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EDITORIAL

With the multiplicity of journals and internet sources in existence, one might ask, "Why a new journal called "European Journal of Musculoskeletal Diseases, EJMD"?

The answer is straightforward. European Journal of Musculoskeletal Diseases (EJMD) is a new international peer-reviewed journal, committed to publishing high scientific quality clinical papers. The aim of the journal, that will be focused on publishing all new discoveries on diagnostic, clinical and therapeutic topics about musculoskeletal diseases, with sections of rheumatology, clinical immunology, orthopedics, endocrinology, sports medicine, physical rehabilitation and imaging, is to create a platform for high-quality scientific exchange between the different specialists interested in musculoskeletal diseases, and to promote health education, research, and practice in the osteoarticular field with a wide international scope.

Musculoskeletal diseases were the most known and the most common cause of chronic diseases, with high potential for disability and handicap. The joint disease (degenerative or inflammatory), back pain, osteoporosis involving, as it is known, a huge impact of the individual, society and healthcare systems and society. To date, it is estimated that the world has hundreds of millions of people who are suffering from these diseases, and it is reasonable to assume that in the future this number will increase. It predicts that in 2020, the number of people who have an age above 50 years will double the current one. The intention in launching this journal is not to compete with existing scientific journals, but rather to complement traditional journals in bone and joint diseases by providing a forum for discussion, analysis, and improvement of all aspects regarding the management of musculoskeletal diseases. The main topics will be intrinsic musculoskeletal diseases, characterization, diagnosis and clinical manifestations, biological therapies and novel therapeutic targets for muscle-skeletal diseases, different intra-articular treatments, osteoarthritis prevention and management, epidemiology, indicators of disease risk and progression, clinical options for osteoporosis therapy, prevention of the onset or delaying the progression of joint and bone diseases, predictors of therapy outcome, pharmacoeconomics aspects. Moreover, we hope to make an impact by placing emphasis on the integration of knowledge that allows science to be translated into real clinical practice. We will promote interdisciplinary approaches, the diffusion of innovations and best practices, and draw attention to emerging new issues. Particular attention will be given to all new contributions and advances in intra-articular (IA) therapy. Despite the wide range of many official journals in the field of joint diseases, there is currently no single medical journal available, which specializes on the issue of intra-articular management. In our opinion, this imbalance appears unjustified, since intra-articular treatments represent a significant contributing skill to care joint disorders. An evidence-based approach to quality improvement in IA care must include the analysis of new and innovative products, dosing regimens, outcome predictive factors, long term results and eventual adverse events. The journal should complement traditional journals in intra-articular therapy by filling an essential void, through providing a forum for discussion, analysis, and work-up in the management of this kind of patients. One of the most relevant aims of this new journal is to increase the debate and the level of evidence in the field of IA management. These areas will be addressed through original research (full papers and concise rapid reports), systematic reviews, RCTs, cohort studies, data from registries, pilot studies, preliminary data, short communications, expert opinion and commentary, letters to the editor, case reports and case series. Our renowned international

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

Editorial Board will ensure a high quality peer review by qualified experts in the field. Each submitted manuscript will first be screened by the Editors-in-Chief for suitability. All manuscripts deemed suitable for peer review will be assigned to at least two expert referees. The journal aims to provide the most complete and reliable source of information about current developments in the field.

This is the first journal's issue, and it'll be published every six months by BIOLIFE s.a.s. (Managing Editor Prof. Pio Conti). The publishers are confident of the journal's rapid success, similarly to titles already published by BIOLIFE s.a.s. (www.biolifesas.org), also because can rely on a solid network's relationships with leading researchers and scientific value to ensure quality and plenty of content.

This journal is direct to a wide array of physicians, rheumatologists, immunologists, orthopedics, endocrinologists, radiologists, and other clinical specialists working directly on clinical and biomedical applications for the management of musculoskeletal diseases or wishing to maintain an up to date knowledge on this exciting field. We would like to see this publication in regular use for updating purposes as well as an aide to clinicians so keeps up with current trends in a dynamic, rapidly-evolving field.

There is a pressing need to look ahead to the emerging health needs of Europe and the larger world, taking into account all the dimensions that provide opportunities for optimizing our populations' health. We will promote interdisciplinary approaches and draw attention to emerging health diseases.

Our editorial policy will be to present articles that are not only reporting concrete evidence, but also anticipatory or prescriptive, describing evidence-based innovative best practices for advancing the field of musculoskeletal disease in Europe and globally. We intend this journal to have a balanced view, providing opportunities for everyone, particularly Europeans, to contribute.

We urge you to consider submitting your next research article about the topic to our new journal.

Wish us good luck, and join us!

ALBERTO MIGLIORE.
Editor in Chief

A CONSECUTIVE CONTROLLED CLINICAL SERIES COMPARING THE EFFICACY OF HIGH MOLECULAR WEIGHT HYALURONAN INJECTIONS PERFORMED IN A BLIND FASHION AND UNDER ULTRASOUND-GUIDANCE IN THE TREATMENT OF KNEE OSTEOARTHRITIS

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Viscosupplementation consists of injecting exogenous hyaluronan (HA) into the synovial joints. Efficacy might be related to the rheological properties and molecular weight (MW) of the hyaluronan preparations. This open-label consecutive clinical assessment was aimed at comparing the effect of using ultrasound-guidance in order to improve the efficacy, and safety of injections of high-molecular weight hyaluronate. Fifty-one patients undergoing blind injections were compared with thirty-four patients undergoing ultrasound-guided injections. All underwent three weekly injections, and there were no drop-outs. Follow-up VAS pain score was assessed up to one year after treatment. The groups appeared to be similar in demographics including: age, gender, presence of diabetes and pre-treatment pain score as well as WOMAC score. The patients were mostly over-weight and slightly more so in the blind injection group. Any increase of VAS score two days after the first injection was considered as an adverse event termed 'pain reaction'. There were significantly fewer pain reactions in the ultrasound-guided group (8%) as compared with the blind-injection group (37%). In the ultrasound guided group first needle insertion was intra-synovial in about a third of cases. Extra-capsular needle misplacement did not occur in this study. In conclusion, it appears that ultrasound guided injections are less likely to be associated with pain reaction, and there is a trend for better clinical results in this group than in the blind injection group.

Osteoarthritis (OA) is characterized by changes in all joint tissues[1, 2] including cartilage fibrillation and eventual loss, synovial hypertrophy and capsular thickening as well as abnormal muscle function. Concomitantly, a decrease in rheological properties of synovial fluid occurs. It is hypothesized that synovial fluid property changes are related to the result of reduction in molecular size and concentration of hyaluronan in it[3].

It is hypothesized that the hyaluronan matrix acts as a fluid shock absorber and inhibit the formation and release of prostaglandins following osteoarthritis induction by meniscectomy in a rabbit model[4].

Unfortunately injected hyaluronan is eliminated quickly from the joint affecting its effectiveness. Chemically cross-linked hyaluronan were produced to improve the efficacy of viscosupplementation therapy of OA but seem to be associated with some pseudo-septic reactions, including joint effusion and pain[5]. As the results of the viscosupplementation therapy might be expected to depend also upon the rheological properties and molecular weight of the hyaluronan preparation, use of high-molecular weight, non cross-linked hyaluronan of non-animal

Key words: Viscosupplementation - Hyaluronic acid - Knee osteoarthritis - Ultrasound-guided injections

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origin, might be advantageous[6]. Another problem with hyaluronate, relates to the possibility of extra-capsular injection or intra-synovial intra-capsular injection that might create a pain reaction and lower overall treatment efficacy.

The aim of this consecutive clinical series using two different injection techniques, was to compare the efficacy of a high-molecular weight viscosupplement, Euflexxa 1% (Ferring Pharmaceuticals Inc.), in the management of knee osteoarthritis injected blindly or under ultra-sonographic guidance.

PATIENTS AND METHODS

Patient Selection Process

This is a consecutive series, made possible by the introduction of an ultrasonography system at one of the authors (D.R.) practices on April 1st 2010. Due to this device introduction, the treatment protocol of patients undergoing intra-articular injections changed. Thus, patients treated until March 31st, 2010 were compared with those treated after April 1st, 2010. Retrospective analysis of patient's records was approved by the Rabin ethical committee.

Inclusion Criteria

Patients undergoing knee injection due to osteoarthritis were considered for inclusion in the study according to the following criteria:

1. Patients with knee OA according to American College of Rheumatology criteria[7], with Kellgren–Lawrence grades II–III radiographic evidence of knee OA
2. Each patient consented verbally for the treatment, after reading a data sheet explaining the purpose of the treatment, its potential risks and possible benefits. In patients with bilateral disease the more painful knee was treated. No bilateral injections were included in this study.

Exclusion Criteria

Exclusion criteria were:

1. Refusal to undergo knee injections
2. Renal and hepatic comorbidity
3. Treatment with immunosuppressives, including methotrexate, chemotherapeutic agents and systemic steroid therapy
4. Intra-articular injection of hyaluronic acid or steroids within the past 12 months
5. Known congenital bleeding diathesis or warfarin treatment.

Power Analysis

Recent study has reported a 19% adverse events rate in post-meniscectomy knees[5]. Thus, a power analysis was conducted assuming 20% pain reactions in the blind knee injections versus a 5% pain reaction in the ultrasound guided knee injections, for a significance level of $p = 0.05$ and a power of > 0.90, the sample size was calculated as 20 patients per treatment group (Lenth, 2009).

Injection Technique

The first part of the study was performed prior to introduction into one of the authors (D.R.) offices of an ultrasound scanner and injections were performed in a blind fashion. The second part of the study was performed using cross-plane visualization technique to ensure needle placement in the synovial cavity. This part of the study required the use of 2 milliliters of lignocaine hydrochloride 1% in order to allow pain-free needle manipulation into the synovial cavity.

Three-weekly injections and ultrasound visualization were performed by one physician (D.R.) in an anterolateral approach (along the patellar tendon) with the knee in 70° flexion. Knees in the first group received hyaluronan injected blindly via the inferior-lateral arthroscopic portal with the patient lying supine and the knee flexed to about 70 degrees.

Patients of the second group underwent visualization of the anterior knee joint cavity by placing a linear transducer over the patellar tendon of the 70°-flexed knee. The needle was inserted via the inferior-lateral arthroscopic portal and two milliliters of lignocaine hydrochloride 1% were injected. The needle tip was visualized and assured that it is located intra-articularly. In case of intra-synovial placement of the needle, it was manipulated in order to ensure an intra-articular location of the tip.

Outcome Assessment

Primary outcome measures included the Western Ontario and Mc Master Universities Osteoarthritis Index (WOMAC) for pain, stiffness and function

scores, and patient-filled VAS assessment (0–10 scale)[8]. The possible range for WOMAC pain score was 0-20; the possible range for the stiffness score and function score were 0-8 and 0-68 respectively. For patient global pain VAS assessments, the question was: "How do you grade the severity of your (or the patients) knee osteoarthritis according to a 0–10 scale, 10 being the worst?"

One of the authors (D.R.) filled the WOMAC evaluation with the patient pre-injection and after 24 weeks. An evaluator unaware of each patient's treatment group — not present at the place of each weekly injection — assessed the patients for their symptoms and adverse reactions at baseline and at 2 days after the first injection and weekly for three weeks, according to a standardized VAS scale, and the consumption of analgesics was noted. An aggravation in VAS score of even one grade was considered a worsening event. Follow-up assessments were made at weekly intervals during the first 3 weeks and at 8 weeks, 12 weeks, 24 weeks and 52 weeks after the first injection.

There were no drop-outs and all patients received the three weekly injections.

Statistical Analysis

Statistical analysis was performed using the Analyse-it program (Analyse-it for Microsoft Excel (version 2.20), Analyse-it Software, Ltd. http://www.analyse-it.com/; 2009), Student's t-test was used to compare the two groups for continuous parameters and Mann-Whitney's test for non-parametric tests. Repeated measures ANOVA was performed for evaluation of the VAS scores over time. The latter test was calculated using SPSS statistical analysis program by the Tel Aviv University Center for Calculations, Faculty of Exact Sciences.

RESULTS

Fifty one patients were included in the first group (blind injection via a inferior-lateral portal) and thirty-four patients were included in the second group (ultrasound guided injection). The groups were comparable in age, gender, presence of diabetes mellitus, BMI and pre-injection VAS scores (Table 1).

Local adverse events such as transient pain at injection site or warm knee lasting for one night were recorded in three patients (8%) of the ultrasound-guided group and in 19 patients of the blind injection group (37%). This difference was found to be statistically-significant (Mann-Whitney test, $p<0.01$).

Improvement from baseline was observed for both treatment groups after the first injection and the following visits (repeated measures ANOVA, $p<0.01$). The degree of improvement was significantly higher in the ultrasound-guided injections than in the blind injection group (1-way ANOVA, F statistic 21.3, $p<0.0001$) (Figure 1). Compared with baseline, the degree of VAS improvement after one year was similar after one year in the ultrasound guided group (1.5±2) as compared with the blind injection group (1.3 ±1.4, t-test $p>0.05$). The presence of diabetes did not affect VAS scores, either when baseline levels were evaluated (Average 7.3±0.5 non-diabetics vs. 7.2±0.4 in diabetics, t-test, $p>0.05$) or to the results of therapy after one year (Average 6±0.3 non-diabetics vs. 6.2±0.4 in diabetics, t-test, $p>0.05$). Obesity (defined as BMI over 35) had no detrimental effect on VAS (average 7.6±0.15), as compared with thin patients (average 7.5±0.18).

