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## EDITORIAL

**MAY REGENERATIVE MEDICINE EXERT A ROLE IN THE INTRA-ARTICULAR MANAGEMENT OF ARTHRITIDES?**

Regenerative medicine identifies the branch of medicine whose goal is to bring permanent recovery of damaged tissues and organs exploiting the regenerative potential of stem cells. Advances regenerative medicine are so closely related to the progress of knowledge on stem cells' biology as stem cells and their specialized derivatives, natural or engineered, provide the functional components of a regenerative regimen. Two are the intervention strategies of regenerative medicine: the in-vivo approach, that is based on pharmacological stimulation of the resident stem cells of endogenous tissues in order to stimulate the regenerative potential; the ex-vivo approach aimed at stem cells or progenitor transplantation, expanded and/or genetically modified in vitro, than able to colonize the area of interest and support the regenerative-reparative process. These approaches theoretically can be used for intra-articular treatments. The first challenge related to the use of stem cells or progenitor transplantation is the tissue source of cells. Both differentiated and undifferentiated stem cells have been used as the starting cell type in cell-based therapies. While primary differentiated cells, such as chondrocytes, are often limited in quantity, adult mesenchymal stem cells (MSCs) can be easily obtained from a bone marrow aspirate or other mesenchymal tissues. MSCs have a high expansion capacity, the potential to differentiate along all mesenchymal lineages, and have emerged as a candidate cell type with high potential for cell-based musculoskeletal regeneration.

Mesenchymal stem cells (MSCs) are partially defined by their ability to differentiate into tissues including bone, cartilage and adipose in vitro, but it is their trophic, paracrine and immunomodulatory functions that may have the greatest therapeutic impact in vivo. MSCs are commonly isolated by adherence to cell culture plastic or density-gradient

fractionation, and MSC cultures thus generally represent a heterogeneous population of cells. While the exact mechanisms that guide tissue homing of delivered MSCs are not known, it is clear that MSCs themselves secrete a broad spectrum of bioactive molecules that have immunoregulatory and/or regenerative activities. The secreted bioactive factors have been shown to inhibit tissue scarring, suppress apoptosis, stimulate angiogenesis, and enhance mitosis of tissue-intrinsic stem or progenitor cells. This complex, multifaceted, "pro-regenerative" activity of the secretory function of MSCs has been referred to as "trophic activity", distinct from the capacity of MSCs to differentiate. MSCs are potent modulators of immune response, exhibiting antiproliferative capacities. The immunosuppressive activity of MSCs is induced by a combination of inflammatory cytokines including interferon (IFN)- $\gamma$ , tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\alpha$  and -1 $\beta$ . Also, MSCs can inhibit the proliferation and activation of B cells, similar to their effect on T cells. These features might play a key role especially for the treatment of musculoskeletal diseases, such as OA or Rheumatoid Arthritis (RA). Targeted gene therapy might further enhance the activities of MSCs. Direct injection of vectors can be used for in vivo gene delivery. Also, stem or differentiated cells can be used as vehicles for ex vivo gene delivery. The transduced cells can be applied either by injection as a cell suspension or seeded within a scaffold material. Both ubiquitous and local transgene expression can be induced by the ex vivo genetically modified cells or resident cells can be transduced via vector application into the musculoskeletal tissue. In recent years more and more interest is being given to the use of stem cells in clinical practice. Cell-based therapies and regenerative medicine, more and more based on progress of stem cells biology, have begun to lay the foundations of the future clinical

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practice. The challenges still open in order to exploit the full potential of stem cells as well as tissue transplantation, however, are many and require an integrated multidisciplinary approach.

We are pleased to publish in this issue of European Journal of Musculoskeletal Diseases, EJMD, a review on the use of adipose mesenchimal stem cells in the treatment of musculoskeletal diseases.

## VISCOSUPPLEMENTATION WITH HANOX-M-XL IS EFFECTIVE IN MODERATE HIP OSTEOARTHRITIS BUT IS NOT AN ALTERNATIVE TO HIP JOINT SURGERY IN PATIENTS WITH SEVERE DISEASE. RESULTS OF A CLINICAL SURVEY IN 191 PATIENTS TREATED IN DAILY PRACTICE

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Viscosupplementation with hyaluronic acid (HA) or its derivatives for the symptomatic relief of osteoarthritis (OA) of the hip joint have been studied in open-label and placebo-controlled trials with conflicting results. The objective of this survey was to obtain data from the daily practice on efficacy and safety of HANOX M-XL, a novel viscosupplement made of non-animal cross-linked HA and high concentration of mannitol (HappyCross®) administered through a single injection regimen in patients suffering from hip OA. Multicenter retrospective clinical survey using a standardized questionnaire in which one hundred ninety one consecutive patients treated with a single intra-articular injection of HANOX M-XL for symptomatic hip OA were included. Demographic data, imaging guidance, pain on a 10 point Likert scale (LS), patient's self-evaluation of efficacy, satisfaction with the treatment and tolerability were obtained. According to the patient's own decision, which was taken prior to the injection, patients were classified into two groups: those for which the viscosupplementation was the last resort before total hip arthroplasty (THA group) and those who would not consider surgery in the short term (Non Surgery- NS group). Tolerability was very good/good in 165 patients (86.4%), moderate in 14 (7.3%) and poor in 12 (6.3%) cases. In the total population, the percentage of patients very satisfied/satisfied and not really satisfied/not satisfied with the treatment was 24.6%, 27.7% and 22.5% and 25.1% respectively. The efficacy was considered as very good/good in 51.8%, moderate in 23.6% and poor in 24.6% of the cases respectively. Efficacy was unrelated to gender, age, and guidance but was highly correlated with pain on LS ( $p < 0.0001$ ). Efficacy was significantly different with regard to the clinical severity: 66.6% of the NS group patients were satisfied with the treatment versus only 25% of those belonging to the THA group ( $p < 0.0001$ ). In satisfied patients the decrease of analgesics/NSAIDs consumption was  $>75\%$  in 60.5% of cases. These data suggest that despite HANOX M-XL is a safe and efficient intra-articular treatment of hip OA, it is not a valuable alternative to surgery in advanced disease.

Osteoarthritis (OA) is the most common musculoskeletal condition and one of the major cause of disablement in elderly. As a cause of disability affecting large joints, hip OA is second only to knee OA and its prevalence is estimated between 3 and 11

% in subjects aged over 35 years (1, 2)

Current treatment for hip OA is made of a combination of non-pharmacological and pharmacological modalities (3, 6) including viscosupplementation (VS). VS is a therapeutic

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consisting in intra-articular injections of hyaluronic acid (HA) or its derivatives (7). The aim of VS is to reduce joint pain and improve function, possibly by restoring the physiological and rheological state of arthritic joints (7). *In vitro* and *in vivo* studies have also suggested that HA could have protective effects on cartilage (8-12). VS is widely used in the symptomatic treatment of knee OA (13) and several studies have suggested it could also be useful as a safe and well tolerated adjuvant treatment in patients with symptomatic hip OA, not adequately relieved with conventional therapy (analgesics, non-steroidal anti-inflammatory drugs –NSAIDs, physiotherapy) (14, 15). However clinical trials showed controversial results regarding the effectiveness of VS in hip OA, a number of open-label trials have reported promising results (16-20), though no definitive conclusions can be drawn from these studies in the absence of a placebo group. On the opposite Qvistgaard et al (21) as well as Richette et al (22) did not show evidence of efficacy in randomised double blind, placebo-controlled trials. Nevertheless there are several possible explanations for these conflicting results. These could result from the difference of design of the studies (small samples with lack of statistical power), the choice of the regimen (single or serial injections), possible differences of efficacy between HA derivatives (linear or cross-linked, HA concentration and molecular weight) (13), and/or from the selected population (moderate to severe disease). Furthermore, if the efficacy is usefully observed in about 50% of the patients, no predictive factors of response has been clearly identified, excepted, in some studies, the Kellgren-Lawrence grade (23, 24).

HANOX-M-XL (marketed as HappyCross®, Laboratoire LABRHA, Lyon, France) is a new viscosupplement, specifically designed to treat hip OA, that combines a high molecular weight cross-linked sodium hyaluronate (15.5g/L) of non-animal origin with a high concentration (3.5%) of mannitol, a polyol known for its antioxidant properties by scavenging radical oxygen species (ROS). The *in vitro* effectiveness of mannitol to protect HA against ROS mediated depolymerization has been widely demonstrated (25, 26) suggesting that the addition of mannitol to HA might increase the intra-articular residence time of the latter and consequently might

allow to use a single injection regimen. The aim of the survey was to assess the efficacy of a single injection of HANOX-M-XL in patients with symptomatic hip OA in daily practice, by comparing those in who viscosupplementation was the last resort before total hip replacement and those with a less severe symptomatology, irrespective to the radiological grade of the disease.

## MATERIALS AND METHODS

One hundred and ninety-one patients, who have been referred to a rheumatologist for symptomatic hip OA, and who received a single injection of HANOX-M-XL into the hip joint within the 6 previous months were contacted by phone and were interviewed using a 10-item standardized questionnaire. Only 1 patient refused to answer the interview. Demographic data (gender, age), analgesic or NSAIDs consumption, imaging guidance (fluoroscopy, ultrasonography or no guidance), pain on a 10 point Likert scale (LS), patient's self-evaluation of efficacy using a 4 point LS, satisfaction with the treatment (4 pt LS) and tolerability (4 pt LS) were obtained. The goal of the study was to assess whether viscosupplementation could be a valuable alternative to hip replacement in patients who did not wish to be operated or in those who were on waiting list for surgery. Consequently patients were classified into two groups, according to the patient's answer to the question: "Before viscosupplementation, was your own decision taken, to have surgery in case of treatment failure?". The THA group consisted of patients for whom the viscosupplementation was the last resort before total hip arthroplasty; The No Surgery -NS group-consisted of those who would not consider surgery in the short term. The classification was made according to the patient and physician decision irrespective to the radiological features. The patients gave the informed consent prior being included into the survey. The survey was performed in agreement with the French Conseil National Informatique et libertés (CNIL N°1583599V0).

