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Oggi, il mondo di domani è l'impegno ad agire per un presente responsabile ed un futuro sostenibile. Per Bristol-Myers Squibb significa innanzitutto sviluppare farmaci che realmente possano fare la differenza nella vita delle persone per prolungare e migliorare la vita umana. Ma significa anche avere la piena consapevolezza degli obblighi verso la comunità locale e globale, trasformandoli in impegno concreto. Il nostro impegno guarda al futuro e alle realtà più lontane ma inizia nel presente e dai luoghi a noi più vicini. **Oggi, per il domani.**

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*EDITORIAL*

# WHAT IS THE BEST WAY TO MEASURE THE IMPACT OF INTRA-ARTICULAR THERAPY ON OSTEOARTHRITIS?

ALBERTO MIGLIORE

One of the main issues when assessing the effectiveness of intra-articular treatments is the correct measurement of its impact. Clinical parameters and structural parameters are used to this end. Other fundamental aspects that must be taken into account are the observation time spent in order to measure the evolution of the parameters and the drug dosage employed. Most of the available studies and literature on the subject only focus on the evaluation of the parameters themselves and then, regrettably, only for short term observations (2-3 months). The clinical parameters are mostly measured against pain scales or articular functions. However, one must take into account the relevant effect of injected placebo, which, in combination with the nature of the pathology itself - which is chronic and characterized by cyclic acute phases - results in some studies reporting a measurement of the effectiveness of hylauronic-acid based intra-articular therapy as slightly above the placebo, if not completely devoid of any statistical relevance. One must point out, however, that these randomized studies tracked the clinical parameters during an observation period limited to about 3 to 6 months. On the other hand, we are very much aware that the osteoarthritic disease is a degenerative chronic condition that is characterized by acutization and remission phases. The randomized controlled studies have so far not considered, not even as a secondary objective, the frequency and sheer intensity of the acutization phases, or better yet, of the episodes of reactivation throughout the osteoarthritic disease, for a period of at least one or two years. We have developed observational cohort studies (12-36 months) that have highlighted a linear behavior of the symptomatology, without acute phases, with an overall reduction of 40 to 60% of the symptoms following the ultra-sound guided infiltration treatment of hip osteoarthritis. This percentage was a constant throughout the cyclic repetition of the injective treatments. Unfortunately these observational studies did not include a control group, for which we would have doubtlessly recorded a much higher number of acutizations of the disease. In other words, more research should be done, in the form of controlled randomized as well as cohort studies with control groups, in order to best evaluate the clinical parameters as well as the frequency and sheer intensity of inflammatory reacutizations throughout the injection treatment, and to highlight the significant statistical differences with respect to classic os therapy. In one particular study of ours "Hip viscosupplementation under ultra-sound guidance reduces NSAID consumption in symptomatic hip osteoarthritis patients in a long follow-up. Eur Rev Med Pharmacol Sci. 2011 Jan;15(1):25-34", we highlight the significant reduction of anti-inflammatory drug intake by those patients which were treated with ultrasound guided injective therapy, a reduction that was consistent in time and that therefore seems to indicate, even though indirectly, the evidence of clinical stabilization with an absence of poussees of inflammatory reacutization. The premises so far discussed can also extend to the evaluation of structural parameters, meaning the methods of diagnostic highlighting of the structural articular damages of osteoarthritis. Most of the afore mentioned methods include a standard radiological study, even though magnetic resonance is progressively becoming more popular in current studies, given its superiority as an image diagnostic instrument for the evalution of the therapy

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**DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.**

induced inhibition of the progressive structural damage suffered by the joints. We believe that the analysis of these parameters should also be conducted through controlled randomized studies as well as cohort studies which include control groups treated with standard therapy, for a reasonable period of 12 months at least (though 24-36 months of observations would be best) in order to evaluate the effectiveness of intra-articular hylauronic acid therapy for joints suffering from degenerative osteoarthritis. Clearly, every study should take into account drug dosages. There is a clear evidence of a pharmacological dosage that ultimately determines a clinical reduction in the symptoms, meaning of a symptomatic treatment. However, we can not presently know whether the dosage needed to affect the symptoms is the same as that which might eventually slow or even stop the progression of the degenerative disease, meaning of the disease-modifying dosage. This is a particularly interesting field of study, because there is a chance that increasing the frequency or dosage of the injections might result in a greater structural benefit.

Another variable that must be taken into account is the volume of the dosage that is to be injected, which as per our own experience, varies according to the joint and on the state of advancement of the articular damage itself. All these open issues are a call to increase our research efforts, to conduct such studies and work with rigorous and appropriate methods, in order to push our knowledge of this fascinating and promising field of intra-articular treatment of rheumatic diseases.

# LONG-TERM SAFETY OF ANTI-TNF ETANERCEPT IN PATIENTS WITH PSORIATIC ARTHRITIS AND CONCURRENT HBV INFECTION: A CASE SERIES OF 10 PATIENTS

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**Immunosuppressive drugs used in the treatment of psoriatic arthritis, for their mechanism of action, make treated patients more susceptible to bacterial, fungal or viral infections. Not only because they increase the risk of new infections, but also act by altering the natural course of pre-existing infections. While numerous data are available in the literature regarding the reactivation of tuberculosis infection, limited and poor information are available about the risk of HVB and HCV reactivation or exacerbation during biologics treatment. Furthermore, the duration of biological therapy in reported series regards short periods of follow-up and this is not very reliable as regards the risk of reactivation in the long-term treatment. Our study evaluated patients with a history of hepatitis B and psoriatic arthritis treated with etanercept monitored for up to six years. Throughout the observation period, in all patients, treatment was effective and well tolerated and liver function tests and viral load levels remained unchanged.**

Immunosuppressive agents used in psoriatic arthritis might have an effect on the natural course of coexisting infectious diseases or new developed infections. An association between the use of anti-TNF-α and an increased risk of severe bacterial infections and the reactivation of tuberculosis has been investigated. (1) Long-term safety and efficacy of anti-TNF-α agents in patients with chronic hepatitis B or hepatitis C are less known. (1) Case reports and a small prospective study indicate that anti-TNF therapy may be safe, and even beneficial, to use in chronic HCV. (2-6) In contrast to HCV, TNF-α may play a role in clearing and controlling HBV. Elevated levels of TNF-α are seen in both the serum and hepatocytes of patients with chronic HBV. (7-8) TNF-α is secreted by HBV-specific cytotoxic T lymphocytes (CTL) and seem to be synergizing with interferons in suppressing viral replication. (9) Animal studies show that TNF-α knockout mice have defects in the proliferative capacity of the HBV specific CTL, suggesting that TNF-α may play a role in clearing or controlling HBV. (10) Therefore, inactivation of TNF-α could theoretically lead to enhanced viral replication and reactivate or worsen the disease. (11)

The risk of HBV reactivation appear to be linked both to the phase of immuno-suppression and to that of immune-reconstitution. The risk of clinical events is mainly observed in active carriers of HBV, but can also develop in the "occult" condition of infection which has been widely described in the literature of the last decade. (Tab I) (9,12) For patients with a known history of HBV who are HBsAg-negative, the risk of reactivation appears to be significantly

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less, but cannot be totally ruled out. (13) In the rheumatic field, reports regarding the reactivation of HBV during the course of hydroxychloroquine, azathioprine, methotrexate and conventional DMARDs are episodic. (14) Safety and efficacy of anti-TNF-α agents in chronic hepatitis B are not known and data available on reactivation of these viral infections are conflicting. (15)

## PATIENTS AND METHODS

Since 2006, we enrolled ten patients, four females and six males, aged between 40 and 70 years, all suffering from psoriatic arthritis resistant and/or intolerant to DMARDs. The average value of DAS 28 and PASI was respectively 6.5 and 11. None of the patients was aware of previous HBV infection nor of the period of contagion. All patients were HCV-negative.

Before starting immunosuppressive therapy, serological markers of HBV were evaluated in each patient. The cases in this study included 1 case of an inactive HBsAg carrier, and 9 cases of HBcAb positive cases, occult carrier, that can be considered as 'past HBV infection'. All patients were negative for HBeAg. Patients' characteristics at baseline are shown in Table 2.

All patients started the treatment with the anti-TNF etanercept (Enbrel, Wyeth); only the inactive carrier was subjected to prophylactic therapy with lamivudine 100 mg/day, (16) which was started 1 month before starting anti-TNF. Etanercept was administered at the standard dose of 50 mg every week.

## RESULTS

During the treatment period we have carried out the evaluation of HBV markers and liver function initially every three months and thereafter at six month; in all patients, throughout the treatment period, values have remained normal. After the first six months every patient achieved clinical remission of both skin lesions and joint symptoms defined as DAS28<2.6 and 75% improvement in the PASI score.

The inactive carrier achieved clinical remission after the first few months of therapy with etanercept; treatment was continued for 24 months and then stopped for the potential risk of reactivation of the viral disease and also because of the stable regression of psoriatic disease. Lamivudine was continued for further three months after the suspension of

**Table I.** *Virologic categories of hepatitis B infection.*

| Markers | Active Carrier | Inactive Carrier | Occult Carrier |
|---|---|---|---|
| HbsAg | + | + | - |
| HbeAg | +/- | - | - |
| HBV-DNA | >2000 UI/ml | <2000 UI/ml | - |
| HBcAb IgM | +/- | - | - |
| HbcAb | + | + | + |
| HbeAb | +/- | +/- | +/- |
| HbsAb | - | - | +/- |

**Table II.** *Patient's baseline characteristics.*

| Pt | **Sex** | **Age** | **HBV-DNA** | **HBsAg** | **HBsAb** | **HBcAb** | **HCV Ab** | **ALT** | **Drug** | **Class** | **Year of starting ETA** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | M | 55 | Neg | Neg | Pos | Pos | Neg | Neg | ETA | OC (PI) | 2006 |
| **2** | M | 61 | Neg | Pos | Neg | Pos | Neg | Neg | ETA | IC | 2006 |
| **3** | M | 55 | Neg | Neg | Pos | Pos | Neg | Neg | ETA | OC (PI) | 2007 |
| **4** | M | 67 | Neg | Neg | Pos | Pos | Neg | Neg | ETA | OC (PI) | 2008 |
| **5** | F | 65 | Neg | Neg | Pos | Pos | Neg | Neg | ETA | OC (PI) | 2008 |
| **6** | F | 62 | Neg | Neg | Pos | Pos | Neg | Neg | ETA | OC (PI) | 2008 |
| **7** | F | 37 | Neg | Neg | Pos | Pos | Neg | Neg | ETA | OC (PI) | 2008 |
| **8** | F | 47 | Neg | Neg | Pos | Pos | Neg | Neg | ETA | OC (PI) | 2009 |
| **9** | F | 62 | Neg | Neg | Pos | Pos | Neg | Neg | ETA | OC (PI) | 2010 |
| **10** | F | 36 | Neg | Neg | Pos | Pos | Neg | Neg | ETA | OC (PI) | 2010 |

*Pt, patient; Class, classification; ETA, Etanercept; OC, occult carrier; PI, past infection; IC, inactive carrier.*

etanercept.

## DISCUSSION

To date, only few studies have been carried out regarding the use, efficacy and tolerability of anti-TNF α in patients with previous HBV infection. (17-20)

In clinical practice the guidelines for the use of biological drugs in PsA published in 2005 are usually considered (21). According to these, HBV infection is considered a relative contraindication (21). Therefore, the treatment is considered when absolutely necessary after evaluation of the risk of viral reactivation.

In fact, there are several reported cases of transient viral reactivation regressed with antiviral therapy and although rare cases of fulminant hepatitis, particularly in patients treated with infliximab. (22-24)

It seems that the risk of viral reactivation during immunosuppressive therapy changes according to the categories of patients assessed by serological category. (25-29)

We can distinguish active carriers (HBsAg pos, HBV-DNA> 2000 IU / ml), inactive carriers (HBsAg pos, HBV-DNA <2000 IU / ml), occult carriers (HBsAg neg, HBcAb pos) and patients with past HBV infection (HBsAg neg, HBcAb pos, HBsAb pos).

The risk of reactivation appears to be greater in HBsAg positive patients, and lower, not exceeding

5%, in HBsAg-negative HBcAb-positive patients (carriers or not of HBsAb). (19,30)

Data in literature is still conflicting, as it exists a report of 266 patients who showed a higher incidence of abnormal liver function in 88 patients, occult carriers, treated with etanercept, infliximab and adalimumab, compared with the control group.(31)

Data regarding the possibility of biological treatment in patients with a history of hepatitis are contrasting, and it depends not only by the small number of patients treated, but also and especially by the limited period of treatment reported.

Our study represents a series of patients with a history of hepatitis B treated for psoriatic arthritis with etanercept for up to six years. Since 2006 we followed 10 patients observing the serological pattern, liver function and viral load throughout the treatment period.

Patients' serological pattern was not uniform: a patient was inactive carrier, other patients were occult carriers, that could be considered as past HBV-infection. In all patients the viral load was negative and liver function was normal. In accordance with the guidelines of the European Association for the Study of Liver, HBsAg positive patient was subjected to prophylaxis with lamivudine, which began before immunosuppression and continued for three months after therapy suspension. (27)

The 9 occult carriers enrolled had negative viral load and they didn't receive any prophylactic drug as previously reported. (11,32) To date, only the inactive carrier, which obtained stable clinical remission, discontinued treatment after 24 months, for the potential risk of viral disease reactivation. The other 9 patients are still in treatment and show no signs of viral reactivation or flare of joint or skin disease.

In our study, etanercept administered for a long period has been proved effective and well tolerated in patients suffering from PsA. All the patients, at the beginning of anti-TNF-α treatment, exhibited normal liver function and a negative viral load (<200 IU / ml) due to a resolved viral disease. These conditions seem to be necessary to begin safe treatment with anti-TNF-α. However other authors reported cases of reactivation in patients with the same characteristics.

To date, there is no certainty about the risk of reactivation, but, according to our findings, the relative safety of anti-TNF-α and the lack of direct hepatotoxicity encourage its use in PsA patients resistant or intolerant to conventional DMARDs and with occult or inactive viral disease. The limitations of our study concern the small number of patients, that require studies on larger sample size.

