APM depends on the intensity and frequency of mechanical symptoms, as well as a thorough clinical examination.

Improvement of functional outcomes can be expected after APM. However, most of the randomized controlled trials found no difference in terms of clinical outcomes after surgery compared to non-operative treatment. While mid-term outcomes may be similar, short-term outcomes (<12 months) might be better with APM than with non-operative treatment. Three to six percent of patients will require another surgical procedure in the year following APM. Various predictive factors of poor results or treatment failures have been described in the current literature, such as increased BMI, lateral side, chondral damage, bone marrow edema, meniscus extrusion and total or subtotal meniscectomy.

In conclusion, surgery should not be proposed as a first line of treatment of DMLs. APM may be proposed after 3 months and persistent pain and/or mechanical symptoms related to a DML with normal X-rays but an abnormal MRI. The patient has to be informed about chances of successful outcomes and risks of either method. Surgery can be proposed earlier for patients presenting considerable mechanical symptoms. The patient has to be informed of chances and risks of either method. Arthroscopic surgery should be avoided in patients with DML and advanced OA on weight-bearing radiographs.

ROLE OF HYALURONIC ACID IN MENISCAL HEALING

Hyaluronic acid (HA) is a glycosaminoglycan that contributes to the viscoelasticity of the synovial fluid, and it is already used to provide additional shock absorption and prevent cartilage degeneration in OA (80). Investigations have continued to reveal a variety of actions of HA, such as anti-inflammatory and analgesic functions (43, 81). It has been demonstrated that human synovial cells stimulated with HA increase the expression of Transforming Growth Factor beta 1

(TGF-β1) and Vascular-Endothelial Growth Factor (VEGF). Furthermore, in pathological human chondrocytes and synoviocytes stimulated by Interleukin 1 beta (IL-1β), HA was able to reduce the gene expression of degradative enzymes and inflammatory cytokines (82). In inflammatory or degenerative joints, HA increased the synthesis of chondroitin sulfate and proteoglycans (83, 84) and reduced the production and activity of Matrix Metallopeptidases (MMPs) and A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS) (85-87).

The possibility of increasing levels of growth factors and counterbalance catabolic cytokine may open up new treatment option for patients with DMLs.

The potential role of HA in meniscal healing has been described in several in vivo studies (44, 88, 89). Ishima et al and Suzuki et al found a significant increase in the healing rate of artificial tears injected with HA (88, 89). Ishima et al produced a longitudinal tear in the peripheral region of the medial meniscus and injected HA in the study group and saline solution in the control group (88). The HA group had a higher healing rate than the control group at 12 weeks.

Suzuki et al generated a cylindrical lesion on the lateral meniscus and injected HA in the study group and phosphate buffer in the control group. The HA group showed a significant increase in the rate of filling of the defect (89). The cell population of the repaired tissue shifted from fibroblast-like cells to chondrocyte-like cells. At six weeks, the ratio of chondrocyte-like cells to all cells was higher in the HA group, inducing authors to deduce that the healing rate was increased by HA.

Only recently, the underlying mechanism of HA in the meniscal healing process has been clarified. HA promoted human meniscus regeneration by inhibiting apoptosis, promoting cell migration, and accelerating cell proliferation, potentially through the phosphatidylinositol 3-kinase (PI3K)/ mitogen-activated protein kinase (MAPK) pathway via the CD44 receptor (90). Murakami et al analyzed the effects of HA on prostaglandin E2 (PGE2)-induced apoptosis and gene expression in meniscus cells (90). They showed that HA increases cell migration and proliferation in a concentration-dependent manner in both inner and outer meniscus cells. An anti-CD44 antibody blocked these effects. HA activated the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways, and this effect was also blocked by an anti-CD44 antibody. Furthermore, HA upregulated the level of

Collagen Type II Alpha 1 Chain (COL2A1) and ACAN mRNA of inner meniscus cells. Authors concluded that HA is expected to have clinical application in the management of meniscal injuries.

In the clinical setting, evidences about the efficacy of HA in the management of degenerative meniscus lesions are still scant. Only one prospective randomized trial on 50 subjects with DMLs, demonstrated that patients treated with a hydrogel based on a non-crosslinked HA alkylamide (HYADD4®; Fidia Farmaceutici SPA) had a significant reduction in Visual Analogue Scale (VAS) pain and reduction in length and depth of the meniscal lesion (91). However, further studies are needed to corroborate this result.

QUANTITATIVE MR IMAGING OF THE MENISCUS: T2 MAPPING

The challenge in studying the healing of the meniscus in humans is to identify a non-invasive and objective method to evaluate changes in meniscus composition. Conventional Magnetic Resonance Imaging (MRI) sequences are used to assess anatomy and detect morphological changes of the knee. An increase in the intrameniscal signal or intra-substance tear is commonly seen in asymptomatic subjects and represents an early stage in meniscus degeneration culminating in a meniscal tear most commonly seen in the posterior horn of the medial meniscus (29, 92, 93).

Both radiography and morphologic MRI of the knee are not able to detect matrix alterations in DMLs and the response to infiltrative therapies. Using conventional MRI, measuring changes in meniscal tissue composition prior to surface breakdown is challenging. Several recent studies attest the role of quantitative MR imaging, such as T2 mapping, that is commonly used in the research field of knee OA (94, 95).

T2 mapping is based on T2 relaxation time measurements and it has been extensively used to evaluate cartilage degeneration (96). T2 measures the decay caused by spin-spin interactions and has been associated with hydration and collagen content and organization (97-99). Collagen matrix degradation increases water mobility, decreasing the spin-spin interactions and increasing the T2 relaxation time. Mechanical properties are also affected by compositional and structural changes in the extracellular matrix, and T2 has shown negative correlations with various

mechanical measures (100, 101). Based on properties of biochemical tissue components, quantitative analysis of T2 relaxation times can reveal the composition of extracellular matrix, without the need of contrast or special MRI hardware.

Numerous studies have examined relationships between cartilage composition and T2 relaxation times, and a progressively increasing number of studies is focusing on the meniscus. Meniscus consists of a macromolecular framework of collagen fibers, proteoglycan, and water, albeit in different concentrations. Type I collagen represents 98%, and proteoglycan only 1 % (102, 103). T2 is sensitive to interactions between water molecules and the macromolecular concentration and structure of the extracellular matrix (104-106), especially such interactions based on the content, orientation, and anisotropy of collagen (107, 108). The correlation of T2 with PG, however, remains controversial. Although Watrin- Pinzano et al found significantly increased T2 with hyaluronidase-induced PG degeneration (109), in other studies, the depletion of PG had little influence on T2 (106, 110, 111). Compared with articular cartilage, which has a type 2 collagen concentration of 10%–20%, the meniscus has a higher type 1 collagen concentration: 15%–25% (112). The higher collagen concentration in the meniscus may in part also explain the lower meniscal T2 values compared with the cartilage T2 values.

Compared to cartilage, the meniscus is difficult to image due to its short T2 relaxation time, on the order of 8-18ms (113, 114), which causes rapid transverse signal decay. The complex, heterogeneous regional patterns of tissue composition and mechanical function pose another challenge in the meniscus, requiring higher resolution to detect these differences. The radially inner, avascular region experiences high compressive stresses under functional loading and has a relatively high proteoglycans content (8). The radially outer, vascularized region, experiences high circumferential tension and has a relatively low proteoglycans content with the collagen fiber bundles oriented primarily in the circumferential direction (115). In addition, differences in the shape of the meniscus and position within the knee between medial and lateral menisci and anterior and posterior horns contribute to regional differences in loading (116). Son et al demonstrated that T2 times reflect regional variations in tissue composition and functional properties, with increased T2 in regions with greater water content and lower GAGs content, collagen content and

mechanical properties (7). T2 relaxation times varied depending on region in both the radial and circumferential direction. The central region showed shorter T2 relaxation times than either the anterior or the posterior region. In the radial direction, relaxation time was lower in the middle region than in the inner region. Small mediolateral differences were observed in T2, which showed higher relaxation times in the medial meniscus. Biochemical and histological analysis showed that water content, GAGs per dry mass (dm) and collagen per dry mass did not show much variation among anterior, central, and posterior regions but varied in the radial direction. The outer and middle regions showed higher GAGs/dm than the inner region, and the outer region had higher collagen/dm than the other regions. Water content was lowest in the middle region. Mediolateral differences in composition were also detected, with medial menisci showing higher GAGs/dm and lower collagen/dm and water content. GAGs and collagen content normalized by wet mass (wm) showed slightly different patterns. Collagen/wm showed little regional variation. GAGs/wm was higher in the medial meniscus than in the lateral meniscus for the anterior and central regions but not the posterior region. However, it did not exhibit significant circumferential variation in either medial or lateral mensci. GAGs/wm was highest in the radially middle region, followed by the outer and inner regions. Safranin-O staining for GAGs showed the greatest intensity in the middle region and the least in the inner regions (Figure 6).

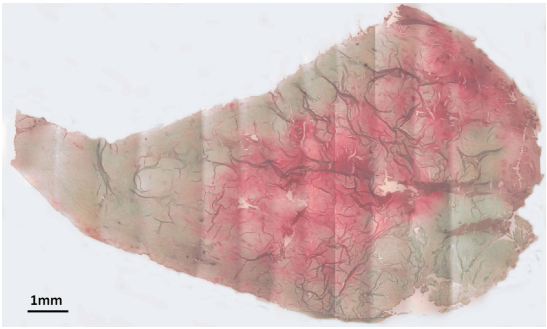

Figure 6. Representative Safranin-O stained meniscal cross-section from the central region created by tiling 50 images. Red staining indicates regions of higher sGAG content. Consistent with quantitative measurements of sGAG content, staining was most intense in the radially middle region and least intense in the radially inner region. *From Son et al. Regional Variation in T1ρ and T2 Times in Osteoarthritic Human Menisci: Correlation with Mechanical Properties and Matrix Composition. Osteoarthritis Cartilage. 2013.*

The differences in meniscal T2 between ages, gender, location, and zone have been investigated by Chiang et al, who measured T2 relaxation times in the posterior horns of knee menisci in asymptomatic subjects (15). Study population consisted of 30 men and 30 women that were divided into three different age groups: 20–34, 35–49 and 50–70 years. The inclusion criteria were BMI 30 kg/cm^2, normalized Western Ontario and McMaster Universities (WOMAC) pain score of zero, and no evidence of meniscal and ligamentous abnormalities on routine knee MR imaging. The T2 values of the posterior meniscal horns increased with increasing age in women and were higher in women than in men.