In order to examine the effect of US on the VAS improvement during 52 weeks, a two-way anova was conducted. We found a significant effect for the US guidance on the VAS improvement, beyond the time of measurement [$F(1,83) = 5.23$, $p<0.05$], so that patients who had the injection without US guidance had a lower mean of VAS improvement ($M =6.26$, $Sd =0.18$) than patients who had the injection under US guidance ($M =6.78$, $Sd =0.14$) (Figure 2).

We also found a significant effect for the time of VAS improvement measurement, beyond the US guidance [$F(8,664) =29.43$, $p<0.001$]. The differences between the different times of VAS improvement measurement are delineated in Table 2.

There was no significant interaction between the US guidance and the time of measurement on the VAS improvement [$F(8,664) = 0.61$, n.s.].

In order to examine the effect of injection on the WOMAC score pre-injection and 24 weeks after-injection, a paired sample's t-test was conducted. We found a significant difference in the WOMAC score pre-injection and 24 weeks after-injection [$t(84)= 12.58$, $p < 0.001$], so that the mean WOMAC score pre-injection was higher ($M =46.18$, $SD =5.00$) than the mean WOMAC score 24 weeks after-injection

Table 1. *Pre-injection assessment of Demographic characteristics of the patients.*

Parameter	**Group 1: Blind Injection**	**Group 2: Ultrasound guided injection**	**Significance (n.s. – nonsignificant)**
Number	N=51	N=34	
Gender	53% males	56% males	n.s.
Age	65.9±10.5 years	64.2±11 years	n.s.
BMI*	33±5	31±6	$p<0.05$
Diabetes	22% diabetics	31% diabetics	n.s.
Side Injected	Right 33%	Right 47%	n.s.
Pre-Injection VAS**	7.2±1.1	7.7±1.2	n.s.
WOMAC Pain	9.5±1.4	9.6±1.6	n.s.
WOMAC Stiffness	3.2±1.2	3.4±1.4	n.s.
WOMAC Function	42.8±4.9	45.3±9	n.s.

**The groups were very similar, except for the blind injection group being slightly more obese than the ultrasound-guided group. **The ultrasound guided group trended to be stiffer than the blind injection group but this difference was not statistically significant.*

(M =40.45, SD =5.82) (Figure 3).

Finally, in order to examine the effect of injection on the pain, stiffness and function score pre-injection and 24 weeks after-injection, a paired sample's t-test was conducted. We found significant differences in the pain, stiffness and function pre-injection and 24 weeks after-injection [$t(84)= 8.00$, $p<0.001$], so that the mean pain, stiffness and function score pre-injection was higher (M =23.38, SD =2.43) than the mean pain, stiffness and function score 24 weeks after-injection (M =21.15, SD =2.85) (Figure 4).

The patients in whom ultrasound revealed either an extra-capsular location or an intra-articular intra-synovial initial needle placement, were recorded (Figure 5). Extra-articular injection does not seem to occur, using this portal; however intra-synovial

Table 2. *Means and standard deviations of VAS improvement over time. (VAS range 0-10)*

Time From injection	Mean	SD	Pre	2 days	1 week	2 week	3 week	8 w	12 w	24 w	52 w
Pre	7.50	0.13									
2 days	7.33	0.13	n.s.								
1 week	7.07	0.13	P<0.001	n.s.							
2 weeks	6.43	0.13	P<0.001	P<0.001	P<0.001						
3 weeks	6.30	0.16	P<0.001	P<0.001	P<0.001	n.s.					
8 weeks	5.90	0.17	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001				
12 weeks	6.30	0.17	P<0.001	P<0.001	P<0.001	P=0.064	n.s.	P<0.01			
24 weeks	5.97	0.17	P<0.001	P<0.001	P<0.001	P<0.001	P<0.005	n.s.	P<0.005		
52 weeks	5.90	0.17	P<0.001	P<0.001	P<0.001	P<0.001	P<0.005	n.s.	P<0.005	n.s.	

placement is common.

DISCUSSION

Our aim was to compare the effect of hyaluronate injection under ultrasound guidance and compare it with injection in a blind fashion, as is currently quite commonly used. Previous studies have shown that injections into the biceps tendon and into the peroneal tendon are preferably done under ultrasound guidance[9, 10]. It is also important to use image-guidance when injecting the hip joint[11]. However, to date most authors reported on hyaluronate injections into the knee joint that were performed blindly.

Electrophysiological studies in animals receiving hylans confirm that high molecular weight is necessary to produce an analgesic effect[12]. Thus, the hyaluronan used should be high molecular weight. Unfortunately, it is possible that the hyaluronic acid preparation used, being high-molecular weight when injected intra-synovial might act as a filler. It is known that macrophage-activation is related to adverse events of filler's injection[13]. Hyaluronan in-situ acts to increase the osmotic pressure within the tissue by a volume exclusion effect. The increase is larger the algebraic sum of the components[14], probably due to the collagen and hyaluronan acting as a two-phase system. Intra-synovial injection might be responsible for the pain reaction observed in our study though definitive proof for this theory is not currently available. Granulomatous reaction can ensue following injection of hyaluronate[15], though such reactions are fortunately rare.

The rate of pain reaction following intra-articular hyaluronan injection varies in different studies. Altman and coworkers have reported a rate of over 13 percent of limb pain of various sorts using Euflexxa[6, 16]. A frequency of up to 22 percent of patients have been reported for other hyaluronan preparations[17]. Other authors have reported a much lower rate of 2-4% local pain[18], perhaps due to fewer stringent definitions. In the current study, a high rate of pain was observed in the blind injection group. This might be due to the more stringent criteria of even one VAS grade increase being considered a pain aggravation event. The difference between the ultrasound-guided injection and the blind injection group was significant. This reduced incidence of pain events might be related

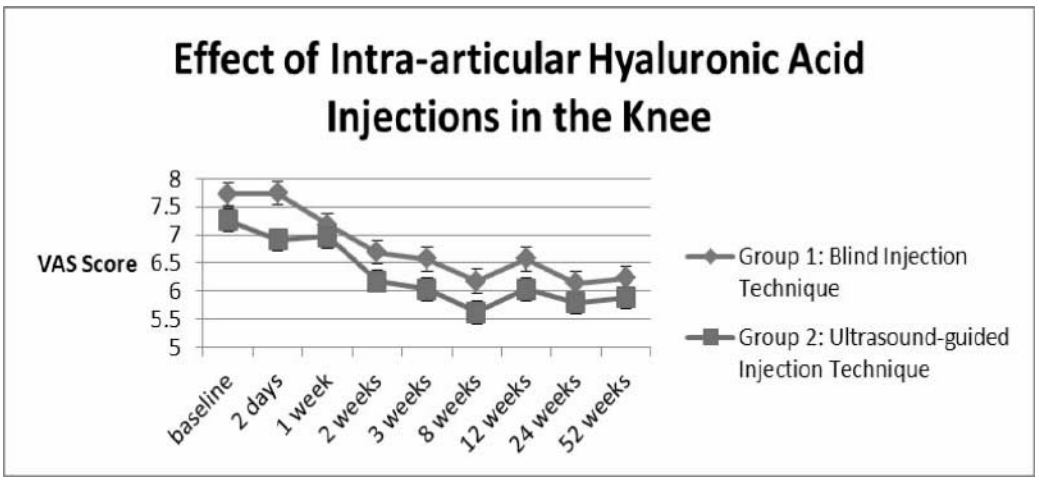

Fig. 1. *Effect over-time of intra-articular injections of the knee, with and without ultrasound guidance.*

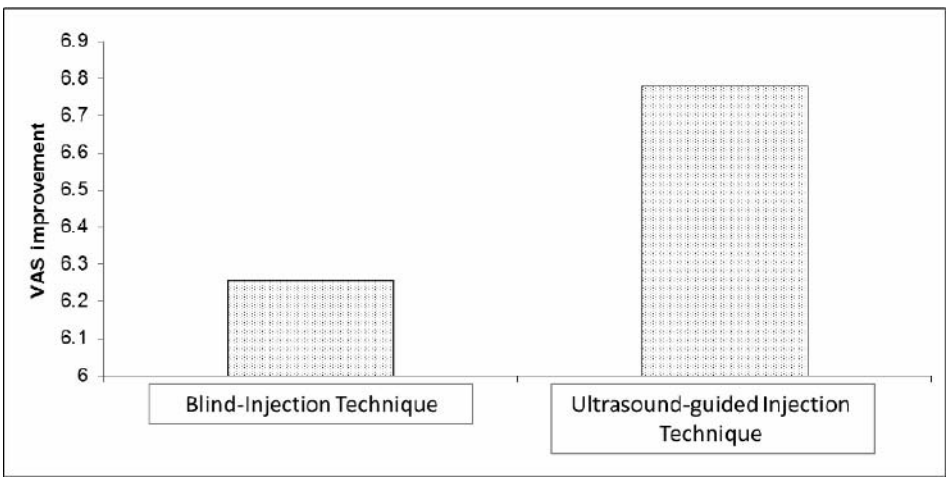

Fig. 2. *Mean VAS improvement score as a function of the injection technique used.*

to a more precise needle placement. The frequency of misplacement of the needle during first insertion under ultrasound guidance is high (35.2%) and similar to the rate of pain reactions in the blind injection group (38%). These further supports our hypothesis that intra-synovial injection might be responsible for pain after hyaluronate injection in the knee.

Studies tend to show that symptoms such as pain are better influenced by hyaluronate than clinical signs, and that the best results are obtained in patients with mild to moderate radiographical changes, and with no or only small effusions[18]. In the current study, mild cases (K/L grade I) and severe cases (K/L grade IV) were excluded. This might perhaps explain the overall positive effect of hyaluronate seen in this study. The current study appears to indicate that hyaluronate injections are similarly effective in diabetic and obese patients as well as non-diabetic and non-obese patients. The effect appears to last for at least a year. Sonographic-guided injections appear to be associated with fewer pain reactions, though their overall effectiveness is similar to blinded injections at further time-points.

In conclusion, it appears that ultrasound guidance reduces the pain-events associated with intra-articular knee injections of hyaluronate. It is recommended that such injections be performed using ultrasound-guidance as it is radiation-free and relatively cheap to use, and improves the success rate of intra-articular therapy at least in the short term.

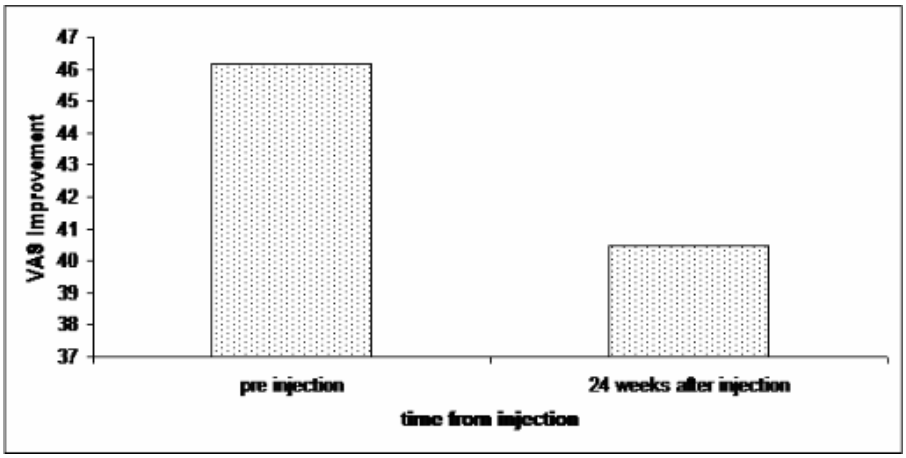

Fig. 3. *Mean WOMAC score as a function of time from injection.*

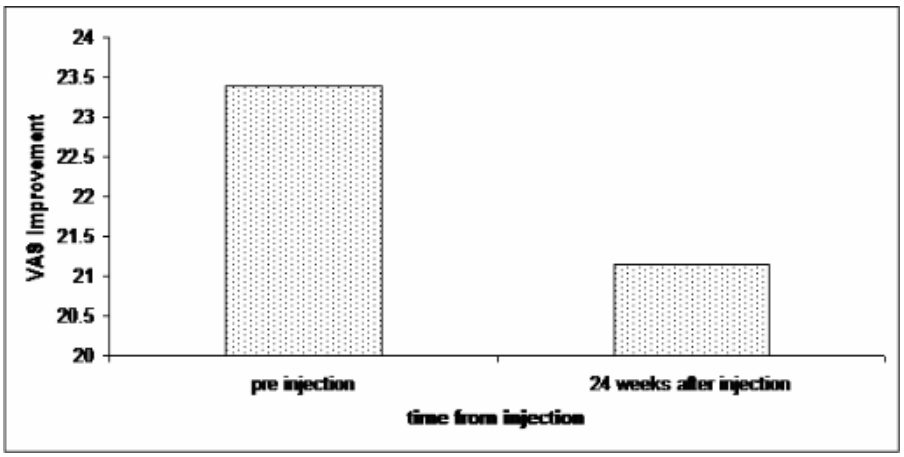

Fig. 4. *Mean pain, stiffness and function score as a function of time from injection.*