Our results, however, confirm the efficacy and safety of etanercept, administered for long periods, in patients with PsA and past HBV infection.

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# RECENT ADVANCES IN THE GENETIC SUSCEPTIBILITY TO OSTEOARTHRITIS

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**Osteoarthritis (OA) is a multifactorial disease characterized by progressive destruction of joint articular cartilage that causes structural damage, articular swelling, functional limitation and mechanical pain. Recent progress in the genetics of OA suggests that this disorder is genetically heterogeneous with multiple risk variants. In this review, we discuss the role of genetics on OA, focusing both on previous findings in the field and on the most recent advances in OA biomarkers discovery development.**

Osteoarthritis (OA) is a multifactorial disease characterized by progressive destruction of joint articular cartilage that causes structural damage, articular swollen, functional limitation and mechanical pain. Incidence of OA is higher among older population and it is estimated that this disease affects about 40% of people over 70 years of age and it is more common in women (1). OA primarily involves the joints of the knee, hip, hand, foot and spine. Both genetic and environmental factors are involved in its pathogenesis. In the past few years, there has been a considerable increase of genetic studies in order to indentify OA susceptibility genetic factors (2-3). Here, we discuss the role of genetic on osteoarthritis, focusing both on the first evidences revealed in the field and on the most recent advances in OA biomarkers discovery development.

## TWIN STUDIES AND FAMILIAL AGGREGATION

Several classic twin studies and familial aggregation studies have investigated among the years the contribution of genetic factors to the development of osteoarthritis. The first studies took place in the 1940s, when Stecher RM et al. (4) investigated the hereditary component of hypertrophic arthritis in finger joints. In 1996, a study investigating a female twins cohort revealed that there was an higher incidence of hands and knees OA in identical twins than in non-identical twins (5). Classic twin studies and familial aggregation studies have also investigated the genetic implications on cartilage volume changes in knee structure and progression of disease, showing that all these aspects have heritable traits (6-7). In 2007 McDonnell et al. (8) studied 112 patients with primary anteromedial osteoarthritis of the knee and their families. The presence of symptomatic osteoarthritis was determined using an Oxford knee score of $\geq$ 29 supported by a Kellgren and Lawrence radiological score of II or greater (9). The Oxford knee score is a 12-item joint-specific self-administered questionnaire; it was developed for the assessment of pain and function in patients with osteoarthritis of the knee (9). The study observed a significant increased risk of anteromedial osteoarthritis (OA) in comparison with the control group ($p = 0.031$). The recurrence

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risk of anteromedial OA in siblings was 3.21 (95% confidence interval 1.12 to 9.27), suggesting that genetic factors may play an important role in the development of anteromedial OA of the knee.(8)

## LINKAGE ANALYSIS AND CANDIDATE GENE STUDIES

Several genome-wide linkage analysises have been performed in order to identify OA susceptibility biomarkers. In a study of 2010 (10) a large multigenerational study of extended family investigated five OA-related serum biomarkers: c-propeptide of type II procollagen (CPII), N-propeptide of type IIA collagen (PIIANP), hyaluronan (HA), cartilage oligomeric matrix protein (COMP), type II collagen neoepitope (C2C). Four of these five serum biomarkers showed a significant heritability after adjusting for age and sex (PHANP<0.57, HA 0.49, COMP 0.43, C2C 0.30; P 0.01 for all). Meulenbelt et al. (11) published a genome-wide linkage scan and combined linkage association analysis of 179 affected siblings and four trios with generalized osteoarthritis. Suggested evidence for linkage in this study was observed on chromosome 14q32.11 (log of odds = 3.03, P = 1.9 x $10^{(-4)}$). Genotyping of single-nucleotide polymorphisms (SNPs) of three important candidate genes mapping in this region (*CALM1, FLRT2, DIO2*) was performed. *CALM1* gene encodes a member of the EF-hand calcium-binding protein family, *FLRT2* encodes a transmembrane protein involved in cell adhesion and/or receptor signalling and *DIO2* gene encodes an intracellular enzyme that converts the inactive form of thyroid hormone (T4) in the active one (T3). The active thyroid hormone is essential for chondrocyte proliferation's control. The study revealed no evidence of association with OA of SNPs in *CALM1* and in *FLRT2* genes, but, a significant positive correlation was found for the C allele of SNP rs225014 within the *DIO2* gene (iodothyronine-deiodinase enzyme type 2) (OMIM #601413 ). The study also revealed a protective association with the T allele of the *DIO2* gene (rs12885300).

Another locus identified by linkage studies that contributes to OA pathogenesis is the interleukin-1 (*IL-1*) gene cluster on chromosome 2q13; this cluster contains the *IL1A, IL1B* and *IL1RN* genes. Like other pro-inflammatory cytokines, the IL-1 family of molecules plays an important role in joint destruction, mediated by the production of proteases that degrade cartilage extracellular matrix proteins (12). In 2002 Loughlin et al. (13) demonstrated an association between the *IL-1* gene cluster with knee OA but not with hip OA in UK population (13-14). In a different case-control study performed within the UK population, Smith et al. (15-16) were able to characterize two haplotypes associated with the risk of the disease (P=0.02), and a protective haplotype including alleles of the *IL1B-IL1RN* cluster conferring to a five-fold reduced risk of OA (P=0.0000008). Another case-control study has recently been performed to verify the association between the *IL-1* gene cluster with the knee OA susceptibility in the Chinese Han population; in this study, it has been shown that the polymorphisms within the genes encoding IL-1 and IL-1 receptor antagonist were not associated with the risk of developing knee OA (17). In 2011 a case-control study in the Croatian population, compared allelic and genotipic frequencies in the *IL1B* SNP between healthy blood donors and patients with knee joint replacement surgery, previously diagnosed with OA. No significant associations were seen within the *IL1* gene cluster (*IL1B-IL1RN*) polymorphisms in patients with severe knee OA. However, two genotypes at IL1B G -511A-IL1RN (VNTR) (1-2/1-2 and 2-1/2-2) showed a trend toward an association with susceptibility to disease (18).

DVWA (double von Willebrand factor A) is another candidate gene that has been implicated in OA. This gene encodes a protein known to bind cartilage-specific tubulin chain. In the Japanese and Chinese population a single nucleotide polymorphism (SNP) (rs7639618) was discovered within the *DVWA* gene in cohorts with knee OA (19). However, a 2011 study in the Korean population with OA failed to confirm this SNP (20).

Another candidate gene growth differentiation factor 5 gene (*GDF5*) has been the subject of several other studies (21). The GDF5 protein is an extracellular signalling molecule that is a member of the TGF-superfamily and which participates in the development, maintenance and repair of bone, cartilage and other tissues of the synovial joint

(22). In 2007 a SNP (rs143383) of this gene was identified to be associated with OA (23). Independent reports confirmed this SNP association in a range of different ethnic groups (24-27). Other candidate genes have been identified after the *GDF5*; in 2010 it was reported that SNP rs12901499, located on the *SMAD3* gene, is associated to hip and knee OA (28). SMAD3 is an intracellular molecule that links the extracellular TGF signal with changes in gene transcription. It seems that a reduction of its activity leads to an osteoarthritis phenotype in model systems (29).

## GENOME-WIDE ASSOCIATION STUDIES (GWAS)

Some interesting genes have emerged from GWA studies. The first large-scale (500.000 markers) GWAs was performed on knee osteoarthritis, and the analysis focused on the possible role of the cyclooxygenase 2 enzyme in the disease. This enzyme, encoded by *PTGS2* gene, is involved with inflammation and mitogenesis, and was found to be related to the pathogenesis of the disease. Anyway, even if this study did not achieved genome-wide significant association, it unveiled the importance of this signalling pathway in the pathogenesis of knee OA (30). In 2008 a Japanese group identified two coding variants in the *DVWA* gene, rs11718863 and rs7639618, that were strongly associated with an increased risk of knee OA in Chinese and Japanese populations (19). However, subsequent studies revealed these two SNPs do not have any effect in the susceptibility to hip and knee OA in caucasian populations (31-32). In 2010, through a genome-wide association study and a replication on a Japanese population, two other single nucleotide polymorphisms in the *DVWA* gene (SNPs) (rs7775228 and rs10947262) have been identified as associated with susceptibility to knee OA (33). The two SNPs were contained in a region containing HLA class II/III genes and their association reached genome-wide significance (combined $P = 2.43x10^{(-8)}$ for rs7775228 and $6.73x10^{(-8)}$ for rs10947262). These results suggest the possibility that an immunologic mechanism is implicated in the etiology of OA (33). However this association was not seen among European and Chinese cohorts (34). Recently the arcOGEN consortium performed a genome-wide association study for 3177 knee and hip osteoarthritis cases and 4894 controls from the UK (35). No signal reached the genome-wide significance; the strongest signal for knee and/or hip OA was for the SNP rs2277831 ($P=2.3x10^{(-5)}$) located within the *MICAL3* gene. The protein encoded by this gene belongs to flavoprotein monooxygenase protein family and interacts with the cytoskeleton, cytoskeletal adaptor proteins, and other signalling proteins (36). In 2010 a genome-wide association study identified an OA susceptibility locus on chromosome 7q22 within a Dutch population (37). In particular, the minor allele of the SNP rs38115148 was associated with a 1.14-fold increased risk for knee and/or hand osteoarthritis and also with increased risk for knee osteoarthritis progression. Moreover, another SNP identified at this locus, resulted in a strong association with GPR22 expression levels in lymphocytes. This SNP is almost in complete linkage disequilibrium with rs3815148. *GPR22* encodes a G-protein receptor whose ligand is unknown. GPR22 is absent in normal mouse articular cartilage or synovium by immunohistochemical staining, but it is expressed in chondrocytes of the articular cartilage of mouse knee joints treated by papain in vivo, or in the presence of interleukin-1, suggesting a role of *GPR22* on OA(37).

Since OA is a phenotypically heterogeneous disease and involves the articular districts with variable severity, in different patients, in the following paragraphs we summarize the principal results regarding association studies with the three most involved articular districts: knee, hip and hand.

## KNEE OA SUSCEPTIBILITY LOCI

The largest OA GWA performed, showed a strong association between a locus on chromosome 7q22 and knee OA in European patients (37). Althought the specific function of this locus is unknown, this locus reached a GWA significance also in a meta-analysis of GWAs performed in European populations (38). The strongest genetic association with knee OA identified by GWA was found with DVWA in Asian populations (19,33). The association of DVWA variants with OA was not observed, however, in European populations (32, 39).

**Table I.** *Principal OA susceptibility identified loci*

| Locus | OMIM number | Chromosome Location | Associated Gene | Population | References |
|---|---|---|---|---|---|
| OS1 | 165720 | 2q32.1 | *FRZB* | European | Evangelou et al. (24) |
| OS2 | 140600 | 2p24.1 | *MATN3* | Icelanders | S.E. Stefansson et al. (62) |
| OS3 | 607850 | 9q22.31 | *ASPN* | Chinese, Korean, Japanese | Kizawa H. et al. (43)<br>Jiang Q et al. (44)<br>Shi D et al. (45)<br>Song JH et al. (46) |
| OS4 | 610839 | 2q33.3 | | Caucasian | Meulenbelt et al. (66) |
| OS5 | 612400 | 20q11.22 | *GDF5* | Japanese, Chinese, Caucasian | Miyamoto Y. et al. (21, 35)<br>Loughlin et al (19)<br>Khan IM et al (20) |
| OS6 | 612401 | 3p24.3 | | Japanese | Miyamoto et al. (17) |

An important association was found with the SNP rs143383 in the *GDF5* gene. This gene encodes a bone morphogenetic protein involved in joint formation. The rs143383 SNP was found to be related to knee OA in Japanese and Chinese subjects, and it seems to cause a reduction in GDF-5 mRNA expression (23, 40); it was also found to be associated with knee OA in Caucasian populations (25, 39,41-43).

Several genetic association studies have investigated the role of the TGF signalling pathway in OA pathogenesis. In particular in 2005, a Japanese group identified an association between a polymorphism in the aspartic acid repeat of the *ASPN* gene and knee OA (44). This gene encodes for asporin, a protein that binds TGF-1 *in vivo*. Over-expression of asporin encoding gene (ASPN) seems to cause a decrease in TGF-1-mediated chondrogenesis *in vitro*. This association was also reported in Chinese (45-46) and Korean cohorts (47) but was not observed in other populations (48).

Inflammatory and immunomodulatory proteins are another class of molecules that seems to be involved with OA. A Japanese GWA identified two MHC-related SNPs, strongly associated with knee OA (33). The first SNP, rs7775228, mapped to the MHC class II gene *DQB1*, showed a significant genome-wide association with knee OA in Japanese subjects. The other identified SNP (rs10947262) was mapped within the *BTNL2* gene, which is involved in T-cell response. Other inflammation genes have been investigated with the aim of finding a significant association with OA, such as the C-reactive protein and the TNF. However, an association with knee OA has not been shown (49-50).

## HIP OA SUSCEPTIBILITY LOCI

Hip osteoarthitis is a major source of pain and

disability in the elderly community, although its prevalence is lower compared to hand and knee OA (51). Several candidate gene studies and GWAs have identified genetic associations shared also by knee OA. Miyamoto et al. (23) identified the rs143383 SNP in the promoter region of *GDF5* gene as the SNP having the strongest association with hip OA. As reported above, genes of the iodothyronine deiodinase family have been identified as OA susceptibility genes. Two SNPs in DIO2 gene have been associated with hip OA ( rs225014, rs12885300) and a SNP in the DIO3 gene (deiodinase, iodothyronine, type III) was reported to be associated most significantly with knee and/or hip OA (39). TGF-SMAD3 signalling is essential for maintaining articular cartilage; and a SNP mapping to intron 1 of the *SMAD3* gene has been reported to be associated with both knee and hip OA in European cohorts (52). The involvement with OA is due to the fact that the TGF-SMAD3 signalling is essential for maintaining articular cartilage. Another gene identified as implicated in OA is the *ANP32A* gene. This gene encodes a tumor suppressor molecule that has a regulatory role in apoptosis, and interferes with Wnt signalling *in vitro* and is associated with hip OA in women (53).