The age-related rise of T2 values appeared to be more severe in medial menisci than in lateral menisci. This location-dependent variation may be attributed to the presence of less collagen, less proteoglycans, and more water in the medial meniscus than the lateral meniscus (117, 118). The scarcity of proteoglycan in the medial menisci leads to a concomitant increase in the space inside the porous matrix, which in turn is filled by an influx of water. As the mobility and the amount of water increase, the regional T2 value increases (113, 119), suggesting susceptibility to degenerative damage is elevated in the medial menisci.

T2 values also depended on zone. Due to zonal differences in meniscal composition and vascularization (120), T2 values is significantly greater in the white and red/white zones than in the red zone. This is consistent with the fact that common degenerative tears, as the horizontal-cleavage tear, usually begins near the inner margin of the meniscus and extends out toward the periphery (121, 122). Thus, the white zone may be more prone to early degenerative tears than the red-white and red zones. This difference was observed also in the age-related variation and it was especially evident in women (15).

Gender difference in T2 values was reported by Chiang et al T2 values in the posterior meniscal horns were higher in women than in men regardless of age (15). The underlying reason is complicated and controversial. Several studies evaluated the effect of gender on meniscal morphology and biomechanics (123-125). Kerrigan et al indicated that a gender difference in walking pattern could result in a difference in mechanical stress and could thereby account for the difference in OA prevalence (126). Webber et al examining gender differences in meniscal fibrochondrocyte

quantity and proliferation showed that their number was greater in women than in men, regardless of age (127). Further investigations are needed for gender difference during meniscal degeneration.

Recent studies have shown the potential of T2 relaxation time as biomarker to quantify meniscal degeneration in patients with knee OA (128). In vivo T2 mapping of the human meniscus correlates strongly with its degeneration, suggesting that T2 mapping enables detection and quantification of early compositional changes of the meniscus **(129)**.

In a prospective histology-based study, Eijgenraam et al showed that in vivo T2 mapping of the human meniscus correlates strongly with histological degeneration (129) (Figure 7).

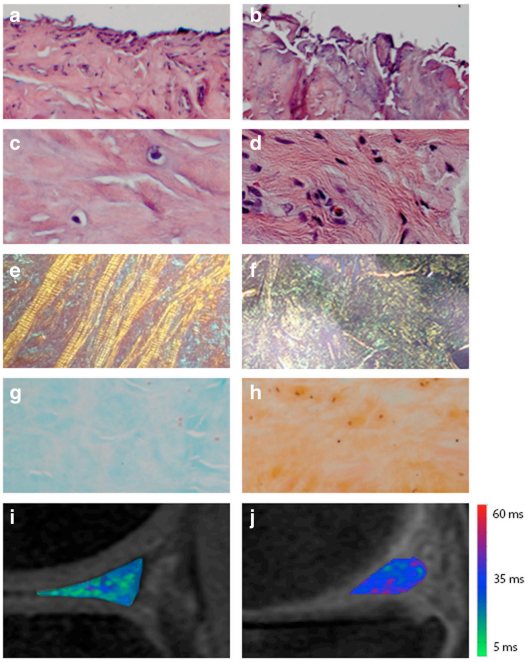

Figure 7. Representative images of histological findings in meniscal tissue and corresponding T2 mapping images. a, c, e, g Posterior horn of lateral meniscus of a 67-year-old female with knee OA (Kellgren and Lawrence grade 3), with a mean T2 relaxation time of 18.6 ms and a histological score of 5. b, d, f, h Posterior horn of medial meniscus of a 66-year-old female with knee A (Kellgren and Lawrence grade 4) with a mean T2 relaxation time of 26.9 ms and a histological score of 13. a, b Surface integrity (HE staining, Å~ 10 zoom). c, d Cellularity (HE staining, Å~ 40 zoom). e, f Collagen organization (Picrosirius red staining, Å~ 5 zoom). g, h Collagen matrix staining intensity, a decreased intensity of green staining indicates disruption in collagen matrix (Saf O-green staining, Å~ 10 zoom). i, j Corresponding noncontrast sagittal T2 mapping images with color map of the meniscus. The color bar on the right shows the range of T2 relaxation times. *From Eijgenraam et al. T2 mapping of the meniscus is a biomarker for early osteoarthritis. European Radiology. 2019.*

They used 13 menisci obtained during total knee replacement surgery from 7 patients (median age 67 years, three males) previously undergone to 3-T MR scanner with T2 mapping pulse sequence with multiple echoes (129). For both T2 relaxation times and histological scores, higher values were found in the medial menisci than in the lateral menisci and the highest were found in the medial anterior horn of the meniscus. Also, no statistically significant differences between the anterior and posterior meniscal horns in T2 relaxation time and histological score were found. A strong correlation between T2 mapping and histology was found. These findings indicate the potential of T2 relaxation times, obtained with in vivo T2 mapping, as non-invasive imaging biomarker for meniscal degeneration.

T2 mapping can differentiate between healthy patients and those with knee OA. Zarins et al investigated the relationship between T2 relaxation measures and the meniscal grades as assessed by the modified Whole-Organ Magnetic Resonance Imaging Score (WORMS) and with clinical WOMAC scores (113). MRI of the knee of 44 osteoarthritis patients and 19 controls, (29 men, 34 women, mean age of 51±13.6 years, mean body mass index (BMI) 26.2±5.3 kg/m2) were performed using a 3T GE Excite Sigma MR Scanner. A T2-weighted fat-saturated FSE sequence (TR/TE: 4300/51 ms, FOV: 14 cm, matrix: 512 x 256, slice thickness (ST): 2.5 mm, gap: 0.5 mm) was used to determine the clinical WORMS measurements for the meniscus. The meniscus compartments analyzed included the following: anterior horn lateral meniscus (AHLAT), anterior horn medial meniscus (AHMED), posterior horn lateral meniscus (PHLAT), and PHMED. The meniscal body was excluded because of partial volume effects. 3D meniscus contours after segmentation were overlaid to T2 maps. Mean T2 values were calculated in defined regions. T2 measurements were then compared between subjects with a meniscal grade of 0, 1, and those with a meniscal tear (grades 2-4). All subjects completed the WOMAC questionnaire to assess pain, stiffness, and function of the knee joint. The overall incidence of meniscal tears was 16% in controls and 57% in OA patients. Among OA patients, 42% of mild OA and 78% of severe OA patients had a meniscal tear. The incidence of a meniscal tear was the highest in the PHMED regardless of Kellgren Lawrence grade. The meniscal grade 2-4 group had higher T2 relaxation times in the PHLAT ($p=0.035$) and the PHMED ($p<0.001$) compared to grade 0. The

PHMED was the only compartment that had statistically different T2 times between more than one meniscal grade. Specifically, the T2 values in the PHMED grade 2-4 were higher than grade 0 ($p<0.001$) and grade 1 ($p< 0.001$). Furthermore, there was a trend for T2 being higher in PHMED grade 1 compared to grade 0 ($p=0.073$). Three of four meniscal compartments (PHMED, AHLAT, and the PHLAT), had significant correlations with the total WOMAC and WOMAC scores (pain, stiffness, function). Meniscal WORMS sum was correlated with WOMAC scores as follows: 0.437 for pain, 0.483 for stiffness, and 0.578 for function ($p<0.001$ for all measures). These findings indicate that meniscal T2 mapping can discriminated between healthy and severe OA, but not between healthy and mild OA, and only in the posterior meniscal horns.

Rauscher and colleagues validated meniscal T2 mapping and T1rho against radiographical and clinical OA (assessed by using WOMAC questionnaires) in 60 subjects without OA, mild OA, and severe OA (119). They observed significant differences in T2 and T1rho values between subject groups and concluded that T2 mapping was more useful than T1rho for differentiating healthy subjects from patients with mild or severe OA (Figure 8). The changes in meniscal T1rho and T2 values with increasing OA severity were supported by significant correlations with the WOMAC scores. The highest such correlation observed was that with combined T2 measurements for the lateral meniscus. This is a complex issue, and it has been hypothesized that patients with more advanced disease, characterized by morphologic cartilage lesions and meniscal and ligamentous abnormalities, could be treated with pain medications and might be able to adapt to more advanced disease (130).