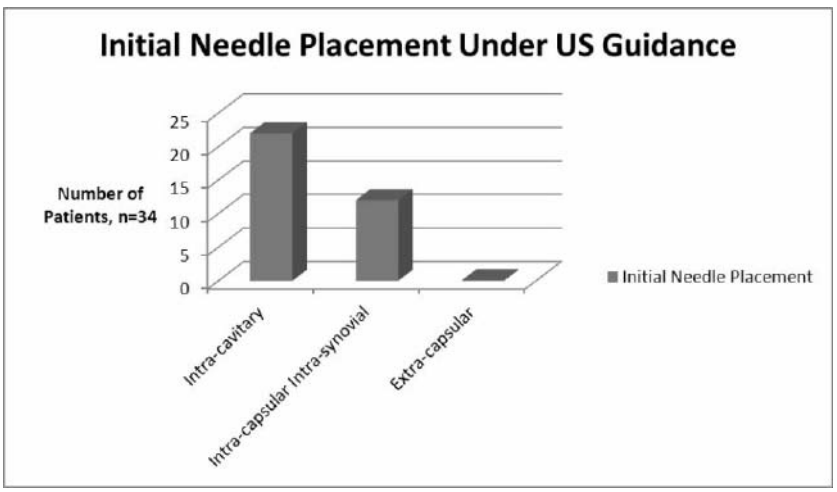

Fig. 5. *Initial Needle Placement Into The Knee Joint Using Ultrasound-Guided Injection Technique.*

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BOSENTAN REVERTED DIGITAL ULCERS IN A PATIENT WITH SYSTEMIC SCLEROSIS NOT RESPONDING TO PROSTACYCLIN – CASE REPORT

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We report a case of a patient with severe digital ischemia secondary to Systemic Sclerosis (SS) who experienced an excellent response to Bosentan, a dual endothelin-1 receptor antagonist. Endothelin-1 is a powerful vasoconstrictor with both proliferative and fibrotic effects. The objective of this case report is to describe the effectiveness and safety of Bosentan for healing digital ulcers in a patient with SS over the long term.

Case report

We report a case of a 39-year old man suffering from limited form of SS presented to our Division in November 2008 with digital ulcerations. He gave an eight months history of Raynaud's phenomenon and was receiving treatment with prostacyclin (Iloprost).

On examination, he presented ulcers in the fingertips of the second and third digit of right hand as well as an extensive fingertip ulcer on the third digit of left hand extending into the bone underneath (Fig. 1). Blood pressure was 100/60 and laboratory findings showed Antinuclear Antibodies (ANA) positivity 1:1280 (centromeric pattern). Routine blood chemistry was within laboratory standards. Echocardiogram showed normal heart valves, chambers, heart wall movement and normal PAPs. Despite the use of methilprednisolone, penicillamine, lansoprazole, pentoxifylline, nifedipine, acetylsalicylic acid plus local medications (escharectomy included), the ulcers progressively worsened. Iloprost has been stopped and in March 2009, Bosentan has been introduced at 62.5 mg twice a day (increasing to 125 mg twice a day after one month). To assess the functional impairment and deterioration in patients' health-related quality of life (HR-QoL) the Visual Analogic Scale for pain (VAS) and two validate questionnaires, the Medical Outcomes Study Short Form-36 (SF36) and The Health Assessment Questionnaire (HAQ) have been performed. Before Bosentan introduction, HAQ score was 1.25/3, the SF-36 physical and mental component summary scores (PCS and MCS) were 32/100 and 41/100 respectively and VAS was 70/100. The patient's clinical conditions have been controlled monthly including the routine blood biochemistry.

Lung spirometry conducted in April 2009 showed conserved respiratory function and normality of the CO diffusion capacity. X-ray of hands performed in April 2009 showed acrolysis of second-third right digit and third left digit, with partial resorption of the distal bone phalangeal tuft.

By May 2009, the patient reported a significant pain reduction of digital ulcers.

By September 2009, six months from starting treatment with Bosentan, the ulcers healed completely. (Fig. 2) Routine blood biochemistry was normal. The HAQ decreased to 0/3, the PCS

Key words: Scleroderma, Digital ulcers, Digital ischemia, Endothelin-1 receptor antagonist

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

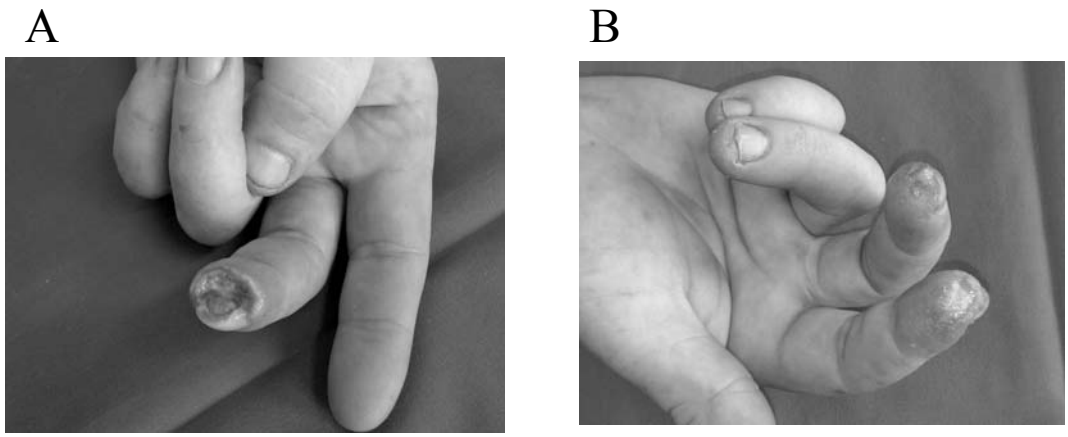

Fig. 1. *Extensive fingertip ulcer of third digit of the left hand showing underneath bone (panel A); moderate fingertip ulcers of the second and third digit of the right hand (panel B)*

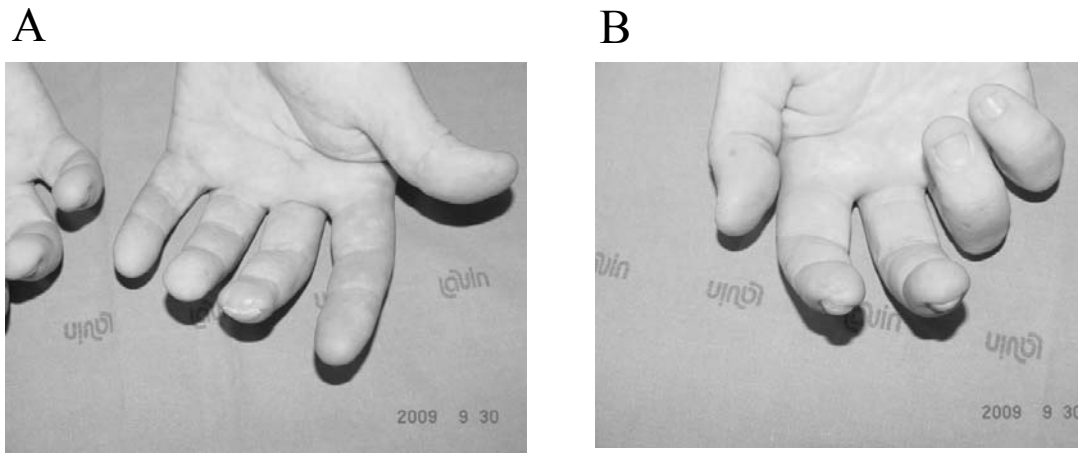

Fig. 2. *Disappearance of the fingertip ulcer of third digit of the left hand (panel A) and of the second and third digit of the right hand (panel B) after treatment with Bosentan*

and MCS SF-36 improved to 46/100 and 58/100 respectively, and the VAS reduced to 20/100.

DISCUSSION

The digital ulcers presented in this patient were serious; therefore the result obtained with Bosentan appears to be of huge importance.

SS is a complex autoimmune connective tissue disease characterized by cutaneous and visceral fibrosis and widespread vascular pathology.[1]

The most frequent expression of the vascular pathology in SS is Raynaud's phenomenon with complications, including digital ulceration and infarction.

The primum movens in the development of digital ulcers seems to be the lack of oxygen due to vasoconstriction, thickening of vessel walls and disrupted microvasculature that leads to vessels alteration and skin fibrosis.[2] Endothelin-1 is a potent endogenous vasoconstrictor reported to be increased in patients with systemic sclerosis; evidence suggests that it play a significant role in the pathogenesis of collagen vascular disease and it increases skin fibrosis.[3]

Digital ulcers remain a serious and frequent (30%) complication for many SS patients [4] that can reflect in a huge impact on patients' everyday life causing local pain and functional impairment.[2] Treatment with hydrocolloids, used to heal up wounds, NSAIDs, antibiotics and vasodilatators such as alpha-adrenergic inhibitors, ACE inhibitors, prostacyclin had all been used with uncertain results.[4]

Bosentan works by competitively binding at specific receptor sites in the endothelium and vascular smooth muscle. In so binding, it prevents the neurohormone endothelin-1 (ET-1) from interacting to the same receptor sites and triggering vasoconstriction.[5] Bosentan has been successfully used in the treatment of pulmonary artery hypertension (PAH)[6] furthermore, recent reports describe promising results for digital ulcers in patients with SS. The RAPIDS1 study demonstrated a relevant decrease in the appearance of new digital ulcers in 122 patients with SS treated with Bosentan for 16 weeks.[4] More recently, the RAPIDS2 study showed pain reduction, functional improvement and decreased occurrence of new digital ulcers.[7] Nevertheless, both Bosentan studies showed no benefit in healing digital ulcers.[2]

On the other hand, several case series showed that Bosentan can be effective in treating digital ulcers. Launay reported a significant improvement in severe active digital ulcers in nine patients after eight weeks therapy[5]; Tsifetaki et al., observed digital ulcers healing in 17/26 (65%) of patients during a 36 months follow up[8]; Nagai et al., indicate Bosentan as an effective treatment for refractory digital ulcers associated with SSc but with a tight monitor of liver function [9].

The reduction and subsequent healing of digital ulcers by Bosentan observed in this patient correlated with a marked improvement in the HAQ, VAS pain and SF-36; in particular the SF-36 showed a sensible amelioration not only in the physical activities but mostly in the mental/psychological areas, underlining the impact of digital ulcers resolution in all aspects of QoL.

In conclusion, Bosentan represented the unique opportunity to resolve digital ulcers resistant to conventional treatments in our patient with a good safety profile. The patient could experience an almost resolution of pain, a marked improvement in joint function and consequently, a significant amelioration of general well being. Other studies are needed to better investigate the role of Bosentan in this clinical condition.

Written consent was obtained from the patient for publications of his clinical history.

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INTRA-ARTICULAR ADMINISTRATION OF ADALIMUMAB: CASE SERIES OF 12 PATIENTS AFFECTED BY MONOARTHRITIS UNRESPONSIVE TO DMARDS.

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Diseases such as Rheumatoid Arthritis (RA), Ankylosing Spondylitis (AS) and Psoriatic Arthritis (PsA) and other pathologies often involving organs and apparatuses other than joints alone are usually treated with a systemic approach. When arthritis is not responsive to traditional treatments, and especially in the few cases where only joint involvement is observed, intra-articular (IA) therapy may represent a useful therapeutic tool. Moreover, patients who are not eligible for systemic therapies with anti-TNF or other biological agents or DMARDs could benefit from the use the intra-articular (IA) injection therapy, as well as patients with an arthritis involving a single joint, as knee or hip joint. In this article we report our experience with twelve patients affected by joint inflammatory diseases reporting monoarthritis, not responding to traditional DMARDs-based therapies or biological therapies or not eligible to systemic use of biological who underwent to ultrasound-guided IA injection of Adalimumab after failure of intra-articular administration of steroids. 8 out of 12 patients showed a positive and long-lasting response to treatment with IA Adalimumab. Safety profile was good according to literature data. This is also the first report on intra-articular use of Adalimumab in the hip joint.

Inflammatory chronic joint diseases represent a relevant and widespreading problem for public health and also represent one of the most important causes of morbidity in the world.

Disease-modifying antirheumatic drugs (DMARDs) such as Methotrexate (MTX), Sulfasalazine and A-Cyclosporin, aim to slow down the progression of articular damage and the eventual multi-organ involvement. Recently, the development of "biologicals", anti Tumor Necrosis Factor alpha agents, such as Infliximab, Adalimumab, Certolizumab, Golimumab and Etanercept, as well as other biological agents such anti Abatacept, Tocilizumab, Rituximab, strongly impacted on the management of patients affected by chronic articular inflammatory diseases, such as RA or AS[1-3]. These agents showed good safety profiles as well as outstanding capacities in stopping or slowing radiological progression and clinical symptoms and signs of joint inflammation, also when compared with DMARDs. Nevertheless, in some patients, monoarthritis unresponsive to systemic therapy

Keywords: Adalimumab, Arthritis, Intra-articular injection, ultrasonographic guide

List of abbreviations: TNF = Tumor Necrosis Factor, DMARDs = Disease Modifying Anti Rheumatic Drugs, RA = Rheumatoid Arthritis, AS = Ankylosing Spondylitis, PsA, = Psoriatic Arthritis, MTX = Methotrexate, ACY = A-Cyclosporin, PDN = Prendisone, SLZ = Sulfasalazine, CLX = Celecoxib, IA = intra-articular, ACR = American College of Rheumatology.

