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Ultra-Low Electromagnetic Fields Application on In Vitro Cartilage Regeneration: A Pilot Study to Improve Treatment of Osteoarticular Diseases

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Abstract: Extremely low-frequency and low-intensity electromagnetic fields show positive effects on the treatment of several osteoarticular diseases, such as osteoarthritis, and are currently applied in the clinical setting with promising results on tissue regeneration. However, the biological mechanisms underlying the beneficial effects triggered by this type of physical stimulation still need to be deciphered. We tested the hypothesis that ultra-low complex electromagnetic fields stimulation using an innovative medical device could enhance chondrogenesis in human adipose-derived stem cells (ADSCs), and analyzed its biological effects. Chondrogenic lineage markers, like ACAN, SOX9, RUNX2, COL2A1, and COL10A1, were evaluated after 21 days of treatment. Thus far, we have provided preliminary evidence that a dedicated pattern of ultra-weak complex electromagnetic sequences emitted by a cutting-edge technology can promote cartilage regeneration, inducing the chondrogenic differentiation and maturity of ADSCs.

Keywords: ultra-low electromagnetic fields; osteoarthritis; collagen; adipose-derived stem cells; cartilage regeneration; chondrogenesis markers

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1. Introduction

Articular hyaline cartilage is composed of chondrocytes included within an extracellular matrix (ECM) set as a framework of macromolecules like collagens, glycosaminoglycans (GAGs), proteoglycans, glycoproteins and water. It shows a typical stratified organization of fibrils, mainly collagen type II, which supply tensile strengthening to a highly hydrated proteoglycans gel, resulting in a structure able to support the compressive load and protect the bone surface. During life, articular cartilage is subjected to a physiological inner remodeling through neo-synthesis and replacing ECM components with chondrocytes. Nevertheless, chondral tissue possesses low self-repair potential [1]. Moreover, aging reduces the capability of chondrocytes to maintain such turnover rate and to re-establish functional tissue, increasing the risk of progressive degeneration and damage to the joints' cartilage surface. Age-related and traumatic lesions of articular cartilage, if left untreated, lead to joint pain and impairment, which is clinically defined as osteoarthritis (OA).

Cartilage depletion represents a truly disabling condition, and over 200 million people worldwide suffer from OA [2]. The surgical treatment of these pathological manifestations presents limitations, as mature cell- and tissue-based transplants, such as autologous ex vivo cultured chondrocytes implantation or osteochondral grafts, often fail to restore hyaline cartilage structure and functionality [3,4]. The latter is due to chondrocyte poor

in vitro proliferation and de-differentiation susceptibility. In this scenario, adult stem cells rapidly emerged as a valuable cell source for articular cartilage tissue engineering [5].

In particular, Mesenchymal Stem (and/or Stromal) Cells (MSCs), which are adult mesoderm-derived undifferentiated cells, showed the capability to self-renew together with a multilineage differentiation potential (e.g., chondrogenic, osteogenic, and adipogenic) and have been already employed including cartilage regenerative medicine for a variety of clinical applications [6]. Among MSCs populations, Adipose-Derived Stem Cells (ADSCs) have shown promising potential for chondrogenesis and the advantage of being easily obtainable from liposuction waste [7–10]. Moreover, most in vivo animal studies have reported good results using pre-differentiated or undifferentiated, autologous or allogeneic ASCs to regenerate cartilage in osteochondral defects or surgically induced osteoarthritis.

The application of electromagnetic fields (EMFs) has already shown a positive impact in vitro on enhancing chondrogenesis [11–13]. In particular, recent studies highlighted the high biological activity of some extremely low-frequency magnetoelectric fields (ELF-EMFs) [14]. It has been shown that low-frequency pulsed electromagnetic fields improve patients' recovery both in the short (90 days) and in the long term (3 years), as demonstrated in the results of two level-I clinical studies. The long-term benefit results from biophysical chondroprotection of articular cartilage and prevention of the fibrotic stimuli exerted by pro-inflammatory cytokines on wounded tissue. Regenerative medicine also relies on biophysical stimulation, exerting a protective effect on the repair of tissue from catabolic effects of the inflammatory reaction elicited by the surgical implantation procedure. The microenvironment regulating stem cell differentiation can be cell–matrix adhesions or cell–cell interactions. The microenvironment is certainly helpful for maintaining MSC survival, commitment, and differentiation. It has already been reported that a hyaluronan-enriched microenvironment can both initiate and promote the chondrogenic differentiation of human adipose-derived stem cells (ADSCs) and that single-pulse electromagnetic field (SPEMF) stimulation may promote chondrogenic differentiation and cartilaginous matrix formation thus being applied for articular cartilage tissue engineering. Nevertheless, understanding the biological mechanisms underlying the therapeutic effects of low-intensity electromagnetic field treatment is lacking.