The role of the IL1 gene cluster in hip OA risk is controversial. Several studies among different ethnic groups have been performed. Moos et al. found a positive association between the IL1 polymorphism and the TNF-high-phenotype and between the IL1RA polymorphism and the TNF-low-phenotype in 61 German patients with end-stage hip and knee OA (54). Loughlin et al. (13) genotyped eight common polymorphic variants located in the IL1 ligand gene cluster in a cohorts of Caucasian patients with hip and knee OA, showing that the IL1 gene cluster was associated with a risk of knee OA but not of hip OA (12). In a Dutch study Meulenbelt et al. (55) showed a positive association between IL1 gene cluster and radiographic signs of hip OA. Moxley et al. (56) conducted an *IL1* region meta-analysis on 1,238 cases with various OA phenotypes and 1,269 controls from four study centres on European descent populations. They concluded that meta-analysis data did not confirm the association, but only suggested that some hand and hip OA risk could be associated with the *IL1* region, particularly with the *IL1B* gene and probably also *IL1R* gene (56). Also a Croatian group investigated the role of *IL1* gene cluster in a cohort with hip OA (57). This group studied the allelic and genotypic frequencies of the *IL1B* gene single nucleotide polymorphism at -511 (G>A; rs16944) and the variable number tandem repeat (VNTR) in the IL1RN gene. The genotype G/A (1/2) at IL1B was significantly associated with a protective effect in respect to the hip OA ($p<0.036$, OR=0.72, 95% CI= 0.52-0.99). The genotype G/G (1/1) had showed only a trend towards the susceptibility ($p=0.053$, OR=1.35, 95% CI=0.98-1.86) toward developing hip OA. None of the haplotypes *IL1B* -511 (G>A) and *IL1RN* (VNTR) were found to be associated with hip OA (57).

Lastly, different studies have investigated the role of FRZB gene, that is a WNT pathway antagonist. The WNT-Beta catenin signalling pathway plays a key role in the homeostasis of bone and cartilage during adult life (58-59). In particular, two studies have found an association between two polymorphisms in the FRZB gene (rs288326 and rs7775) and hip OA (60-61). A recent meta-analysis study did not demonstrate a sizeable association between FRZB variant alleles and hip OA. Another study, however, has demonstrated an association between the two SNPs and the shape of the proximal femur. In particular, the rs288326 seems to alter the relation between proximal femur shape and incident radiographic hip OA (62).

## HAND OA SUSCEPTIBILITY LOCI

Digital OA heritability has been estimated to occur between 48 to 65% of the population (63). Digital OA is a multifactorial condition, and except in the case of Heberden's nodes it has been shown a mendelian inheritance (64). Early genetic association studies on digital OA focused on the HLA class I and II genes. In a British study of patients with interphalangeal osteoarthritis an association was found with HLA-A1B8 (65). On the contrary, an Italian study showed that primary hand OA was associated with HLA-B35, B40, DQ1 and CW4 alleles but not with HLA-A1B8 (66). In a Dutch case-control study, it was reported a negative association of distal interphalangeal OA with HLA-DR4 (suggesting a protective role of this allele) and a positive association with HLA-DR2 (67). Despite all these reported associations between

HLA alleles and hand OA, there is no evidence of linkage with the HLA-A locus on the short arm of chromosome 6; interestigly, a locus at 6p12 has been associated with knee and hip OA. Several genome-wide-association studies identified SNPs linked to various manifestations of hand OA. In a cohort of patients from Iceland, a polymorphism (T303N) was found within the *MATN3* gene (matrilin 3 gene); this polymorphism was found to be present in more than 2% of Icelanders with hand OA and showed a relative risk of 2.1 also in healthy subjects (68). This gene is involved in the formation of the extracellular matrix. Genome-wide scans using microsatellite markers identified numerous loci related to hand OA, these loci are located on chromosomes 2p, 2q, 3p, 4q and 7p (68). In 2009 a GWA study performed by Zhai et al. (69) suggested an association between hand OA and a SNP on the ataxin 2 binding protein gene (*A2BP1*). Many other candidate genes have been investigated by several other studies, but the results of these studies did not seem to be reproducible; among these studies, a polymorphism on the *IL-6* gene was found to be associated with both susceptibility and severity of distal inter-phalangeal OA (70). An interesting study focusing on telomeres length, conducted in groups of female twins, was performed by a British group. In this study the women with hand osteoarthritis had significantly shorter telomeres than the women with normal hand radiographic exams (71).

## CONCLUSIONS

OA is a highly polygenic and phenotypically heterogeneous disease. Recent progress in the genetics of OA suggests that this disorder is genetically heterogeneous with multiple risk variants. This concept complicates the identification of OA susceptibility genes. In fact since OA is a multifactorial disease, many genetic and environmental factors contribute to its variability, each one with a modest contribution. Most of the published genetic association studies on OA are underpowered in terms of their ability to detect the minor contributing role of common alleles. This could be due also to the phenotypic heterogeneity of the disease that complicates the replication of the findings of previous studies. Therefore, it is clear that to identify susceptibility OA genes is necessary to analyse large-scale samples size and well-defined phenotypes to minimize heterogeneity. An additional confounding factor in these genetic studies may be the extreme ethnic difference observed in this disorder and the presence of different susceptibility loci in different populations.

However, significant evidence suggest that the following genes are considered susceptibility genes to osteoarthritis: *FRZB* (OMIM #165720; OS1) on chromosome 2q31-q33; *MATN3* (OMIM #140600; OS2) on chromosome 2p24-p23; *ASPN* (OMIM #607850; OS3) on chromosome 9q21.3-q22; and *GDF5* (OMIM #61240; OS5) on chromosome 20q11.2 (Table1). Interestingly, other susceptibility loci such as OS4 (72) and OS6 (19) have been mapped but no specific gene mapping within these regions were identified. Unfortunately, none of these susceptibility loci can be used as genomic biomarker at the present time. Further studies are needed to define the role of each identified at risk allele in the pathogenesis of OA. New additional studies, including larger sample sets, well-characterized phenotypes, and extensive re-sequencing of patient's DNAs searching for rare predisposing alleles are necessary to provide a better insight into the etiology of this common degenerative joint disease. Moreover, new genomics approaches such as Next Generation Sequencing, epigenetics studies and microRNA studies may allow to better understand the genetic basis of OA in the next future.

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# SILDENAFIL AND RAYNAUD'S PHENOMENON (RP) IN SYSTEMIC SCLEROSIS (SSC): RESULTS FROM A RETROSPECTIVE STUDY.

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**This is a retrospective analysis of the effect of sildenafil on Raynaud's phenomenon and of the safety profile of this drug. The chart of 45 patients with RP secondary to SSc were reviewed and divided into 3 groups: 15 treated with sildenafil (20 mg three times daily), 15 with combination therapy (sildenafil 20 mg three times daily and PGE i.v 60 mcg once week) and 15 with PGE i.v. only (60 mcg once week). Symptoms were assessed by diary cards including a 10-point Raynaud's Condition Score (RCS), Medical Outcomes Study 36-Item Short Form Health Survey (SF-36), Scleroderma Health Assessment Questionnaire (S-HAQ) and videocapillaroscopy (NVC) were performed in all patients at baseline and every 3 months. Results were analyzed with the Wilcoxon Mann-Whitney test. After 6 months of treatment the comparison between the three groups did not show any significant difference in almost all parameters examined. After 6 months RCS was significantly lower in the combination therapy and PGE group. Combination therapy was effective immediately in the first three months of treatment, while in PGE group only at the third month of treatment. NVC pattern was unmodified in all patients and HAQ was reduced in combination therapy group only. Four patients reported side effects leading to discontinuation of the sildenafil. Our data show that combination therapy (sildenafil and PGE) may achieve more satisfactory results in the treatment of RP compared to sildenafil monotherapy and confirm that sildenafil is a well-tolerated treatment for SSc patients.**

Raynaud's phenomenon (RP) generally occurs in response to cold or emotion. It is due to the closure of the digital arteries, pre-capillary arterioles and subcutaneous venous shunts, leading to the triphasic clinical finding of sharply demarcated cutaneous pallor, followed by cyanosis and/or erythema. (1,2) Secondary RP occurs in connective tissue disease and it is characterized by episodes of intense, painful, asymmetric vasoconstriction, autoantibody positivity, videocapillaroscopy abnormality (1) and it can be complicated by digital ulceration, soft tissue or bone infection, and critical ischemia or gangrene (3,4). RP and digital ulcers are early and prominent features of systemic sclerosis (SSc) (5). Non pharmacologic therapy includes avoidance of cold temperatures, emotional stress, and smoking (6) while pharmacological therapy includes dihdropiridine-type calcium antagonists as first line therapy and intravenous prostanoid when calcium antagonists have failed; however in clinical practice there are many other therapies that are in use for the treatment of SSc-RP (adrenergic receptor blockers,

*Key words: Sildenafil, Raynaud's phenomenon, systemic sclerosis*

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or angiotensin II receptor antagonists topical nitroglycerin and pentoxifylline). (7) Sildenafil and other phosphodiesterase type 5 (PDE-5) inhibitors are attractive candidates for therapy in patients with RP secondary to SSc. In fact sildenafil enhances the effect of NO by inhibiting phosphodiesterase type 5, which is responsible for degradation of c-GMP, elevating c-GMP which leads to smooth muscle relaxation and vasodilatation (8). Sildenafil has been successfully used for primary and secondary pulmonary hypertension (9,10) and digital ulcers (11) and its efficacy for RP has been reported in several studies and controlled trials (12).

Fries et al (13) showed a substantial reduction of frequency and duration of RP attacks and healing of chronic digital ulcerations in all patients treated with it. A recent double-blind, placebo-controlled study reported a greater percentage reduction in RP attacks in the gruop treated with modified release sildenafil (100 mg for 3 days followed by 200 mg daily) compared to the placebo group (14). Shenoy et al reported benefit of tadalafil treatment in secondary RP resistant to vasodilator therapy in term of frequency and duration of attacks, and Raynaud's Condition Score (15).

In our SSc patients, we analyzed retrospectively whether there is a difference on clinical parameters between different treatments for RP in particular between sildenafil, PGE and combination therapy (sildenafil + PGE).

## METHODS

This retrospective study included 45 patients (2 men and 43 women) with RP secondary to SSc (10 very early SSc, 27 lSSc, 8 dSSc) with RP secondary to SSc who were followed in our outpatient clinic from December 2009 to June 2010.

SSc was classified according to LeRoy et al (16,17) and very early SSc according to provisional criteria proposed by EULAR scleroderma Trial and Research group (EUSTAR). (18) The mean (SD) age of the patients was 55,4 (13) years, and the mean (SD) duration of SSc was 8,6 (6,5) years. The mean (SD) duration of RP was 14 (10,6) years. All patients included did not take calcium channel blockers, alcohol consumption was forbidden while up to 80 mg of caffeine per day was allowed.

The charts of 15 patients treated with sildenafil (20 mg three times daily), 15 with combination therapy (sildenafil 20 mg three times daily and PGE i.v 60 mcg once week) and 15 with PGE i.v. only (60 mcg once week) were analyzed.

All patients were evaluated by diary cards including a 10-point Raynaud's Condition Score (RCS), Medical Outcomes Study 36-Item Short Form Health Survey (SF-36), Scleroderma Health Assessment Questionnaire (S-HAQ). The microvascular modifications (giant capillaries, and microhaemorrhages) were also recorded by videocapillaroscopy. The RCS used in our charts is a clinically validated 11-point scale used to determine the difficulty that patients experience with Raynaud's phenomenon (where 0 _ no difficulty and 10 _extreme difficulty), and it is closely associated with the measures of disability and overall impact and activity of the disease. The SF-36 is a multi-purpose, short-form health survey with only 36 questions. It yields an 8-scale profile of functional health and well-being scores as well as psychometrically-based physical and mental health summary measures and a preference-based health utility index. In the SF-36 scoring system, the scales are assessed quantitatively, each on the basis of answers to two to ten multiple choice questions, and a score between 0 and 100 is then calculated on the basis of well-defined guidelines, with a higher score indicating a better state of health. The HAQ was developed as a comprehensive measure of outcome in patients with a wide variety of rheumatic diseases, including systemic sclerosis. The domain of disability is assessed by the eight categories of dressing, arising, eating, walking, hygiene, reach, grip, and common activities. Discomfort is determined by the presence of pain and its severity.

All these parameters, including videocapillaroscopy were evaluated at baseline and every 3 months.

## STATISTICS:

The three groups were compared in pairs, combination-sildenafil group, sildenafil-PGE group- and PGE-combination group, respectively.