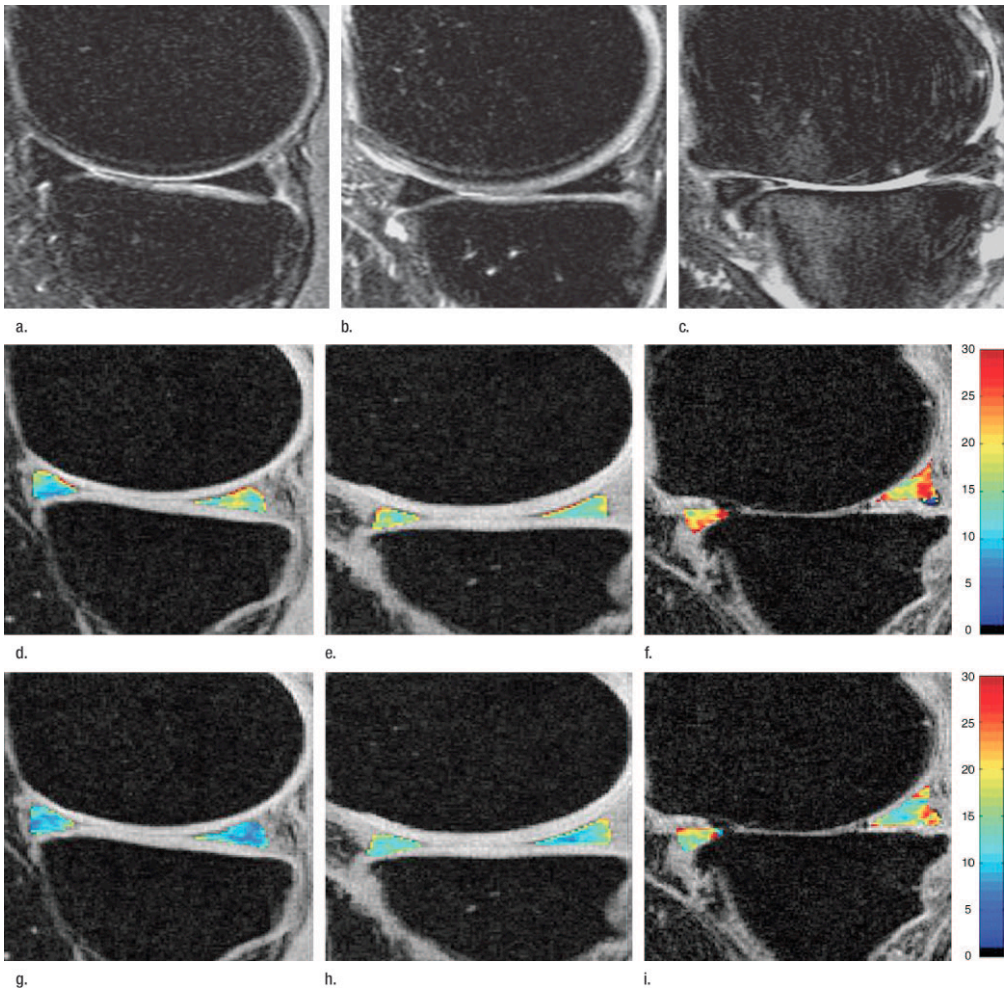

Figure 8. Representative MR images show medial meniscus in (a, d, g) a healthy subject, (b, e, h) a patient with mild OA, and (c, f, i) a patient with severe OA. (a– c) Fast spin-echo images (4300/51) show morphology of the menisci. In a and b, the meniscus appears normal, but c shows a tear in the anterior and posterior horns of the medial meniscus. orresponding (d–f) T1 (in milliseconds) and (g–i) T2 (in milliseconds) color maps overlaid on SPGR images (20/7.5, 12° flip angle) clearly show differences in the meniscal matrix among the three subjects. *From Rauscher et al. Meniscal Measurements of T1rho and T2 at MR Imaging in Healthy Subjects and Patients with Osteoarthritis. Radiology. 2008.*

AIMS

The purpose of this study was to evaluate clinical efficacy and healing effects of conservative management of DMLs with a HA hydrogel (Hymovis® a sterile, non-pyrogenic, viscoelastic hydrogel for intra articular injection, Fidia Farmaceutici SPA). The primary aim of this study was to objectively demonstrate meniscal healing by T2 measurements, providing a quantitative evaluation of qualitative changes in the meniscus. The long-term goal is to be able to treat DMLs conservatively, to avoid APM and prevent the onset of early OA.

MATERIALS AND METHODS

STUDY DESIGN

The study is an open-label prospective pilot study. The study was approved by the local ethics committee (Ethics Committee Campus Bio-Medico University of Rome, Prot 19.17 OSS ComEt CBM). All patients signed informed consent before inclusion.

PARTICIPANTS

Patients were included in the study if they presented the following conditions: male and female ≥35 and ≤65 years of age with DML documented at MRI, without any history of significant acute trauma of the knee; no X-ray or MRI evidence of OA; patients disposed to observe requirements of the study for the whole time period.

Patients were excluded if they presented the following conditions: radiographic evidence of osteoarthritis in the target knee; knee ligament injuries; concurrent pathologies that would prevent the subject to proceed the study or that would interfere with the study results (e.g. rheumatoid arthritis, metabolic bone disease, gout, Paget's disease, symptomatic chondrocalcinosis, etc.); recognized or presumed allergic reactions to hyaluronic formulations; previous operation to the knee or planned surgery throughout the duration of the study; recognized or presumed infection of the joint; unsuitable skin status of the knee such as dermatitis or psoriasis; patient unable to undergo MRI for any reason; patient unable to follow the protocol for the entire length of the study.

INTERVENTION

Patients were subjected to two Hymovis® (HYADD4®, non-crosslinked HA alkylamide, 24 mg/3 mL, Fidia Farmaceutici SPA, Abano Terme Italy), injections two weeks apart (Figure 9).

Hymovis®, highly purified sodium hyaluronate alkylamide obtained from bacterial fermentation, is a CE marked class III medical device, which is indicated for the treatment of pain in osteoarthritic joints and in the conservative treatment of the meniscal lesion of the knee and for the improvement of joint mobility through the enhancement of synovial fluid viscoelasticity.

Participants agree to interrupt NSAIDs consumption at least 24 hours before each office visit for the entire study.

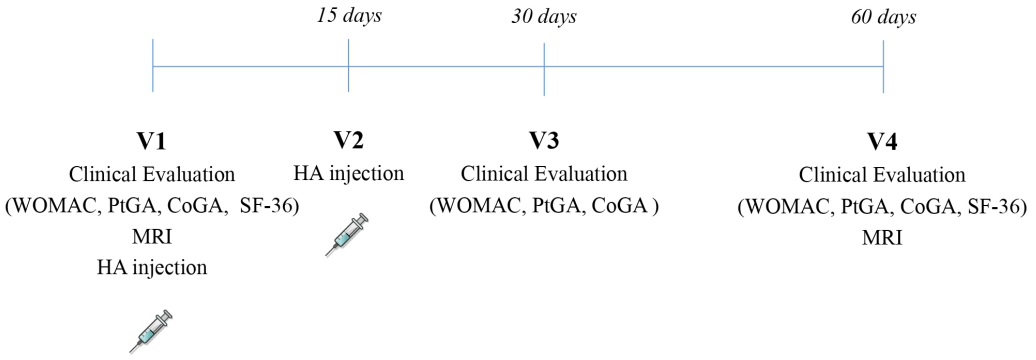

Figure 9: Study's timeline.

EVALUATION OF CLINICAL EFFICACY

Clinical efficacy was assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire (131, 132) (Appendix A) and Patient's Global Assessment (PtGA) and Clinical Observer Global Assessment (CoGA) of the disease. All patients were evaluated at baseline, 30, and 60 days after treatment.

The WOMAC score is a patient-reported outcome measure (PROM), undergone rigorous psychometric validation, and frequently utilized in many clinical trials. It

comprised of 24 items divided into three subscales: pain (WOMAC-A, 5 items), stiffness (WOMAC-B, 2 items), and physical function (WOMAC-C, 17 items). Patients are asked a range of questions about their ability to carry out daily activities such as using the stairs, rising from sitting, lying in bed and conducting light or heavy domestic duties. All the items are scored on a scale of 0-4 (lower scores indicate lower levels of symptoms or physical disability). Values are summed up for a combined WOMAC score. The higher the score, the higher the amount of pain, stiffness, and functional limitation.

The Short Form-36 Health Survey (SF-36) questionnaire (133, 134) was collected at baseline and 60 days after treatment (Appendix B).

The SF-36 is a set of generic, coherent, and easily administered quality-of-life measures. It comprises 36 questions which cover eight domains of health: limitations in physical activities because of health problems; limitations in social activities because of physical or emotional problems; limitations in usual role activities because of physical health problems; bodily pain; general mental health (psychological distress and well-being); limitations in usual role activities because of emotional problems; vitality (energy and fatigue); general health perceptions. The SF-36 is often used as a measure of a person's quality of life (QOL).

One year after treatment, patients were called to know whether any of them had undergone APM.

Local tolerability in the site of injection was assessed for redness and pain few minutes after each treatment session; local and systemic adverse events were registered for the duration of the study.

A diary of analgesic medications was required for the duration of the study.

EVALUATION OF HEALING EFFECTS

To objectively evaluate meniscal healing, a quantitative estimation of the meniscus was conducted using 1.5T MRI with T2 mapping technique at baseline and 60 days after treatment.

We obtained T2 mapping sequence in sagittal planes using a dedicated knee coil. A sagittal 2D multiecho spin-echo sequence with fat saturation was used with the following parameters: TR, 1500 ms; TE1: 10.9 ms; TE2: 21.8 ms; TE3: 32.7 ms; TE4: 43.6 ms; TE5: 54.5 ms; TE6: 65.4 ms; TE7: 76.3 ms; TE8: 87.2 ms; FOV read: 140 mm; FOV phase: 106.3; bandwidth: 372 hertz/pixel. MATRIX 340 × 320 mm; slice thickness 3 mm; distance factor 20% (0.6 mm). The acquisition time was 15 min 03 s.

Meniscal degeneration can be non-invasively quantified with T2 measurements (113). How T2 relaxation works in the meniscus is subject of recent and ongoing researches. There is a correlation between T2 relaxation and collagen orientation and water content (135). Meniscal degeneration is characterized by matrix alterations such as degradation and disorientation of collagen interconnection, decrease of proteoglycans, enhanced water proportion, and penetration of synovial fluid into compromised locations **(136)**. Meniscal degeneration is represented by elevations in meniscal relaxation measures and is correlated with clinical WOMAC score **(113)**. Moreover, the value of T2 is proportional to the gravity of the meniscal degeneration **(113)**. T2 measurements offer an advantage over arthroscopy by examining the entire meniscus rather than the surface areas only.