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can persist even after the use of association of MTX and biological agents; such patients usually undergo to local treatment, with intra-articular injection of different preparations containing slow-release steroids. In case of unresponsiveness to this treatment, intra-articular administration of anti-TNFα Antibodies can be considered. In fact, the local treatment of mono/oligoarthritis unresponsive to common DMARDs and corticosteroids using intra-articular anti-TNFα agents[4-11] is emerging as a possible and promising therapeutic option. Ultrasound guidance was utilized for performing every intra-articular injection. Ultrasonography allows the recognition of joint features, the evaluation of joint disease severity and the detection of eventual presence of bursitis, effusion and intra-articular free bodies[12-14]. Scientific literature reports on the intra-articular use of Etanercept, Infliximab and Adalimumab, on small cohorts of patients or by case-reports. By this study, we aim to add evidence to the use of intra-articular Adalimumab and to report on its use in different joints such as elbow and hip joint.

MATERIALS AND METHODS

Patients affected by Rheumatoid Arthritis (RA), or Ankylosing Spondylitis (AS) or Psoriatic Arthritis (PsA), under therapy with a stable dose of DMARDs lasting for at least 6 months before entering the study, whose clinical condition was characterized by a residual monoarthritis non responding to IA injection of steroids and lasting for at least 6 months, were selected. Only patients who received at least 1 intra-articular injection of steroids previously were enrolled into this study. Intra-articular injection of steroids had to be performed at least six months before entering the study.

In such patients a single ultrasound-guided intra-articular injection of anti-TNFα biological, Adalimumab, was performed in the affected joint. All patients involved in this study underwent to the common screening performed before the use of biological such as TNF inhibitors and signed an informed consent before entering the study. The present study reports data obtained by an open, uncontrolled, non-randomized treatment.

All patients' diagnosis was performed following different classification criteria for Rheumatoid Arthritis, Ankylosing Spondylitis and Psoriatic Arthritis[15-17]. Results obtained have been evaluated in terms of pain VAS and a Synovial thickness evaluation[18] (performed on knee joints and evaluated as suggested by EULAR committee) and Color-Doppler signal evaluation was performed before and after treatment in order to assess eventual amelioration in inflammatory pattern of arthritis, except in case of sacroiliitis-affected patients, where Color-Doppler evaluation was not performed. Moreover, a global physician's assessment was established for every patient at every control visit.

Each patient was evaluated at baseline and then every 2 months. Pain VAS of the injected joint, physician's assessment and Color-Doppler signal were screened at every control visit, as well as synovial thickness in patients who were treated for knee monoarthritis. Color-Doppler signal was evaluated on a 0-4 scale, with 0 meaning absence of synovial Doppler signal and 4 meaning the most intense synovial Doppler signal. Synovial thickness of knee joint was evaluated as suggested by EULAR commettee[18] as pathological when thickness exceeded 4 mm. We considered patients as responders when maintaining for all control visits a reduction in observed parameters of at least 50% when compared with baseline.

Eventual side-effects due to intra-articular treatment were also screened by mean of laboratory analysis commonly performed during follow-up for inflammatory arthritis (liver and kidney profiles, ESR, CRP, blood count), that was performed every 2 months.

RESULTS

12 patients were enrolled in this study. All patients underwent to IA steroids injection previously, but without a benefit. All patients completed the follow-up time of 12 months performing a complete control visit every 2 months. We performed 12 IA injections with 40 mg of Adalimumab in 12 joints (2 hips, 5 knees, 2 ankles, 2 elbows and 1 sacro-iliac joint); 4 patients were affected by RA, 4 were affected by PsA and 4 by Ankylosing Spondylitis (AS).

A demographic summary has been provided for all patients treated (Table 1)

Results in terms of pain VAS and Color-Doppler signal are reported in Table 2 and Table 3, while results in terms of global psysician's assessment are reported in table 4. Results obtained when analyzing synovial thickness of knee joint are reported in table 5. 7 patients out of 12 reported an improvement of at least 50% when compared with baseline values in pain VAS, global physician's assessment and Color-Doppler signal already at first control visit, performed at 2 months, and lasting for 12 months, 3 patients

Table 1. *Patients' characteristics, with gender, age, injected joint and inflammatory disease. M = Male, F = Female, AS = Ankylosing Spondilitis, RA = Rheumatoid Arthritis, PsA = Psoriatic Arthritis. CLX = Celecoxib, PDN = Prednisone, SLZ = Sulfasalazine, MTX = Methotrexate, ACY = A-Cyclosporin, MPDN = Metil-Prednisolone, TAC = Triamcinolone acetonide.*

Patient n.	Gender	Age	Injected Joint	Disease	Duration of monoarthritis (months)	Treatmennt	Number of previous steroids I.A. injections	Steroids injected
1	M	43	hip	AS	6	CLX,PDN	1	MPDN, 40mg
2	M	38	hip	AS	6	CLX, SLZ, PDN	1	MPDN, 40mg
3	F	62	knee	RA	7	MTX	2	TAC, 40mg
4	M	54	knee	AS	6	CLX	1	TAC, 40mg
5	F	48	knee	RA	7	MTX	2	TAC, 40mg
6	M	61	knee	AS	6	CLX, PDN	1	MPDN, 40mg
7	F	36	knee	RA	7	MTX	2	TAC, 40mg
8	F	44	elbow	RA	7	MTX	1	MPDN, 20mg
9	F	42	elbow	PsA	7	ACY	1	TAC, 20mg
10	M	53	ankle	PsA	11	ACY, PDN	1	TAC, 20mg
11	F	48	ankle	RA	8	ACY	1	MPDN, 20mg
12	M	40	sacro-iliac	AS	10	CLX	1	TAC, 20mg

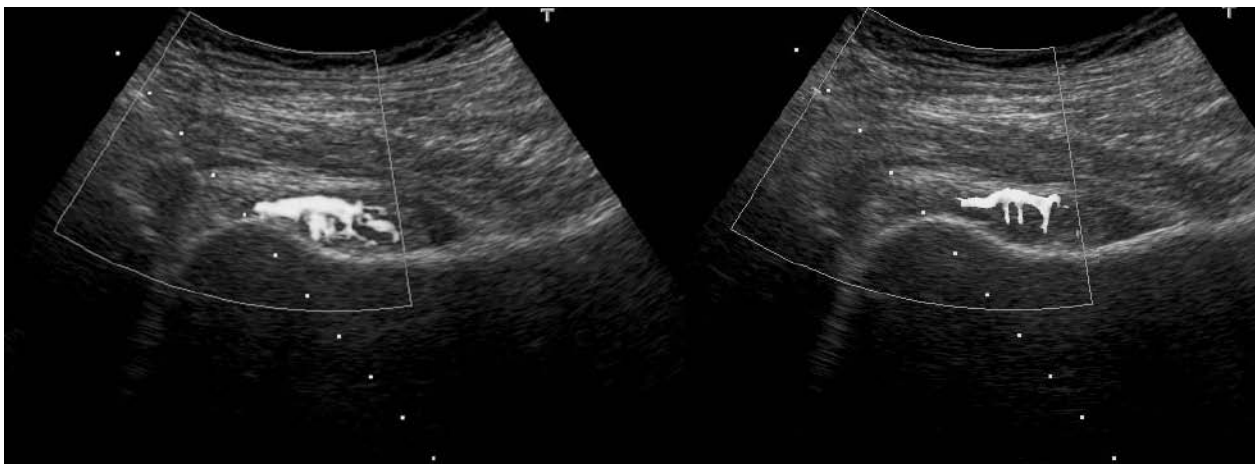

Fig.1. *Ultrasonographic images of hip joint in patient 2, at baseline and at 6 months follow-up visit.*

reported an improvement in joint condition starting at 4 months control visit and lasting until last control visit, and 2 patients, both affected by RA, never showed any improvement. Ankle joint ultrasonographic images from patient 10 are shown in Figure 1.

No side effects were reported by patients or observed by laboratory analysis. 2 patients, who received intra-articular injection on elbow joint, reported a local transient pain that lasted for 24 hours at the site of injection that regressed without need of adjunctive medication.

DISCUSSION

At now, only few and small experiences about intra-articular use of anti-TNF alpha agents in patients affected by Rheumatoid Arthritis or Spondyloarthropaties are reported. No randomized multicentric placebo controlled studies are reported. It is possible to find, from 2001 to 2011, only some

Table 2. *Pain VAS referred to the injected joint as reported by patients at baseline and at every control visit, performed every 2 months.*

	Pain VAS						
Patient n.	baseline	2 months	4 months	6 months	8 months	10 months	12 months
1	7	1	1	1	1	1	1
2	8	4	3	3	1	1	1
3	7	6	3	3	3	3	4
4	10	2	3	4	3	4	4
5	8	2	3	3	3	3	3
6	7	1	1	0	0	0	0
7	9	7	9	9	9	9	9
8	7	5	3	3	3	3	3
9	10	8	3	3	2	3	2
10	8	2	4	3	3	4	2
11	10	7	10	10	10	10	10
12	10	2	3	3	4	3	3

Table 3. *Color-Doppler signalling referred to synovial layer as observed for every patient in the interested joint, at baseline and at every control visit, performed every 2 months. V Patient n12, affected by sacroiliitis, was not assesses with Color-Doppler signalling evaluation due to the kind of affected joint. NP = not performed.*

	Color-Doppler synovial signal						
Patient n.	baseline	2 months	4 months	6 months	8 months	10 months	12 months
1	3	1	1	1	1	2	1
2	3	1	1	2	0	0	0
3	4	3	1	1	1	1	1
4	3	1	1	1	1	1	1
5	2	0	0	1	0	0	0
6	2	1	1	1	1	1	1
7	3	2	3	3	3	3	3
8	3	1	2	1	1	1	1
9	4	3	1	0	2	1	2
10	2	1	1	1	0	0	1
11	4	3	4	4	4	4	4
12	NP	NP	NP	NP	NP	NP	NP

positive or negative reports in patients affected by RA treated with intra-articular Infliximab[4-6] with a small number of patients and with a very short follow-up. Furthermore, IA treatment with Etanercept[7,8] was performed with good results in very small cohorts of patients, affected by RA or Sarcoidosis; similarly, in these reported cases the follow-up period was short. Etanercept showed a good safety profile, but one case of severe side effects after IA treatment is reported[9].

In patients affected by AS, intra-articular treatment with Anti-TNF alpha was performed in sacro-iliac joints[10] and in the knees[11] but similarly, the good clinical results reported regarded few patients

Table 4. *Global physician's assessment as established at every control visit for all patients.*

	Global physician's assessment						
patient number	baseline	2 months	4 months	6 months	8 months	10 months	12 months
1	8	3	3	3	3	3	3
2	8	3	2	1	1	1	1
3	10	3	1	4	2	2	2
4	7	3	2	1	1	1	1
5	9	4	3	3	3	3	3
6	9	3	2	2	2	2	2
7	9	6	8	8	8	8	8
8	8	5	3	3	4	3	3
9	9	5	1	4	2	1	1
10	8	4	2	4	2	2	4
11	8	8	8	8	8	8	8
12	9	3	3	3	3	3	3

Table 5. *Synovial thickness as evaluated in knee joints by ultrasound performed during Adalimumab injection.*

	Synovial thickness as revealed by ultrasound performed for intra-articular injection guidance						
Patient number	baseline	2 months	4 months	6 months	8 months	10 months	12 months
3	>4mm	3mm	2mm	2mm	2mm	2mm	2mm
4	>4mm	2mm	2mm	3mm	3mm	2mm	3mm
5	>4mm	2mm	2mm	2mm	2mm	2mm	2mm
6	>4mm	2mm	2mm	2mm	<2mm	<2mm	<2mm
7	>4mm	>4mm	>4mm	>4mm	>4mm	>4mm	>4mm

and the follow-up time was shorter than six months.

Recently, Haroon et al.[19] reported their experience on the use of the combination of intra-articular corticosteroids and anti-TNF agents in monoarthritis of the knee in five patients. Their experience also added to the knowledge of the use of intra-articular anti-TNF.

We describe our experience about US-guided IA therapy with Adalimumab, in twelve patients affected by different inflammatory rheumatic diseases. None of the patients showed or reported severe adverse events after the IA injections of Adalimumab.

As highlighted in the introduction, local injection of biologic agents seems to be a valid therapeutic option only in cases of mono or oligo-arthritis that are unresponsive to common DMARDs and local steroids.

Furthermore, there are several conditions where biological drugs are contraindicated or must be administered with caution, such as acute infective conditions or active tubercular infections, occurrence of a malignant tumour (present or appeared within the last five years) and severe congestive heart failure.