The patients gave informed consent prior being included into the study. The study was authorized by the local ethical committee and was performed in accordance with the Ethical standards of the 1964 Declaration of Helsinki as revised in 2000.

A descriptive analysis was performed on all the collected data. Qualitative variables were described using frequencies and percentages. Quantitative variables were describes using mean, standard deviation and some characteristics of their distribution (minimum, maximum and median). Univariate analysis was performed using chi-2 test or Fischer's exact test, or Mann-Whitney test as

appropriate. All statistical tests were carried out two tailed at the 5% level of significance. The statistical analysis was carried out using Statview software version 5.0 (SAS institute Inc).

## RESULTS

Data are summarized in Table I. Among the 191 patients, 82 were men and 109 women. Patients mean age (SD) was 65.2 (12.7) and the average follow-up since the injection was 15.3 (11.7) weeks. Intra-articular injection was performed under fluoroscopic guidance in 167 cases and ultrasonography in 24 cases. Sixty-six percent of the subjects were analgesics and/or NSAIDs regular users. Patients from the THA group (N= 68) were more frequently men than women ( $p=0.004$ ) and NSAIDs consumers (75% versus 60.9%,  $p=0.002$ ) but did not differ significantly regarding age ( $p=0.25$ ) compared with those of the NS group (N= 123).

In the total population, the percentage of patients very satisfied/satisfied/not really satisfied/not satisfied at all with the treatment was 24.6%, 27.7%, 22.5% and 25.1% respectively. The efficacy was considered as very good/good in 51.8%, moderate in 23.6% and poor in 24.6% of the cases respectively.

Efficacy was unrelated to sex ( $p=0.29$ ), age ( $p=0.53$ ), and guidance ( $p=0.20$ ). Efficacy was highly

correlated with pain on LS ( $p<0.0001$ ) at the time of interview. In satisfied patients the average pain on LS (SD) was 3.0 (2.3) while it was 7.6 (1.9) in not satisfied subjects. In satisfied patients the patients self-assessed decrease of analgesics/NSAIDs consumption was >50% in 79% and >75% in 60.5% of the cases. When patients were stratified according the surgical decision, 66.6% of the NS group patients were satisfied with the treatment versus only 25% of those belonging to the THA group ( $p<0.0001$ ).

Tolerability was very good/good in 165 patients (86.4%), moderate in 14 (7.3%) and poor in 12 (6.3%) cases, without any difference between THA and NS groups ( $p=0.95$ ). The only reported side-effect was a transient increase of hip pain, that lasted from 1 to 7 days after the injection, unrelated to the imaging guidance ( $p=0.39$ ).

## DISCUSSION

Despite its limitations, which will be discussed below, this retrospective survey provides interesting data. Indeed, they come from real life but not from a particular recruitment and effectiveness as tolerance are evaluated by patients themselves. The average age was consistent with that of usual clinical trials (19, 20, 22) and the patients self-reported success rate, self- about 50% - was similar to that obtained

**Table I.** Characteristics of 191 patients with hip osteoarthritis treated with a single injection of HANOX-M-XL.

Population	All patients N=191	No Surgery N=123	THR N=68	p NS vsTHR
Age (SD)	65.2 (12.7)	65.7 (13.4)	64.5 (9.9)	0.25
Gender (M/F)	82/109	48/75	34/34	0.004
VAS pain mm ( SD)	5.3 (3.0)	4.4 (3.02)	6.8 (2.4)	<0.0001
Time since injection (weeks)	15.3 (11.7)	15.2 (12.8)	15.5 (8.9)	0.64
Guidance (Fluo/US)	167/24	106/17	61/7	0.94
Satisfied (Yes/No)	100/91	82/41	18/50	<0.0001
Efficacy (Yes/No)	99/92	82/41	17/51	<0.0001
NSAIDs/ analgesics baseline (Yes/No)	126/65	51/45	51/17	0.002
Tolerability (good/moderate/poor)	165/14/12	107/10/6	58/4/6	0.95

THR: total hip replacement; VAS: Visual analogue scale; Fluo: fluoroscopy; US: Ultra-sound; NSAIDs: Non steroidal anti inflammatory drugs.

in an open-label study with hylan GF-20, in which the percentage of responders according to the OMERACT-OARSI response criteria was 53.6% (24). The main lesson to be drawn from this survey is the very low rate of satisfaction with the treatments in the group of patients in whom viscosupplementation was considered as the last resort before total hip replacement. Only 1 out of 4 patients waiting for surgery was satisfied with viscosupplementation. In contrast, those who do not consider surgery in the short term had a high satisfaction rate, similar to that of patients fulfilling the Minimal Clinically Important Improvement (27) in an uncontrolled trial, performed in patients with mild to moderate hip OA (20). On the other hand, in patients with less severe disease, the results of viscosupplementation were satisfactory in two third of the patients. Furthermore those who were regularly taking NSAIDs and who were satisfied with the treatment, have dramatically reduced their NSAIDs consumption in more than 3/4 of cases. The main limitations of the present study is the lack of radiographic data and the fact to classify patients according to the future decision to have surgery or not. However, radiographic examination, although essential for the surgeon for the pre-operative planning and for the choice of the type of prosthesis, often does not provide essential information in the surgical decision since it has been demonstrated that the radiological severity is not related to the functional impairment (28). Patient's willingness to undergo surgery is, of course, mainly due to pain and functional impairment but other parameters are to be taken in consideration such as their socio-economic status, their age, geographical variation in access to joint replacement, the patient lifestyle, and chiefly the advice of their doctor (28-30). If age is considered as a complex factor in predicting whether patients will undergo arthroplasty and previous studies have found that subjects older than 65 to be less willing to operate (30), we did not find any difference regarding age in patients waiting or not for surgery. In our population, men were more likely than women to say they want to have surgery. This is consistent with data of the literature showing that women older than 54 had lower rates of surgery than men of the same age and similar self-reported co-morbidities, and are less likely to have discussed the option of arthroplasty with a primary care doctor

(30, 31). The failure of treatment in advanced forms of arthritis can be easily explained although the mechanisms by which viscosupplementation reduces pain are still imperfectly known. It seems that hyaluronic acid is acting by reducing the cartilage matrix degradation (32-37) and prostaglandin E2 (38, 39) and bradikinin production (39) and consequently by decreasing the reactional synovitis. Now osteoarthritic pain is not only related to inflammatory processes and there is evidence for the sensitization of pain pathways, involving changes in both joint nociceptors and nociceptive processing in the spinal cord, brainstem, and thalamocortical system (40), particularly at late stages of the disease. Herein, HA does not seem to be able to act on these components. In summary, the present survey showed that three months after a single injection of HANOX M-XL in the target hip of patients with hip OA, more than 1 patient out of 2 was satisfied with the treatment, with no particular or unexpected safety concern. The proportion of satisfied patients reached 66% in subjects with moderate disease, while it was only of 15.5% in those waiting for hip replacement. These data suggest that HANOX M-XL is a safe and efficient intra-articular treatment of hip OA but must not to be considered as an alternative to surgery in advanced disease. A large scale prospective trial with MRI and radiological assessment is already in progress to determine with certainty the predictive factors of response to HA injection in patients with hip OA.

Conflict of interest: One author received fees as a consultant and/or board member from Labrha, Anteis, Genevrier, Sanofi-Genzyme.

All the other authors declare no conflict of interest

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## ADIPOSE MESENCHYMAL STEM CELLS AND “REGENERATIVE ADIPOSE TISSUE GRAFT” (LIPOGEMS™) FOR MUSCULOSKELETAL REGENERATION

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Regenerative medicine is a high-potential sector of strategic developments in medicine and health industry. The perspective to cure diseases up to now relied on medical treatments of long duration and limited effectiveness, and the possibility to avoid organ transplantation renders regenerative medicine attractive. In recent years, basic and translation research held great hope for this new field with significant progress in the modulation of stem cell commitment *in vitro* and providing protocols for targeted clinical applications. In line with this approach, mesenchymal stem cells (MSCs) have been introduced as potential therapeutic tools to correct the breakdown of musculoskeletal disorders. MSCs are able to secrete a large number of trophic factors capable of repairing the recipient tissue through angiogenic, anti-apoptotic and anti-fibrotic mechanisms. In this context, adipose tissue is emerging as a clinically relevant and easy to harvest source of multipotent progenitors to develop regenerative therapies. The present review focuses on the clinical application of MSCs, and in particular of adipose-derived stem cells, in the musculoskeletal disorders and on the current scientific challenges. In this perspective, we discuss future developments of an innovative system (Lipogems) for musculoskeletal regeneration, yielding a non-expanded and ready-to-use microfractured fat tissue product harbouring MSCs and pericytes within a preserved stromal vascular niche. The Lipogems system may also pave the way for future off-the-shelf and large-scale approaches for reconstructive procedures and regenerative medicine.

Musculoskeletal diseases are common conditions, including more than 150 different diseases. According to the World Health Organization, these disorders are the most common cause of severe long-term pain and physical disability (1). With aging of the population, the incidence of musculoskeletal diseases is rising and will be a significant socio-economic burden on society.

The complex nature of these conditions generally means that treatment options are limited to managing symptoms, rather than prevention and cure (2). Therefore, there is an urgent need to develop new and effective therapeutic approaches for these age-related disabling pathologies. In this context, great strides have been made in regenerative therapies thanks to

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stem cell technology. More in detail, recent research has extensively shown the potential of mesenchymal stem cells (MSCs), a class of multipotent stromal stem cells, for reparative/regenerative medicine even in musculoskeletal system (3). Indeed, MSCs isolated from various tissues can differentiate into relevant cell types, thus representing an attractive option for cell-based therapy (4). Moreover, MSCs hold a great promise in tissue restoration thanks to their ability to secrete a wide range of growth factors, which have trophic effects on surrounding host cells, stimulating reparative responses (5). Furthermore, MSCs injected in pathological tissues contribute to favour physiological process by acting as reservoirs of repair cells or immunomodulatory sentinel to reduce inflammation (6). Several early-stage clinical trials are testing the delivery of MSCs in musculoskeletal disease, such as tendon injury, knee osteoarthritis, rheumatoid arthritis. However, despite the excellent potential of MSCs in regenerative medicine, many challenge must be overcome before they can be clinically used (7). In particular, it is essential to standardize protocols of isolation, expansion, and transplantation and to better understand MSC biology. The present review focuses on the clinical application of MSCs in the musculoskeletal disorders, and on the current scientific challenges, in which the difficulty of ex vivo expansion and the complexity of the current Good Manufacturing Practice (cGMP) requirements for expanded cells prompt the development of novel approaches in the autologous use of MSCs. In this perspective, we discuss future developments of an innovative system (Lipogems) for musculoskeletal regeneration, yielding a non-expanded and ready-to-use microfractured fat tissue product harbouring MSCs and pericytes within a preserved stromal vascular niche.