The following meniscus compartments were analyzed according to International Society of Arthroscopy, Knee Surgery and Orthopedic Sports Medicine (ISAKOS) classification (137): anterior horn lateral meniscus (AHLAT), anterior horn medial meniscus (AHMED), posterior horn lateral meniscus (PHLAT), and posterior horn medial meniscus (PHMED). The meniscal body was excluded because of partial volume effects (Figure 10).

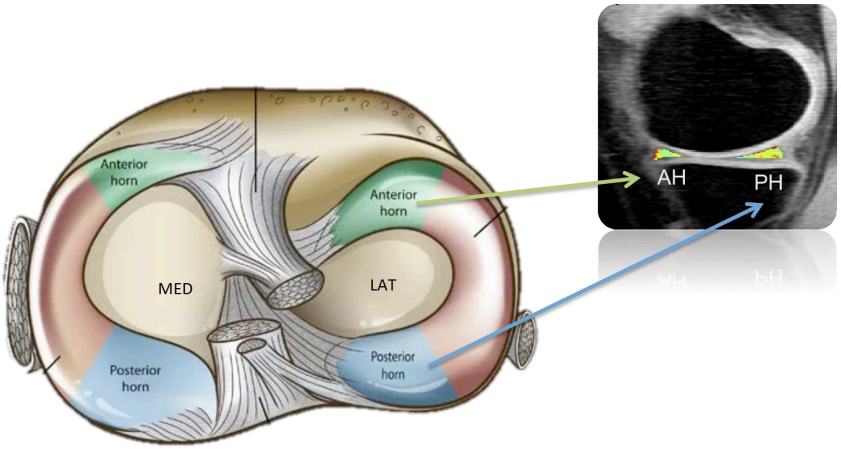

Figure 10: Meniscus compartments.

As the vascularity and cell profiling are different between the inner and outer meniscus, each compartment was divided into three zones: red, red-white, white (Figure 11).

An expert radiologist evaluated MRIs to define the degenerated meniscus compartment. Another radiologist performed T2 measurements in each zone of each compartment. Both radiologists were blind to patients' data.

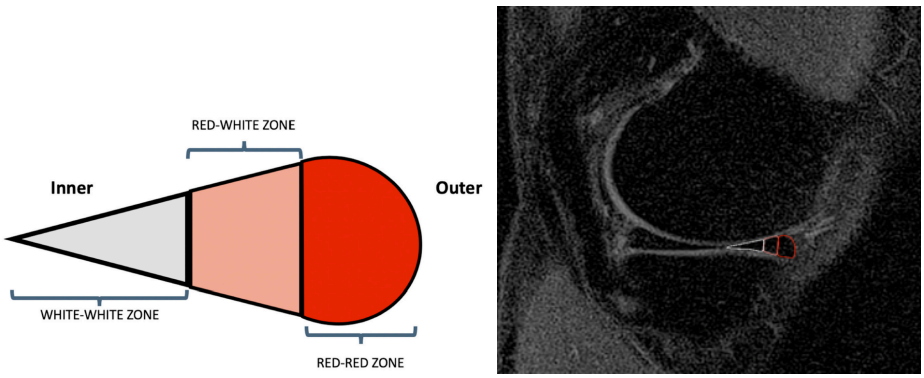

Figure 11. Red,red-white, and white zones of the meniscus.

CORRELATION BETWEEN CLINICAL SCORES AND T2 MEASUREMENTS

The relationship between clinical evaluation and meniscal healing was assessed.

The Pearson's correlation test was performed to correlate pretreatment (V1) clinical scores (WOMAC; WOMAC Section A, B, and C; PtGA; CoGA; and SF36) with pretreatment (V1) T2 measurements of each zone of each meniscus compartment. The same analysis was performed to correlate posttreatment (V3 and V4) clinical scores with posttreatment (V3 and V4) T2 measurements.

DATA ANALYSIS

A sample size of 40 participants was required to achieve statistical significance in the WOMAC score at a 0.05 level with 95% power.

Descriptive statistics were performed for each study time point for each variable collected: median, minimum, and maximum were determined for continuous variables and number and percentage of patients in each category for categorical data.

Non-parametric tests (Fisher's and Wilcoxon test) were conducted to compare pre- and posttreatment data. p-values <0.05 were considered statistically significant.

RESULTS

DEMOGRAPHICS

Forty patients were enrolled in the study from July 2017 to September 2018 (M: 24, F: 16, mean age: 47 years, range: 35–65 years, mean male body weight 74 kg, mean male Body Mass Index (BMI) 25.1, mean female body weight 63 kg, mean female BMI 23.1). All patients completed the treatment, but one patient was lost at 60 days and another at 1-year follow-up.

CLINICAL EFFICACY

The WOMAC score and physical function subscale (Section C) improved after treatment showing a statistically significant difference between baseline and 30 (p = 0,024 WOMAC, p = 0,04 WOMAC-C) and 60 days (p = 0,024 WOMAC, p = 0,02 WOMAC-C) follow-up (Figure 12).

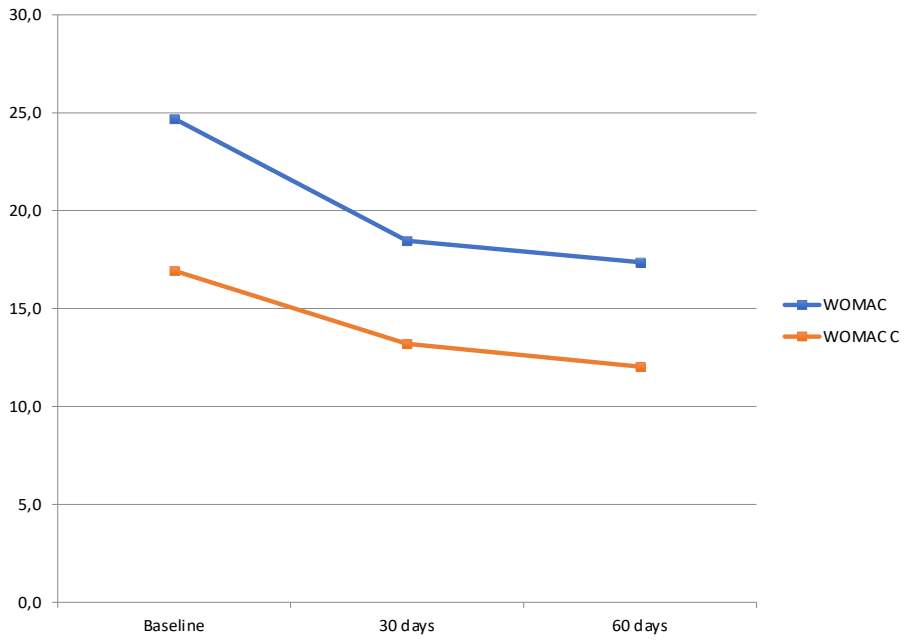

Figure 12. The WOMAC score and physical function subscale (WOMAC-C) showed a statistically significant difference between baseline and 30 and 60 days follow-up.

PtGA and CoGA of the disease revealed an improvement over time. The Wilcoxon test showed a statistically significant difference between baseline and 30 (p = 0.008 PtGA, p < 0.001 CoGA) and 60 days (p = 0.001 PtGA, p < 0.001 CoGA) follow-up (Figure 13).

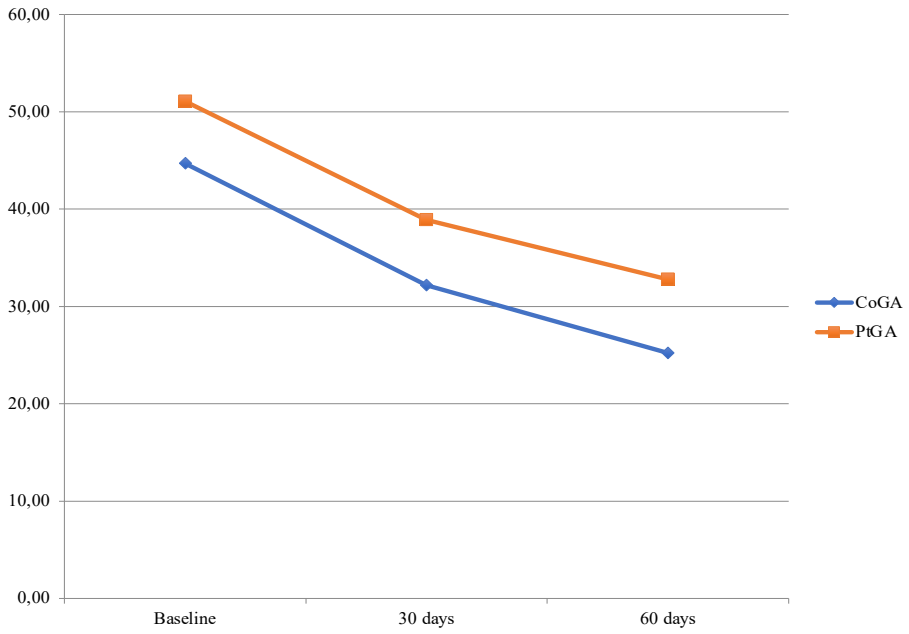

Figure 13. The PtGA, and CoGA showed a statistically significant difference between baseline and 30 and 60 days follow-up.

The SF-36 physical functioning score and pain score showed an improvement with a statistically significant difference between baseline evaluation and 60 days follows-up (p = 0.01 SF-36 physical functioning, p = 0,03 SF-36 pain score) (Figure 14).