Non parametric tests have been used to compare each group with the others (Mann Whitey test, for

**Table I.** *Parameters value in the three groups*

| | Combination | Sildenafil | PGE |
|---|---|---|---|
| ***PhysicalActivity_t0*** | 67.94 ± 16.40 | 67.50 ± 30.30 | 69.00 ± 14.10 |
| ***PhysicalActivity_t3*** | 69.41 ± 22.63 | 67.27 ± 30.50 | 76.50 ± 12.26 |
| ***PhysicalActivity_t6*** | 61.76 ± 27.72 | 72.04 ± 25.20 | 74.50 ± 12.12 |
| ***PhysicalLimitations_t0*** | 27.94 ± 34.10 | 45.45 ± 42.00 | 47.50 ± 38.10 |
| ***PhysicalLimitations_t3*** | 48.52 ± 40.95 | 61.36 ± 42.77 | 42.50 ± 31.29 |
| ***PhysicalLimitations_t6*** | 36.76 ± 38.65 | 50.00 ± 42.26 | 47.50 ± 36.23 |
| ***Pain_t0*** | 42.76 ± 17.03 | 52.00 ± 29.17 | 53.40 ± 18.30 |
| ***Pain_t3*** | 45.47 ± 16.63 | 53.18 ± 28.18 | 55.90 ± 20.01 |
| ***Pain_t6*** | 52.41 ± 16.68 | 54.68 ± 27.78 | 57.50 ± 17.67 |
| ***HealthStatus_t0*** | 38.82 ± 20.75 | 45.00 ± 19.79 | 39.30 ± 18.32 |
| ***HealthStatus_t3*** | 40.05 ± 18.39 | 45.36 ± 18.23 | 41.00 ± 18.07 |
| ***HealthStatus_t6*** | 37.41 ± 17.60 | 47.14 ± 17.21 | 46.50 ± 17.93 |
| ***Vitality_t0*** | 48.82 ± 16.35 | 52.27 ± 22.92 | 47.00 ± 16.86 |
| ***Vitality_t3*** | 52.35 ± 16.97 | 51.59 ± 19.90 | 52.00 ± 15.67 |
| ***Vitality_t6*** | 50.29 ± 13.40 | 53.86 ± 17.79 | 55.00 ± 14.34 |
| ***SocialActivity_t0*** | 60.76 ± 22.50 | 61.64 ± 21.98 | 64.70 ± 16.42 |
| ***SocialActivity_t3*** | 59.23 ± 24.51 | 58.36 ± 17.41 | 68.60 ± 22.17 |
| ***SocialActivity_t6*** | 57.35 ± 15.84 | 60.64 ± 18.54 | 69.90 ± 18.72 |
| ***EmotionalLimitations_t0*** | 39.00 ±37.63 | 46.86 ± 46.72 | 43.00 ± 35.14 |
| ***EmotionalLimitations_t3*** | 52.70 ± 40.87 | 52.95 ± 47.86 | 59.70 ± 37.82 |
| ***EmotionalLimitations_t6*** | 39.12 ± 45.95 | 45.36 ± 46.61 | 70.60 ± 33.23 |
| ***MentalStatus_t0*** | 58.59 ± 20.34 | 61.09 ± 18.60 | 57.90 ± 20.50 |
| ***MentalStatus_t3*** | 55.76 ± 20.71 | 57.73 ± 14.82 | 61.80 ± 20.79 |
| ***MentalStatus_t6*** | 53.18 ± 15.15 | 61.18 ± 14.08 | 62.20 ± 19.99 |
| ***RCS_t0*** | 6.35 ± 1.97 | 5.27 ± 2.31 | 5.10 ± 1.59 |
| ***RCS_t3*** | 4.88 ± 1.58 | 4.91 ± 2.02 | 4.40 ± 1.90 |
| ***RCS_t6*** | 5.06 ± 1.56 | 5.41 ± 2.01 | 3.90 ± 1.66 |
| ***NVC_t0*** | 2.47 ± 0.80 | 1.64 ± 1.18 | 1.70 ± 1.16 |
| ***NVC_t3*** | 2.47 ± 0.80 | 1.64 ± 1.18 | 1.70 ± 1.16 |
| ***NVC_t6*** | 2.47 ± 0.80 | 1.64 ± 1.18 | 1.70 ± 1.16 |
| ***HAQ_t0*** | 0.64 ± 0.34 | 0.48 ± 0.60 | 0.46 ± 0.26 |
| ***HAQ_t3*** | 0.63 ± 0.46 | 0.64 ± 0.61 | 0.44 ± 0.22 |
| ***HAQ_t6*** | 0.54 ± 0.37 | 0.60 ± 0.56 | 0.50 ± 0.24 |

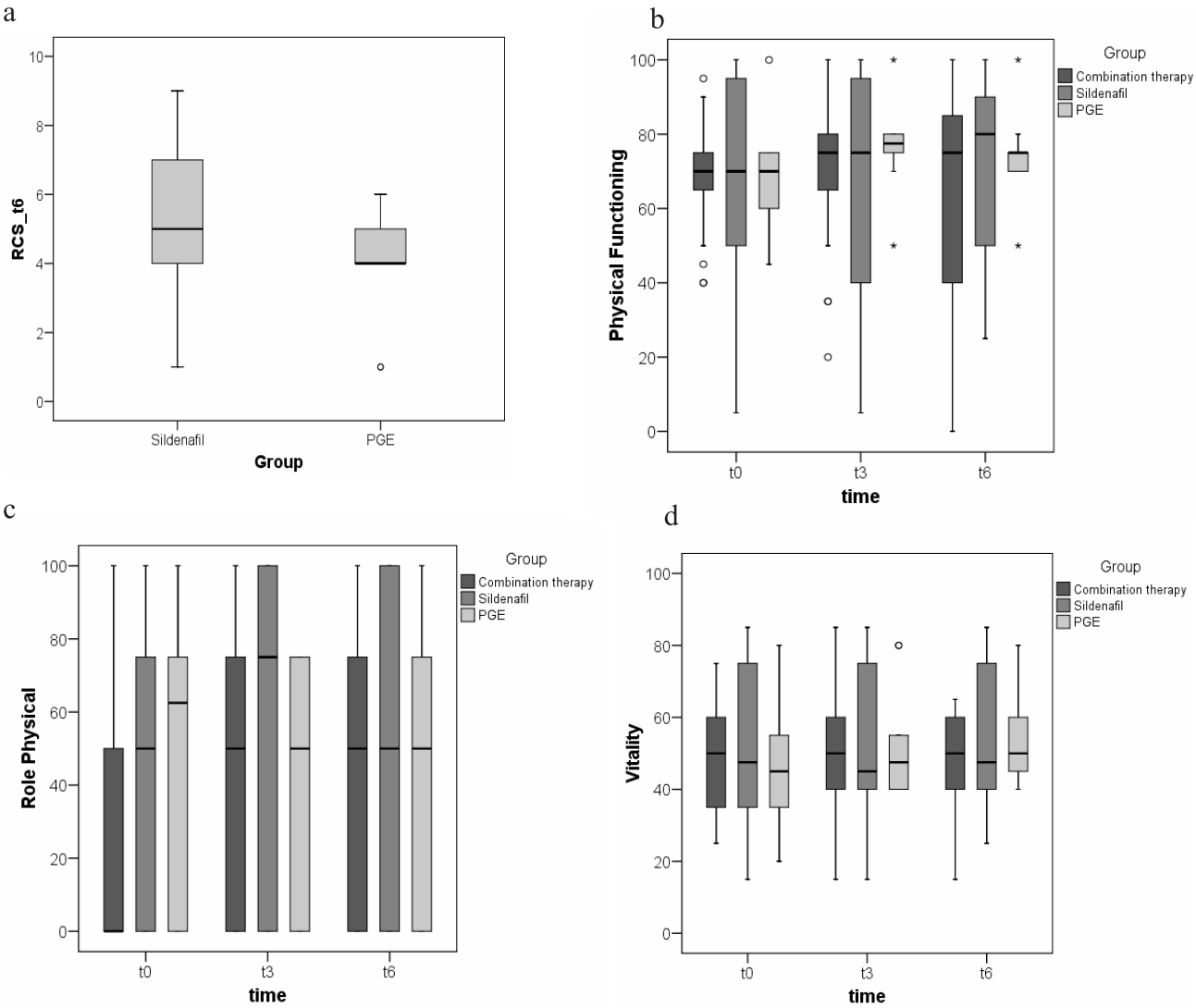


**Fig. 1.** ***a)*** *RCS comparison between PGE and sildenafil group;* ***b)*** *Physical functioning (SF-36) comparison between PGE and sildenafil and combination therapy group;* ***c)*** *Role Physical comparison (SF-36) between PGE and sildenafil and combination therapy group;* ***d)*** *Vitality comparison (SF-36) between PGE and sildenafil and combination therapy group.*

unpaired samples) and to evaluate differences over time for each subject in each group (Wilcoxon test, for paired samples).

Differences were considered significant when probability values were less than 0.05.

## RESULTS

The comparison between the PGE group, combination group and sildenafil group did not show any significant differences.

Only RCS was significantly lower after six months in PGE group in respect to the sildenafil group (RCS in PGE:3.90 ± 1.66; RCS in sildenafil: 5.41 ± 2.01; Mann Whitney test, p= 0.005).

In each group the change of every measurement from baseline to month 3 and then from months 3 and 6 was also evaluated. In SF-36, only three multi-item scales resulted significantly changed after 6 months. Physical functioning increased significantly only in PGE group (Wilcoxon test, p=0.014; values in Table I). After three months of treatment, physical limitations increased significantly either in the combination group (Wilcoxon test, p=0.030; values

in Table I) or in sildenafil group (Wilcoxon test, p=0.010 values in Table I) (no significant in the PGE group), but did not improve further up to 6 months. Vitality increased significantly only from the third to the sixth month in Sildenafil group (Wilcoxon test, p-value=0.041; values in Table I).

After 6 months, changes over time of parameters used to assess RP showed a significantly lowering of RCS in the combination therapy group (Wilcoxon test, p=0.008; values in Table I) and PGE group (Wilcoxon test, p=0.008; values in Table I). In combination therapy group this effect was detected already at the first three months of treatment (Wilcoxon test, p=0.005; values in Table I), while in PGE group only after 6 months of treatment (Wilcoxon test, p=0.025; values in Table I).

During the first three months of treatment HAQ did not show any significant reduction in each group, but reduced further up to the six month in the combination therapy group (Wilcoxon test, p-value=0.041; values in Table I); only even in respect to baseline (Wilcoxon test, p=0.021; values in Table I). NVC pattern was not modified during of the treatment either in mono or combination therapy.

In the sildenafil group the most frequent adverse events were mild or moderate like headache, flushing, peripheral oedema and hypotension.

Treatment-related adverse events leading to discontinuation of treatment in 1 patient in the combination group included headache and peripheral oedema. Three patients in the sildenafil group discontinued the drug because of severe headache, peripheral oedema and flushing.

## DISCUSSION

Sildenafil, a selective inhibitor of cGMP specific phosphodiesterase type 5, provides cGMP-dependent microvascular and macrovascular dilation.

This retrospective analysis of the differences on clinical parameters between different treatment for RP as sildenafil, PGE and sildenafil combined with PGE in SSc patients showed that the only parameter which change significantly after 6 months of treatment was RCS in particular in combination therapy group and PGE group. This effect was obtained earlier in combination group (first three months) compared to PGE group.

After six months of treatment we also observed that only three scales of the SF-36 changed significantly (Physical functioning, role phisical and vitality). Physical functioning increased significantly only in the PGE group while role physical increased significantly in the combination group and sildenafil group but only in the first 3 months. Vitality increased significantly only in the sildenafil group at the sixth month. No changes were found in SHAQ and nailfold videocapillarocopy.

Our results do not agree with those reported in the literature about the efficacy of sildenafil in the treatment of RP (11-14,19,20). In fact, our data do not indicate a superiority of sildenafil monotherapy at the dose of 20 mg three times a day over the prostanoid monotherapy. Probably, this could be related to the low dosage of sildenafil used compared to other published data in which higher doses of drug were used (up to 200 mg daily). However, a major bias of this study may consist in the heterogeneity of the enrolled SSc patients as far as the natural history of their disease is considered. In fact within the three groups there were patients in different phases and subset of SSc. For this reason, the results on the efficacy of sildenafil for the treatment of RP may be distorted by a different stage of disease of patients enrolled.

In conclusion, our data show that sildenafil, in a combination therapy, achieves better results when compared to monotherapy and this may suggest that sildenafil and PGE may enhance each other; data also confirmed that sildenafil is a well-tolerated treatment for SSc patients. Then in our experience combination therapy represents an important therapeutic option in SSc, in particular in an early phase of disease, to control the dysregulation of vascular tone control and to reduce significantly the loss of quality of life and the disability secondary to RP.

In the future, randomized, placebo controlled, double blind studies are mandatory to confirm these hypothesis, to better investigate sildenafil effects on other outcome measures and to determine its optimal efficacious dose.

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# FEASIBILITY OF ULTRASOUND-GUIDED INJECTION OF PLATELET-RICH PLASMA TO TREAT PATELLAR TENDINOPATHY IN ATHLETES: PRELIMINARY RESULTS

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**Purpose: The efficacy of platelet-rich plasma (PRP) in the treatment and healing of chronic tendinopathy through stimulation of cell proliferation and total collagen production has been demonstrated by both in vitro and in vivo studies. The aim of this study is to evaluate the effectiveness of ultrasound (US)-guided autologous PRP injections in patellar tendinopathy. Materials and Methods: Autologous PRP was injected under US-guidance into 28 patellar tendons in 24 prospectively selected patients (14 males, 10 females, mean age 37.4 years, range 21-56 years). All patients were previously evaluated according to the Victoria Institute of Sport Assessment (VISA) scale, which assessed pain and activity level, and they all underwent US of the tendon before treatment and at follow-up after 20 days and 6 months. Statistical analysis was performed with Chi-square and Wilcoxon tests. Results: 20 days after PRP injection the patients presented a non-significant improvement of clinical symptoms, that was conversely found at 6-month follow-up. Tendon thickness did not significantly reduce at 20-day follow-up compared to baseline, while it was significantly reduced when evaluated at six months. Intratendinous vascularity was significantly increased both at 20-day and 6-month follow-up compared to baseline. Conclusion: PRP injection in patellar tendinopathy results in a significant and delayed improvement of clinical symptoms and leads to recovery of the tendon matrix potentially helping to prevent degenerative lesions. US-guidance allows PRP injection into the tendon with great accuracy.**

Chronic musculoskeletal diseases are the most common cause of severe long-term pain and physical disability (1). Patellar tendinopathy is a degeneration of tendon enthesis caused by a repetitive chronic overload that exceeds the adaptive capacity of the tendon, resulting in multiple micro-tears that do not heal because of poor intrinsic tendon vascularity (2). The histological features in the early stages of the disease are focal areas of asymptomatic intratendineous damage without signs of inflammation (3). The histological appearance of degenerative damage begins and progresses long before symptom onset. This delays treatment, resulting in less success of late therapeutic approaches (4). When degeneration is located at the proximal enthesis, the pathologic condition is named *jumper's knee*. The disease mostly affects young athletes practicing running, jumping, soccer, and

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volleyball (3). The prevalence was estimated to be around 40% - 50% among professional volleyball players and 35% -40% among basketball players (5). Some intrinsic – such as gender, age, overweight – and extrinsic factors – such as functional overload and errors in sport action – play an important role in the development of chronic tendinopathy (3,6). At present, patients affected by jumper's knee are primarily treated with rest, local ice, physical treatment, drug therapy with anti-inflammatory drugs (NSAIDs), and steroids injections, although there is no evidence of the effectiveness of these therapies. Recently other therapies such as laser and shock waves have been developed but all required additional research before their use can be recommended (7).