## MESENCHYMAL STEM CELLS

### *Biology*

Even if the exact location and function in the tissue of origin is not fully understood, mesenchymal stem cells are defined as multipotent and self-renewable cells with the ability in vitro to adhere to plastic and to differentiate into multiple lineages, including osteogenic, chondrogenic and adipogenic ones (8). Indeed, the International Society for Cellular Therapy

define MSCs as a heterogeneous population of progenitor cells expressing a pattern of characteristic, but not specific, surface markers, including CD73, CD90, and CD105, but lacking the expression of hematopoietic markers CD34, CD45, CD14 or CD11, CD79a or CD19, and HLA class II (4). MSCs can be extracted from several body districts, including the adipose and synovial tissues, peripheral blood, skeletal muscle, umbilical cord blood, placenta, and bone marrow. However, an optimal source of MSCs in tissue regeneration and repair has not been yet identified. Indeed, MSCs deriving from different sources have similar, but not equal ability to differentiate into a specific kind of specialized cells. Moreover, the number of cells obtained may depend on the donor age and co-morbid conditions.

### *Secretoma and stromal-vascular niche*

The participation of MSCs in tissue regeneration has been largely investigated according to the notion that these cells can themselves differentiate into some cell types, including bone, cartilage, muscle, adipocytes, stroma, fibroblasts and endothelial cells (9-11). Recent studies suggest that MSCs could participate in tissue repair, not only differentiating into cells of the target tissue, but also releasing several factors, contributing to restorative processes, including angiogenic ones (11-13). Indeed, the secreted trophic factors, participate to tissue rescue through pro-angiogenic and anti-fibrotic mechanisms (14-20), anti-inflammatory and immunomodulatory properties (21-29), anti-apoptotic and antimicrobial characteristics (30-38). Recent studies show a direct correlation between the occurrence of MSCs and the blood vessel density in stromal vascularized tissues (14,39,40). The niche is the morpho-functional unit where stem cells live and reproduce themselves. It is a particular kind of tissue within each tissue, in which a huge network of messages is fashioned through the product of the overall paracrine activity of the embedded cells, the so-called "secretome" (41,42). The regenerative potency of MSCs depends mostly on their ability to afford a timely modulation in the composition of the secretome (43). Further understanding of the molecular pathways involved in growth factor production will be very helpful to develop better strategies for MSC-based therapies. In this new vision, the preservation of the niche is fundamental to consider MSCs as a patient-

specific “molecular biology laboratory” adapting over time to the environmental cues released by the injured tissues (44).

#### *Immunomodulatory effects*

One of the most interesting characteristic of MSCs is their inherent immunomodulatory properties. When transplanted *in vivo*, MSCs do not elicit an immune response, allowing them to be used in allogenic stem cell therapy. In particular, they produce anti-inflammatory cytokines and suppress the proliferation, differentiation and function of immune cells *in vitro* (45). To take full advantage of the unique properties of MSCs for tissue engineering applications, a critical issue will be gaining further insights on the mechanisms controlling their self-renewal and differentiation, which will potentially result in the chance to modulate the behaviour of these cells for therapeutic purposes. Several characteristics of MSCs are purported to impart immune privilege, thereby allowing MSCs to avoid immune rejection in certain situations, which may facilitate the clinical use of allogenic MSCs. MSCs do not express class II Major Histocompatibility Complex (MHC) or costimulator molecules and express low levels of class I MHC (46). One of the first evidence of MSCs role in modulating immune reactions shows that activated MSCs inhibit T-cell expansion in mixed lymphocyte reactions (47). Moreover, MSCs influence the immune system through the secretion of a variety of soluble factors including indoleamine 2,3-dioxygenase (48), nitric oxide (49), transforming growth factor beta (TGF- $\beta$ ), prostaglandin E2 (PGE2) (50), and tumor necrosis factor stimulated gene- Protein (TSG-6) (51). Therefore, in addition to the direct regenerative effect of transplanted MSCs, these cells could interfere with microenvironment to stimulate physiological regeneration. However the precise mechanism of MSC action is not fully elucidate. A deeper comprehension of MSC biology both *in vitro* and *in vivo* could lead to the development of trustworthy strategies for clinical application of MSCs.

#### ADIPOSE MESENCHYMAL STEM CELLS

Adipose tissue is a connective tissue derived from embryonic mesoderm, consisting of a heterogeneous population of cells like adipocytes, preadipocytes,

smooth muscle cells, endothelial cells, mast cells, fibroblasts immune cells. About the 10% of the adipocyte population is annually renewed (52). From adipose tissue manipulation it is possible to isolate the so called stromal vascular fraction (SVF), containing, among others, mesenchymal stem cells (53). SVF is easy harvested with minimal donor site morbidity commonly through lipoaspiration followed by *in vitro* cell isolation (54). The efficiency of SVF isolation is strictly related to donor general condition, such as age and obesity (55). In addition, compared to bone marrow, 1 g of adipose tissue contains 500 times more pluripotent cells than 1 g of bone marrow aspirate (54,56). Moreover, besides showing phenotypic and transcriptional profiles similar to that of the other MSCs, ADSCs present some peculiar characteristics. In particular, ADSCs express CD34 glycoprotein (57), stromal markers (CD13, CD29, CD44, CD63, CD73, CD90, and CD166) and endothelial cell markers (CD31, CD144, VEGFR2, and von Willebrand factor) (58). Moreover, ADSCs have a higher yield upon isolation and a greater proliferative rate in culture when compare with MSCs isolated from other sources (59,60). Of note, it has been demonstrated that these cells, besides the multiple mesodermal potential, are also able to differentiate in cells of ectodermal and endodermal origin, considering them as pluripotent stem cells (54,61). Therefore, adipose tissue represents a promising and clinically relevant source of multipotent progenitors to develop regenerative therapies. However, some challenges must be overcome before their use in tissue engineering (7). One of the most relevant problem in the use of ADSCs is their expansion for several weeks *in vitro* prior implantation, that can modify their pluripotent potential and precluding their use in emergency circumstances (62). Moreover, emerging evidence indicates that culturing ADSCs the risk of infection, immunogenicity, genetic instability, and tumorigenicity could increase (63,64). Therefore, there is an urgent need to assess the safety of ADSC implantation in humans, and to identify novel protocols for ADSC isolation, with minimal manipulation.

#### APPLICATION OF ADSCS FOR MUSCULOSKELETAL REGENERATION

The potential applications of MSCs in

regenerative medicine, and, in particular, in musculoskeletal regeneration, show encouraging results for possible clinical applications. However, only few clinical studies use MSC-based approaches for such purposes, due to medical, research, and regulatory reasons (65). However, due to the lack of specific MSC markers, little is known on the *in vivo* ability of MSCs to differentiate. Indeed, different *in vivo* studies show that MSCs could participate to regeneration in various injury models, however, any evidence of clonal expansion, the true differentiation, and regenerative potential does not exist. Therefore, more studies are necessary to elucidate the mechanisms and biological properties of MSCs in determining their therapeutic efficacy in various diseases. Here we summarize the current application of MSCs for musculoskeletal diseases.

#### *Cartilage repair*

Cartilage repair represents a clinical challenge due to the intrinsic limited ability to repair itself. The current treatment of cartilage defects includes intra-articular administration of hyaluronic acid, treatment with platelet-rich-plasma, that contains bioactive proteins such as chemokines, cytokines and growth factors, and bone marrow stimulation, that include subchondral drilling, abrasion, and microfracture. Up to now, the only approved cellular-based therapy for cartilage restoration is based on the autologous chondrocyte implantation after *in vitro* expansion (66). Despite the promising initial results, the limited expansion of chondrocytes *ex vivo*, and donor site morbidity limit the beneficial effects of this technique (66,67). Alternative cellular therapies focused on progenitor cell populations bone marrow stem cells, that once transplanted ameliorated pain and movement ability (68,69). Recently, ADSCs are emerging as a less invasive source of progenitors that can be differentiated into chondrocytes *in vitro* in a 3-dimensional environment (70), in presence of growth factors (TGF- $\beta$  superfamily) (71) and then implanted to restore cartilage tissue (72). Moreover, it has been observed that also uninduced ADSCs were able to fully restore cartilage in ear auricle defects (73) and in patellofemoral joints (74). These promising data suggest, therefore, that a minimal *ex vivo* manipulation of ADSCs, thanks to the intrinsic ability of these cells to adapt to their environment

*in vivo*, could allow the development of an easy and effective clinical treatment to restore cartilage defects.

#### *Bone regeneration*

Bone graft is extensively used in a wide range of orthopaedic, plastic oral, dental, and neurocranial surgical procedures (75). However, the use of autograft bone is often associated to morbidity and scarce regenerative properties. Indeed the harvest of bone is associated to pain, haematoma formation, infections, and fractures (76). Moreover, aging reduce the availability of bone marrow stem cells (77). Therefore, research efforts in the field of orthopedics have been directed to ameliorate treatments for reconstructing bony defects, including traumas, tumors, infections, aseptic loosening or nonunions. These investigations have led to the development of improved treatments with strong osteogenic potency, as the recombinant bone morphogenetic proteins (BMPs). BMPs induce and promote critical steps of mostly endochondral bone formation and have been approved for clinical use in spinal fusion, non-unions and severely compromised long bone fractures. However, BMP technology has some therapeutic limitations when the microenvironment is compromised with poor or no vascularization. Therefore, to improve the therapeutic efficacy of this method, BMP administration has been successfully applied together with biomaterials and stem cells (78). In this regard, ADSCs, thanks to their osteogenic potential, have proven as good candidates both *in vitro* and *in vivo* (79,80). Moreover, growing evidence indicate that ADSCs alone, in absence of exogenous growth factors, are able to restore bone defects. In particular, it has been shown that ADSC transplantation could represent a good therapeutic option to treat craniofacial defects (81-83). Surprising results were obtained in the repair of the calvarium, that is unable to ossify after the first two years of life. ADSCs, administered in presence of milled autologous cancellous bone and fibrin glue, or seeded in  $\beta$ -tricalcium phosphate granules, were able to completely repair calvarial defect, resulting in new bone formation and near complete ossification of the preoperative defect (81,83). However, the use of multiple concomitant treatments limits the comprehension of the therapeutic effect

of ADSCs. Indeed, implanted cells could enhance bone regeneration through direct differentiation into mature osteoblasts and by paracrine effects, releasing osteogenic and vasculogenic molecules and growth factors, which facilitate migration and differentiation of resident precursors (84). Nevertheless the promising results obtained with ADSCs in bony defects restorations suggest that cell therapy represents an attractive alternative to traditional treatments like core decompression, osteotomy and total joint replacement, and provide a relatively simple method of autologous bony reconstruction with little donor site morbidity.