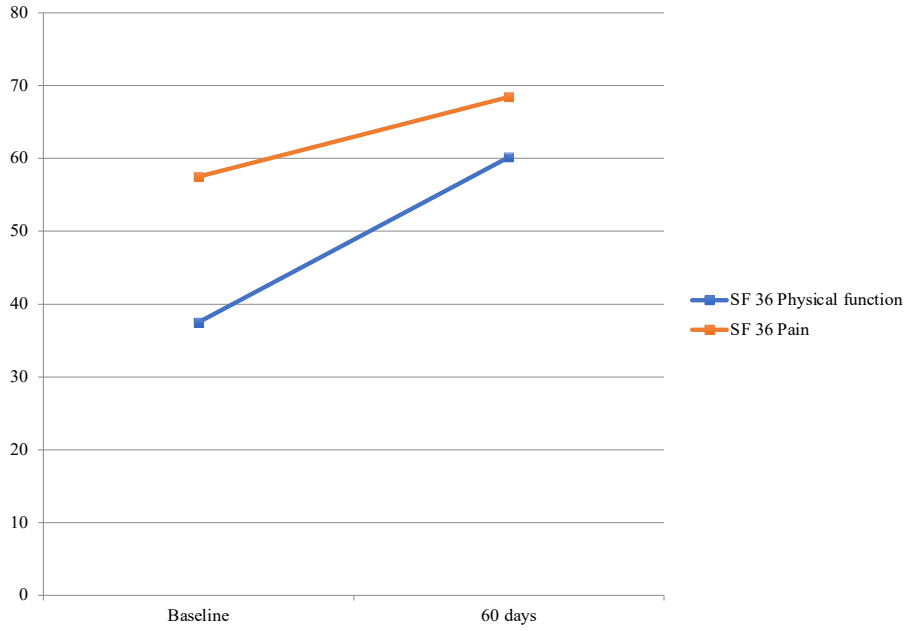

Figure 14. The SF-36 physical functioning score and pain score showed a statistically significant difference between baseline evaluation and 60 days follows-up.

One year after treatment, only one patient had undergone APM. He had a BMI higher than the average of the patients.

Treatment showed good local tolerability, as no redness or pain was detected in the site of injection few minutes after each treatment session. No local or systemic adverse events were registered for the duration of the study.

Only 4 patients required analgesic medications from 3 to 10 days after the first or second injection (1 patient ibuprofen 800 mg × 5 days after the first injection, 1 patient ketoprofen 80 mg × 3 days after the first injection, 1 patient ketoprofen × 5 days) after the first injection; 1 patient paracetamol 1000 mg × 3 days after the second injection).

HEALING EFFECTS

At MRI, the PHMED was degenerated in 33 cases, the PHLAT in 20 cases, the AHMED in 4 cases, and the AHLAT in 7 cases (Figure 15).

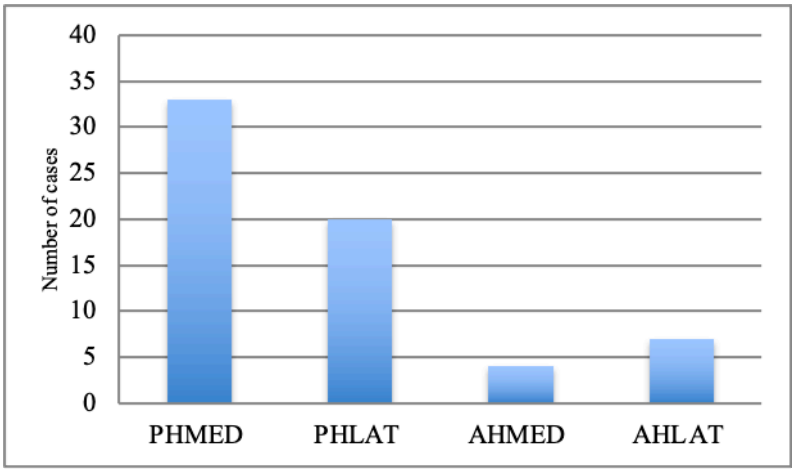

Figure 15: Location of DMLs in the study population.

MRIs of 4 patients were excluded because of artifacts that altered T2 measurements. Decrease in T2 measurement after treatment was detected in 13/33 (39%) cases in both the red and red–white zone of the PHMED, in 20/33 (60%) cases in the white zone of the PHMED, in 11/20 (55%) cases in both the red and white zone of the PHLAT, and in 13/20 (65%) cases in the red–white zone of the PHLAT. Only for the latter, there was a statistically significant difference between baseline and posttreatment T2 measurements (p = 0,03) (Figure 16).

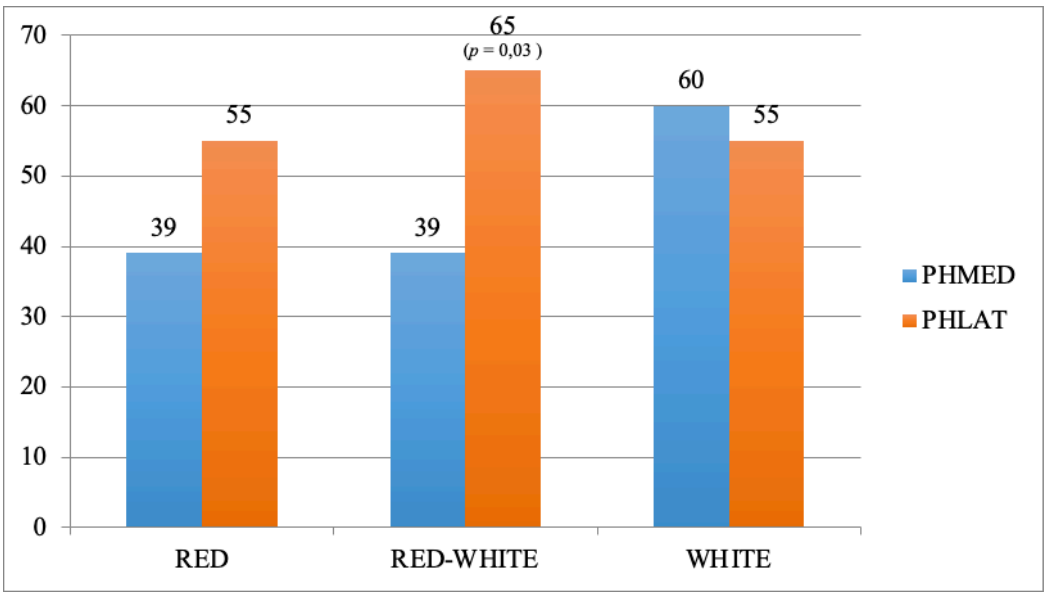

Figure 16. Percentage of cases for each zone of the PHMED and PHLAT that showed a decrease in T2 measurement after treatment. A statistically significant difference between baseline and posttreatment T2 measurements was observed only for the red-white zone of the PHLAT.

CORRELATION BETWEEN CLINICAL SCORES AND T2 MEASUREMENTS

A statistically significant correlation was observed between pretreatment (V1) CoGA and all the zones of the AHMED (red zone $p = 0.030$, red and white zone $p = 0.020$, white zone $p = 0.054$), and between pretreatment (V1) PtGA and the red and white zone of the AHMED ($p = 0.067$). Pretreatment (V1) SF36 mental health score was significantly correlated with the red zone ($p = 0.036$) and red and white zone ($p = 0.018$) of the PHMED; SF36 physical functioning score was significantly correlated with the white zone of the PHMED ($p = 0.048$).

Posttreatment (V4) SF36 emotional role functioning was significantly correlated with the white zone of the AHLAT ($p = 0.047$), and SF36 physical functioning score was significantly correlated with the red zone of the PHLAT ($p = 0.038$).

DISCUSSION

The most important finding of this preliminary study is that HA is a valuable biologic solution for meniscal healing. It demonstrates clinical efficacy and healing effects of conservative management of DMLs with a HA derivative hydrogel (Hymovis®, HYADD4®, Fidia Farmaceutici SPA). The treatment of DMLs with HA showed a marked improvement in patient-reported outcomes that was maintained 60 days after treatment. For the first time, the healing effect of HA on DMLs has been objectively demonstrated, thanks to T2 mapping technique. A high percentage of cases showed a decrease in meniscal relaxation measures, with statistically significant differences in the red–white zone of the PHLAT. This finding can be related with improvements in macromolecular structure of collagens, proteoglycan, and water. One year after treatment, none except one patient needed APM.

The management of DMLs deals with the contradiction between "science" vs. "daily practice". Basic research has demonstrated the key role of the meniscus in the homeostasis of the knee. The meniscus is vital for stability, shock absorption, and transmission of load across the knee joint (138). Injury or dysfunction of the meniscus alters knee mechanics and may initiate or accelerate the pathological cascade leading to OA (64). Osteoarthritis and meniscus tears, particularly degenerative meniscus tears, share many of the same risk factors and are associated with many of the same biological processes, such as cartilage defects and alterations in bone size (139). Thus, it is difficult to determine if one condition precedes the other or if they both occur independently and/or simultaneously.

It has also been shown that certain meniscus lesions are able to heal (140). The healing potential depends on the location, type, length and stability of the tear. In a retrospective study, 65% of longitudinal tears reviewed by arthroscopy showed complete healing after 26 months (122).

The precise mechanism by which meniscal regeneration occurs remains unknown, it is, however, thought to occur via both extrinsic and intrinsic pathways (141-143).

The extrinsic pathway is dependent on the tear site vascularity, where undifferentiated MSCs and growth factors can encourage the repair. The more direct intrinsic pathway occurs via the self-healing capability of the meniscal tissue and is not always a strong contributor to repair (144). It is known that after meniscal injury the number of MSCs in the synovial fluid increases providing endogenous cells required for repair (145). As with all healing, angiogenesis is a vital factor in meniscal tear repair too, promoting repair by supplying growth factors and inflammatory processes. The significance of angiogenesis has been demonstrated in a rabbit meniscal defect model where angiogenin treated defects had significantly better healing rates than the control group (146). This is following other studies that have shown good healing rates in the vascular rich red zone of the meniscus (56). Some literature has also shown synovium to contribute some element of vascularity to injured meniscal sites (147, 148).