Our experience shows a positive and rapid response to treatment with local Adalimumab injection with an acceptable safety profile in 7 of 12 patients treated. However, the mean period of follow-up is short (12 months). 3 patients showed an improvement in all parameters observed but

starting from 4 months control visit and lasting for 12 months. Patients showing a late response were affected by RA (2 patients) and PsA (1 patient) and were all females of different age who received different numbers of different steroids by intra-articular injection. The reasons for this late response remain unclear, probably due to the small number of patients observed.

Articular damage caused by long-lasting systemic or local inflammation is irreversible, and the effects of this injury can be responsible for a residual symptomatology even if inflammation is quenched by the proper therapy. The local treatment with Adalimumab was aimed to reduce symptoms and, if possible, to slow down progression of articular damage caused by persisting inflammation, but we did not expect that patient could recover anatomic injury.

Two patients, both affected by RA, although showing an initial positive response to Adalimumab intra-articular administration, soon returned to previous clinical conditions.

Two patients never showed signs of amelioration both in symptoms and in ultrasound pattern of synovia.

From the present data, we could argue that IA Adalimumab was more effective in patients affected by Ankylosing Spondylitis or Psoriatic Arthritis than in patients affected by RA, with a better and more prolonged result, even if further studies are necessary to confirm it.

The short follow-up time and the small number of patients treated can not lead to definitive conclusions. We should also consider the relevance of correct timing in the choice of IA therapy, which could be one of the reasons causing the failure of IA Adalimumab administration in the last two patients. For such reason, we suggest to act during the early active inflammatory phase, in order to arrest the evolution of the pathology and then arrest or slow down subsequent radiographic progression and consequently to prevent irreversible damage of the joints[21,22].

According to the study of Conti et al.[20] that used a scintigraphy with 99mTc-Infliximab to predict response to IA infliximab, a scintigraphy with 99mTc-Adalimumab could be useful to select patients with best probability of a good response to IA Adalimumab treatment.

Another open question regards what may be the right dosage for the IA use of anti-TNFα agents. In literature, previous experiences are reported on the use of 10 mg of Infliximab in inter-phalangeal joints up to 200 mg for knee joints (two 100 mg injections separated by a 24-hours interval). Etanercept doses used can also range from 2 mg to 25 mg[23,24].

Bokarewa et al.[6] reported data on low responses and even on exacerbation of arthritis symptoms after IA injection, suggesting that low doses of TNFα blocking agent injected into the joint could be insufficient to bind a large amount of TNFα continuously produced *in loco* by inflamed joint tissues.

In the case of IA biological treatment, eventual systemic absorption and IA half-life of the injected biological agent must also be considered as possible conditioning factors. As previously performed for steroids, it could be necessary to produce slow-releasing formulations of biological agents in order to maintain the active form of the drug inside the joint for the time needed to induce the desired effects.

Finally, it is important to emphasize how that the effectiveness and safety of each IA injection of different anti-TNFα agents depend on the correct positioning of the needle, and consequently, of the drug, within the joint; it has been reported that in more than 50% of conventional IA injections the needle is not correctly inserted[25-27]. Therefore, it is necessary to use image guidance such as ultrasound scan, as a safe guidance allows clinicians to inject the product exactly inside the joint. Furthermore, a real-time monitoring of needle progression reduces the risks of injecting vessels, tendons or nerves and also allows to visualize that the therapeutic compound is being injected into the joint, during the injection procedure, especially in joints that are difficult to reach, such as hip joint[13].

CONCLUSIONS

Our data suggest that IA therapy with Adalimumab may be a safe and effective approach for selected cases of patients affected by mono/oligoarthritis unresponsive to traditional therapies and not suitable for systemic use of biologicals.

Larger, randomized, controlled, double-blind

studies are needed, not only to validate our initial positive observations, but also to establish the right dose to be used for IA treatment and to possibly develop new slow-releasing formulations of drugs, that may grant the achieving of an effective and durable response.

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EFFECTIVENESS AND SAFETY OF INJECTABLE ENDPROSTHETICS OF SINOVIAL FLUID BY CROSS-LINKED POLIMER NOLTREX FOR TREATMENT OA KNEE.

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Osteoarthritis – this is the diagnosis of the most numerous group of patients of any orthopedic clinical department nowadays. It is considered to affect from 6.4 to 12% of population and the number keeps growing. Disability, caused by osteoarthritis, takes the first place by the number of cases among all orthopedic pathologies. Arthritis occurs in more than 50% of people over 60 years old. Social significance of the disease determines the urgency of developing the new effective methods of treatment and prevention of arthritis. Today, there are three most popular ways of treatment for osteoarthritis (OA) of the knee: intra-articular hyaluronan viscosupplementation injections; oral glucosamine, chondroitin or the combination; furthermore, arthroscopic lavage or debridement. Intra-articular hyaluronan (IA-HA) injections are now licensed worldwide for the treatment of osteoarthritis (OA). A course of treatment consists of series of one to five weekly intra-articular injections with a viscoelastic solution of hyaluronan or its derivatives. It is believed that such a mechanism should have an impact on the ability to suspend joint cartilage degeneration, significantly improve the quality of life of the patient's oppression pain syndrome, which is particularly important in patients with early stage of arthrosis, as well as in people which should refrain from disseminating radical treatment - a total replacement joint. This method does not require special conditions for usage and is accessible to several specialists as this new drug may be useful for many different kinds of specialists such as orthopedics, rheumatologists, pain medicine practitioners and so on.

Recently, the effectiveness of the treatment of OA knee joint with intra-articular injection hialuronic acid products seem more than disputable. In published report "Viscosupplementation for the treatment of osteoarthritis of the knee"[1] the authors came to the conclusion that this type of treatment prove effective only for 13 weeks. In the Evidence Report/Technology Assessment Number 157 "Treatment of Primary and Secondary Osteoarthritis of the Knee"[2] prepared for the Agency for Healthcare Research and Quality U.S. Department of Health and Human Services authors gathered: "The three interventions (intraarticular hyaluronan viscosupplementation injections; oral glucosamine, chondroitin or the combination; and arthroscopic lavage or debridement) reviewed in this report are widely used in the treatment of OA of the knee, yet the best available evidence does not clearly demonstrate clinical benefit. Uncertainty regarding the clinical benefit can be resolved only by rigorous, multicenter RCTs. In addition, given the public health impact of OA of the knee, research on new approaches to prevention and treatment should be given high priority."

Currently, there are a lot of HA products for viscosupplementation available in the market. Here are some of them: Hyalgan (Fidia SpA, Padua, Italy), Supartz (Seikagaku Corporation, Tokyo, Japan), Orthovisc (Anika Therapeutics, Woburn, MA) and

Key words: Knee osteoarthritis - Viscosupplementation - Intra-articular injection - Hyaluronic acid

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

Synvisc (GenzymeCorporation, Cambridge, MA). Four of the IA-HA products currently available in the United States are produced from chicken combs and therefore require the removal of inflammatory and immunogenic impurities endogenous to the avian tissue source[3]. Efficacy trials comparing HA injections with saline injections demonstrate a statistically significant difference over a 3 -month period, depending on the trial design[4-7].

The main objective of our study is to confirm the effectiveness and safety of OA treatment by intraarticular injectable endoprosthetics of synovial fluid with new 100% synthetic polymer material ("Noltrex") which has antibacterial properties and provides a long-lasting effect. Our study was initiated in 2002, when the material was introduced to the Russian market, currently the product is approved for use in the EU, as well as in other countries around the world.

METHODS

This was a multicenter, prospective, cohort study conducted in adult patients with symptomatic OA of the knee. Noltrex™ – high viscosity gel, a unique form of water-based three-dimensional biopolymer with silver ions. Noltrex ™ - fully synthetic material with viscoelastic-elastic characteristics of synovial fluid. It does not contain substances of animal origin. It has an antibacterial effect. The product was administered as a course of three 2,5 ml injections weekly. Before each injection, the synovial fluid presented in the knee was aspirated. Patients were advised to rest for 24 h following each injection, consistent with the label instructions for most IA-HA products. Assessments were performed at screening, at baseline (prior to the first injection), and at 13, 26, 39, 52, 78 and 104 weeks after the initial injection. Only acetaminophen was permitted for rescue analgesia, up to 4 g daily, with usage quantified by pill counts. Non-steroidal anti-inflammatory drugs (NSAIDs) and other non-acetaminophen pain medications were prohibited during the study, and patients taking such agents were considered dropouts from the point of medication usage. The study was carried out in accordance with the International Conference on Hormonization (ICH) Guidelines for Good Clinical Practice (May 1, 1996, amended September 1997) and the Declaration of Helsinki concerning medical research in humans (1966).

PATIENTS

Patients were enrolled at 3 sites across Russia. The study protocol and informed consent form were approved by the local ethics committees. The study was open to patients of either sex, age 50-80 years, with confirmed OA in one or both knees. OA diagnosis date and radiological diagnosis date for the study knee and other knee were recorded in the study. In patients with bilateral OA, the more symptomatic knee was assigned as the study knee at the screening visit based on the investigator's clinical judgment.

Criteria for inclusion were as follows: clinical and radiological evidence of chronic idiopathic OA of the study knee according to Altman's criteria; radiologically verified OA of the study knee of grade 1 to 4 according to a modification of Kellgren-Lawrence grading system (grade 1 defined as osteophytes with some joint space narrowing; grade 2 defined as definite osteophytes with unimpaired joint space; grade 3 defined as definite osteophytes with moderate joint space narrowing; grade 4 defined as definite osteophytes with moderate bone attrition[8]; symptoms in the study knee for at least 1 year; willingness to discontinue all OA treatments other than acetaminophen; and moderate-to-severe knee pain as reflected by a subjective - analog scale pain score of 1 to 3 (on a scale of 0 [no pain] to 3 [worst pain]) for the average of the four pain questions. The four questions in pain scale are regarding pain during (1) walking on a flat surface, (2) going up and down stairs, (3) rest at night, (4) standing upright. Patients were excluded from the study if they had secondary OA originating from a known injury to the knee, rheumatoid arthritis, history of joint infection, dermatologic disorders or skin infection in proximity to the study knee, osteonecrosis, chronic active fibromyalgia, any inflammatory or metabolic arthritides and known hypersensitivity to acetaminophen or polyacrilamide. Patients were also excluded if they had hyaluronan injections to the study knee within 6 months of the screening visit, corticosteroid injections and surgery or arthroscopy to the study knee within 3 months of screening.

OUTCOME MEASURES

The primary effectiveness outcome measure

was the change in patients' average score on the four questions in the 4-points pain scale. For the patient global evaluations, subjects were asked to respond to the question ''Are you satisfied with the results of the injections?'' and to grade their response on the four-point ordinal scale: (1) dissatisfied, (2) slightly satisfied, (3) satisfied or (4) very satisfied. These provided the core outcomes measures of pain, function, and global assessment suggested for OA clinical trials[9,10] Safety was assessed by collecting adverse event data at each study visit, or whenever reported by patients. Adverse events were defined as any emergent sign or symptom, whether related or unrelated to the study treatment. A serious adverse event was defined as one that (1) was fatal, (2) was life threatening, (3) resulted in persistent or significant disability/incapacity, (4) required or prolonged inpatient hospitalization or (5) was a congenital anomaly or birth defect. Adverse events were coded using MedDRA (Medical Dictionary for Regulatory Activities) terminology, with coding performed before unblinding the study. Patients' blood chemistry and haematological parameters were assessed at the beginning and end of the study, and vital signs were monitored for 30 min after each injection.

STATISTICAL METHODS

The analyses presented were performed per protocol, defined as all patients to treatment and receiving the course of injections. Patients using prohibited pain medications during the trial were considered dropouts from the point of medication usage. The primary effectiveness outcome measure of the study was the improvement in the pain score from baseline (week 0) to the follow-up patient visits (weeks 13,26,39,52,78 and 104). The value of 0.3 , which is less than the minimal clinically important difference of 0.36 – 0.4831, was selected as the criterion for non-inferiority. Patients with a score of <1.0 were defined as ''symptom free'' because a 0-1.0 score on the pain subscale would correspond to a ''none'' rating on the pain subscale[11]. Patient global overall assessments evaluated at the end of the study were analyzed using Wilcoxon's twosample test.

SAMPLE SIZE CALCULATION

Assuming a standard deviation of 1.0 for the primary outcome measure, the required sample size for the study was estimated to be at least 360 patients for a power of 0.8 and a significance level of 0.05 (one sided). Anticipating protocol violators and early discontinuations for 30%, it was projected that 515 patients should be enrolled.