### *Arthritides*

Arthritides, such as rheumatoid arthritis or osteoarthritis, are systemic and disabling autoimmune pathologies characterized by chronic joint inflammation and bone erosion. The crucial process underlying arthritides initiation is the abnormal activation of dendritic cells, T cells, B cells, macrophages, and neutrophils (85). The current treatments for these diseases are mainly addressed to manage symptoms and inflammation. However, a treatment that solves definitively the arthritides does not exist. Some studies focused their attention on cell-based approaches, taking into account that is a systemic defect, differently from focal articular defects, and the underlying disease process. In this context, MSCs, as previously mentioned, hold an immunoregulatory capacity, and elicit immunosuppressive effects, that can be used for several autoimmune diseases, including arthritides. In vitro studies on MSCs derived from umbilical cord demonstrated an anti-proliferative effect on synoviocytes, key players in inflammation and joint destruction in rheumatoid arthritis. This effect was mediated by the release of IL-10, 1-methyl-DLtryptophan, TGF- $\beta$ 1, and by the down-regulation of mixed metalloproteinase 9 (86). These results are in line with the in vivo studies on an arthritis rodent model, in which ADSC systemic injection reduced the levels of inflammatory cytokines and chemokines and the ratios of Th1/Th17 cells. Moreover, ADSC transplantation was able to increase the production of the anti-inflammatory IL-10 in lymph nodes and joints, and de novo generation of antigen-specific Treg cells (87,88). Similar results were obtained

in the same mouse model after the intraperitoneal infusion of umbilical cord-derived MSCs: arthritis severity was reduced, the levels of proinflammatory cytokines and chemokines were decreased, and IL-10 levels were increased (86). Therefore, taking together these data suggest that ADSCs could ameliorate Arthritis pathogenesis decreasing the production of inflammatory cytokines and activating Treg cells. However, contradictory data was reported in adjuvant-induced and spontaneous arthritis model in which MSCs were effective only when injected before disease onset (89). Therefore, the anti-inflammatory function of stem cells may be effective in preventing or delaying arthritis if delivered at early stages of the disease process. More compelling investigations are needed to improve the specific targeting and the retention of MSCs at the cartilage surface in order to maximize their potential effect.

### *Tendon*

Tendon injuries are often associated with significant dysfunction and disability, due to the limited self-repair capacity and propensity for scar formation of tendinous tissue. Despite the improvements in conventional treatment, such as transplant, clinical outcomes in tendon treatment are still variable. Moreover the use of allograft can lead to immune response and rejection (90), and the use of autografts is related to donor-site morbidity (91). Therefore, new strategies have been devised, such as tissue engineering techniques. Considering the fact that fibroblasts are involved in tendon healing by producing collagen, in an initial pilot study in humans, ex vivo expanded autologous dermal fibroblasts have been successfully used for refractory lateral elbow epicondylitis (92). Recently, with the progression in MSC characteristic identification, tissue engineering relying on these cells has been proposed to enhance tendon healing. In a rat experimental model of patellar tendon window defects, the injection of bone marrow stromal cells in a liquid fibrin matrix stimulated histological, ultrastructural, molecular, and biomechanical parameters of patellar tendon healing, whereas injection of fibroblasts in fibrin matrix had only minor effects (93). Others in vivo studies showed that bone marrow MSCs were effective on tendon-bone healing. In a model of rat Achille's tendon damage, in which the enthesis

(bone-tendon junction) was destroyed, the effect of chondrocytes or bone marrow MSCs were compared with not treated rats. Noteworthy, the bone marrow MSCs group showed an enthesis most similar to the pre-morbid state (94). Similar results were obtained in rabbits undergoing anterior cruciate ligament repair (95). These studies support the application of cell-based therapies for the regeneration of tendon tissues. However, these strategies have been investigated only in pre-clinical studies and the role of stem cells needs to be confirmed.

#### THE CHALLENGE OFFERED BY LIPOGEMS™-DERIVED ADSCs

Lipogems (PCT/IB2011/052204), represent a new completely closed tool to harvest, wash, process, and reinject human (or animal) lipoaspirates. Briefly, the surgical procedure consists in two steps: the infiltration step, in which adrenalin, in a saline solution, and very diluted lidocaine are injected to induce vasoconstriction and local anesthesia, facilitating the subsequent lipoaspiration; the aspiration step, in which a standard liposuction technique is performed. Afterwards, lipoaspirate is processed, by mild mechanical forces, passing floating adipose clusters through different reduction filters 59. Lipogems product is, therefore, a fat tissue derivative minimally manipulated that can be readily injected in an autologous fashion. The fact that Lipogems product is composed of completely normal pericytes (although beginning to detach from the vessels as in NORMAL response to trauma injury from the washing and cluster reduction process) and no cell expansion is done before injection is an extremely safe issue that make this technique just as a standard fat grafting but with less concern regarding the presence of oil and blood contaminations and with smaller size tissue clusters which makes the whole procedure less traumatic.

This device ameliorates the classical Coleman lipofilling technique (96), eliminating oil and blood residues lipoaspirate and reducing the size of the clusters of adipose tissue (97). These technical improvements have important biologic implications. In islet transplantation for diabetes treatment, the density of loading transplanted tissue and the size of clusters are important predictors of cell transplant

outcome, in terms of inflammation, vascularization, and, therefore, in post-transplant engraftment (98). These promising results are probably due to the gentle fat tissue harvesting technique used in Lipogems. Differently from the lipoaspirate, which causes an imbalance in the cytoarchitectonics, Lipogems technique is not traumatic for cells and preserves vascular/stromal architecture with a high percentages of mature and a low amount of hematopoietic-like elements.

In an effort to characterized MSCs resident in fat tissue derivative, it has been observed that Lipogems-derived cells and lipoaspirate cells show a similar tissue architecture. Indeed, any differences in vimentin and fatty acid binding protein 4 distribution, as markers of adipose tissue, and in Ki-67 positivity, as marker of proliferation, were observed (99). Moreover, Bianchi and coworkers demonstrated that the in vitro culture of Lipogems derivatives allows the isolation of a population of ADSCs with a high degree of purity, than Lipoaspirate. These cells fulfilled the definition of mesenchymal stem cells, being able to differentiate mesoderm-type of cells, including osteogenic, chondrogenic, and adipogenic ones, like lipoaspirate cells (59). Nevertheless, intriguingly, Lipogems procedure was able to promote the expression of different antigens and to favour the multipotent potential with respect to lipoaspirate cells. In this regard, Carelli and colleagues demonstrated that Lipogems-ADSCs show a higher expression of self-renewal antigens, including OCT4, SOX2, NANOG, and of neural phenotype genes, such as  $\beta$ -tubulin III, NEUROD1, PAX6, and SOX3 (99). Moreover, Lipogems-derived ADSCs, in response to pro-vasculogenic molecules, show a more pronounced expression of angiogenic genes, such as VEGF, KDR, and HGF (59). Furthermore, the treatment with a radio electric asymmetric conveyed field, was able to promote the transcription of genes involved in the commitment towards cardiac, vascular, neuronal, and skeletal muscle lineages, and of stemness related genes, including Nanog, Sox-2, and Oct-4 (100). The possibility to obtain a final cell product containing viable adipocyte, preadipocyte, and stem cells, eliminating problems related to enzymatic digestion and other manipulations (8), exhibits a great appeal not only for its application in plastic

and reconstructive medicine, but also in research, and regenerative medicine. Differently from lipoaspirate, the Lipogems product preserves the stromal vascular fraction also after cryopreservation, bypassing the difficulty of *ex vivo* expansion and the complexity of current Good Manufacturing Practice requirements for expanded cells. Of interest, the “earlier” cryopreservation of adipose tissue-derived stem cells represents an attractive challenge to treat different degenerative age-related pathologies, as younger adipose tissue progenitors and stem cells have a higher regenerative, tissue remodelling, and therapeutic potential (97,101,102). Based on its properties, the Lipogems product may pave the way to novel approaches and paradigms in the rescue of diseased tissues, within the context of both a personalized and a large-scale regenerative medicine (8).

### CONCLUSIONS

Understanding the dynamics that regulate MSCs homeostasis, especially their anti-inflammatory effect and immunomodulatory capacity, has led to challenge a number of consolidated beliefs on their therapeutic mechanisms. Today, EMA (European Medicines Agency) and FDA (Food and Drug Administration), which had in the past significantly different views in conceiving stem cell biology, efficacy and storage conditions, have developed very similar rules. Nevertheless, these rules are constantly changing, and this is the reason why both Regulatory Agencies refers to “current Good Manufacturing Practice (cGMP)” as the set of guidelines and regulations that should dynamically ensure the currently best available standard for manufacturing medicinal products, including stem cells. However, the emergence of regenerative medicine raises new questions about the best ways to maintain quality. Regenerative medicine therapies involve new kinds of medical products, such as lab-grown or genetically modified cells. A therapy that uses living cells cannot be standardized in the same way as a conventional pill, and this makes sometimes very difficult to make statements and considerations that may remain valid for more than a few months (94,95). In this complex scenario, the challenge offered by Lipogems represents an attractive technique to

obtain minimally manipulated fat tissue product that retains the intact microenvironment in which MSCs live, being amenable for the use in different clinical settings. Moreover, the multipotency of Lipogems-derived MSCs has been shown to be optimized by cell exposure or physical energy providing future off-the-shelf and large scale approaches for reconstructive procedures and regenerative medicine strategy.