Nevertheless arthroscopic meniscectomy is still one of the most common orthopaedic procedures. APM may provide a temporary relief of symptoms, but it will not restore the normal biomechanical function of the meniscus. The removal of meniscus tissue alters the mechanics of the knee, leading to the possible development of OA (149). The surgeon should evaluate the candidate for intervention, the lesion's entity, and the preservation of as much meniscal tissue as possible.

Numbers from Sweden and Denmark confirm that meniscal procedures are the most common arthroscopic knee procedures. Thorlund et al examined data from the Danish National Patient Register, observing a large increase in the incidence of arthroscopic meniscal procedures in middle-aged and older individuals between 2000 and 2011 (45). According to a recent survey, approximately 500,000 APMs are performed every year in the United States, with a considerable proportion resulting in arthroscopic degeneration in the following years (150).

In contrast with the increasing incidence of APMs, several studies demonstrated a higher risk of developing early OA. In a multicenter study by the French Arthroscopy Society, the prevalence of joint line narrowing was 22% in the medial meniscus and 40% in the lateral meniscus at a mean 13 years' follow-up (151). Hulet et al (152), at 20 years' follow-up, found 56% osteoarthritis following lateral meniscectomy. Our research group evaluated the progression of OA after meniscal

resection (55). We assessed clinical and radiographic changes in 57 patients (38 males and 19 females) at a follow-up ranging from 5.1 to 12.1 years (mean: 8.1). The entire cohort had a significant progression postoperatively. The progression of knee OA ranged from 17.2% preoperatively to 65.95% postoperatively in the medial compartment and from 17.64% preoperatively to 58.82% postoperatively in the lateral compartment. The progression of knee OA in the patellofemoral compartment ranged from 5.26 to 42.10%. Obese, overweight patients and those with degenerative tears had a greater predisposition to OA in the tibiofemoral and patellofemoral compartments after meniscectomy. A recent study reported that 14% of patients had a total knee arthroplasty 1 year after APM (55, 153).

Beside the concern about development of early OA, the increasing incidence of APM is surprising in light of several high-quality randomized controlled trials that failed to show any benefit of arthroscopic interventions. APM was not better than placebo surgery, physiotherapy alone, or physiotherapy in combination with other medical treatments for patients in the same age groups, with or without features of knee osteoarthritis (47, 53). Further consolidating these results, 3 more recent randomized controlled trials and an extended follow-up of a previous randomized controlled trial showed no additional benefit of APM in combination with physiotherapy compared to physiotherapy alone for patients with meniscal tears and knee osteoarthritis (154), no superior effect of APM in comparison to 3 weeks of supervised exercise for middle aged patients with meniscal tears (50), and no difference between APM and placebo surgery in middle-aged patients with meniscal tears and no features of knee OA (52). The recent study by Sihvonen et al (2013) extended previous findings by showing that there was no benefit of APM for middle-aged or older patients with degenerative meniscal tears even in the absence of radiographic signs of osteoarthritis (52).

To answer the need for a uniform and clear message for the treatment of DML, the European Society of Sports Traumatology, Knee Surgery & Arthroscopy (ESSKA) realized the Meniscus Consensus Project. More than 80 European physicians, surgeons and scientists were involved with the goal to propose a “framework” that could take into account both scientific evidence and clinical expertise.

Recommendations stated that “surgery shouldn’t be proposed as a first line of treatment of DMLs (Grade A)”. Non-operative treatment can include rehabilitation, nonsteroidal anti-inflammatory drugs (NSAIDs), and intra-articular injections; however, there is “no evidence of which time/type of non-operative treatment should be proposed”.

Nonoperative treatment with exercise therapy has been demonstrated to be as effective as meniscectomy in terms of improving knee pain, mobility, and quality of life (47, 69). According to the present clinical praxis, patients should undergo physiotherapy for at least 2-3 months before a possible knee arthroscopy. Aichroth recommended mobility and strengthening exercises and gradually increased resistance training (155). Several authors (156, 157), pointed out the importance of exercise programs tailored for each individual in order to reach the best clinical outcome. It is suggested to start with supervised training sessions to optimize the performance of the exercises with core stability and control of the knee and to develop the exercises according to the patient’s former experience.

Literature is scantily of studies focused on intra-articular injections for the treatment of meniscal lesions. This is the first study to evaluate the healing of DMLs treated with hyaluronic acid injection in vivo with an objective method such as T2 mapping.

Intra-articular injections of hyaluronic acid has been a mainstay of arthritis treatment since initial trials of such therapies more than 30 years ago (158, 159) but studies about DML are still few. The use of such products for OA is widespread, and annual sales are expected to surpass $2.6 billion in the near future. The FDA classifies HA injections as class III medical devices, implying that a primary mode of action is mechanical. Addition of HA is known to lower friction coefficients of whole joints and in ex vivo studies of cartilage-on-cartilage and cartilage-on-glass interfaces (160-163). A recent study showed that frictional characterization of injectable hyaluronic acids is predictive of clinical outcomes more than traditional rheological or viscoelastic characterization (164). On the other hand, potential biological mechanisms exist. Many studies report that hyaluronic acid has a protective effect on cartilage explants and chondrocytes. As a critical component of the cartilage extracellular matrix, hyaluronic acid interacts with chondrocytes

through the CD44 receptor (165). This binding is thought to be both anabolic and anti-catabolic, inhibiting expression and activity of inflammatory cytokines and degradative proteinases in vitro (86, 166), and reducing matrix damage (167), fibrosis (168), and expression of inflammatory markers in vivo (169). Only recently, researches focused on the effects of HA on the meniscus.

The recent knowledge about the effects of HA on proliferation and migration of human meniscus cells, and the underlying healing mechanisms, can explain the result of this study. Murakami et al demonstrated that HA increases cell migration and proliferation in both inner and outer meniscus cells in a concentration-dependent manner (90) . Meniscus cells from the outer and inner menisci were collected from 18 lateral menisci of patients who underwent total knee arthroplasty and were treated with HA or chondroitin sulfate. Prostaglandin E2 (PGE2)-induced apoptosis and gene expression were evaluated. Cell proliferation was induced by activating the CD44 receptor and PI3K and MAPK signaling. Moreover, HA also inhibited PGE2-induced apoptosis and increased the expression of extracellular matrix elements, particularly type II collagen and aggrecan. This effect was notably evident in inner avascular meniscus cells, whose profiling is similar to those of chondrocytes. Chondroitin sulfate down-regulated MMP13 mRNA of both inner and outer meniscus cells, suggesting that chondroitin sulfate suppresses the inflammatory reaction rather than providing meniscal restoration.

The rationale of using HA on DMLs is sustained by some in vitro studies. Two preclinical studies have been conducted in rabbits with meniscal lesions (88, 89). Ishima et al created a longitudinal tear in the peripheral region of the medial meniscus (88). The target joint received HA injection once a week for 5 weeks (HA group), while the contralateral knee received saline injection (control group). Assessment was conducted 6 and 12 weeks after surgery. Meniscal healing was evident in both groups on gross morphological examination, but the HA group showed a significantly higher healing rate than the control group at 12 weeks. Meniscal healing on histological evaluation moved from the tibial portion in both groups at 6 weeks and proceeded toward the femoral surface at 12 weeks in the HA group. In summary, meniscal injury in the peripheral region showed evidence of healing without suturing and HA maintained the healing process of the injured

meniscus, especially in the femoral surface, for up to 12 weeks after the injury. Suzuki et al (89) produced a cylindrical lesion on the lateral meniscus and injected the knee with HA once a week. The meniscus was compared with control, injected with phosphate buffer, at 1 and 6 weeks. A significant increase in the rate of filling of the defect was detected in the HA group. As the repair progressed, the cell population of the repaired tissue shifted from fibroblast-like cells to chondrocyte-like cells. At six weeks, the ratio of chondrocyte-like cells to all cells was higher in the HA group, inducing authors to deduce that the healing rate was increased by HA.

In humans, the meniscus' potential to heal is greater and depends on the location, type, length, and stability of the tear. In a retrospective evaluation of 3612 arthroscopic procedures, Weiss et al (122) selected 80 stable meniscal tears (in 75 patients). At an average of 26 months, a new arthroscopy was performed on 32 patients (26 of whom had a longitudinal tear and 6 of whom had a radial tear). Complete healing was observed in 17 of the 26 longitudinal tears.

Our findings agree with previous studies. The incidence of meniscal tear was higher in the PHMED as reported in many studies (113). The medial meniscus has greater susceptibility to degenerative damage because of the lower content of collagen and proteoglycans, and the higher content of water compared to the lateral meniscus (117, 118). It is highlighted by higher T2 values.

The efficacy of HA in the management DMLs was evaluated in a prospective randomized clinical trial on fifty subjects by Zorzi et al (91). Patients were clinically evaluated at baseline and after 14, 30, and 60 days to assess pain (Visual Analog Scale), knee functionality (WOMAC questionnaire), and health status (SF-36). By comparing the follow-up MR images with the baseline images, the presence or absence of a reduction in length and depth of the lesion was identified. Authors found a significant reduction in VAS pain and reduction in length and depth of the meniscal lesion after treatment. In our study, we used an objective method to evaluate meniscal degeneration and the healing effects of HA. T2 measurements allow to quantify meniscal composition because of the correlation between T2 relaxation and biochemical tissue components (135). Increased T2 relaxation times indicate damage to the collagen network and an increase in water content (129, 170).