New theories on injuries repair have been focused on the role of platelets, developing different therapeutic strategies in the treatment of chronic tendinopathy (8). Being involved in the mechanism of thrombus formation, platelets participate in the healing process removing necrotic tissues and stimulating the regeneration of tissues and their repair (9,10). Platelet-rich plasma (PRP), prepared with a high concentration of platelets, has been used in cardiac, maxillo-facial, plastic, neuro, and orthopedic surgery (11-13). In more recent times, it has found application in injectable form to treat muscle, tendon, and cartilage tears (14,15). In our preliminary study, we evaluated the effectiveness of ultrasound-guided intratendineous injection of PRP to treat jumper's knee in a population of young and amateurs athletes.

## MATERIALS AND METHODS

### *Patients*

Institutional Review Board approval and volunteer informed consent were obtained. Twenty-eight patellar tendons in 24 patients (14 men, 10 women) were prospectively evaluated. Patients were aged between 21 and 56 years (mean age 37.4 years), practicing sports at a competitive level or amateur, with a clinical diagnosis of patellar tendinopathy. The inclusion criteria for the study were the presence of patellar tendinopathy, pain at palpation and during physical activity for at least three months, and ultrasound or magnetic resonance imaging evidence of tendon degeneration.

All patients previously underwent other treatments (NSAIDs, ultrasound, shock waves, local injection of corticosteroids) and 10 out of 24 patients had phisical therapy (eccentric lengthening) with unsatisfactory outcome.

Exclusion criteria were systemic diseases (diabetes, rheumatoid arthritis, coagulopathies, severe cardiovascular disease, infections, immunosuppression), antiplatelet therapy in progress, NSAIDs consumption 5 days prior to procedure, hemoglobin <11g/dl and platelets <150.000 / mmc.

### *Pre-treatment clinical and ultrasound evaluation*

Pre-treatment clinical evaluation was performed using the Victoria Institute of Sport Assessment questionnaire for knee (VISA-P) (16) adapted to the Italian language (17). The VISA-P questionnaire consists of eight questions, of which six concern pain experienced during a range of everyday activities. Two questions deal with the ability to perform sport activities. All questions are answered on separate scales (16,17), where a higher score indicates a lower level of pain or impairment. The maximum VISA-P score for an asymptomatic subject who habitually exercise is 100 points and the theoretical minimum is 0 points.

Ultrasound examination was performed using an ultrasound equipment (MyLab 70 XvG, Esaote) with high-resolution linear transducer (13-6 MHz). For each patient, patellar tendon thickness, presence/absence of hypoechoic areas, and vascularity on power Doppler (PD) according to a semiquantitative scale (0=absent; 1=few spots; 2=moderate; 3=intense) were recorded (Figure 1a, 2a).

### *Percutaneous ultrasound-guided injection of PRP*

In all patients, after thorough disinfection of the skin and local subcutaneous anesthesia (4 mL of 2% mepivacaine), ultrasound-guided scarification of the insertional and pre-insertional tendon region was performed using a 21G needle in order to induce spontaneous bleeding.

Afterwards, using the same needle, 6 mL of autologous PRP from a previous sampling (110 mL of venous blood taken from the patient a few hours before the procedure, centrifuged, and activated in the laboratory), were injected within areas of tendon degeneration under ultrasound guidance (Figure 1a, 1b).

In all patients, two treatments were performed at a distance of 3 ± 0.52 (mean ± SD) weeks.

Patients were asked to minimize their physical activity for 48 hours after treatment and to avoid the maximum effort during the next two weeks. Thereafter, they were recommended to start a physiokinesitherapy program and a gradual return to sports practice. Patients were also requested to avoid the use of drug therapies, with

particular reference to corticosteroids and NSAIDs.

*Follow-up*

Pain occurred between first and second PRP injection was assessed using a visual analogue scale (VAS). In all patients, clinical evaluation was performed using the VISA-P questionnaire while ultrasound evaluation included patellar tendon thickness, presence/absence of hypoechoic areas, and vascularity on power Doppler (similarly to baseline) at 20 days and 6 months after the second treatment (Figure 2, 3a, 3b). At six-month follow-up, patients were also asked about their overall satisfaction of the treatment, compared to baseline, using a semiquantitative scale (poor, good, excellent).

*Statistical Analysis*

VISA-P score and patellar tendon thickness at baseline and at different timepoints (20 days and 6 months) were compared using the Wilcoxon test. Presence/absence of hypoechoic areas at ultrasound analysis at different timepoints compared to baseline was assessed using the Chi-square test. Vascularity on PD at different timepoints was assessed using the two-way ANOVA test with post-hoc analysis. A P-value less than 0.05 was considered as significant. The Bonferroni correction was applied when needed.

## RESULTS

No peri-procedural complications were observed.

In regard to post-procedural pain, patients experienced a moderate pain in 27/28 tendons (96%) lasted in 3 ± 2 days after the injection (mean VAS = 4.8), treated with local application of ice. Among them, in one case (1/27; 4%) we observed an intense pain response on the day following the treatment (VAS = 8), lasted for about 2 weeks.

Twenty-day follow-up was available for all patients, while 6-month follow-up was available for 18 patients (20 tendons). In all treated cases, there was a gradual return to sport practice in an average time of 6 weeks from the second treatment.

Regarding the VISA-P score, we observed a non-significant minimal improvement at 20 days and a significant improvement at six months, compared to baseline. At 20 days, we detected a non-significant reduction of number of patients in whom focal hypoechoic areas were detected, that became significant at six-month follow-up (Figure 4). Tendon thickness did not significantly reduce

**Table I.** *Clinical and ultrasound evaluation of 28 tendons in 24 patients affected by jumper's knee who underwent ultrasound-guided injection of platelet-rich plasma*

| | **Baseline** | **Twenty days** | **Six months** |
|---|---|---|---|
| Victoria Institute Sport Assessment - P | 56 ± 18 | 60 ± 19 | 74 ± 14 |
| *P* | | 0.644* | 0.044* |
| Tendon thickness; mean ± standard deviation (mm) | 17 ± 8 | 16 ± 9 | 11 ± 5 |
| *P* | | 0.925* | 0.031* |
| Hypoechoic areas; number | 28/28 | 26/28 | 15/28 |
| *P* | | 0.874§ | 0.021§ |
| Power Doppler; median ($25^{th}$-$75^{th}$ percentile) | 2 (2-3) | 3 (2-3) | 1 (1-2) |
| *P* | | 0.411# | 0.001# |

*Note. P values are calculated in comparison to baseline. *Wilcoxon test; §Chi-square test; #two-way ANOVA test.*

at 20-day follow-up compared to baseline, while it was significantly reduced when evaluated at six months. Intratendinous vascularity was significantly increased both at 20-day and 6-month follow-up compared to baseline. Detailed data are reported in Table I.

Overall satisfaction at 6-month follow-up was rated by patients as positive in 20 tendons (71%; excellent = 8/28 tendons, good = 12/28 tendons) and poor in 8 tendons (29%).

## DISCUSSION

Jumper's knee is a common condition in elite and amateur sport players, as a result of overuse during sports activity, that is characterized by severe stress injuries of insertional patellar tendon (19). The repetition of the stresses on the tendon insertion stimulates the local release of cytokines that result in a modification of the reparative activity (20).

Several treating options are available for this condition (21). Physiotherapy and steroid injections were proved to have no long-term efficacy (22, 23). Shock waves may be effective but are painful when performed in acute conditions (24), while surgical debridement is effective but expensive and invasive (25).

Recently, use of autologous growth factors has been proposed to treat different pathologic conditions (8), with the aim to promote and support the biological reparative response of tissues to complete repair.

The therapeutic effect of PRP has been proposed based on the knowledge of the effects of platelet-derived growth factors (GFs) in the stages of the reparative cascade (26, 27). In vitro studies

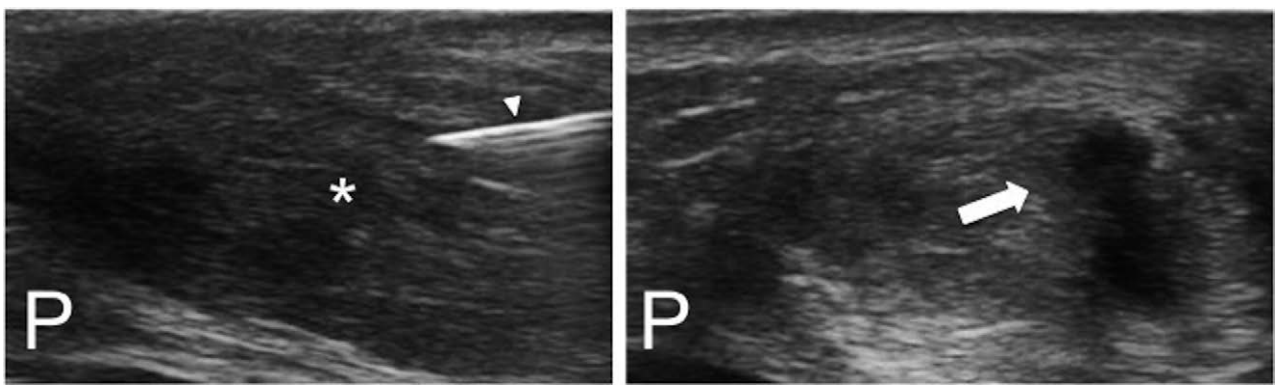


**Fig. 1.** *(a) Platelet rich plasma (PRP) injection within areas of patellar tendon degeneration (asterisk) under ultrasound guidance. (b) Patellar tendon immediately after PRP injection (arrow). P=patella; arrowhead=needle.*

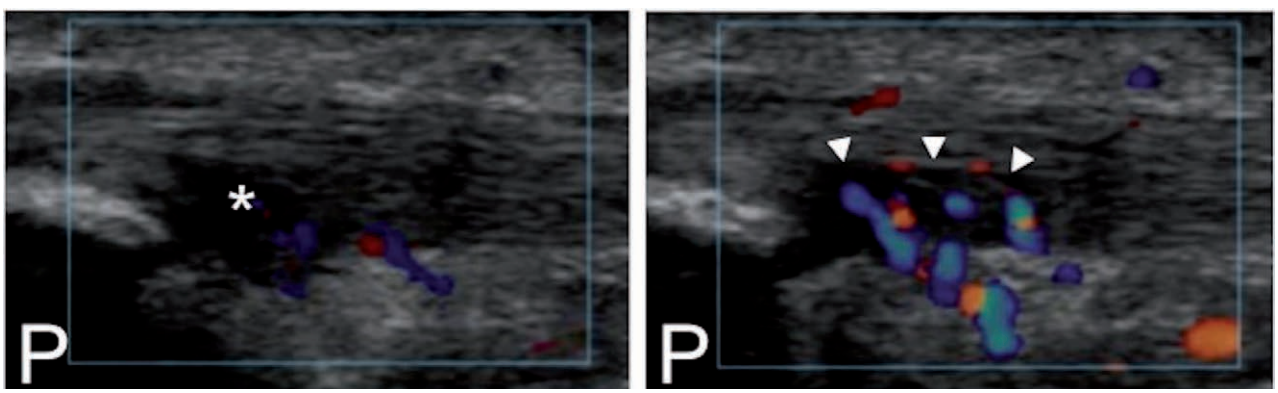


**Fig. 2.** *Jumper's knee in a 22-year-old football player. (a) power Doppler (PD) evaluation shows very low patellar tendon vascularity at baseline (asterisk). (b) six months after ultrasound-guided platelet-rich plasma injection, PD shows a significant increase of vascularity (arrowheads). P=patella.*

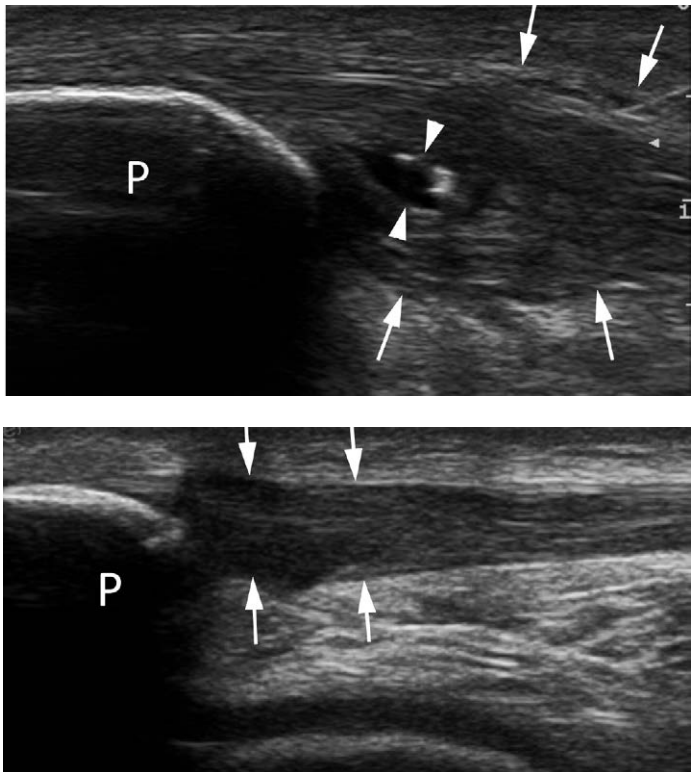


**Fig. 3.** *Jumper's knee in a 22-year-old football player. (a) the tendon is thickened (arrows) with a focal hypoechoic area (arrowheads). (b) six months after ultrasound-guided platelet-rich plasma injection, tendon thickness (arrows) is markedly decreased compared to baseline. The enthesis is homogeneously hypoechoic but no focal degeneration area can be seen. P=patella.*

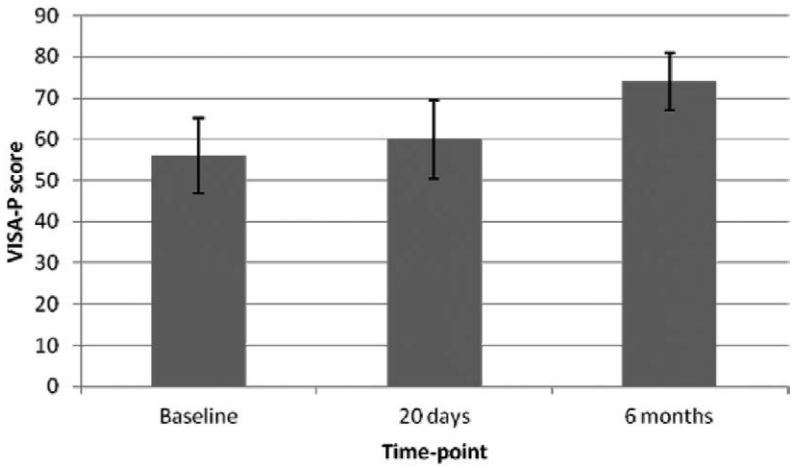


**Fig. 4.** *Victoria Institute Sport Assessment evaluation of patellar tendinopathy at baseline and at 20 days and 6 months after ultrasound-guided percutaneous injection of platelet-rich plasma.*

shown that PRP may increase gene expression for protein coding of the extracellular matrix, collagen production, and tenocytes proliferation (28-30). Tendon tissue is known to repair slower than connective tissues, because of its intrinsic poor vascularization (31). No inflammatory response is

observed in histological samples taken from chronic tendon injuries, but rather an abnormal response to fibroblast and vascular degeneration, known as *angiofibroblastic hyperplasia* (32). Based on these data, new therapeutic strategies have been proposed. These were aimed at stimulating inflammation rather than to suppress it, trying to modify the poor intrinsic reparative capacity of tendons.