**DISCLOSURES.** Prof. C. Tremolada is owner of patents and president of Lipogems International srl.

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## TOPAZ MICRO-RADIOFREQUENCY ABLATION ACHIEVES SIMILAR RESULTS TO ENDOSCOPIC PLANTAR FASCIA RELEASE

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**Plantar fasciitis is usually responsive to conservative treatment. Recalcitrant cases might require surgery. The current study is a retrospective evaluation of two surgical techniques used to treat plantar fasciitis. Endoscopic plantar fascia release and micro-radiofrequency ablation (TOPAZ) were used in our department over the last decade. The patients were all treated conservatively according to a standardized protocol. Failed cases were operated using either technique. The current study evaluates the results of the treatment. There were 14 TOPAZ procedures and 22 endoscopic plantar fascia release. Pain levels declined from  $6.8 \pm 1.5$  to  $1.8 \pm 1.5$  on the VAS scale. The results in both groups were similar. It is possible to conclude that surgical treatment of plantar fasciitis is an efficient procedure with relatively gratifying results. Complications tend to be slightly though not significantly lower in the TOPAZ group.**

Plantar fasciitis lifetime prevalence is approximately 10% of the US population creating a significant burden to the health care delivery system. Approximately 90% of patients diagnosed with plantar fasciitis respond to nonsurgical therapy and do not require surgical intervention (1). However it has a significant deleterious effect on both foot function and general health overall functional score (2). Suspected etiologies (3-5) include obesity, overuse due to walking or running or standing, excessive subtalar pronation, seronegative arthritis, and limited dorsiflexion of the ankle joint. Conservative therapy usually entails the use of foot orthoses, stretching exercises, local corticosteroid injection, oral nonsteroidal anti-inflammatory drugs and other physical therapy modalities, and nonweight-bearing status and rest. The small subset that requires surgery are patients in whom 6 months or more of conservative therapy have not led to remission. Open fasciotomy, has a high

satisfaction rate but a prolonged recovery period (6). Endoscopic plantar fasciotomy allows faster return to regular activities in comparison with open surgical approaches (6). Risks associated with plantar fascia surgery include complex regional pain syndrome (7), nerve damage and lateral column collapse (7, 8). These complications of surgical approaches, create an interest in minimal invasive interventions, these include extracorporeal shockwave therapy that is similar in its efficacy to placebo (9), cryosurgery appears to be somewhat more successful with a 76% success rate (10). The current study was performed in order to try to determine the effectiveness and safety of minimally invasive percutaneous bipolar radiofrequency plantar fasciotomy for the treatment of recalcitrant plantar fasciitis (fasciosis). The study compared a consecutive series of patients treated by either endoscopic assisted fasciotomy or with radiofrequency ablation of the plantar fascia. The study hypothesis was that

*Key words: fasciitis, radiofrequency*

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percutaneous radiofrequency plantar fasciotomy would demonstrate effectiveness without excessive postoperative complications and with rapid recovery in comparison with an endoscopic surgical approach to this common pedal pathology.

## MATERIALS AND METHODS

### *Patient population*

The patients were a consecutive series of patients selected from the authors' surgical records from January 2007 to May 2012 at a public hospital. The diagnosis of recalcitrant plantar fasciitis (fasciosis) was made based on each individual patient's history and physical examination, sonographic evaluation of the plantar fascia, as well as standard foot radiographs. All patients had pain that was at least 5 on the VAS pain scale after a minimum of 6 months of conservative therapy before they had surgery, including plantar fascia rest taping, calf and arch stretching, functional foot orthotics, two local corticosteroid injections, and at least a two weeks course of oral etoricoxib 90 mg per day and topical diclofenac cream 1%. Confirmatory physical findings included 1. focal proximal plantar fascia tenderness upon deep palpation, 2. plantar medial heel pain without distinct neuritic radiation, 3. Thickening of the plantar fascia insertion more than 5 mm on ultrasound. Exclusion criteria entailed positive straight leg raise, signs of peripheral neuropathy such as loss of sensation in the toes or the foot, known systemic arthritis, calcaneal bone tumor, positive tarsal tunnel Tinnel's test.

The surgeons evaluated the participants throughout the postoperative period and procured the data used in the study analyses. Each patient provided answers to the American Orthopaedic Foot & Ankle Society (AOFAS) hindfoot scoring scale (15) and a Visual Analogue Score, and the answers to 2 additional questions were also required of each patient, specifically: "Would you recommend this surgery to a family member or friend?" and "Would you do the surgery over again, if the heel pain returned?"

### *Radiofrequency ablation surgical procedure*

Radiofrequency plantar fasciotomy was performed as an isolated surgical procedure in every case. Before administration of anesthesia, the area of maximum tenderness was marked on the foot. After the area was marked, an ankle block was performed using local anesthetic (20 ml bupivacaine 0.25% and 20 ml esracaine 1%). The foot was then prepped and draped in the usual sterile manner, after which a skin marker was used to draw out a grid of 16 to 20 dots over the proximal

plantar fascia, encompassing the point of maximum tenderness, each mark being 3 to 4 mm apart. Thereafter, an 18-gauge needle was used to puncture through the skin and superficial fascia and subcutaneous fat layers down to the level of the deep fascia. A Mosquito Clamp was used to enlarge the channel and prevent skin injury. After creation of the channels, the bipolar radiofrequency unit (Stryker SERFAS Energy RF Ablation System, San Jose, CA 95138) was set to a power level of 4 for 20 seconds. Thereafter, the probe was inserted sequentially into each needle channel, to the level of the plantar fascia, which is identified by palpable resistance to penetration by the probe. Once the deep fascia was contacted, the radiofrequency energy was applied, and the probe felt to pierce the deep plantar fascia, thereby effecting micro fasciotomy (Fig. 1) while the great toe was held at maximal dorsiflexion to tighten the fascia.

Care was taken to avoid penetration through the intrinsic musculature deep to the plantar fascia, and also to avoid application of the radiofrequency energy to the skin. After completion of the micro fasciotomy, a sterile Coban™ dressing was applied and the patient placed in a postop shoe and allowed to bear weight to tolerance for 4 weeks postoperatively, regular shoe gear and weight-bearing activities were allowed by 4 weeks postop.

### *Endoscopic plantar fasciotomy*

Endoscopic plantar fasciotomy was performed as an isolated surgical procedure in every case. Before administration of anesthesia, the area of maximum tenderness was marked on the foot. After the area was marked, an ankle block was performed using local anesthetic (20 ml bupivacaine 0.25% and 20 ml esracaine 1%). The foot was then prepped and draped in the usual sterile manner. Thereafter, an 18-gauge needle was used to define the suprafascial plane on the medial side of the heel. A medial portal for 2.7 mm arthroscope was created and the camera inserted. A potential space is inflated with 40 ml of saline in order to allow visualization. Camera rotation allows appreciation of an operative muscle filled potential triangular space. The apex is composed of the bony spur, one side is the plantar fascia (internal surface) and the other is the calcaneal bone (inferior surface). Insertion of a hook allows definition of the width of the plantar fascia and measurement of the width of the medial two thirds of the fascia. The hook is retrieved and a meniscotome is inserted to the measured depth. The arthroscope is used to evaluate the completeness of the fascia release. The great toe was held at maximal dorsiflexion to tighten the fascia. Care was taken to avoid penetration through the intrinsic musculature deep to the plantar fascia. After completion of the micro fasciotomy, a single suture is used to close the portal, a sterile dressing was applied and the patient

placed in a postop shoe and allowed to bear weight to tolerance for 4 weeks postoperatively regular shoe gear and weight-bearing activities were allowed by 4 weeks post-op.

#### Statistical analysis

Quantitative variables were compared using the Analyse-it<sup>3</sup> software program. The Student's *t*-test was used, and a significant difference was defined at the 0.05 level.

## RESULTS

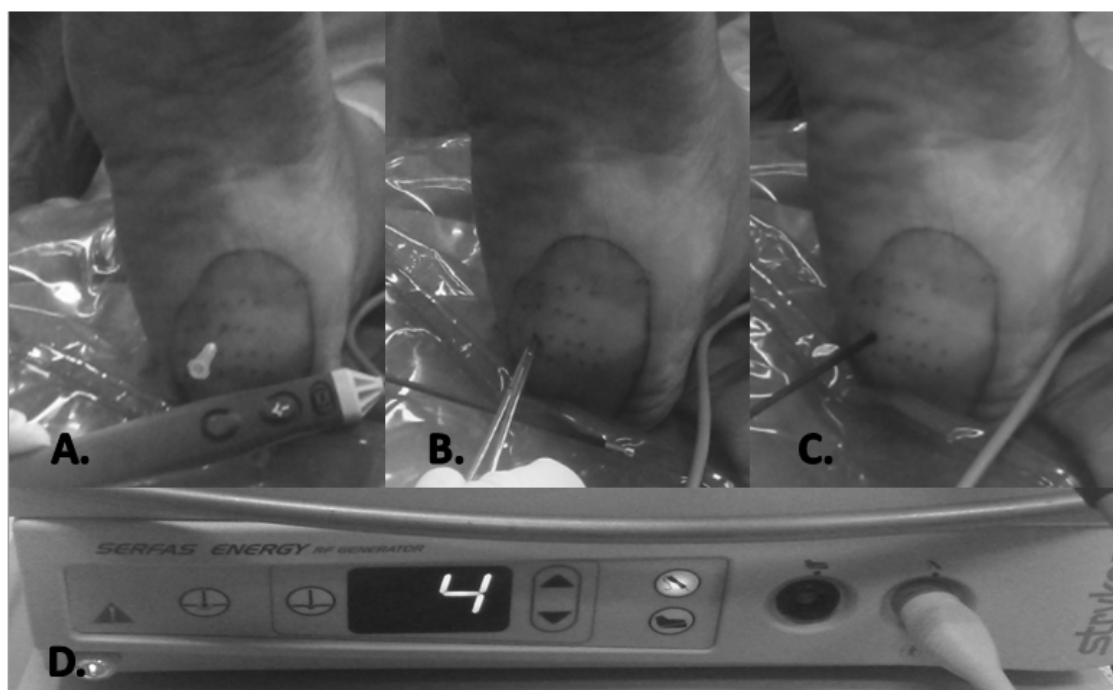
A total of 36 eligible patients were identified and their records assessed in this retrospective study. All patients were followed up at the foot & ankle clinic. A statistical description of the results is shown in Table I. The mean age of the patients was 55 years (range, 25–84 years). In regard to gender, 29 of the patients were female (81%) and 7 (19%) were male. No bilateral procedures were performed however six