T2 measurements are non-invasive and offer an advantage over arthroscopy by examining the entire meniscus rather than the surface areas only. T2 mapping provides a useful tool for clinicians in radiological analysis of the meniscus. The value of standard radiography is extremely limited for the assessment of meniscal pathology; it may be indicated to obtain a differential diagnosis and to estimate the grade of OA. Ultrasound is rarely used as a diagnostic tool for meniscal pathologies, and its accuracy is operator dependent. CT arthrography with multiplanar reconstructions can detect meniscus tears that are not visible on MRI, but it is an invasive technique. Due to its non-invasiveness, superior soft tissue contrast and absence of ionizing radiation, MRI is clearly the most powerful and versatile imaging method for diagnosing meniscal lesions. Clinical-standard morphological MRI is the modality of choice in the evaluation of meniscal lesion with high diagnostic accuracy in the assessment of gross meniscus pathologies (171). However, evaluation is subjective and solely based on morphological aspects such as surface integrity and intra-tissue signal intensities. MRI is also limited when detecting smaller lesions and changes prior to surface breakdown (172, 173). It is not suitable to detect matrix changes in response to treatment. Quantitative MRI techniques, such as T2 mapping, provide spatially resolved measures of tissue (ultra)structure and composition beyond mere morphology and have been applied to assess meniscus in health and disease (7, 16, 119, 128). Quantitative T2 and structural assessment of the meniscus with T2 mapping show significant correlations with the reference standard and is a useful technic to quantitatively evaluate qualitative changes of the meniscus after treatment. T2 measurements are sensitive to the content of water and concentration of macromolecules in the extracellular matrix and to interaction between biochemical components that is related with the content, orientation, and anisotropy of the collagen (119, 174).

One of the limits of T2 mapping is the susceptibility to the magnetic angle effect. T2 values may be artificially elevated in specific regions depending on reciprocal orientation of meniscal components and the main magnetic field. Some studies suggest that T2 values are greater when the region of interest is oriented at 55° to B0 and lower in regions oriented at 180° (0°) to B0 (175). We tried to reduce this effect using the same positioning in relation to the magnet for all patients.

When interpreting T2 mapping results, it is important to remember that the T2 value changes according to the strength of the magnetic field B0. Lower T2 values were observed at higher field strengths. In addition, sequence type, coil architecture, and calculation method of T2 mapping affect T2 results. A larger population and histological correlations with meniscal anatomic structure are needed to assess this.

Regarding the relationships between T2 in menisci and clinical findings, it should be noted that, to our knowledge, the results of only smaller-scale studies are available. Clearly, larger-scale studies are required. This study shows a statistically significant correlation between some clinical scores and some T2 measurements in different zones of the meniscus. We did not find any correlation between WOMAC and T2 measurements. Conversely, a previous study reported a correlation between meniscal compartments and total WOMAC and WOMAC scores (pain, stiffness, function) (113). This finding can be interpreted as an inconstant relationship between clinical symptoms and degenerative characteristics of the meniscus that is already known. It is well established that DMLs are often incidental findings at MRI, and it is difficult to understand whether pain is directly produced by DML even if the lesion is unstable (176). Incidentally, the progression of symptoms of DMLs is not clear. It has been hypothesized that patients with more advanced disease could be treated with pain medications and might be able to adapt to more advanced disease (130). Data should have been stratified according to the degree of meniscal degeneration and patients' BMI because of potential influence on the outcomes. Further studies are needed to investigate this aspect.

In this study, one year after treatment, none except one patient needed APM. In other Level I studies, the need of arthroscopy after conservative treatment without HA knee infiltrations was reported to be over 30%, and it was required at an interval of 3 to 6 months (177).

Findings of this study should be considered as preliminary because of the small study group. Results should be interpreted carefully, and larger confirmatory studies are needed.

The limitations of this pilot study include the absence of a control group because ethical issues preclude preforming placebo intra-articular injections. Placebo intra-articular injections face with potential complications such as septic joint. The needle

can inoculate a joint by depositing a core of tissue within the joint. The consequence of septic arthritis can be devastating. Fifty percent of adults with septic arthritis have significant sequelae of decreased range of motion or chronic pain after infection. Complications include dysfunctional joints, osteomyelitis, and sepsis. From an ethical point of view, this risk is not acceptable.

As an alternative to the control group, we can refer to the history of symptomatic DMLs treated with conventional conservative therapy. This comparison allows adhering to relevant international ethical standards avoiding unnecessary risk related with placebo injections.

Conservative treatment includes rehabilitation and nonsteroidal anti-inflammatory drugs (NSAIDs). Conservative treatment of patients with mechanical symptoms is associated with poor outcomes, and over 30% of patients need arthroscopy at the second stage (177). Failure percentage of conservative treatment is higher than the percentage observed in this study in patients treated with hyaluronic acid injections.

Another limitation of this study is the omission of evaluating treatment outcomes in proportion to the degree of meniscal degeneration. The healing effect of HA can be proportional to the degree of meniscal degeneration. A high degree of meniscal degeneration in enrolled patients could explain the results. It will be the subject of future endeavors.

The results of this study refer only to patients with symptomatic DMLs. We did not consider traumatic meniscal tears for the following reasons. Traumatic meniscal lesions are generally associated with acute onset of pain, swelling, catching, and locking of the knee. Patients affected by traumatic meniscal tears have usually a poor compliance to conservative treatment because of pain intensity and activity restriction. Those patients need to be surgically treated in a short time to relieve pain and regain physical function. In the clinical setting, there is no evidence about the efficacy of HA injections for traumatic meniscal lesions, representing an ethical issue.

In this study a quantitative estimation of the meniscus was conducted using 1.5 T MRI with T2 mapping technique. Traditionally, 1.5-T MRI has been the standard for evaluating articular lesions (178). More recently, some studies have reported that 3.0-T MRI provides better visualization of the knee lesions compared with 1.5-T

MRI, with increased sensitivity and specificity in animal and cadaver models (179-181). The major advantage of high-field strength MRI is the improvement in the signal-to-noise ratio, which can be used to either increase image resolution or decrease scan time. Nevertheless, 3.0 T MRI presents a certain image quality reduction due to chemical shift artefacts.

A recent meta-analysis revealed that 3.0-T MRI of the knee does not yield a significantly higher diagnostic accuracy than 1.5-T MRI for detecting meniscal and ligament tears (182). Although these results may seem surprising at first glance, they are not completely unexpected. First, the evaluation of meniscal and ligament pathology with standard magnetic field strengths (<1.5 T) has been generally successful. Thus, any further improvement with higher-field strength systems is likely to be small. Second, image quality and diagnostic accuracy are not determined only by magnetic field strength; other factors, such as imaging planes and coil technology, also play critical roles in the ultimate diagnostic accuracy of the MRI examination. In an in vivo study, Lutterbey et al. compared the diagnostic performance of knee MRI performed with the body coil at 3.0 T and a dedicated knee coil at 1.5 T (183). These investigators found a significant decrease in image quality at 3.0 T with a visible and measurable signal loss. In our study, high-resolution imaging sequences, obtained with thin slice thickness and a dedicated knee coil, have been used to improve the results.

The performance of 3.0 T versus 1.5 T MRI using dedicated research protocols, such as T2 mapping technique, has not been evaluated. We can hypotize that 3.0 T MRI would allow analyzing the complex, heterogeneous regional patterns of tissue composition more in detail but should no change substantially the results of the study.

The key strength of this study is the use of T2 measurements as an objective method to assess the healing process of the meniscus. T2 mapping has been extensively studied to differentiate healthy and degenerative menisci in OA patients. Studies by Rauscher et al and Zarins et al found significantly higher meniscal T2 in OA patients than asymptomatic volunteers and in torn menisci than intact menisci (113, 119). Meniscal degeneration is characterized by increased water content and decreased collagen and proteoglycan content of the tissue. Son et al compared

quantitative MRI parameters of meniscus in patients with OA with biochemical parameters and found that T2 was strongly correlated with water content but only weakly correlated with proteoglycan and collagen content (7). If T2 is effective to differentiate between healthy and degenerative menisci, it can be used to identify treatment effects on meniscal substance.

To our knowledge, this is the first study to use T2 mapping to detect changes induced by treatment in the menisci. T2 measurements provide a quantitative evaluation of qualitative changes in the meniscus after treatment. The healing process involves matrix change that cannot be otherwise evaluated in vivo. Clinical scores resent of patient's perception of the disease and expectation about the treatment, as well as external factors that can affect pain and function, such as medications and physical activity. The combined use of both T2 mapping and clinical scores provide a complete overview of treatment effects.

Another strength of the study is the analysis of both menisci considering regional variations. It has been demonstrated that T2 relaxation times varied depending on region in both the radial and circumferential direction (7). In the study of Son et al, the central region showed shorter T2 relaxation times than either the anterior or the posterior region (7). In the radial direction, relaxation times were lower in the middle region than in the inner region. Small mediolateral differences were observed in T2, which showed higher relaxation times in the medial meniscus. We subdivided each meniscus in 4 compartments according to the ISAKOS classification (137) (AHLAT, AHMED, PHLAT, PHMED) and each compartment into three zones (red, red-white, white). This allowed taking into consideration regional variation in T2 relaxation times due to anatomic subregions of the menisci and their response to treatment that could be affected by vascularity and cellular characteristics.

One of the strengths of this study is that all patients completed the treatment, and only two patients were lost at follow-up (at 60 days and 1 year respectively) because of personal reasons. This means that the management of DMLs with hyaluronic acid is associated with good patients' compliance and can be easily used in the clinical practice.

Although findings of this study should be interpreted with caution, this investigation is of clinical relevance because opens new prospective in the

management of DMLs. The use of hyaluronic acid can be recommended for this type of patients, reducing healthcare costs and early OA correlated with APMs. According to the European consensus in the treatment of patients with a symptomatic knee and a DML, APM should not be proposed as a first-line treatment (176). The main reason is that patient's symptoms may not necessarily relate to the actual DML but to more unspecific joint or joint line pain related to early onset osteoarthritis. On the other hand, there is "no evidence of which time/type of non-operative treatment should be proposed". Literature is mainly focused on rehabilitation protocols, even if non-operative treatment can include nonsteroidal anti-inflammatory drugs (NSAIDs), and intra-articular injections. Unfortunately, there are no studies on this topic.