Some studies regarding PRP use in tendinopathies demonstrated the validity of such a therapeutic option in unresponsive cases to conservative therapy alone (27). However, other studies on large series reported that the treatment of tendinopathies with PRP is not more effective than placebo (33). Thus, this issue remains challenging and requires further studies to clearly define its effectiveness.

In our experience, ultrasound guided injection of PRP in combination to dry needling has been shown to improve symptoms and tendon structure at 6-month follow-up. The observed significant improvement in VISA-P score at 6 months follow-up is consistent with previous results (28,34). Also, tendon thickness and hypoechoic areas reduction may represent a sign of tendon reparation, as collagen fibers returns to be more packed as they are in normal tendons. Also, the increase of local PD signal both at 20-day and 6-month follow-up represent a sign of the induced vascular response needed to improve tendon reparation.

The absence of peri-and post-procedural complications proves the safety of PRP injections. The only case of severe post procedure pain could be probably due to stimulation of the body's natural response to mediators of inflammation.

In order to reduce this kind of reactions we have performed local anesthesia before PRP injection in all patients. Although some authors reported that this kind of drugs are characterized by intrinsic teno-myotoxicity and could slow PRP induced healing processes we remark that in our series local anaesthetic is injected only in the subcutaneous tissues in order to reduce patient disconfort and not inside the tendon were can slow healing processes.

As previously described by other authors (36), any proposed therapy for tendinopathy of the patellar tendon is effective only if, in the following period, is associated with a sport activity interruption, followed by eccentric rehabilitation therapy and to a subsequent gradual return to sport activity. Thus, jumper's knee treatment using PRP should be avoided when demanding activity is foreseen in the short period. In all treated cases in our series, we suggested a gradual return to sport practice in an average time of 6 weeks from the second injection.

The main limitation of our work is the lack of a control group. This is particularly important, as we do not know whether the effectiveness of this treatment is due to PRP injection or to the concurrent performance of dry needling. However, our preliminary results were mainly focused on testing the feasibility of this procedure in our clinical setting. Also, the sample size is relatively small.

## CONCLUSIONS

The study shows that ultrasound-guided treatment of jumper's knee using PRP is feasible and represents an effective and minimally invasive treatment option. Further randomized controlled studies performed on a larger sample size are warranted to confirm our preliminary results.

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# THE RATIONALE OF ANALGESIC INTRA-ARTICULAR INJECTIONS IN OSTEOARTHRITIS PAIN

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**Osteoarthritis (OA) is a common and chronic disease causing frequently pain and motor disability. Furthermore, joint pain in OA is associated with substantial disability and consequently inflicts considerable burden on affected individuals and society. Symptomatic relief from pain and reducing functional disability are the main goals in the treatment strategies of OA. Various non-pharmacological and oral pharmacological treatments constitute the core medical care and first-line options in the management of symptomatic OA. However, in many cases the OA related pain is not adequately controlled with these options; or certain oral analgesic and anti-inflammatory drugs are contraindicated due to their risks and side-effects.**

The intra-articular (IA) treatment for OA pain is a well-established and widely used adjunctive method. Its main advantage is being local treatment method, with a substance injected directly into an affected joint and thus, minimising the risk of systemic effects of these drugs. In general, the IA injections are recommended for OA patients with moderate or severe pain in addition to their core medical care if non-pharmacological and oral pharmacological options do not achieve sufficient pain reduction. This article reviews some of the intra-articularly administered substances and explains the rationale underlying these injections. We review the most commonly used intra-articularly injectable medications and some medications, the administration of which to the joint milieu is not yet as wide-spread. The efficacy and safety studies are reviewed from the published clinical trials.

*Joint pain in osteoarthritis*

Synovial joints are one of the most common loci of pain in all age groups. Joint pain is the leading cause of physical disability at older age and with aging of populations its impact on functional impairment is growing (1,2). Unquestionably, the highest prevalence that elicit the clinical symptom of joint pain, has osteoarthritis or degenerative arthritis (1,3,4). According to the American College of Rheumatology (ACR) the term OA is applied to heterogenous group of conditions that lead to joint signs and symptoms, which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins (5,6).

The definition of osteoarthritis underlines some central features of OA. First, neither the direct cause of OA nor the exact origin of the symptoms

*Key words: intra-articular injections, pain, osteoarthritis*

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is precisely known. However, several risk-factors and mechanisms of OA have been outlined and may contribute to its progression, to the occurrence and severity of symptoms. Thus, as according to the ACR, OA is a heterogenous and multi-factorial disorder. Secondly, OA is a chronic disorder in which loss of normal articular cartilage and remodelling of the subchondral bone are the most characteristic structural changes and successively may lead to the symptomatic course of OA. In addition to these, during the course and the progression of the disorder, all components of synovial joints may be affected, including joint capsule with synovial lining and surrounding muscles and ligaments.

Frequently the term "wear-and-tear" is used to describe the degenerative disease of joints, highlighting the association with older age and subsequent deterioration of joints and accumulation of structural changes in time. OA and age are strongly associated, as the condition is less common before the age of 40, but its frequency rises in older age groups (1,6) . In fact, the majority of individuals over the age of 65 have OA, as confirmed with clinical and/or radiological evidence (7). The small joints of hands, followed by knee and hip are most commonly affected. Knee OA is present in approximately 37% of persons in the age group of >60 years (8). Although, the prevalence of OA in the small hand joints is higher than the prevalence of knee OA, the latter has more frequently symptomatic course and results in higher functional disability.

The cardinal symptoms in OA are pain, stiffness and reduced movement range. Pain is usually dull and poorly localized. It generally increases with active joint use and reduces with rest. However, in advanced stage, the pain maybe present also during the rest and at night. In addition to these, the signs of deformity, swelling, crepitus, and instability of the affected joint may be present. The symptoms and signs of OA indicate the structural and functional deterioration of affected joints and are associated with significant functional impairment and disability. For example, knee OA, with its high prevalence and frequent symptomatic course, accounts for more difficulty with climbing stairs and walking than any other disease, including the cardiovascular diseases (9). Approximately 23% of adults with knee OA cannot perform their major activities of daily life due to severe pain and disability (3). Thus, the OA inflicts a substantial burden on affected individuals, families and society.

Several features in the mechanism of OA pain have been outlined, although the precise pathogenesis of joint pain is unclear. It is postulated that OA pain is provoked by recurring mild inflammation episodes of joints and at later stage surrounding capsular fibrosis and muscle contracture contribute to pain symptom (10). The inflammation component is supported by imaging studies, which show synovial hypertrophy and effusions in the affected joints, and cytological studies with evidence of inflammatory cells at sites (10). These inflammation episodes in turn are thought to be responsible for the hypersensitivity of joint nerve fibers. Joints contain Aβ, Aδ and C-fibers and normal mechanical and noxious stimuli are transmitted with these sensory fibers. Most of Aβ fibers respond to harmless movements of the joint and give the information of the joint position (10). The thinly myelinated Aδ and C-fibers respond mainly to noxious mechanical, thermal and chemical stimuli. Furthermore, there is also a large group of C-fibers which do not transmit noxious stimuli in the normal joint, but respond in inflamed ones (10). The structural deterioration of joint and inflammation episodes increase the response of these articular nociceptors and thus, lead to the hypersensitivity of these joint fibers. The nociceptive mechanism is mediated by various substances, including bradykinin, prostaglandins, serotonin, substance P, glutamate and neuropeptide Y etc (10). Besides the previously described peripheral nociceptive mechanism in joint pain there is also evidence of central sensitisation with hyperexcitable spinal neurons.

OA pain is the most frequent reason for patients to seek medical advice and treatment. The main goal for the OA management is to reduce pain and stiffness, and to improve the general functional status of the patient. The OA treatments can be divided into non-pharmacological, systemic- and local pharmacological treatments. According to the National Institute for Care and Health Excellence (NICE) guidelines the non-pharmacological treatment, such as patient education, weight loss, physical therapy, are classified as core medical care, which should be considered in every person with OA

according to the relevance of these suggestion to the individual patient (3). For example, weight loss is advised for overweight patients, which would help to reduce the mechanical overload to joints or specific exercises for muscle strengthening in patients with insufficient muscle use.

The next consideration after core treatments should be given to relatively safe pharmacological treatments of pain with paracetamol or topical non-steroidal anti-inflammatory drugs (NSAIDs) (3). Subsequent considerations should be given to the so-called adjunctive treatments, which include pharmacological treatments with oral NSAIDs and opioids, intra-articular injections, surgery and other less efficient non-pharmacologic options. The adjunctive methods are often considered for patients with moderate or severe OA pain, which does not respond to the core treatments and/or topical NSAIDs or paracetamol. Each of these alternative treatment strategies has certain aspects in favour of their use and certain disadvantages. The decision of their use depends on various factors, including patient co-operation, comorbidities, severity of pain and functional disability, response to the previously chosen treatments or patient's preferences. The use of oral NSAIDs, for example, does not control sufficiently pain in all patients and the NSAIDs have well-known side-effects on gastrointestinal or renal system, the risk of which is increased in certain groups of patients. On the hand, the joint replacement surgery is considered in the presence of substantial functional disability, but is not recommended for patients with significant comorbidities. Thus, the management of OA pain should be individualised by taking into account various patient and disease related aspects.

The intra-articular injections are favoured by many physicians as the administration of substance is site-specific and avoids the systemic side effects of drugs. Furthermore, the direct local administration helps to achieve better the appropriate drug concentration at the site (11). The IA injection treatment option has also relatively few contra-indications. Absolute contraindications for IA treatment include systemic infection, local infection or fracture or tumour near the injection site, coagulopathy and unstable joint. Relative contra-indications are the following: severe immunodeficiency, anticoagulant therapy, poor response to prior injection, uncontrolled diabetes and severe obesity. We review the most commonly used intra-articularly injectable substances in the treatment of symptomatic OA and some medications, the administration of which to the joint milieu is not yet as prevalent. The potential mechanism of action of these substances, the evidences of their efficiency and safety in the light of the published clinical trials is discussed.

*Glucocorticoid injections*

Glucocorticoids (GC) exhibit strong anti-inflammatory and also immunosuppressive effect. Currently, the GCs are the most common drugs that are administered into joint space for chronic pain. The intra-articular GC causes the reduction of pro-inflammatory and pain mediators, including bradykinins, histamine leukotrienes and prostaglandins (11,12). These mediators cause pain by stimulating the nociceptors and have shown to sensitise the nociceptors (12). There are different formulations of glucocorticoids that can be administered into joint spaces. Some of them are suspensions and some are solutions with a wide range of concentration of active substance.

The intra-articular GC injections (IAGC) are recommended for OA patients with moderate or severe knee pain in addition to their core medical care (3,13). The IAGC has been used for treating OA pain for decades in clinical practice and positive results in pain management and improved function has been found in the treatment of knee, hip, shoulder, carpo-metacarpal and tarso-metatarsal-joints. Over the years various efficacy studies of IAGC injections in comparison with placebo or with hyaluronic acid injections have confirmed the reduced pain intensities and improved functional scores in treated OA joints with follow-up evaluations (3,12,14). However, the randomised and comparative studies with placebo have mainly evaluated the pain relief and other outcome measures in patients with knee or hip OA. The efficiency of IAGC in other joints have not proved with placebo-controlled clinical trials (11). The evidence affirms mainly short term improvement of symptoms from IAGC for osteoarthritis pain, as significant difference in pain scores between the IAGC and placebo is generally shown in between one and four weeks (3). The

follow-up evaluations of longer time periods of 12 and/or 24 weeks have not shown consistently effect on pain measures (3).

The IAGC injections for knee OA are common, as they are simple to administer with relatively few side-effects. To minimise the risk of side-effects there are some contraindications for this treatment method. These include local infection at the injection site, anticoagulant therapy and severe joint destruction. One of the main reported adverse events is post-injection flares. These are starting usually 6-12h after injection and are commonly self limiting within 3 days (12). Some data has been published indicating the negative effect of IAGC injections on articular cartilage. Specifically, in animal studies and in some human studies, the IAGC administration has been associated with destructive cartilage changes (11,12). However, based on more recent findings the risk of cartilage destruction is minimal if the number of injections in the given joint is kept to 3 or 4 times in a given year (12).