To test the hypothesis, we have investigated the effects of Limfa® Therapy on an in vitro model of ADSC cells to study their differentiation capability towards cartilage lineage. Limfa is based on a non-invasive technology centered on the application of ultra-weak magnetoelectric fields, emerged as one of the most innovative and promising medical devices in electromagnetic therapy. The experimental study had the aim to decipher the molecular mechanism underlying the Limfa functions. This research aims to biologically characterize the specific action mechanism of "connective tissue regeneration" Limfa® Sequence on adipose stem cells chondrogenesis, to better enlighten and validate its efficacy and therapeutic effect.

2. Materials and Methods

2.1. ADSCs Cell Culture

Commercial human ADSCs (ATCC PCS-500-011) from different batches were cultured in Mesenchymal Stem Cell Basal Medium (ATCC PCS-500-030) supplemented with Mesenchymal Stem Cell Growth Kit for Adipose-Derived MSCs (ATCC PCS-500-040) following the manufacturer instructions. Given that all experiments have been performed on three independent cell batches, it is possible to assume that different cell types have been used. After cell expansion, 1×10^5 cells were seeded in p60 culture dishes and maintained either in Basal medium (Basal) or in Chondrogenic Differentiation Medium (Diff) (Chondrocyte Differentiation Tool, ATCC) for 21 days at 37 °C, 5% CO_2.

2.2. Limfa® Therapy Treatment

The Limfa® Therapy system (Eywa Srl, Rimini, Italy) (CE c.n. DD 60155923) was used to create extremely low-frequency (2–80 Hz), low-intensity (1–100 μT) complex variable electromagnetic fields, delivered as a predesigned module of patented wave sequences.

Specifically, the Limfa® Therapy apparatus is equipped with an electromedical computer allowing the emission of low multi-frequency signals, available as of specific pre-set treatment programs, free of 99% electromagnetic noise and thermal effect once they reach the targeted tissues. Several sequences of complex variable ultra-weak EMFs have been developed, tested and patented (Limfa® Sequences), showing a biologically active and extremely positive effect. Compared to traditional magnetotherapy, where ELF-EMFs are also exploited, their signals are a combination of one or two pulsed waveforms with the same geometry; Limfa® Therapy operates through several wave geometry shapes and frequencies, integrated into defined sequences that have a tissue-specific and -targeted therapeutic action.

To apply the magnetoelectric field emitted by the Limfa® Therapy transductor to the cell culture vessels, we used an Okolab Mini Stage-Top H301 Incubator (Okolab Srl, Naples, Italy,) allowing optimal cell exposure to the wave sequences pattern while still maintaining standard in vitro culture conditions. The Limfa® Therapy patented pre-loaded program—"connective tissues regeneration"—was applied for the entire standardized duration of 50 min. Eleven treatment sessions were performed every other day for a total duration of 21 days from cell seeding to mimic the standard schedules used in the clinical setting (+Limfa). Cells were examined to monitor cell morphology every day with microscopy evaluation. In contrast, half of the cell cultures did not undergo electromagnetic therapy and were evaluated as the negative control (−Limfa).

2.3. Morphological Cellular Evaluation

Images were acquired using an EVOS microscope (ThermoFisher, Waltham, MA, USA) using 40× magnification. The evaluation was performed by two independent operators, JI and GB. A scoring system was applied as follows: N, non-differentiated; +, weak differentiation; ++, moderate differentiation; +++, strong differentiation.

2.4. Cell Viability Assay

Cell viability was measured through the Trypan Blue exclusion test. Cells were harvested from plates after treatment with Limfa® Therapy in the complete medium; the treatment was performed following the protocol described in the previous paragraph.