RESULTS

Patients

Patient disposition for the study is presented in Fig. 1. Of 612 patients screened at study entry, a total of 527 patients met the criteria for study entry. Of the 85 patients not meeting the trial's inclusion or exclusion criteria, 27 were excluded because they did not meet the inclusion criteria for pain scores at baseline. Of the 527 patients receiving at least one injection, 408 (77%) completed the final study visit, with two patients discontinued because of an adverse event (effusion in the injected knee, possibly treatment related). 70 patients were lost for follow-up (probably by reason of prolonged 2 years follow up period and presumably they had got satisfactory results and therefore, decide not to waste time for visits, during treatment 65 of them noticed improvement), 20 patients decided to withdraw 1 year after the course of injection (they evaluate the same pain as before treatment) and 27 patients decided to withdraw from the study for unknown reasons. The analyses presented here were performed per protocol, defined as all patients and receiving the course of injections. Baseline characteristics for the patients are provided in Table I. The patients were predominately female (3:1 ratio), with a mean age of 57.2 years and a mean duration of OA in the study knee of 63.9 months. Kellgren and Lawrence radiologic grades were distributed between grades as following: I -17% (70 patients), II – 37% (151 patients), III – 38% (155 patients) and IV – 8% (32 patients). All the patients met at least four of the six Altman criteria for the diagnosis of knee OA. During the physical examination prior to treatment, patients were evaluated with respect to presence of effusion in the study knee (in ml) by performing effusion drainage before injecting the drug.

Primary outcome measure

Table III provides the absolute change and percent

improvement from baseline for the groups divided by grade for each question in the pain subscale and for the average of the four pain questions (the primary outcome measure). All groups experienced statistically significant and clinically important improvements from baseline (P<0.0001). At the study end point, the mean improvement in the primary effectiveness measure for the Grade I group was 1.25 (73% improvement from baseline) in 13 weeks interval , the same result 1.25 (73% improvement) for 26 weeks, and 1.05 (61% improvement) for 1 year and 0.55 (32% improvement) for two years interval. Primary effectiveness measure for the Grade II group was 1.225 (61% improvement from baseline) in 13 weeks interval , the same result 1.225 (61% improvement) for 26 weeks, and 1.1 (55% improvement) for 1 year and 0.325 (16% improvement) for two years interval. Primary effectiveness measure for the Grade III group was 1.525 (63% improvement from baseline) in 13 weeks interval , the same result 1.525 (63% improvement) for 26 weeks, and 1.35 (55% improvement) for 1 year and 0.325 (13% improvement) for two years interval. Primary effectiveness measure for the Grade IV group was 1.3 (46% improvement from

Fig. 1. *Flow chart of patient disposition.*

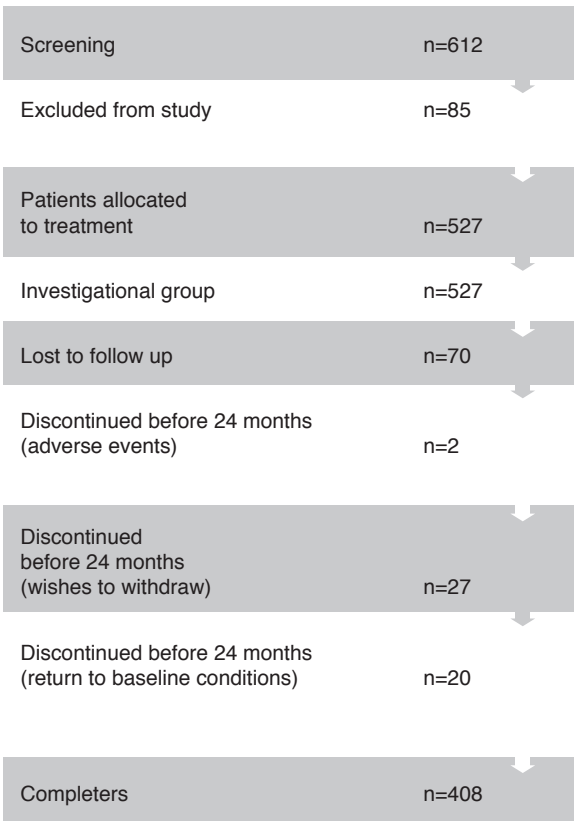

Table I. *Patient demographic baseline characteristics*

	Parameter Number of patients (%)
Gender	
Female	305(74.8%)
Male	103(25.2%)
Age (years; mean+Standard Deviation (SD))	57,2±7.3
Study knee	
Left 196	(48.0%)
Right 212	(52.0%)
Kellgren and Lawrence grading system	
(Grade 1)	70 (17.2%)
(Grade 2)	151(37.0%)
(Grade 3)	155 (38.0%)
(Grade 4)	32 (7.8%)
Clinical symptomatology	
Knee pain	408 (100%)
Stiffness <30 minutes	384 (94.1%)
Crepitus	399 (97.8%)
Bony tenderness	352 (86.3%)
Bony enlargement	193 (47.3%)
No palpable warmth	387(94.8%)
Duration of OA in study knee	
Months prior to enrollment (meanGSD)	63.9±49.8

Table II. *Mean baseline score*

	Mean baseline score (+SE)		
Everage score for 4 Questions (0-3 points)	Grade I	Grade II	Grade III
	1.7±0.1	2.0±0.1	2.4±0.1

Table III. *Reduction from baseline in individual questions. Mean* (%) reduction from baseline (+Standard Error (SE))*

Weeks	13	26	39	52	78	104
Grade I (Average Score)	**1.25**	**1.25**	**1.05**	**1.05**	**0.75**	**0.55**
walking on a flat surface	1.3 ±0.2 (76 ± 11)	1.3 ±0.2 (76 ± 11)	1.1 ±0.2 (64 ± 11)	1.1 ±0.2 (64 ± 11)	0.8 ±0.1 (47 ± 6)	0.6 ±0.1 (35 ± 6)
going up and down stairs	1.3 ±0.2 (76 ± 11)	1.3 ±0.2 (76 ± 11)	1.1 ±0.2 (64 ± 11)	1.1 ±0.2 (64 ± 11)	0.8 ±0.1 (47 ± 6)	0.6 ±0.1 (35 ± 6)
rest at night	1.2 ±0.2 (70 ± 11)	1.2 ±0.2 (70 ± 11)	1.0 ±0.15 (59 ± 9)	1.0 ±0.15 (59 ± 9)	0.7 ±0.1 (41 ± 6)	0.5 ±0.1 (29 ± 6)
standing upright	1.2 ±0.2 (70 ± 11)	1.2 ±0.2 (70 ± 11)	1.0 ±0.15 (59 ± 9)	1.0 ±0.15 (59 ± 9)	0.7 ±0.1 (41 ± 6)	0.5 ±0.1 (29 ± 6)
Grade II (Average Score)	**1.225**	**1.225**	**1.1**	**1.1**	**0.875**	**0.325**
walking on a flat surface	1.3 ±0.2 (65 ± 10)	1.3 ±0.2 (65 ± 10)	1.2 ±0.15 (60 ± 7)	1.2 ±0.15 (60 ± 7)	0.9 ±0.15 (45 ± 7)	0.5 ±0.1 (25 ± 5)
going up and down stairs	1.3 ±0.2 (65 ± 10)	1.3 ±0.2 (65 ± 10)	1.2 ±0.15 (60 ± 7)	1.2 ±0.15 (60 ± 7)	0.9 ±0.15 (45 ± 7)	0.4 ±0.1 (20± 5)
rest at night	1.2 ±0.1 (60 ± 5)	1.2 ±0.1 (60 ± 5)	1.1 ±0.1 (55 ± 5)	1.1 ±0.1 (55 ± 5)	0.9 ±0.15 (45 ± 10)	0.2 ±0.1 (10 ± 5)
standing upright	1.1 ±0.1 (55 ± 5)	1.1 ±0.1 (55 ± 5)	0.9 ±0.1 (45 ± 5)	0.9 ±0.1 (45 ± 5)	0.8 ±0.2 (45 ± 5)	0.2 ±0.1 (10 ± 5)
Grade III (Average Score)	**1.525**	**1.525**	**1.35**	**1.35**	**0.775**	**0.325**
walking on a flat surface	1.6 ±0.2 (66 ± 8)	1.6 ±0.2 (66 ± 8)	1.5±0.15 (62 ± 6)	1.3±0.15 (54 ± 6)	0.9 ±0.1 (37± 4)	0.5 ±0.1 (20 ± 4)
going up and down stairs	1.6 ±0.2 (66 ± 8)	1.6 ±0.2 (66 ± 8)	1.5 ±0.15 (62 ± 6)	1.3 ±0.15 (54 ± 6)	0.9 ±0.1 (37 ± 4)	0.4 ±0.1 (17 ± 4)
rest at night	1.5 ±0.1 (62 ± 4)	1.5 ±0.1 (62 ± 4)	1.3 ±0.1 (54± 4)	1.1 ±0.1 (46± 4)	0.7 ±0.1 (29 ± 4)	0.2 ±0.1 (8 ± 4)
standing upright	1.4 ±0.1 (58 ± 4)	1.4 ±0.1 (58 ± 4)	1.1 ±0.1 (46 ± 4)	1.0 ±0.1 (42 ± 4)	0.6 ±0.1 (25 ± 5)	0.2 ±0.1 (8 ± 4)
Grade IV (Average Score)	**1.3**	**1.3**	**0.9**	**0.65**	**0.4**	
walking on a flat surface	(50 ± 4)	(50 ± 4)	(36 ± 4)	(25 ± 4)	(18 ± 4)	
going up and down stairs	1.4 ±0.1 (50 ± 4)	1.4 ±0.1 (50 ± 4)	1.0 ±0.1 (36 ± 4)	0.7 ±0.1 (25 ± 4)	0.5 ±0.1 (18 ± 4)	
rest at night	1.2±0.1 (42 ± 4)	1.2±0.1 (42 ± 4)	0.8±0.1 (29 ± 4)	0.6±0.1 (21 ± 4)	0.3±0.1 (11 ± 4)	
standing upright	1.2 ±0.1 (42 ± 4)	1.2 ±0.1 (42 ± 4)	0.8 ±0.1 (29 ± 4)	0.6 ±0.1 (21 ± 4)	0.3 ±0.1 (11 ± 4)	

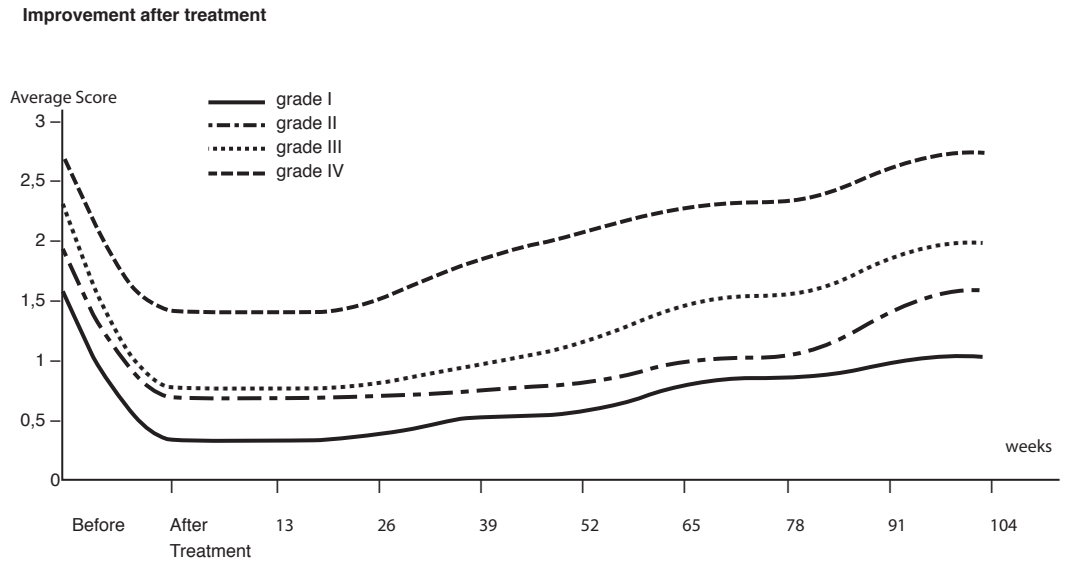

Fig. 2. *Mean change in the primary efficacy outcome during the study.*

baseline) in 13 weeks interval , the same result 1.3 (46% improvement) for 26 weeks, and 0.65 (23% improvement) for 1 year and no result for two years interval.

Figure 2 displays the mean change in the score for the primary effectiveness measure during the full study period, illustrating that clinically important reductions in pain continued after completion of the 15-day injection period for patients in all treatment groups. By the 26-week study interval, the mean score for the pain average had dropped to approximately 1.0 in treatment groups.

Those patients who had a total score <1,0 of four pain questions (defined earlier as symptom-free patients) were compared (In percent) with *post-hoc* analysis treatment groups.

As shown in Table IV the percentage of symptom-free patients has decreased from the beginning of the study to the final stage of testing. For the group Grade I the decline was from 65 patients (93%) at 13 week of the study to 53 patients (75%) at 104 week of the study. For groups Grade II and III the results were as follows: 124 (82%) patient after 13 weeks, 112 (74%) after 52 weeks and 120 (77%) patients after 13 weeks, 95 (61%) patients after 52 weeks respectively. In the group Grade IV there were no symptom-free patients.

Secondary outcome measures

The results of the patient global assessments performed at the study end point (104 weeks) are illustrated in Table V. Despite the fact that the condition of patients in 104 weeks was the same or close to the condition before treatment, approximately 92% of patients reported some degree of satisfaction with treatment and approximately 80% reported being either satisfied or very satisfied in all study groups.