patients (20%) underwent the intervention on both feet each at a separate surgical setting (with at least a 3-months inter-procedure temporal displacement). The mean duration of pre-operative symptoms was 9 months (range, 6–36 months). The mean duration of postoperative follow-up was 30 months (range 24-50 months), and 35/36 of the cases returned to regular shoe gear by 4 weeks after the operation (the one exception was an endoscopic release case with nerve injury). In regard to the outcome measures, the overall mean of both groups preoperative AOFAS hindfoot-ankle score was  $57 \pm 13$  (range, 42–84). The pre-operative VAS score (0=no pain, 10=maximal possible pain) was  $6.8 \pm 1.5$  (range, 9-5). At the latest follow-up visit (minimum 24 months) the average AOFAS score was  $94.6 \pm 6$  (range, 100-73) and the VAS was  $1.8 \pm 1.5$  (range, 5-0). Regarding recommendation to friends and family, 29 (81%) of the patients indicated that they would recommend the procedure to a family member or friend, and

**Table I.** Demographic data of patients undergoing plantar fascia release and results.

Parameter	Endoscopic Group	Micro-radiofrequency Ablation Group	Statistical Significance
Patient Number	22	14	
Age (years±S.D.)	51.5±7	61±8	n.s.
BMI	33±7	36±9	n.s.
Gender	18% male	21% male	n.s.
Pre-Op AFAOS Score	63±11	48±10	p<0.002
Pre-Op VAS pain Severity Score (10-maximal pain)	6.1±2.1	7.2±1.8	p<0.005
24 Months AFAOS Score	95±5	92±6	n.s.
24 Months VAS pain Severity Score	1.9±1.3	1.5±0.8	n.s.
AFAOS Score Delta Change	31±9.8	43±8.6	P<0.001
% of patients who recommend the procedure to a friend	81%	68%	
% of patients who would choose to undergo the procedure again if symptoms recur	78%	64%	

BMI: Body Mass Index; VAS: Visual Analogue Scale: 0=no pain, 10 maximal pain.



**Fig. 1.** The operative sequence of the TOPAZ radiofrequency micro-fasciotomy consists of marking the painful area (A) and filling the area with dots with about 4 mm separation, an 18 gauge needle is used to penetrate the skin at every dot. To minimize the chance for skin burn a mosquito clamp is inserted (B), then the radiofrequency probe is inserted (C) at number 4 setting (D) Serfas system, by Stryker).

24 (67%) of the patients indicated that they would undergo the procedure again should their symptoms recur (the intergroup responses were similar, Fisher exact test). In regard to complications, the most prevalent complaint expressed by the patients in the postoperative period was that of loss of sensation or electric currents like feeling distal to the operative site in the endoscopy group (2/22, 9%). None of the TOPAZ patients, however, developed such symptoms (n.s., Fisher's exact test, 2 \* 1-sided p-value 0.51). Lateral side foot pain occurred in 2 patients in the endoscopy group and in none of the patients in the TOPAZ group.

## DISCUSSION

Plantar fasciitis as a cause of heel pain is associated with thickening of the plantar fascia (11). It has been suggested that this thickening can best be observed on a non-weight bearing lateral

foot radiograph (12), though ultrasonography is often used (as in this study) due to the ability to quantitatively define the plantar fascia thickness (13). This disease entity has had significant influence on the quality of life of afflicted individuals (2). Due to the level of symptoms the surgeon's attention is warranted. Conventional surgery is often associated with relatively high morbidity (6). This led to the development of multiple procedures for symptomatic relief, including cryosurgery (10), botulinum toxin injection (14), extra-corporeal shock wave therapy (9). Currently a commonly used technique is endoscopic release of the fascia (8), but recently a percutaneous novel radiofrequency based technique has been described (15). The main advantage of the percutaneous technique might be reduction in the occurrence of neuropraxia after open and endoscopic interventions (16), CRPS (7), lateral column syndrome, as well as iatrogenic pes planus (17). The current study confirms that none of these

complications was observed after micro fasciotomy via bipolar radiofrequency. Weil and colleagues (15) conducted the first study using bipolar radiofrequency for the treatment of chronic plantar fasciitis. In that limited number prospective study, the AOFAS score changed from a mean of 57.40 in the preoperative period to 88.50 in the postoperative period, with a minimum follow-up duration of 12 months without any notable postoperative complications. The current study for radiofrequency ablation of the plantar fascia is a little more conservative. Similar to the Weil et al. report, our patients were able to weight bear on the operated foot in a surgical boot immediately after the surgery. But our patients resumed normal shoes wear by 4 weeks after surgery as compared with 2 weeks in the Weil et al. series. A similar protocol has been reported by Sorenson et al. (18). In comparison with historical controls, recovery time after open surgery of plantar fasciotomy has been documented to range from 4 to 8 months (16). As in many retrospective studies, we realize that our investigation was flawed by a number of methodological shortcomings that could threaten the validity of our conclusions. In particular, selection bias could have influenced our results, even though we described the outcomes in consecutive patients. Moreover, the treating surgeons determined the outcomes, although two outcome measures, namely the AOFAS score and VAS, with known subjective validity were used (19, 20). In conclusion, we believe that percutaneous bipolar radiofrequency plantar micro-fasciotomy is an effective, minimally invasive, and safe surgical option for the treatment of recalcitrant plantar fasciitis (fasciosis) in adults of at least equal efficacy to the endoscopic procedure, which is also a highly successful procedure. It appears to be slightly safer than the endoscopic procedure due to fewer cases of nerve damage. The limitations of this study include its retrospective nature as well as the non-randomized manner of treatment choice. The groups were thus slightly different demographically with the endoscopy group slightly but not significantly younger. Gender distribution was similar as well as the BMI but the micro-radiofrequency group were significantly more limited pre-operatively. At the latest follow-up results were similar in both groups. Due to these limitations the results should be validated in a double blind randomized study.

It does seem to be possible to conclude that the micro-radiofrequency treatment is not inferior to the endoscopic release of the plantar fascia.

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## RADIOFREQUENCY DENERVATION IN LUMBAR FACET JOINT PAIN: OUR PRELIMINARY OBSERVATIONS

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Facet syndrome is characterized by a dull ache, due to degeneration of facet joints and a segmental instability with variations in alignment and leanings of zygoapophyseal joints, through our experience with pulsed and continuous radiofrequency treatment in patients with lumbar facet syndrome. Within 12 months we treated 36 patients, 20 males and 16 females, ages 43 to 84 years (mean:  $64\pm 8,53$ ) suffering from facet syndrome. All patients had, previously, joint infiltration with anesthetics and corticosteroids that produced a transient pain relief. All patients had pulsed and continuous radiofrequency neurotomy at one or more lumbar joints with fluoroscopy guidance. They were in a day-hospital setting. After the procedure we kept them under observation for about 120 to 240 minutes. Their hospital release was made in the presence of a caregiver, and the patients were told they needed bed rest for 24 hours. The follow-up occurred after 15, 30, 45 and 60 days evaluating pain through the NRS scale and disability through the Oswestry Index. No patients had any major complications. Mean NRS before the procedure was 6.7. It was  $4.3\pm 1.11$  at first check up and  $2.3\pm 0.45$  at the end of follow-up. The mean Oswestry Index before the procedure was  $65\pm 5.03$ , and  $32\pm 7.01$  at 60 days. We observed that radiofrequency neurotomy is a safe and relatively easy technique, following the evidence based guidelines. Side effects were transient. Latter analgesia data could be more significant with a statistically significant number of procedures.

Chronic low back pain has been described as a source of disability and work absence (1). Among the causes of chronic low back pain, lumbar facet joint (LFJ) related pain is reported to have a prevalence of 15% to 45% (2). The LFJ form the posterior lateral articulations connecting the vertebral arch of one vertebra to the arch of the adjacent vertebra. As true synovial joints, each LFJ has a distinct joint space capable of containing between 1 to 1.5 ml of fluid, a synovial membrane, hyaline cartilage surfaces, and a fibrous capsule (3). The LFJ capsule and surrounding structures are innervated by small medial branches of dorsal rami. Each facet joint is supplied by two medial branches. The course over the transverses processes at the levels constituting the joint (4). Chemical or mechanical stimulation of

the LFJ and the nerve supply elicits back pain (5). LFJ pain is predominantly caused by repetitive stress and/or cumulative low level trauma; the resulting osteoarthritis leads to inflammation, which can cause the facet joint to be filled with fluid and swell, therefore stretching the joint capsule and causing pain. The most frequent complaint is axial low back pain. Sometimes pain may be also felt in the groin or thigh area. Lumbar paravertebral tenderness is indicative of facetogenetic pain (6). When pain gets worse by flexion and extension, it may be considered a pathology of the lowest lumbar segments. LFJ pain has been managed by intra-articular injection, nerve blocks, and neurolysis of the medial branches (7). The use of intra-articular corticosteroid injections is controversial. Radiofrequency (RF) treatment of the

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facet joints was given 1B+ score in recently published practice guidelines (8). This score implicates a positive recommendation. We have observed pulsed plus continuous RF denervation of medial rami of lumbar facet joints.

## MATERIALS AND METHODS

### *Patient characteristics*

We studied consecutive patients, presenting chronic low back pain, within a 12 month period. Inclusion criteria were: chronic low back pain of > 6 months duration and not relieved by conservative treatment; pain diagram suggesting facet low back pain; MR or tomography excluding other causes of low back pain. All patients underwent a diagnostic block under fluoroscopic guidance of the affected joint with lignocaine 40 mg (2% 2 ml) before proceeding to RF. Patients who had consistent pain relief (NRS reduction > 5 points) were considered to be eligible for RF procedure.