2.5. Real-Time PCR (RT-qPCR)

Real-time PCR was used to evaluate the expression of the main chondrogenesis markers, including ACAN, SOX9, RUNX2, COL2A1, and COL10A1 [6]. At the end of the 21-day culture, RNA extraction of the cellular samples was performed using TRIzol Reagent (ThermoFisher Scientific), following the manufacturer protocol. RNA was quantified using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific), and 1 μg RNA was retrotranscribed using the SuperScript IV Reverse transcription kit (Invitrogen, Waltham, MA, USA) following the manufacturer's instructions. RT-qPCR was carried out using 1 μL of the obtained cDNA and 100 μM of gene-specific reverse and forward primers (Eurofins Genomics, listed in Table 1. PCR was carried out using SYBR green chemistry (Applied Biosystems, Waltham, MA, USA). Amplification was performed using a 7500 real-time PCR system and software (Applied Biosystems, Waltham, MA, USA). Samples were held at 50 °C for 2 min and 95 °C for 10 min, then amplified at 95 °C for 15 s and 60 °C for 1 min for 40 cycles. The specificity of the PCR amplification was checked with a continuous heat dissociation curve (measured between 60–95 °C) performed subsequently to the final PCR cycle. Gene expression levels were standardized using GAPDH as an internal control. Quantification analysis was performed using the comparative ΔΔCt method [15], and gene

expression was expressed as a fold change relative to the control's untreated Basal medium samples. All experiments were conducted in triplicate on three different cell batches.

Table 1. Primer sequences used in RT-qPCR experiments. GAPDH was used as an endogenous normalizer in relative expression quantification.

Gene	Forward 5′	Reverse 5′
RUNX2 (Runt-related transcription factor 2)	GGT CAG ATG CAG GCG GCC	TAC GTG TGG TAG CGC GCT
SOX9 (Sex-determining region Y-box 9)	AGA CAG CCC CCT ATC GAC TT	CGG CAG GTA CTG GTC AAA CT
ACAN (Aggrecan)	TAC ACT GGC GAG CAC TGT AAC	CAG TGG CCC TGG TAC TTG TT
COL2A1 (Collagen type-II alpha 1 chain)	GTG AAC CTG GTG TCT CTG GTC	TTT CCA GGT TTT CCA GCT TC
COL10A1 (Collagen type-X alpha 1 chain)	CAC CTT CTG CAC TGC TCA TC	GGC AGC ATA TTC TCA GAT GGA
GAPDH (Normalizer)	ACC CAG AAG ACT GTG GAT GG	TTC TAG ACG GCA GGT CAG GT-

2.6. Western Blot

Western blot was performed to evaluate collagen type-II and collagen type-I protein levels, considering their key role in defining chondrogenesis progression. Western blot was carried out on whole-cell lysates using sequence-specific antibodies directed against collagen I and collagen II (ab138492 and ab188570, respectively, Abcam, 1:1000 in blocking solution), as in Duranti et al., 2021 [16]. All experiments were performed on three different cell batches. Briefly, cells were gently collected by mild scraping and resuspended in ice-cold PBS. Protein extraction was performed using the lysis buffer with the following composition: NP40 (150 mM), NaCl (150 mM), Tris-HCl pH 8 (50 mM), EDTA pH 8 (5 mM), NaF (10 mM), Na4P2O7 (10 mM), Na3VO4 (0.4 mM), and protease inhibitor cocktail (Complete Mini-Roche, Mannheim, Germany).

2.7. Protein Quantification

Data were analyzed with ImageJ and graphs were plotted with OriginPro 8. When quantifying protein variations, the signal was normalized to the signal of the corresponding protein in the total lysate.

2.8. Statistical Analysis

Statistical and graphical data analysis was carried out using Origin V.8 (OriginLab Corporation, Northampton, MA, USA) and GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). Results were expressed as mean ± SEM. Since data were normally distributed, statistical comparisons between multiple groups were performed using a one-way ANOVA test. For all tests, differences with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effects of Limfa® Therapy on Cell Morphology

The morphology of ADSCs was examined every day during the 21 days of treatment. From the images obtained by inversion microscopy, we can observe that cells in the control appear different compared to those after Limfa treatment when considered in Basal conditions with a stretched shape and longer protrusions. In this regard, cells in the Basal condition tend to resemble the ones in the control condition of the differentiation set. It is clear how ADSC differentiation in chondrogenic conditioned medium (Diff) led to the deposition of abundant extracellular matrix, generating interconnected structures where single-cell shape appears to be undetectable. On the contrary, cells cultured in Basal medium (Basal) maintained a fibroblast-like single-cell appearance throughout the entire period of culture (Figure 1A). In Figure 1B, we have reported images taken after 10 days

of treatment, showing a differentiated morphology of the cells. Figure 1C shows a graph reporting cell viability after 21 days of treatment, with a percentage of survival of 100%.