Baseline scores for the pain, stiffness, and physical function scales are shown in Table II.

Use of rescue medication

The use of rescue medication (acetaminophen) in the study population is detailed in Table VI for all evaluation time points. For the full study population, 40% (165/408) patients required rescue medication at some point during the study. It was prohibited to use rescue medication 2 weeks before the visit.

Table IV. *Number of symptom-free patients (score below 1.0) in four pain questions (individual categories and average scores) during the study*

	13	26	39	52	78	104
Grade I	65 (93%)	65 (93%)	63 (90%)	62 (88%)	54 (77%)	53 (75%)
walking on a flat surface	65 (93%)	65 (93%)	64 (91%)	63 (90%)	57 (81%)	55 (78%)
going up and down stairs	63 (90%)	63 (90%)	61 (87%)	61 (87%)	52 (74%)	51 (72%)
rest at night	67 (96%)	67 (96%)	65 (93%)	64 (91%)	54 (77%)	54 (77%)
standing upright	65 (93%)	65 (93%)	62 (88%)	62 (88%)	53 (75%)	52 (74%)
Grade II	124 (82%)	124 (82%)	116 (77%)	112 (74%)	69 (45%)	
walking on a flat surface	127 (84%)	127 (84%)	117 (77%)	114 (75%)	72 (48%)	-
going up and down stairs	116 (77%)	116 (77%)	107 (70%)	102 (67%)	64 (42%)-	
rest at night	131 (87%)	131 (87%)	125 (82%)	122 (81%)	77 (51%)	
standing upright	123 (81%)	123 (81%)	115 (76%)	111 (73%)	65 (43%)-	
Grade III	120 (77%)	120 (77%)	96 (62%)	95 (61%)		
walking on a flat surface	121 (78%)	121 (78%)	97 (62%)	95 (61%)		
going up and down stairs	109 (70%)	109 (70%)	77 (49%)	76 (49%)		
rest at night	132 (85%)	132 (85%)	113 (73%)	111 (71%)		
standing upright	119 (76%)	119 (76%)	99 (64%)	97 (63%)		

Safety outcome

Adverse events were coded using MedDRA terminology. A total of 134 treatment-emergent adverse events were reported in 84 patients. The severity of these adverse events was coded by the investigator as mild or moderate in 93/134 cases (69.4%). There were no deaths in treatment groups. Six serious adverse events were reported during the trial; none of these events were considered related to the study treatment, and none resulted in withdrawal of the patient from the study. No significant systemic reactions were noted. There were no significant within or between-group changes in mean values for clinical laboratory evaluations or vital signs over the course of the study. Adverse events for more than 5% of patients are listed in Table VII. The most commonly reported events were burning pain (during the course of injections and week after), arthralgia (unrelated to the treatment, just consequences of the disease) and joint effusion. Two patients were reported to have a strong joint effusion (possibly attributed to study therapy); these patients withdrew from the study.

DISCUSSION

Several IA-HA products are currently available in the United States for the treatment of knee OA, and more than 20 similar products are available in other parts of the world. These preparations can differ significantly in their molecular weight, purity, and concentration[12-14]. Efficiency of treatment OA by HA products is proven by numerous researches, but

Table V. *Subjective patient assessment of treatment. Overall patient assessment of treatment*

	Grade I	Grade II	Grade II	Grade IV	Total
Patients assessed	70 (100%)	151 (100%)	155(100%)	32 (100%)	408 (100%)
Dissatisfied	3 (4,2%)	8 (5,2%)	12 (7,7%)	9 (28,1%)	32 (7,9%)
Slightly satisfied	7 (10%)	13 (8,6%)	23 (14,8%)	10 (31,2%)	53 (13%)
Satisfied	15 (21,4%)	48 (31,8%)	33 (21,4%)	7 (21,9%)	103 (25,2%)
Very satisfied	45 (64,4%)	82 (54,4%)	87 (56,1%)	6 (18,8%)	220 (53,9%)

Table VI. *Number of patients required rescue medication*

Time	Grade I	Grade II	Grade III	Grade IV	Total
During the course of injections					
and 1 week after	4	40	60	18	132
Week 13	2	15	26	22	65
Week 13-26	2	25	33	24	84
Week 26-52	7	32	42	27	117
Week 52-78	8	38	51	27	124
Week 78-104	8	41	57	32	138
During the study	11	52	70	32	165

duration of effect from treatment longs no more than 13 weeks[1] and some recent reports of acute local inflammatory reactions to CL-HA injection[15-20]. This trial shows that Noltrex, a product based not on the Hyaluronic acid, but on the base of three-dimensional crosslinked polymer, can be successfully applied for treatment of knee OA. Thus, the trial was designed to indicate the efficiency of Noltrex in the treatment of knee OA and to establish the duration of treatment effect in clinical practice. Dramatically positive results of treatment were reached at groups with the first, second and third grade of the disease, here duration of the effec last more than 52 weeks which is substantially longer, than duration of treatment effect of HA products, reported from other bibliographies on HA studies[1, 2] The results of the patient overall assessments performed at the study end point proved approximately 80% of patients being either satisfied or very satisfied in all study groups. These results are also very important for assessing the benifits of the injectable endoprosthetics of synovial fluid with Noltrex. Safety of the product was also confirmed by this trial. Managing patients with OA of the knee presents a growing challenge to clinicians and health policy decision makers.

Table VII

Adverse event	Number of events	Number of patients (%)
Burning pain	36	28 (6,8%)
Arthralgia	41	38 (9,3%)
Joint effusion	3	2 (0,05%)
Total	93	57 (14%)

Pharmacotherapy with NSAIDs remains a mainstay of therapy, despite the iatrogenic morbidities of long-term NSAID administration[21, 22].

Safety concerns associated with the use of systemic medications are exacerbated by comorbidities in the affected population and potentially dangerous drug interactions. Treatment with HA products is sufficient only for short period of time.The trial data presented, suggest that Noltrex can reduce pain and improve function in patients with knee OA for 1 year period without any iatrogenic local reactions associated with some viscosupplementation products. The injectable endoprosthetics of synovial fluid can help phisicians to manage patients with OA of the knee more efficiently and patients can get chance to defer joint replacement operation.

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POSSIBLE ROLE OF NT-PROBNP IN DIAGNOSIS OF PULMONARY HYPERTENSION ASSOCIATED WITH SYSTEMIC SCLEROSIS

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Object: Pulmonary hypertension (PAH) is a common complication in systemic sclerosis (SSc) and NT-proBNP appears to be an early diagnostic marker of PAH, and highly predictive of severity of the disease and survival. Methods: We enrolled 98 SSc patients (F92; M6), 55 without PAH and 43 with PAH in all functionally NYHA classes. Results: A significant correlation was observed at diagnosis between NT-proBNP values and PAP (r^2 0,254 - $p< 0.0001$). Using values above 35 mm Hg of pulmonary arterial pressure (PAP) as cut-off for echocardiographic diagnosis of PAH, and a cut-off above 70% of predicted DLCO as a pulmonary function test, NT-proBNP values correlate directly with PAH and indirectly with decrease DLCO. According to a bivariate regression model, joint NT-proBNP and DLCO appeared predictors of PAP (r^2 0.42, $p< 0.001$). Considering the presence or absence of pulmonary fibrosis in relation to NT-proBNP and DLCO, in the subgroup of patients with fibrosis we found a trend of better performance of NT-proBNP levels. Conclusions: Our data confirm that NT-proBNP and DLCO levels can significantly predict PAP values, and we suggest in SSc patients, in the presence of reduced DLCO, to associate the measure of serum NT-proBNP to early select a cluster of patients with bad prognosis, strong candidates to develop PAH, to submit to a stricter follow up and eventually more aggressive therapies. An advantage of this approach can be represented by simplicity, availability and low cost of both methods.

Systemic sclerosis (SSc) is a connective tissue disease characterized by vascular damage in skin and connective tissues, and immunological abnormalities leading to fibrosis of skin and internal organs[1]. Among vascular disturbances Pulmonary Arterial Hypertension (PAH) is the major complication; it occurs in 8-15% of SSc patients[2-6] both in limited (lSSc) and diffuse (dSSc) subsets, and is associated with poor prognosis and survival[2-8].

Once established, PAH is poorly responsive to treatments in scleroderma patients, moreover, early symptoms are non specific, mimicking other pulmonary and cardiac aspects of sclerodermic features, and they may occur also in the absence of pulmonary specific involvement.

Many recent studies have stressed the role of N terminal pro-B-natriuretic peptide (NT-proBNP) as a marker of cardiac dysfunction[9-10], also in PAH patients[11-14]. In SSc patients NT-proBNP was reported to be an early diagnostic marker of PAH[12,13], and highly predictive of severity of the disease and survival[15-16-17].

Key words: NT-proBNP, pulmonary hypertension, systemic sclerosis

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Recent studies[18-23] also showed a negative correlation between serum levels of NT-proBNP and diffusing capacity for carbon monoxide (DLCO), therefore suggesting another possibility of use of this marker in association with DLCO as potential early detector of PAH in scleroderma patients.

Aim of our study was o further evaluate the utility of serum NT-proBNP as a non invasive tool to check cardiac and pulmonary involvement in a large group of SSc patients.

PATIENTS AND METHODS

Patients affected by SSc subtype diffuse or limited according to LeRoy et al. criteria[1] were enrolled in three Centres, all in Italy Internal Medicine Department of Legnano Hospital, Gaetano Pini Institute of Milan (hospital day of rheumatology), Referral Centre for Systemic Autoimmune Diseases. Fondazione IRCCS. Ospedale Maggiore Policlinico Milan.

All patients had been diagnosed at least 12 months before inclusion in the study and had been evaluated for pulmonary, cardiac, renal, skin, scleroderma involvement.

Global assessment disease gravity score**,** as usually in rheumatological diagnostic, was determined according to Medseger TA et al. severity scale[24].

None of the patients had renal insufficiency, and at baseline, none was taking calcium channel blocker or endothelin receptor antagonist (ETRA). All patients received monthly intravenous therapy with iloprost , but the therapy was stopped one month before the NT-proBNP determination.

All patients gave informed consent and the study protocol was approved by Local Ethical Committees.

Assay of NT-proBNP. Serum NT-proBNP was determined trough the ECLIA method using Modular E170 (Roche Diagnostic, Basel Switzerland) equipment. The 95 percentiles of normal values, as determined by the manufacturer (1411 healthy subjects) according to sex and age group, are as follows: in women 178 pg/mL for those < 45 years, 192 pg/mL for those 45-54 years, 256 pg/mL, for 55-64 years, 353 pg/mL for those 65-74 and 624 for those > 75 years; in men 97 pg/mL for those < 45 years, 137 pg/mL for those 45-54 years, 177 pg/mL, for 55-64 years, 229 pg/mL for those 65-74 and 852 for those > 75 years. The total coefficient of variation, both declared by manufacturer and obtained by internal quality control, was below 5%. The NT-proBNP values are determined by means of three sequentially determinations.

Other Laboratory findings: in all serum samples, Antinuclear Antibodies (ANA) were determined by indirect immunofluorescence (IFI) on Hep2 cells substrate (Euroimmun) and Estraible Nuclear Antigen using FEIA technology on UNICAP 100 instrument (Phadia).

Cardiac assessment: The left ventricular ejection fraction was determined by dual dimensional echocardiography Echocolor Doppler (echocolor doppler General Electric System Five 3,5 MHz) and pulmonary systolic arterial pressure (PAP) was determined by regurgitation of tricuspidal or pulmonary valves adding 5, 10 or 15 mm Hg for auricular pressure by Continous Doppler (CW) 2Mhz at 100 mm/sec and acceleration time of pulmonary valve[25]. As usually, during the exam, right atrial size measure (normal value Td <29 mm), TAPSE (tricuspid anular systolic plane excursion, normal value 18 ±4 mm, considering as pathological values < 16 mm), right ventricular myocardial performance index (RVMPI) were determined, to avoid other possible biases of right heart disfunction. The left function test was normal in all the patients and between 50-65% (mean 56%± 3) (median 59 %±3). The diastolic ventricular left function was tested considering E/A relation mitral tested on values 0.9-1 suspected by diastolic ventricular dysfunction. Each patient was submitted to three determination of PAP by the same cardiologist in different, but tight time after 30 min of bedrest. We chose a value of 35 mm Hg of PAP as cut-off for patients description. The functional class of PAH is expressed according to NYHA. All patients underwent the 6-minute walking test (6MWT). All patients with PAH refused to be subjected to right–heart catheterization.