The benefits of IAGC are not evident in all patients and the predictors of the response remain unclear. The main emphasis in currently on-going clinical trials is to characterise better the group or groups of patients which would benefit from the injections of GC. Thus, several disease-, patient- and treatment-related factors are evaluated, to define those which would predict better the magnitude or the duration of pain relief. The review by Maricar *et al.* focused on publications of IAGC injections in knee OA, which tried to bring out prognostic factors of treatment response (15). The main focus in this review was on such factors as the presence of synovitis and effusion, the radiological stage of OA, aspiration during the treatment, the baseline pain severity, duration of symptoms and injection delivery method. The conclusion of the authors was that certain factors could be prognostic, such that the absence of synovitis, presence of effusion, injection under the ultrasound guidance and greater symptoms at baseline could be associated with greater response in pain scales (15). Similarly, another review article by Hirsch *et al.* found the presence of effusion and aspiration of synovial fluid at the baseline to have positive effect on the overall clinical response (16). However, the positive or negative contribution of these factors to the pain measures were not consistent in all of the reviewed studies and large-scale clinical trials should be performed with standardised methods to enable the investigation of these prognostic factors in more detail. Thus, there is still relatively sparse data of certain patient- or disease-related factors on pain relief and the overall results from published trials are currently in this respect inconclusive.

*Hyaluronic acid injections*

The intra-articular administration of hyaluronic acid (IAHA) in the management of osteoarthritic pain is increasing. However, the debate about its usefulness is still on-going, as not all results of clinical trials confirm its effectiveness. Hyaluronic acid belongs to the glycosaminoglycans' class of polysaccharides. This large molecule is present in all tissues of the body and connective tissues, including skin, ligaments and joints, have abundant amount of HA. HA is produced by synoviocytes, fibroblasts and chondrocytes and is the major chemical component of the synovial fluid (17). Besides joint fluid HA is also a fundamental part of extracellular matrix in articular cartilage (17). Its function in joint milieu is to provide viscosity, elasticity and lubrication. But not only, as HA stabilises proteoglycans in the extracellular matrix, maintaining therefore also the normal cartilage structure (18). In addition, HA in the joint milieu is believed to have anti-inflammatory and anti-nociceptive effects (17).

The functions of HA in healthy joint and its alternations in OA pathology have led the theory of using exogenous HA for the treatment of OA pain. HA in osteoarthritis pathology is depolymerised and cleared faster than in normal joints, thus degenerative joints have reduced amount of endogenous HA with reduced molecular weight (19). The deficiency and changes of HA in OA are consequently associated with the increased vulnerability of articular cartilage. The idea behind the injection of exogenous HA into the intra-articular space is therefore to restore the viscoelasticity properties of the joint, to help to restore the synthesis of endogenous HA and to reduce the joint pain (20,21). As the IAHA injection is thought to aid to balance the components of synovial fluid and to provide better viscoelasticity, the treatment is also termed viscosupplementation. Currently, there are various hyaluronic acid and hylan preparations available. The latter are cross-linked hyaluronic

acids and have higher molecular weight with distinct pharmacokinetics and pharmacodynamics (22,23).

Most clinical trials have evaluated the efficiency of IAHA treatment for knee OA. Other injection sites, such as hip, ankle, shoulder, metatarso-phalangeal, carpo-metacarpal and temporo-mandibular joints have been used and only a few studies have described their clinical benefit (17,24). The latest review article of IAHA by Colen *et al.* included into the analyses 73 randomised controlled trials with IAHA treatment in knee OA. Based on these trials the authors concluded that the pain in the HA treatment group is generally reduced by 40-50% compared with baseline (25). However, when the pain reduction in this group was compared with the placebo group, the differences between the groups were not that large. Thus, it is questionable if the HA treatment reaches the minimum clinical important difference compared with placebo (25). Bellamy *et al.* in the Cochrane review had collected data from 76 randomised trials with IAHA treatment in knee OA (20). The results of the Cochrane review were more assuring of the beneficial effect of IAHA on pain, on functional and global assessment. Generally, the pain percentage reduction compared with baseline was reported to be between 28-52% in trials and the improvement in function was 9-32% (20). The positive effect of IAHA in other joints than knee is less apparent. Some articles report the beneficial effect on the OA pain in other joints, but there is only limited evidence of this treatment method to be more effective than placebo and there is no clear evidence IAHA to be better than other therapies (24).

Compared with intra-articular glucocorticoids, the IAHA treatment is characterised by delayed onset of symptomatic benefit, but with longer-lasting effect than the IAGC (13,18). The peak of the effect on pain and functional assessment is between 5-13 weeks after injection with residual effect reported up to 24 weeks (12,26). No specific patient group is found to benefit from the treatment the most and there is little evidence of the superiority of one hyaluronic preparation over the others (25). In the light of the variable reported clinical benefit the recommendations of international organisations for the use of IAHA are relatively modest. The European League Against Rheumatism (EULAR) has endorsed the IAHA treatment as part of non-operative management of knee osteoarthritis pain (13). The NICE has stated in its guidelines that the IAHA is not recommended for the treatment of osteoarthritis (3). The latter recommendation was mainly concluded due to the cost effectiveness analysis, while its effect on outcome measures of pain reduction and functional improvement was considered positive (3).

The administration of IAHA into knee joint is considered relatively safe. Minor adverse events have been reported in 3 to 20% of patients (18). The majority of these reported events are transient local pain in the injection site, which lasts usually for 1 to 2 days; itching in the injection site; headaches or calf pain (12,18). However, there is some concern of safety due to the case reports of post-injection pseudo-sepsis. The cause of this severe joint inflammation is not entirely clear, but the association with cross-linked hylans and not with hyaluronic acid has been postulated (11,18). It has been also associated with consecutively repeated treatment courses (18).

In conclusion, the IAHA injections are relatively safe and have modest positive effect on knee OA pain. However, the future studies should focus on the standardisation of the IAHA treatment and on the evaluation of different prognostic factors. The latter would also help to enlighten the issue of disparities in the published efficiency studies.

*Capsaicin injections*

Capsaicin or 8-methyl-N-vanillyl-noneamid is a natural compound in chilli peppers (27). Most of the pungent flavour or "hot" taste of this plant is due to capsaicin. Although, initially originating from the Americas, chilli peppers have been incorporated in various traditional cuisines in the world to add flavour. Capsaicin can be extracted from chilli peppers or synthesized and the compound is nowadays used not only in the culinary world, but also in medicine, in pepper- or so-called capsicum sprays and in biopesticides.

Capsaicin belongs to the vanillyl functional family and binds to the transient receptor potential vanilloid subfamily member 1 (TRPV1) (28). TRPV1 is one of the 9 temperature-sensitive transient release potential ion channels (thermoTRP) (28,29). The activation of 7 of these thermo-TRP ion channels leads to the sensation of warm or hot temperatures, while 2 channels are activated by cold temperatures. TRPV1

was the first isolated thermoTRPs and is found to be activated not only by heat (more than 43°C), but also by other exogenous and endogenous physical and chemical stimuli, including capsaicin, allyl isothiocyanate (ingredient in mustard, horseradish and wasabi) and low pH (27). Due to the binding of capsaicin to the receptor, TRPV1 is also called capsaicin or vanilloid receptor. Vanilloid receptor is present mainly in neurons with unmyelinated axons (C-fibers), although small amount of myelinated Aδ fibers have also these ion channels (29). Capsaicin binds to the sensory neurons with TRPV1 and causes the initial neuronal excitation, which is perceived as a hot and burning feeling. The neuronal excitation is followed by a relatively long refractory state. In the refractory state the neurons fail to transmit relevant temperature-related stimuli and also various other stimuli, including pain stimuli. Thus, the effect of capsaicin treatment is to desensitise sensory neurons and diminish consecutively the transmission of pain stimuli.

The desensitizing effect of capsaicin on sensory neurons has been used for decades for chronic pain relief. The topical use of capsaicin, as a component in analgesic creams, lotions and patches, started in the 1980s (30). These topical creams contain low-concentration capsaicin of around 0.075%. Clinical trials evaluating the efficiency of capsaicin containing creams have found, however, modest beneficial effect for various pain syndromes, including post-herpetic neuralgia, diabetic neuropathy and chronic musculoskeletal pain (Anand and Bley 2011). The topical application of capsaicin-creams is currently recommended by NICE and EULAR as an adjunctive method to core OA treatments (3,31) .

Recently, the treatment with intra-articularly administered capsaicin has been introduced and the first efficiency studies in the management chronic pain have been published. The injectable capsaicin has been already evaluated in the management of pain in OA, tendonitis, Morton's neuroma, in post-operative pain after hernia repair or after arthroplasty (29). Overall, the first clinical trials in OA pain with intra-articular capsaicin have shown some promising results. For example, the evaluation of efficiency in pain management in patients with end-stage OA with the double-blind randomised clinical trial showed the single intra-articular injection of capsaicin to result in pain reduction of 25-30% compared with baseline (29). In comparison the placebo group had the reduction in pain of only around 3% (29). Another open-label clinical study with multiple intra-articular injections of capsaicin in patients with moderate OA found in post-treatment phase reduction in pain, stiffness and movement difficulty (29). The reported side-effects have been only transient pain in the injected sites.

Although, the initial results have shown certain promising effect on pain and function, there is still very few trials and info to draw more detailed conclusions. More information is needed regarding the capsaicin dose for the intra-articular injections and treatment schedules. The evaluation of efficiency and safety is still in its early stage and randomised placebo-controlled trials with larger populations of patients should be performed.

*Injections of local anesthetics*

Local anesthetics are routinely injected into the joint space with the aim to achieve post-operative analgesic effect. The method is especially used after arthroscopic procedures of the knee, after knee and hip prosthetic joint replacement surgery, following acromioplasty and during rotator cuff repair. Thus, the intra-articular injection of local anesthetics is a common measure for treating acute pain in the post-operative setting. The injection of local anesthetics into the operated joint space reduces the need for oral and intra-venous pain medication (32,33). It also improves the patient satisfaction in post-operative period and results in earlier discharge from the hospital (34). The single injection to the joint space is the most common method, although continuous infusion through a catheter placed into the surgical site is also used.

The most common local anaesthetics for the intra-articular injections are bupivacaine, lidocaine and ropivacaine. The longest experience and the most preferred from these is bupivacaine (35). Both, bupivacaine and ropivacaine are members of the amino-amid group in the local anaesthetics and have found to have similar clinical efficiency in the management of post-operative pain (36). Although, several randomised placebo-controlled studies have found the injections of local anesthetics superior to placebo on pain outcome measures, many of the

clinical trials have found the effect relatively small and over a relatively short period of time (33). In general, 10-30 ml of bupivacaine 0.20% to 0.5% is injected (33). In post-operative pain management regimes in some centres bupivacaine is used in combination with morphine or tramadol.

Local anaesthetics may also be injected into the joint space during the injection of other substances as part of chronic pain management, during the aspiration or lavage. For example, lidocaine is used by some physicians combined with the IAGC treatment (37). In many cases the lidocaine is administered only subcutaneously, but the injection into the joint space is preferred by some to confirm the appropriate site of injection. However, other physicians avoid it, as they claim the local anesthetics dilutes the steroid crystals, although it is unclear whether this has any impact on the IAGC treatment outcome measures (37). Lidocaine has also mild anti-inflammatory effect, but it has relatively short half-life and is not in general recommended as an individual substance for IA treatment (12). The short duration of the effect of intra-articular local anesthetics was confirmed by Creamer *et al*. The authors evaluated the effect of intra-articular bupivacaine and placebo in 20 patients with chronic OA pain. The results showed the short-duration but significant effect on pain intensities (under 24 hours) (38).

The overdose and intravascular administration of local anesthetics may lead to nervous system and cardiovascular toxicity. The central nervous system intoxication exhibits first with muscle tremors and shivering and the further increase in the systemic concentration may lead to generalized convulsions and respiratory distress. However, the intra-articular injection of local anaesthetics avoids their systemic toxicity effects, as the systemic concentration is low enough. There is a concern that the intra-articular use of bupivacaine, lidocaine and ropivacaine may have chondrotoxic effect. This has been observed in several animal studies, in vitro studies with cartilage cultures and in some clinical cases (33). Bupivacaine acutely inhibits the synthesis of articular cartilage, causes inflammation of the cartilage and also synovial changes in several animal studies (35). The clinical reports about the possible association of bupivacaine and the postoperative extensive loss of articular cartilage over a relatively short of time have mainly been reported with shoulder joint and ankle joint (35). The general view is that the effect of local anaesthetics is time-and dose-dependent, cautioning especially about the possible chondrolytic effect of continuous-infusion method (35).

*Morphine injections*

The intra-articular administration of morphine is frequently administered, like local anaesthetics, after arthroscopic joint surgeries. Its analgesic effect alone or in combination with other substances results in reduced post-operative pain intensity measures and its effect in the joint milieu lasts up to 48 hours (39). Locally administered morphine has strong anti-nociceptive effect by inhibiting the excitability of nociceptive terminals and the propagation of action potentials. It is also established that they inhibit the release of excitatory pro-inflammatory compounds and decrease plasma extravasation, having thus anti-inflammatory effects (39,40). Both of these effects are regulated by opioid receptors in peripheral sensory nerve terminals (40).

The administration of morphine in chronic pain has been evaluated only in a small number of studies. Likar *et al.* evaluated 23 patients with knee OA in a cross-over placebo controlled study and compared the effect of intra-articular morphine and saline injection on pain intensity measures. The post-injection pain intensities were measured over 7 days and were found to be significantly decreased in the group treated with morphine injections compared with baseline intensities and were also significantly lower than in the control group (39). The results showed the decreased pain measures remained during the 7 days of post-injection evaluations. Stein *et al.* evaluated the analgesic effect of morphine in 44 patients with knee OA (40). In this randomised double-blind study the effect of morphine, dexamethasone and saline on pain measure was compared. The patients were observed and pain was measured up to 6 days after injection and morphine was found to have similar or even stronger analgesic effect during that time period compared to the glucocorticoid injection (40). The latter study found also that synovial leukocyte counts to be lower in the morphine group than in the saline group, thus confirming the hypothesis that morphine may have anti-inflammatory effects (40).

In the light of these studies it, however, remains

to be investigated how long precisely the analgesic effect of the morphine injections would last. It is also of interest if repeated injections of morphine could be a beneficial treatment option for chronic OA pain.