In future studies, we aim to increase the study population to obtain more solid data to highlight the healing effect of HA and sustain the conservative treatment of DMLs with HA. Patients will be stratified based on the degree of meniscal degeneration and BMI. It is plausible that an early degenerative change may respond better to treatment, as it can happen for patient with low BMI. The tissue may have greater difficulty to recover when the structure is corrupted or a higher load is systematically applied, interfering with its biomechanical and cellular characterization (34).

CONCLUSIONS

This study is a first step in an ongoing investigation of conservative treatments for DMLs. Lately, therapeutic efforts have been aimed at preserving and restoring the damaged meniscus secondary to degeneration. This is of particular relevance as meniscus and cartilage pathologies are closely interrelated. Consequently, meniscus damage and loss are key features of and risk factors for developing OA. Only a few guidelines on treatment options exist, and new evidence is highly warranted (176).

We aimed to evaluate clinical efficacy and healing effects of conservative management of DMLs with a HA hydrogel Hymovis® a sterile, non-pyrogenic, viscoelastic hydrogel for intra articular injection (Fidia Farmaceutici SPA).

Before this treatment is implemented clinically it needs to be further investigated to confirm collected results in a larger population.

The long-term goal is to be able to treat DMLs conservatively, to avoid APM and prevent the onset of early OA.

In conclusion, this study supports the use of HA in the conservative management of DML as it is clinically effective and enhances meniscus healing as demonstrated by T2 measurements. Moreover, it reduces the need for APM at 1-year follow-up, representing a less invasive and cost-effective option compared to APM.

APPENDIX A

ITALIAN-WOMAC QUESTIONNARIE

A. DOLORE

Che intensità prova:

Per ogni voce indicare il grado di dolore secondo la seguente scala

	0 = nessuno	**1 = lieve**	**2 = moderato**	**3 = forte**	**4 = severo**
1. Camminando su una superficie piana					
2. Salendo o scendendo le scale					
3. A letto, durante il sonno notturno (interferisce con il sonno)					
4. Da seduto o in posizione supina					
5. Stando in piedi, in posizione eretta					

B. RIGIDITA'

Quanto è intensa la Sua rigidità:

Per ogni voce indicare il grado di rigidità secondo la seguente scala

	0 = assente	**1 = lieve**	**2 = moderata**	**3 = forte**	**4 = severa**
6. Subito dopo il risveglio al mattino					
7. Dopo essere stato seduto, sdraiato oppure dopo aver riposato, più tardi nel corso della giornata					

C. FUNZIONE FISICA

Qual è il grado di difficoltà che avverte nel:

Per ogni voce indicare il grado di difficoltà secondo la seguente scala

	0 = assente	**1 = lieve**	**2 = moderata**	**3 = forte**	**4 = severa**
8. Scendere le scale					
9. Salire le scale					
10. Alzarsi da seduto					
11. Stare in piedi					
12. Piegarsi verso il pavimento (per raccogliere un oggetto)					
13. Camminare su una superficie piana					
14. Entrare ed uscire da una macchina o salire e scendere da un autobus					
15. Andare a far spese					
16. Mettersi i calzini o le calze					
17. Alzarsi dal letto					
18. Togliersi i calzini o le calze					
19. Stare sdraiato a letto					
20. Entrare ed uscire dalla vasca da bagno					
21. Stare seduto					
22. Sedersi o alzarsi dal water					
23. Fare lavori domestici pesanti					
24. Fare lavori domestici leggeri					

APPENDIX B

SHORT FORM-36 HEALTH SURVEY (SF-36)

Versione italiana ufficiale, di Apolone et al 1997 (progetto IQOLA), dall'originale inglese di Ware and Sherbourne, 1992

Scelga una risposta per ogni domanda

1. In generale direbbe che la Sua salute è…				
Eccellente	**Molto buona**	**Buona**	**Passabile**	**Scadente**
1	**2**	**3**	**4**	**5**

2. **Rispetto a un anno fa**, come giudicherebbe, ora, la Sua salute in generale?				
Decisamente migliore adesso rispetto a un anno fa	**Un po' migliore adesso rispetto a un anno fa**	**Più o meno uguale rispetto a un anno fa**	**Un po' peggiore adesso rispetto a un anno fa**	**Decisamente peggiore adesso rispetto a un anno fa**
1	**2**	**3**	**4**	**5**

Le seguenti domande riguardano alcune attività che potrebbe svolgere nel corso di una qualsiasi giornata. Ci dica, scegliendo una risposta per ogni riga, se attualmente la **Sua salute** La limita nello svolgimento di queste attività.

	Sì, mi limita parecchio	**Si, mi limita parzialmente**	**No, non mi limita per nulla**
3. **Attività fisicamente impegnative**, come correre, sollevare oggetti pesanti, praticare sport faticosi	**1**	**2**	**3**
4. **Attività di moderato impegno fisico**, come spostare un tavolo, usare l'aspirapolvere, giocare a bocce o fare un giretto in bicicletta	**1**	**2**	**3**
5. Sollevare o portare le borse della spesa	**1**	**2**	**3**
6. Salire **qualche** piano di scale	**1**	**2**	**3**
7. Salire **un** piano di scale	**1**	**2**	**3**
8. Piegarsi, inginocchiarsi o chinarsi	**1**	**2**	**3**
9. Camminare **per un chilometro**	**1**	**2**	**3**
10. Camminare **per qualche centinaia di metri**	**1**	**2**	**3**
11. Camminare **per circa cento metri**	**1**	**2**	**3**
12. Fare il bagno o vestirsi da soli	**1**	**2**	**3**

Nelle ultime quattro settimane, ha riscontrato i seguenti problemi sul lavoro o nelle altre attività quotidiane, **a causa della Sua salute fisica**?

Risponda **Si** o **No** a ciascuna domanda	**Sì**	**No**
13. Ha ridotto **il tempo** dedicato al lavoro o ad altre attività	**1**	**2**
14. Ha **reso** meno di quanto avrebbe voluto	**1**	**2**
15. Ha dovuto limitare alcuni **tipi** di lavoro o di altre attività	**1**	**2**
16. Ha avuto difficoltà nell'eseguire il lavoro o altre attività (ad es., ha fatto più fatica	**1**	**2**

Nelle ultime quattro settimane, ha riscontrato i seguenti problemi sul lavoro o nelle altre attività quotidiane, **a causa della Suo stato emotivo** (quale il sentirsi depresso o ansioso)?

Risponda **Si** o **No** a ciascuna domanda	**Sì**	**No**
17. Ha ridotto **il tempo** dedicato al lavoro o ad altre attività	**1**	**2**
18. Ha **reso** meno di quanto avrebbe voluto	**1**	**2**
19. Ha avuto un calo di **concentrazione** sul lavoro o in altre attività	**1**	**2**

20. Nelle ultime quattro settimane, in che misura la Sua salute fisica o il suo stato emotivo hanno interferito con le normali attività sociali con la famiglia, gli amici, i vicini di casa, i gruppi di cui fa parte? (Indichi un numero)

Per nulla	**Leggermente**	**Un pò**	**Molto**	**Moltissimo**
1	**2**	**3**	**4**	**5**

21. Quanto dolore **fisico** ha provato nelle ultime quattro settimane?(Indichi un numero)

Nessuno	**Molto lieve**	**Lieve**	**Moderato**	**Forte**	**Molto forte**
1	**2**	**3**	**4**	**5**	**6**

22. Nelle ultime quattro settimane, in che misura il dolore L'ha ostacolata nel lavoro che svolge abitualmente, sia in casa sia fuori? (Indichi un numero)

Per nulla	**Molto poco**	**Un pò**	**Molto**	**Moltissimo**
1	**2**	**3**	**4**	**5**

Le seguenti domande si riferiscono a come si è sentito nelle ultime quattro settimane. Risponda a ciascuna domanda scegliendo la risposta che più si avvicina al Suo caso.

Per quanto tempo nelle ultime quattro settimane si è sentito…..

	Sempre	**Quasi sempre**	**Molto tempo**	**Una parte del tempo**	**Quasi mai**	**Mai**
23. Vivace e brillante?	**1**	**2**	**3**	**4**	**5**	**6**
24. Molto agitato?	**1**	**2**	**3**	**4**	**5**	**6**
25.Così giù di morale che niente avrebbe potuto tirarla su?	**1**	**2**	**3**	**4**	**5**	**6**
26. Calmo e sereno?	**1**	**2**	**3**	**4**	**5**	**6**
27. Pieno di energia?	**1**	**2**	**3**	**4**	**5**	**6**
28. Scoraggiato e triste?	**1**	**2**	**3**	**4**	**5**	**6**
29. Sfinito?	**1**	**2**	**3**	**4**	**5**	**6**
30. Felice?	**1**	**2**	**3**	**4**	**5**	**6**
31. Stanco?	**1**	**2**	**3**	**4**	**5**	**6**

32. Nelle ultime quattro settimane, per quanto tempo la Sua salute fisica o il suo stato emotivo hanno interferito nelle Sue attività sociali, in famiglia, con gli amici? (Indichi un numero)

Sempre	**Quasi sempre**	**Una parte del tempo**	**Quasi mai**	**Mai**
1	**2**	**3**	**4**	**5**

Scelga , per ogni domanda, la risposta che meglio descrive quanto siano **Vere** o **False** le seguenti affermazioni.

	Certamente vero	**In gran parte vero**	**Non so**	**In gran parte falso**	**Certamente falso**
33. Mi pare di ammalarmi un po' più	**1**	**2**	**3**	**4**	**5**
34. La mia salute è come quella degli altri	**1**	**2**	**3**	**4**	**5**
35. Mi aspetto che la mia salute andrà peggiorando	**1**	**2**	**3**	**4**	**5**
36. Godo di ottima salute	**1**	**2**	**3**	**4**	**5**

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