Pulmonary assessment. All the patients were submitted to chest x-ray and thorax HR CT Scan with determination of alveolitis and fibrosis according to Kazerooni score[26], functional pulmonary tests with determination of forced vital capacity (FVC), forced expiration volume 1 (FEV1), DLCO and DLCO/alveolar volume (DLCO/Va) with correction for

Table 1. *Characteristics of patients*

	SSc all pts (98)	SSc no PAH (55)	SSc + PAH (43) PAP > 35 mm Hg
Age (y) (mean ± SD)	59.28 ± 13.21	56.11 ± 14.1	63.33 ±10.84
Sex (F/M)	F 92 M 6	F50 M5	F42 M 1
Autoantibodies pattern	ACA 36 (36.8%) Scl70 32 (32.7%) ACA+Scl70 1 (1.0%) O 29 (29.6%)	ACA 21 (38.2%) Scl70 13 (23.6%) ACA+Scl70 1 (1.8%) O 20 (36.4%)	ACA 16 (34.9%) Scl70 19 (44.2%) ACA+Scl70 0 (0%) O 9 (20.9%)
DLCO > 70% predicted < 70% predicted of normal range	 44 (44.9%) 54 (55.1%)	 37 (67.3%) 18 (32.7%)	 7 (16.3%) 36 (83.7%)
Modified Rodnan skin score > 14	44 (40.08%)	26 (47.27%)	18 (41.80%)
Pulmonary fibrosis Yes (all stage according Kazerooni score)	60 (70.1%)	41 (77.4%)	20 (46.5%)
NYHA Pulmonary hypertension (class: n° (%) pts)			0: 17 (39.4%) I: 9 (21.2%) II: 2 (6.1%) III:13 (27,3%) IV : 2 (6.1%)

Legend Autoantibodies pattern: ACA: centromeric; O: nucleolar

hemoglobin values, considering pathologic a DLCO < 70% of predicted.

Renal assessment: all patients were submitted to determination of renal function tests, renal ultrasonography with determination of renal resistive index, or renal sequential scintiscan, to exclude patients with renal involvement possibly influencing NT-proBNP.

Skin assessment : the skin thickness assessment was determined by modified Rodnan skin score, considering clinically relevant a score upper 14[27].

We considered also the values of NT-proBNP before and after infusion of iloprost.

STATISTICAL ANALYSIS

Continuous variables are expressed as the mean value ± SD, median, interquartile and maximum range. Unpaired t-test was performed to determine the differences between mean values for continuous variables while Chi-Square test or Fisher exact test, if deemed more appropriate, was used to analyze categorical variables. Log transformation for laboratory data was adopted for some analyses in order to fulfil the gaussian assumption. Relationships of event incidence (pulmonary hypertension) to covariates were investigated with univariate logistic regression models. Pairwise correlations between log-transformed NT-proBNP, PAP and DLCO were computed using the nonparametric Spearman's test. Finally, a bivariate regression model using log-transformed PAP as dependent variable and log-transformed NT-proBNP and DLCO as covariate was employed in order to estimate the joint ability of NT-proBNP and DLCO to predict PAP. SAS version 9.1 (SAS Institute Inc, Cary, NC) was used for data analysis.

RESULTS

A total of 98 patients was enrolled in the study.

Table 2. *Correlation between NT-proBNP or DLCO (numeric and categorical evaluation) and pulmonary hypertension (echocardiographic value of PAP > 35 mm Hg). Univariate analysis. NT-proBNP and DLCO were log-transformed*

	SSc all pts (98)	SSc no PAH (55)	SSc + PAH (43) PAP > 35 mm Hg	Odds Ratio (95% CI)	P*
NT-proBNP pg/ml (numeric)					
Mean ± SD	309.89±532.86	144.53±134.13	521.4±742.3	3.09 (1.79-5.35)	<.0001
Median (25th - 75th)	145.35 (85.35 - 318)	111.4 (52.36 - 193.9)	243.2 (133.3 - 552.1)		
Min - Max	0 - 3822	0 - 775.3	49 - 3822		
NT-proBNP pg/ml (categorical)					
Above normal	29 (29.6%)	9 (16.4%)	20 (46.5%)	4.44 (1.75-11.29)	0.0017
vs	69 (70.4%)	46 (83.6)	23 (53.5 %)		
Normal					
DLCO (numeric)					
Mean ± SD	67.44±22.08	79.51±17.09	51.45±17.35	0.92 (0.88-0.95)	<0.0001
Median (25th - 75th)	68 (49 - 85)	79 (68 - 91)	48 (38 - 64)		
Min - Max	28 - 120	34 - 120	28 - 97		
DLCO (categorical)					
Pathologic	44 (44.9%)	37 (67.3%)	7 (16.3%)	10.56 (3.94-28.33)	<0.0001
VS					
Normal	54 (55.1%)	18 (32.7%)	36 (83.7%)		

P. Fisher exact test for categorical data Chi-Square test for numeric data*

Main characteristics of patients are reported in Table I.

A significant correlation was observed between NT-proBNP values and PAP ($p< 0.0001$) (see Figure 1). Using 35 mm Hg of PAP as cut-off for ecocardiographic diagnosis of PAH, NT-proBNP (directly) and DLCO (inversely), both in numerical and categorical evaluation, were significantly related to PAH at univariate logistic regression analysis (Table II).

Further, according to a bivariate regression model, joint NT-proBNP and DLCO appeared as predictors of PAP (r2 0.42, $p< 0.001$, Figure 1).

In Table III correlations between NT-proBNP and PAP, DLCO and PAP, and NT-proBNP and DLCO are reported, in the two subgroups of patients with or without pulmonary fibrosis. No clear differences were seen between NT-proBNP and DLCO as predictors of PAP, if related to the presence or absence of pulmonary fibrosis. However, a trend was noted indicating NT-proBNP as the parameter more strongly related to PAP in patients with pulmonary fibrosis, and DLCO as more predictive if pulmonary fibrosis was absent.

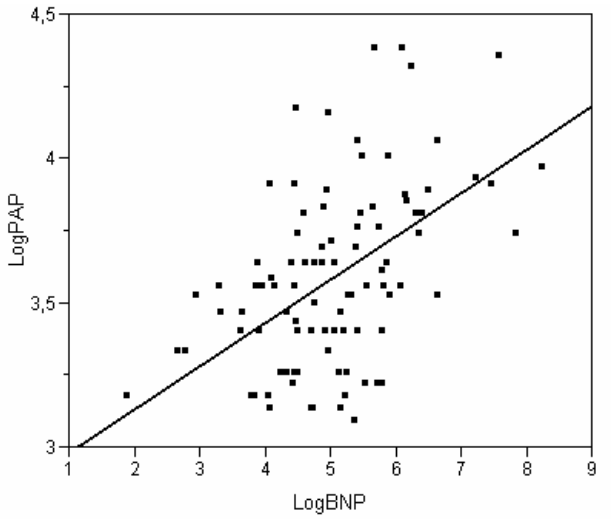
DISCUSSION

The role of NT-pro-BNP as a diagnostic and prognostic marker of cardiac failure and right ventricular dysfunction has been stressed in recent years, and data from many authors have supported its use in the management of patients with PAH. Recently, a few reports have examined the role of NT-pro BNP as an early diagnostic marker of PAH in patients with SSc also without right cardiac failure, and have demonstrated that levels of this parameter correlate with, severity and course of PAH[18-22]. As reported by many authors[16,20-23], the raise of NT-proBNP seems directly related to severity of PAH in scleroderma patients, it is highly predictive of mortality, and determination of this parameter can be very useful in screening of patients with high risk for therapeutic failure.

Moreover, NT-pro BNP in scleroderma seems to be correlated with other parameters of the disease; some authors[20,23-29] demonstrated that levels of NT-

Table 3. *Correlation between PAP, NT-proBNP and DLCO in patients with or without pulmonary fibrosis (logarithmic scale)*

	Pulmonary fibrosis YES (n =26)		Pulmonary fibrosis NO (n =72)	
Variable log scale	**Correlation**	**P value**	**Correlation**	**P value**
NT-proBNP/PAP	0.4761	<0.0139	0.4877	<0.00001
DLCO/PAP	- 0.4048	<0.0402	- 0.6037	<0.00001
DLCO/NT-proBNP	- 0.4831	<0.0124	-0.3950	<0.0009



r2 = 0,254
P = 0,0001

Fig. 1. *Correlation between NT-proBNP and PAP expressed in logarithmic scale*

proBNP are significantly higher in patients with SSc compared to healthy controls and are directly related to aging, skin thickness score, and systolic pulmonary artery pressure, while they correlate negatively with DLCO (particularly in diffuse subset); according to these findings, the authors conclude that NT-proBNP is a useful new biologic marker of skin fibrosis and pulmonary vascular assessment in systemic sclerosis.

Finally, emergent new therapies such as endothelin receptor antagonists seem to have changed the fatal course of primary and secondary PAH[30-33], and to improve functional class in scleroderma PAH patients[34]. Since in PAH secondary to scleroderma pulmonary and cardiac hemodynamic function tests appear to remain stable during the first periods of treatment with these agents, the use of NT-proBNP has been suggested in monitoring the effects of therapy and in screening patients potentially facing a bad prognosis and candidates to aggressive therapies[35].

In our study, both at univariate and multivariate analyses, NT-proBNP levels appear to have a significant correlation with PAP values, and above normal NT-proBNP was significantly related to PAH (above 35 mm Hg of PAP as cut-off): these findings suggest once more the potential utility of this biomarker as early detector of PAH or as a predictor of future development of PAH in patients with SSc. In this paper, we do not report data relative to prospective evaluations of our patients, however preliminary findings in a subgroup of our patients suggest a potential role of NT-proBNP in follow-up and in monitoring therapy.

At support of accuracy and sensibility of reduced DLCO as a marker of PAH, in a recent study Langleben et al[36] reported that a reduced DLCO is present only in patients affected by PAH secondary to connective diseases, and it correlates with functional capillary surface area. Allanore et al[18] firstly demonstrated that

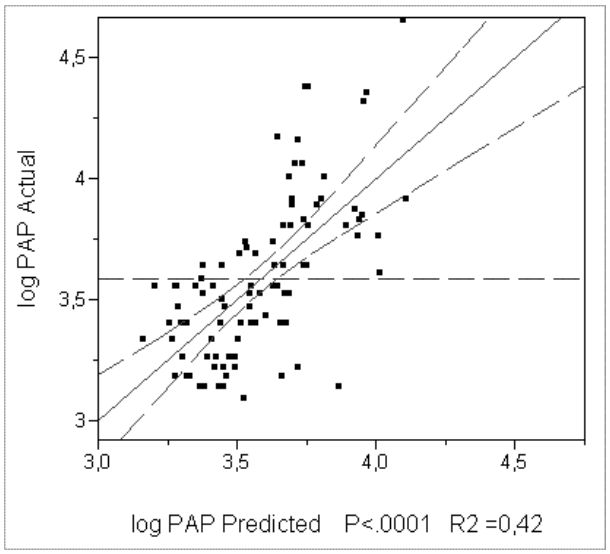


Fig. 2. *Statistical correlation between PAP and NT-proBNP + DLCO (expressed in logarithmic scale)*

the combination of increased serum NT-proBNP levels together with a decrease of DLCO is a strong predictor of occurrence of PAH, and correlates to survival.

Our data confirm that joint NT-proBNP and DLCO levels can significantly predict PAP values. Since the determination of pulmonary function tests with DLCO is usually utilized in screening and follow up of patients with SSc, we suggest, in the presence of reduced DLCO to associate the measure of serum NT-proBNP to early select a cluster of patients with bad prognosis and strong candidates to develop PAH, to submit to a stricter follow up and eventually more aggressive therapies. An advantage of this approach can be represented by simplicity, availability and low cost of both methods.

When we analysed our data considering the presence or absence of pulmonary fibrosis in relation to NT-proBNP and DLCO, in spite of a small number of cases, in the subgroup of patients with fibrosis, we found a trend of better performance of NT-proBNP levels. These data, though preliminary, could be explained by the relative higher prevalence (30-40%) of pulmonary hypertension in interstitial lung disease secondary to connective disease[37], and suggest another potential role of NT-proBNP as early marker of PAH in secondary pulmonary fibrosis.

Our study may present several limitations. Despite the right–heart catheterization remains the gold standard method to assess PAP, we were obliged to evaluate PAH only by echocardiography. However, echocardiography is routinely utilised in screening and follow up of patients with SSc, and it may be a specific method, with high predictive value for diagnosis and follow-up of PAH in patients with SSc[38-40]. Moreover, Ciuzynski et al[41] found that increase of NT-proBNP is directly related to PAH detected by echocardiography, to right ventricular diameters and it negatively correlate with 6MWT, so stressing the role of NT-proBNP and other not invasive methods to screen early PAH and monitor PAP in scleroderma patients; the same report is confermed also by Dhja et al[42]. Data from our study, showing a positive association between PAP determined by echocardiography and NT-proBNP levels, confirm those preliminary findings, and the potential clinical utility of these non invasive methods.

However, our study has some strengths, which may make its results valid. First, our data refer to a quite large patient population, if compared with previous reports on these issues. Further, the three participating centres applied common procedures as for patients' management, and assessment of pivotal parameters (PAP, NT-proBNP, DLCO).

Our study presents a major limit represented by the absence of right–heart catheterization.

In conclusion, our results support available data suggesting that NT-proBNP measurement can be a useful and non invasive method of early identifying patients with SSc at high risk to develop PAH. Significance of this marker as a potential early detector of PAH in scleroderma patients can be enhanced by the association with DLCO, and be particularly relevant in patients with pulmonary fibrosis, but we stressed again that all these data must be confirmed with right-heart catheterization, which still remains the gold standard method for diagnosis of PAH.

Further large-scale studies are needed to confirm our findings.

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