*Platelet-Rich Plasma injections*

The progressive loss and deterioration of articular cartilage is one of the characteristic pathological features of OA. The hyaline cartilage has very little capacity to repair, as the cartilage does not contain nerve and blood vessels. It is postulated that the stimulation of repair mechanisms, however, could stop or at least delay the progression of OA (41). Tissue biology studies have found that various growth factors (GF), cytokines and chemokines play important role in these repair mechanisms (42). These include anabolic factors for cartilage such as transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), fibroplast growth factor (FGF) and hepatocyte growth factor (HGF) (42). The role of several of these growth factors in cartilage regeneration and metabolism repair is widely investigated now in vitro and in vivo. For example, TGF-β1has been found to induce chondrogenic differentiation of mesenchymal stem cells, as well as matrix deposition (41). Platelet-derived GF is found to increase chondrocyte proliferation and to regulate proteoglycane synthesis (41).

The storage pools of these GF are found in platelets. In order to acquire the GF the peripheral blood is collected and centrifuged to separate plasma fraction. The centrifugation brings the platelet concentration to 4-5 times higher than it is in normal peripheral blood (41). This autologous plasma fraction rich in platelets and growth factors is often called also as platelet-rich plasma (PRP). To acquire the PRP is simple and with low-cost. Furthermore, the method is non-immunogenic, as no additional thrombin or other human or animal substances is added. The cytokines and growth factors in PRP intra-articular environment diffuse towards chondrocytes and subchondral bone, where they bind with cell surface receptors (43). The method is thought to aid and enhance cartilage repair with exogenous growth factors and to delay the deterioration of hyaline cartilage.

There are still relatively few clinical human studies evaluating the safety and potential value of PRP injections in disease modified joints. In a retrospective cohort study by Sanchez *et al.* the authors evaluated patients, who had received PRP injections, and patients with IAHA treatment (44). The results in pain and in physical function scores showed bigger percentage of reduction from baseline in PRP group at 5 weeks (44). Another study by Kon *et al*. compared the outcome measure of pain intensity in patients with OA, where one group received the injection of PRP, another low-molecular weight HA and the third group received high-molecular weight HA injections (41). The authors found the PRP group and low-molecular weight HA group to have similar pain intensity scores at 2 months post-treatment, while at 6-months the PRP group showed better results (41). In the more detailed analysis the PRP showed to be more effective than the HA in younger patients with early OA (41). Similar results of PRP effectiveness in patients with less cartilage degeneration compared with advanced OA was obtained by Filardo *et al.* In the study the patients were divided in groups with early or advanced OA. The follow up evaluation at 2, 6 and 12 months confirmed the younger and early OA groups to have better outcome measures of pain and physical function (45). The safety data from these studies indicate that the PRP injections are safe with no reported serious adverse events and only a few cases of post-injection heaviness, swelling or self-limiting pain reactions were reported.

The evaluation of PRP injection treatment is still ongoing and randomised placebo-controlled clinical trials are needed in order to support or contradict the findings of the initial studies. The current issue is also that the products and the treatment schedules differ qualitatively and quantitatively between centres and studies. Thus, it would be of value to standardise the methodology to achieve better comparative information.

*Botulinum neurotoxin type A injections*

Botulinum neurotoxin type A (BoNT/A) is a protein produced by anaerobic Gram-positive bacterium *Clostridium botulinum*. In the 1980s the substance was first approved as the treatment for strabismus and blepharospasm. Over the decades the clinical use due its neuromuscular blocking effect has expanded and it is used for several muscle tone

and activity disorders, including spasticity, tremors and tics (46,47). The substance is also used for smooth muscle disorders of esophageal sphincter achalasia, neurogenic bladder and Hirschsprung disease. In addition, its analgesic effect is used in the management of chronic pain, including headaches and migraine, lower back pain and for joint pain (48).

The BoNT/A treatment of muscle tone and activity disorders relies on the mechanism that the neurotoxin blocks the release of neurotransmitter acetylcholine at the neuromuscular junction and inhibits consequently muscle contraction. The heavy chain of BoNT/A molecule binds to specific receptors on cholinergic nerves and the molecule is then absorbed into the cell (49). Thereafter, the light chain of the neurotoxin acts as an enzyme and cleaves proteins that are responsible for neurotransmitter release from motor nerve terminals (49). By this mechanism of action the signalling process that would result in the contraction of the muscle is blocked. Besides the blocking effect the substance was found to have analgesic effect, which was found to develop earlier and was greater in extent than the region of neuromuscular effects (46). Thus, over the decades the BoNT/A was started to be used for various disorders that included the pain component. The studies to investigate the possible mechanisms underlying the analgesic effect in the intra-articular milieu have found that BoNT/A may inhibit the release of substances, which generate joint pain. Specifically, the BoNT/A has been found to decrease the release of substance P, calcitonin gene-related peptide (CGRP) and glutamate. The decreased amount of these substances in turn result in decrease of peripheral nociceptor activation and sensitisation (48). Thus, the analgesic effect is achieved by the chemodenerervation of articular pain fibers.

The clinical studies about the effects of IA BoNT/A are relatively few. Mahowald *et al.* investigated the pain reduction IA BoNT/A injections in patients with pain in shoulder, knee and ankle joints (50). All of the selected patients in this study had chronic arthritis and severe joint pain that was not responding to the treatment with oral or IA medications of glucocorticoids or HA. These case series found clinically significant reduction in joint pain that lasted in the range of 3 to 12 months (50). After these initial positive cases the authors undertook two pilot controlled randomized studies of IA BoNT/A and saline injections (47). One study included patients with advanced Rheumatoid Arthritis and degenerative osteoarthritis with shoulder pain and the other one patients with knee pain. The patients with chronic shoulder pain showed the IA BoNT/A to result in significant decrease in pain scores compared with baseline and compared with the patients treated with saline injections (47). The positive effect on pain of IA BoNT/A in the study with severe and moderate knee pain, showed however the beneficial effect on pain scores only in the severe pain group (47). Another study evaluated the efficiency of two consecutive IA BoNT/A injections with 3 months interval in patients with knee OA (46). The authors found significant improvement in pain and stiffness compared with baseline, but only 3 months after the initial injection (46). These studies show the analgesic effect of the intra-articular treatment with BoNT/A, but further studies with larger patient groups and optimal injection dose and intervals should be investigated.

*Bisphosphonates injections*

Bisphosphonates are pyrophosphate analogs, traditionally used in the treatment of pathologic conditions associated with abnormal bone metabolism, such as osteoporosis, Paget's disease, and cancer-related bone pain. More recently, results of clinical trials have indicated the potential role of bisphosphonates injections in the treatment of OA (51).

Bisphosphonates may have a chondroprotective effect in patients with knee OA, but the results of clinical trials with oral bisphosphonates have been contradictory (52). Rossini *et al.* in their phase 2 randomized, partially blind clinical trial, tested the efficacy of IA clodronate vs IAHA in patients with primary knee OA. The Authors found that IA injection of either HA or different doses of clodronate in symptomatic knee OA is associated with significant and clinically meaningful progressive improvement in pain and function extending for at least 2 weeks after the last injection. Non-significant differences were detected among the four clodronate treatment regimens, even though a significant dose–response relationship for the three doses of clodronate was found for active movement pain and a trend for

extension and mobility scores and paracetamol consumption. The precise dose regimen and the persistence of the effect remain to be established. The uncertainties still persisting on the real therapeutic value of HA also leave the interpretation of the results reported here with IA clodronate open (51).

*Other novel IA therapies*

The previously reviewed IA injected substances are by no means all potential IA therapies in OA pain. Various other novel experimental substances are evaluated, including orgotein, silicone (53), polynucleoutides gel (54), stem cell injections (55) etc. These novel treatments have shown positive clinical effect in a small number of clinical trials and therefore, the approaches are still in the very early stage of evaluation.

## CONCLUSIONS

There is a wide range of treatment options that is used in the management of OA pain, including a variety of non-pharmacological, systemic and local pharmacological treatments or the option of surgery with prosthetic joint replacement. The guidelines stress the importance of non-pharmacological core treatments, including weight loss and physical therapy, and the relatively safe oral pharmacological tratments, as the first line options in pain management (3). The consideration of other adjunctive options, which are with less proven efficiency or with increased risk to patients, should take into account multifactorial patient-, disease- and symptom-related factors. From these adjunctive treatments the intra-articular injections have relatively few contra-indications and are injected locally into specific painful joints. Thus, the role of IA treatments is to provide better pain management for patients, who have not received adequate pain relief with first-line options alone.

Several substances have been introduced and are used to be injected directly into the joint space to treat pain usually in II/III OA stage patients (eg. IAHA). From these, the IA glucocorticoids and hyaluronic acid have been studied most extensively with several placebo controlled randomized studies. IAGC are generally recommended, as the locally injectable treatment in osteoarthritis patients with moderate to severe pain. The studies evaluating the efficiency with IAHA, however, have found only modest beneficial but longer duration effect than the IAGC. The injections with local anesthetics and morphine are mainly used in post-operative settings due to their relatively short duration of clinical effect. Some centres and physicians may also use local anesthetic in chronic pain treatment, but generally this is done in combination with other substances. Although, the other less extensively used substances, e.g. platelet-rich plasma, botulinum neurotoxin type A, bisphosphonates and capsaicin injections have shown some promising results in some studies, their beneficial effect on pain and function of the affected joints should still be evaluated with larger patients groups and randomized studies. In conclusion, the intra-articular injectable substances are important part in the management of OA pain and the future studies should also define prognostic factors, which would characterise better patients who would benefit from IA treatments and which substance would have the best possible clinical effect.

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*LETTER TO THE EDITOR*

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Dear Editor,

Osteoarthritis (OA), the most common form of arthritis, is a chronic disease characterized by slow degradation of cartilage, pain, and increasing disability. (1) Osteoarthritis of the knee affects up to 10 % of the elderly population. (2) Goals for managing osteoarthritis are to reduce pain, improve and maintain joint mobility, and limit functional impairment. (3)

Treatment of osteoarthritis includes: (4)

A) Non pharmacologic therapy:

Patient's education about disease process, prognosis, management issues, psychological measures, reduction of body weight, physical modalities (as thermal modalities) exercise supportive devices (canes, orthotics) and modifications of activities of daily living.

B) Pharmacologic (drug-based) therapy:

Topical agents, oral (systemic) agents and intra-articular agents.

C) Surgical intervention:

A local (as compared to a systemic) treatment for a painful joint has obvious appeal. (5)

Several palpation-based, anatomical landmark–guided approaches for the administration of therapeutic injections into the knee have been

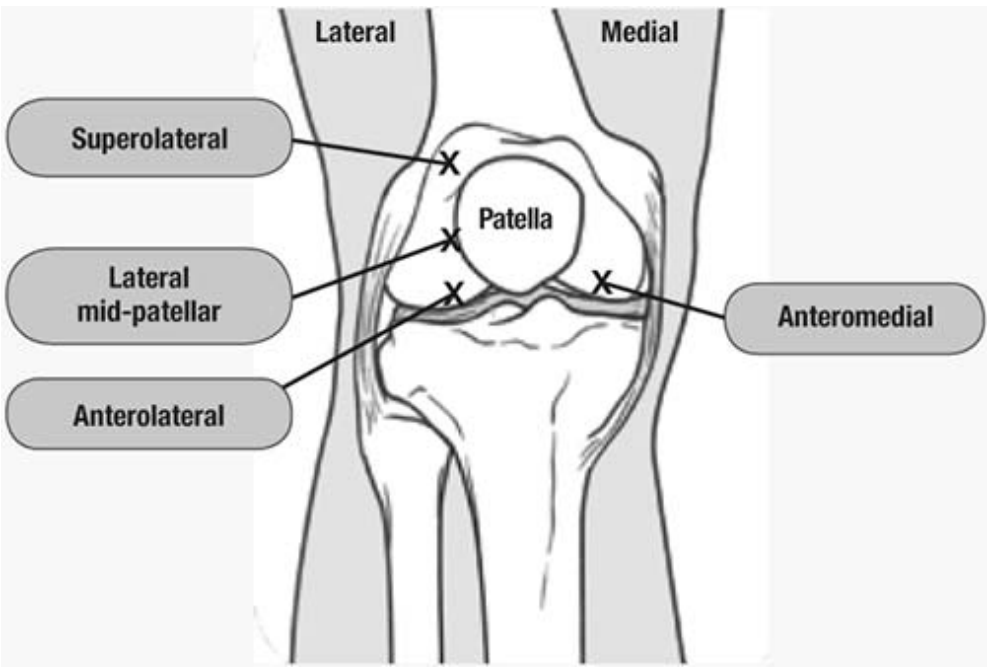


**Fig. 1.** *(9): Diagrammatic representation for different approaches of intra-articular injection of the knee*

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**Fig. 2.** *Allam's approach.*

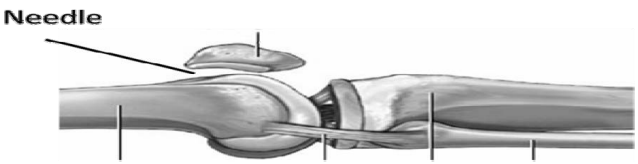


**Fig. 3.** *Diagrammatic representation of needle placement in Allam's approach*

described, including the extended leg lateral mid-patellar (LMP) and medial midpatellar (MMP) portals and the bent leg (flexed to 90°) anteromedial (AM) and anterolateral (AL) portals (6,7). Suprapatellar approach involves insertion of a needle 1 cm above and 1 cm lateral to the superior lateral aspect of the patella at a 45-degree angle (8) (Figure 1).

We propose a new safe effective approach in an attempt to determine a viable alternative to other approaches where this is not practicable:

1. Patient's consent is taken before injection.
2. Patient lies supine with knee exposed, extended and sterilized.
3. Midpoint of upper pole of patella is identified and marked, 24 gauge needle is inserted passing via skin and quadriceps tendon and angled under the post surface of patella between it and femur (figure 2,3).
4. Injection of medication.
5. Post injection care: Plastic adhesive bandages and rest for one day after injection is recommend.

Twenty patients with (mild to moderate) primary osteoarthritis of the knee diagnosed by American College of Rheumatology criteria (clinical, laboratory and radiologic for OA knee) complaining of knee pain were treated by intra-articular steroids through Allam's approach (part of a study not finished yet), reported data shows effectiveness of this approach. Further data will be released later.

However, further studies will be needed to determine whether such approach is less painful and safer than

the others. In addition, comparative studies with ultrasound guidance will be needed to assess and compare with other approaches the achievement of articular space.

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