The number of treated patients was 36 (20 female 16 male), the mean age was 64±8,53.

### *RF lesioning technique*

RF lesioning was performed using an OWL 22G RF insulated Hybrid Cannula, (Length 100 mm Active tip 5 mm) under fluoroscopic guidance and maintaining asepsis. The skin insertion point was infiltrated with lidocaine 2%. Using fluoroscopy the needle was directed

toward the target between the transverse process and superior articular process. Final position was confirmed by 3 fluoroscopic views. After verifying the impedance, sensory stimulation of the target nerve was performed (50 Hz up to 1.0 V), followed by motor stimulation (2 Hz up to 2 V). During sensory stimulation, paraesthesia was elicited, whereas motor stimulation caused palpable and visible twitch of the multifidus muscle of the patient appropriate for the segmental level. Then pulsed RF was started by increasing electrode temperature to 42°C for 120 s. Thereafter, lidocaine 2% (1 ml 20 mg) was injected at tip. Continuous RF was started increasing electrode temperature to 75°C for 120 s.

### *Follow-up*

Treatment outcomes were measured by NRS and Oswestry Disability Index. For measuring NRS a standard 10 cm-scale, where 0 corresponded to “no pain” and 10 corresponded to “worst pain” patients had ever perceived, was used. ODI questionnaire was designed to understand how back or leg pain was affecting the patient’s ability to manage in everyday life. It was completed by the patients and then analyzed. The NRS and ODI were measured before procedure, and after 15, 30, 45 and 60 days.

### *Data analysis*

Numerical data obtained was expressed as mean ± standard deviation. NRS and ODI scores before and after the procedure at different time points were compared using Student Test. A P value <0.05 was considered to be

**Table I.** *Patient characteristics*

Number of patients	36 ( male 16 )
Age (Mean±SD)	64±8,3
Duration of symptoms	1.2y±0.7
Affected joints	Single 4 pts (11%)
	Double 25 pts (69%)
	Triple 7 pts (20%)

**Table II.** *Results of the analgesia.*

Outcome measure	Pretreatment	15 days	30 days	45 days	60 days
NRS (mean ±DS)	6.75±1.15	4.27±1.11	3.38±0.49	2.50±0.56	2.27±0.45
ODI (mean ±DS)	64.83±5.03	49.75±6.53	42.72±5.28	36.77±6.26	31.69±7.01

significant, and a  $P < 0.001$  as highly significant.

## RESULTS

The study interval extended from April 2012 to April 2013. All treated patients satisfied inclusion criteria. Mean NRS before procedure was  $6.7 \pm 1.15$ , at 15 days  $4.27 \pm 1.11$ , at 30 days  $3.38 \pm 0.49$ , at 45 and 60 respectively  $2.5 \pm 0.56$  and  $2.27 \pm 0.45$ . When the scores before procedure and after 15 days were compared the difference was highly significant ( $P < 0.001$ ), but, before procedure and after 60 days the difference was not very significant. Mean ODI before procedure was  $64.83 \pm 5.03$ , at 15 days  $49.75 \pm 6.53$ , at 30 days  $42.72 \pm 5.28$ , at 45 days  $36.77 \pm 6.26$  and at 60 days  $31.69 \pm 7.01$ . When the scores before procedure and after 15 days were compared the difference was highly significant ( $P < 0.001$ ) and before procedure and after 60 days was only significant ( $P < 0.05$ ).

There were no major complications based on the criteria of the Society of Interventional Radiology (9).

## DISCUSSION

We have observed that combined pulsed and continuous RF lesioning resulted in a significant reduction of pain and disability in patients with chronic lumbar facet arthropathy. There are many studies on continuous or pulsed RF alone, but no studies regarding both techniques at the same time.

The clinical results of RF denervation have been shown to be superior compared with sham therapy in double blind studies; other studies did not demonstrate any significant differences with respect to functional improvement and pain relief (10-11). It still remains unclear, from the outcome assessment of different studies, the quantum of pain relief, that can be deemed as a success, attributable to the procedure. Again, the duration of the pain relief required to consider the procedure successful remains ambiguous. (12) Continuous RF treatment was compared with pulsed RF treatment of facetogenetic pain in two randomized trials. Both showed continuous to be superior (13-14). The rationale of our protocol is based on the observation of the use of a lower amount of local anesthetic before continuous RF lesioning, with a

reduction of impedance resulting in a minor spread of radiofrequency with a more close lesion. The present study has some limitations. First of all, the follow-up was only at 60 days while RF is considered successful if its effects last for many months. Patients were under pharmacological treatment, and the scores can be affected by the effects of drugs. We cannot measure the accuracy of the RF lesion of medial branches, that's what we intended to achieve using both RF techniques. In conclusion, pulsed and continuous RF is a safe technique, it provides a reduction of pain and disability in carefully selected patients with lumbar facet joint arthropathy confirmed by diagnostic block. However, a longer follow-up is required to assess the long-term efficacy of this combined procedure.

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## SIGNIFICANCE OF SERUM AND SYNOVIAL FLUID LEVELS OF INTERLEUKIN-33 IN EGYPTIAN PATIENTS WITH RHEUMATOID ARTHRITIS AND ITS POSSIBLE CORRELATION WITH DISEASE ACTIVITY

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This work was designed to evaluate the significance of serum and synovial fluid (SF) levels of Interleukin-33 (IL-33) in patients with rheumatoid arthritis (RA) and to determine the relationship to disease activity. This study included 65 rheumatoid arthritis patients and 25 osteoarthritis (OA) patients serving as the control group. Assessment of disease activity of RA patients was done using the DAS-28 scoring system. The levels of IL-33 was measured by ELISA in sera and synovial fluids of all patients with RA and OA. Both serum and SF IL-33 levels were significantly higher in RA than in OA patients. Serum IL-33 was significantly correlated with duration of morning stiffness, DAS-28, ESR and CRP. On the other hand, SF IL-33 was significantly correlated with duration of morning stiffness, DAS-28, ESR, CRP, RF titer and Immunoglobulins (IgG, IgM and IgA). RA patients had significantly higher levels of IL-33 in serum and synovial fluid than controls. In RA patients, serum and SF IL-33 levels were significantly correlated with disease activity markers (duration of morning stiffness, DAS-28, ESR and CRP). This study supported that IL-33 could have a role in RA pathogenesis. This role might open the door to the development of new therapeutic strategies for RA patients.

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammatory response including excessive production of proinflammatory cytokines and synovial proliferation that leads to destruction in both bone and cartilage. These secretory proinflammatory cytokines are involved in the inflammatory process of rheumatoid arthritis and include TNF- $\alpha$ , IL-1 and IL-6 (1). Blockade of TNF- $\alpha$  activity has been widely used in improving rheumatoid arthritis progression (2). IL-33, a novel member of IL-1 family, is broadly expressed in multiple tissues and organs especially enriched in

the central nervous system and gastrointestinal tract (3). IL-33 has been demonstrated to induce cytokine synthesis and to mediate inflammatory response through its receptor ST2. Moreover, IL-33 is mainly localized in the nucleus but under signal stimulating conditions as inflammation, IL-33 is processed and passively released from necrotic or active secreting cells into the extracellular milieu and binds through its receptor ST2 working as a proinflammatory cytokine that share in the development and progress of many inflammatory diseases including autoimmune diseases as systemic lupus erythematosus and rheumatoid

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arthritis (4) and viral hepatitis (5). The soluble form of ST2, sST2, is considered as a decoy receptor to block the effect of IL-33. IL-33 exacerbates the disease severity of collagen-induced arthritis (CIA) through inhibition of IL-33 signaling pathway by blocking anti-ST2 antibody attenuating the severity of CIA. It was reported that sST2 fusion protein dramatically attenuated severity of the disease through reduction of cellular infiltration in the joints, synovial hyperplasia and erosion of the joint by preventing the release of proinflammatory cytokines comprising IL-6, IL-12, TNF- $\alpha$  and IFN- $\gamma$  (6). The aim of our study is to examine serum and synovial fluid levels of IL-33 in RA patients and to correlate these levels with the disease activity.

## MATERIALS AND METHODS

This study included sixty five rheumatoid arthritis patients (57 females and 8 males). They were all randomly selected from the outpatient clinics and inpatient sections of Rheumatology & Rehabilitation and Internal Medicine departments, Zagazig University Hospitals in the period from October 2013 to March 2014. Patients were diagnosed according to the 1987 revised American Rheumatism Association criteria for the classification of rheumatoid arthritis (7). Twenty-five osteoarthritis patients were enrolled as control group. Patients were diagnosed according to the criteria of American College of Rheumatology (ACR) for osteoarthritis (8). RA patients receiving biologic therapy and those suffering from other diseases known to affect IL-33 level as malignancy were excluded from this study. We followed our Committee's ethical guidelines in Zagazig University and an informed consent was obtained from all patients for their study participation. Patients were subjected to full history taking and thorough clinical examination. RA disease activity was assessed by the Disease Activity Score (DAS-28) (9), with low disease activity defined as  $DAS-28 \leq 3.2$ , moderate as  $3.2 < DAS-28 \leq 5.1$ , high as  $DAS-28 > 5.1$  and remission as  $DAS-28 < 2.6$  (10). Laboratory investigations were carried out in Clinical Pathology Department, Zagazig University Hospitals. Complete blood count (CBC), erythrocyte sedimentation rate (ESR), c-reactive protein (CRP), serum levels of Immunoglobulins (IgG, IgM and IgA), rheumatoid factor (RF) and

anti-cyclic citrullinated peptide antibodies (anti-CCP) were done for RA patients. Level of IL-33 was measured by ELISA in serum and synovial fluid of all patients with rheumatoid arthritis and osteoarthritis.

### *Sample collection*

Venous blood was collected, clotted then centrifuged and the serum was separated and kept at  $-20^{\circ}\text{C}$  until needed.

Synovial fluid was aspirated by joint punctures (arthrocentesis) under complete aseptic conditions then centrifuged and the supernatant was separated and stored at  $-20^{\circ}\text{C}$  until needed.

### *Laboratory procedures*

C-reactive protein (CRP) and Rheumatoid Factor (RF) were quantitatively determined in serum on Cobas Integra 400 autoanalyzer by particle – enhanced Immunoturbidimetric assay (Roche diagnostics). Normal range for CRP is up to 5 mg/L and for RF is up to 15 IU/ml.

Immunoglobulins (IgG, IgM and IgA) were measured quantitatively in serum by immunonephelometry on the BN ProSpec system (Siemens Diagnostics). Reference values for serum IgG, IgM and IgA are 7.0 -16.0, 0.4 -2.3 and 0.7 – 4.0 g / L respectively.

Anti-CCP was estimated using Cobas e 411 autoanalyzer, by electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics GmbH, Mannheim). The principle of test is IgG-capture, where the sample is incubated with both biotinylated cyclic citrullinated peptides (CCP) and monoclonal antibody against human IgG, forming a complex when CCP-specific antibodies are present in the sample. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode. Measuring range is 7-500 U/ml, and the expected normal value is up to 17 U/ml.

IL-33 was estimated in serum and synovial fluid using Boster's human IL-33 ELISA Kit (Catalog No.EK0929) which based on standard sandwich enzyme-linked immunosorbent assay. The 96-well plates of the kit are precoated with monoclonal antibody specific for IL-33.

Before start of the procedure, standards were prepared to have concentrations of 1000 pg/ml ,

**Table I.** Demographic data of both groups.

P - value	OA patients (n = 25)	RA patients (n = 65)	
> 0.05 (NS)	22 / 3	57 / 8	Sex ( F/M)
> 0.05 (NS)	46-75 (61.4 ± 11.3)	27-73 (52.1 ± 14.9)	Age range in years ( Mean ± SD)
> 0.05 (NS)	0.5 – 25 (10.6 ± 7.9)	0.25 – 22 (9.8 ± 7.7)	Disease duration in years ( Mean ± SD)

NS = Non – significant

**Table II.** Comparison between RA patients and OA patients regarding serum and SF levels of IL-33.

P - value	OA patients (n = 25)	RA patients (n = 65)	
< 0.05 (S)	62.45 ± 29.83	146.25 ± 64.51	Serum IL-33 (pg/ml) (Mean ± SD)
< 0.05 (S)	41.66 ± 21.76	107.29 ± 47.37	SF IL-33 (pg/ml) (Mean ± SD)

S = Significant

**Table III.** Correlation between serum and SF levels of IL-33 and clinical and laboratory data of RA patients.

SF IL-33 (pg/ml)	Serum IL-33 (pg/ml)			
p	r	p	r	
> 0.05 (NS)	- 0.171	> 0.05 (NS)	- 0.082	Age
> 0.05 (NS)	- 0.236	> 0.05 (NS)	- 0.195	Disease duration (years)
< 0.05 (S)	0.256	< 0.05 (S)	0.278	Duration of MS (minutes)
> 0.05 (NS)	0.204	> 0.05 (NS)	0.220	Tender joint count
> 0.05 (NS)	0.186	> 0.05 (NS)	0.187	Swollen joint count
> 0.05 (NS)	0.270	> 0.05 (NS)	0.199	VAS (cm)
< 0.05 (S)	0.306	< 0.05 (S)	0.319	DAS-28
< 0.05 (S)	0.334	< 0.05 (S)	0.287	ESR (mm/h)
< 0.05 (S)	0.267	< 0.05 (S)	0.248	CRP (mg/L)
< 0.05 (S)	0.331	> 0.05 (NS)	0.226	RF titre (IU/ml)
> 0.05 (NS)	0.185	> 0.05 (NS)	0.128	Anti – CCP (U/ml)
< 0.05 (S)	0.329	> 0.05 (NS)	0.212	IgG (g/L)
< 0.05 (S)	0.318	> 0.05 (NS)	0.177	IgM (g/L)
< 0.05 (S)	0.286	> 0.05 (NS)	0.136	IgA (g/L)

S = Significant; NS = Non – significant; MS = Morning stiffness; VAS = Visual analogue scale

500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml and 15.6 pg/ml. Serum and SF samples were diluted 1:2 with sample diluent buffer. Standards and test samples were added to the wells; a biotinylated detection polyclonal antibody specific for IL-33 was added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase complex (conjugate) was added and unbound conjugates were washed away with PBS or TBS buffer. Horseradish peroxidase (HRP) substrate TMB (color developing agent) was added and incubated in dark to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue colour product that changed into yellow after adding acidic stop solution. The density of yellow colour is proportional to the human IL-33 amount of sample captured in plate. The optical density (O.D.) absorbance was read at 450 nm in a microplate reader within 30 minutes after adding the stop solution. The standard curve was plotted as the O.D. 450 of each standard solution (Y) vs the respective concentration of the standard solution (X). The IL-33 concentration of the samples was interpolated from the standard curve. As the serum and SF samples were diluted 1:2 with sample diluent buffer, so the dilution factor (2) was multiplied by the concentrations from interpolation to obtain the actual concentrations. The assay range of the kit is 15.6–1000 pg/ml (Web: www. Bosterbio.com).

#### *Statistical analysis*

Statistical presentation and analysis were conducted by SPSS version 17. Numerical measures were represented as means and standard deviation.

Cross tabulation was utilized to describe the relations between variables using the contingency coefficient. Unpaired student t-test was used to compare between two groups in quantitative data, Mann-Whitney test was used to evaluate the statistical difference between RA patients and OA patients regarding the levels of IL-33 in serum and synovial fluid. Spearman's correlation test was used to assess relationships between two variables in one group. P value was considered non-significant if  $P > 0.05$  and significant if  $P < 0.05$ .

## RESULTS

The age of the 65 RA patients (57 females

(87.7%) and 8 males (12.3%)) ranged between 27 and 73 years with a mean of  $52.1 \pm 14.9$  years. Disease duration ranged between 3 months and 22 years with a mean of  $9.8 \pm 7.7$  years. Twenty five OA patients served as the control group. Table I shows the demographic data of both groups.

Serum level of IL-33 in RA patients had a mean of  $146.25 \pm 64.51$  pg/ml while it was  $62.45 \pm 29.83$  pg/ml in OA patients. A statistically significant difference was found between RA patients and OA patients (controls) as regard serum level of IL-33. Regarding synovial fluid level of IL-33, RA patients had a mean of  $107.29 \pm 47.37$  pg/ml while OA patients had a mean of  $41.66 \pm 21.76$  pg/ml. A statistically significant difference was also found between RA patients and OA patients (controls) as regard the synovial fluid level of IL-33. These data are shown in Table II.

Table III demonstrates the correlation between serum and synovial fluid levels of IL-33 and clinical and laboratory data of RA patients. Serum IL-33 was found to be significantly correlated with duration of morning stiffness, DAS-28, ESR and CRP, while SF IL-33 was significantly correlated with duration of morning stiffness, DAS-28, ESR, CRP, RF titers and Immunoglobulins ( IgG , IgM and IgA).

## DISCUSSION

The expression of IL-33 in rheumatoid arthritis and its correlation with disease activity are of great interest because IL-33 may contribute to the pathogenesis of joint inflammation and destruction (3).

In the present study, we found that serum and synovial fluid IL-33 were significantly higher in RA patients than in OA patients. These results are in agreement with the previous findings of Talabot-Ayer et al., 2012 (11) and Tang et al., 2013 (12). This observation supported the hypothesis that IL-33 plays an important role in the pathogenesis of RA. Our study also reported that serum and SF IL-33 levels in RA patients were significantly correlated with disease activity markers (duration of morning stiffness, DAS 28, ESR and CRP). These findings suggest that IL-33 is closely associated with systemic inflammation. This was confirmed and supported by Hong et al., 2011 (13) and Tang et al., 2013 (12).

According to the present study, the SF-IL-33 levels in RA patients were significantly correlated with rheumatoid factor (RF) titre and Immunoglobulins (IgG, IgM and IgA). These results coincide with Mu et al., 2010 (14) and Tang et al., 2013 (12). So, IL-33 may be considered a risk factor for poor prognosis of RA as IL-33 was associated with the production of antibodies (14). The mechanism by which IL-33/ST2 was involved in RA pathogenesis was determined by some studies. Matsuyama et al., 2010 (15) found that the level of serum IL-33 decreased after anti-TNF treatment and correlated with production of IgM and RA-related antibodies including rheumatoid factor and anti-CCP antibodies. Serum and synovial fluid levels of IL-33 has been shown to decrease in patients who respond to anti-TNF treatment while they did not change in non-responders. Kageyama et al., 2012 (16) reported that by administering a TNF- $\alpha$  inhibitor as etanercept, the serum level of IL-33 showed significant correlation with disease activity, C-reactive protein and WBCs count while there is inverse correlation with RBC count and hemoglobin level. Various rheumatologic diseases can have effects on bone including erosion in RA and new bone formation in OA. Mun et al., 2010 (17) showed that IL-33 can stimulate the formation of multi-nuclear osteoclasts from monocyte and enhanced expression of osteoclast and osteoblast differentiation display normal bone formation and had increased bone resorption therapy resulting in low trabecular bone mass.

Saleh et al., 2011 (18) reported that IL-33 mRNA levels are increased in osteoblasts following treatment with bone anabolic factors parathyroid hormone or oncostatin M. Recent studies reported the correlation between IL-33 with rheumatic disease and most of them found that the IL-33 expression levels were consistent with disease activity and development. Furthermore, evidence has indicated that IL-33 related treatment may ameliorate the pathogenic conditions and attenuate disease progression of these rheumatic diseases. Therefore, elucidation of the roles of IL-33 in rheumatic disease would be beneficial to understand the pathogenesis and therapy of these diseases (19). In conclusion, IL-33 levels that increased in RA patients could be considered a sensitive marker of disease activity and also it was significantly correlated with levels

of immunoglobulins and RF titers. So, it can be concluded that IL-33 plays an important role in pathogenesis of RA and it may become a new target of treatment. As there was high correlation between SF levels of IL-33 and its serum levels, it may become a new target of local treatment. Also, therapeutic significance of IL-33 is recommended to undergo further research.

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