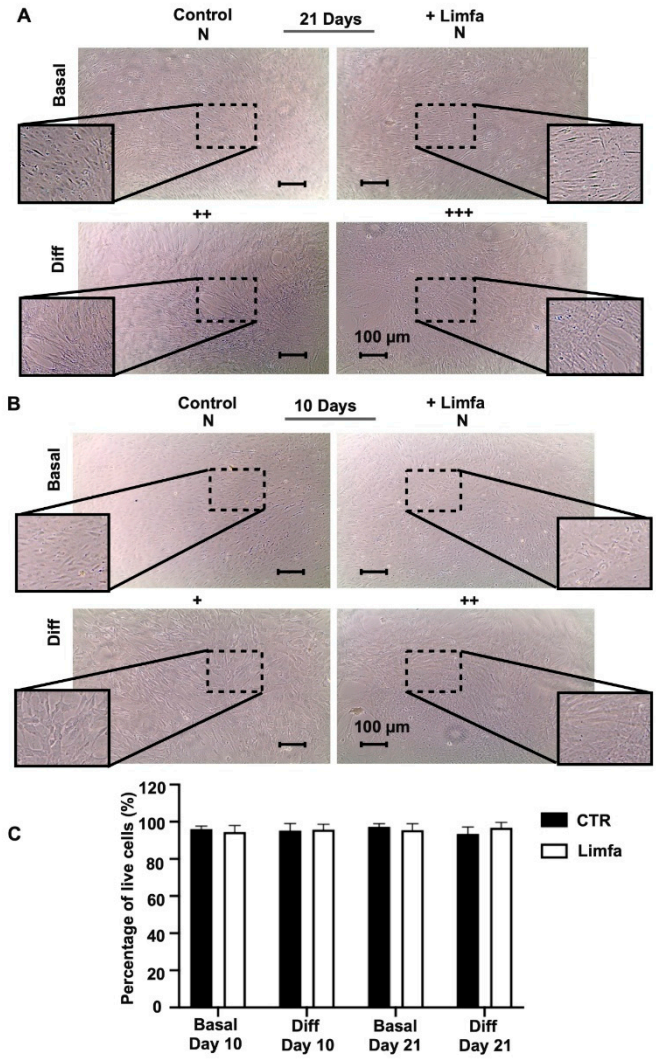

Figure 1. Morphological examination of ADSCs 2D cultures after 10 and 21 days of culture. Cells have been examined under different conditions. Basal, which corresponds to cells cultured in basal adipose stem cells medium; Basal + Limfa, which corresponds to cells cultured in basal adipose stem cells medium and treated with Limfa® Therapy; Diff, which corresponds to cells cultured in chondrogenic differentiation medium; Diff + Limfa, which corresponds to cells cultured in chondrogenic differentiation medium and treatment with Limfa® Therapy. Scoring system: +, weak differentiation; ++, moderate differentiation; +++, strong differentiation. (**A**) cell images after 21 days of Limfa treatment (**B**) cell images after 10 days of Limfa treatment (**C**) Cytotoxicity assay performed after 10 and 21 days of treatment. The scale bar corresponds to 100 µm.

3.2. Effects of Limfa® Therapy on Chondrogenic Molecular Marker Expression

RT-qPCR analysis was then applied to determine the effects of chondrogenic differentiation, as well as Limfa® Therapy treatment, on the following markers of chondrogenesis: SOX9, RUNX2, COL2A1, COL10A1 and ACAN. COL2A1 showed a significant upregulation in its expression rate following the treatment schedule with Limfa® Therapy under defined medium cultures compared to untreated controls.

The RUNX2 marker exhibited a significant increase in Limfa® Therapy-treated cultures, both under Basal and differentiation medium conditions.

ACAN showed significant expression differences between untreated cells maintained in Basal compared to conditioned medium, but no difference was observed due to the Limfa® Therapy treatment per se.

Interestingly, the COL10A1 marker displayed a significant decrease in cells under both Basal and differentiation conditions when treated with Limfa® Therapy, compared to the untreated group. SOX9 also significantly decreased when comparing Basal vs. Diff + Limfa groups.

SOX9 expression only showed a significant reduction in cells treated with Limfa® Therapy cultured in conditioned media (Diff + Limfa) compared to untreated Basal conditions (Figure 2).

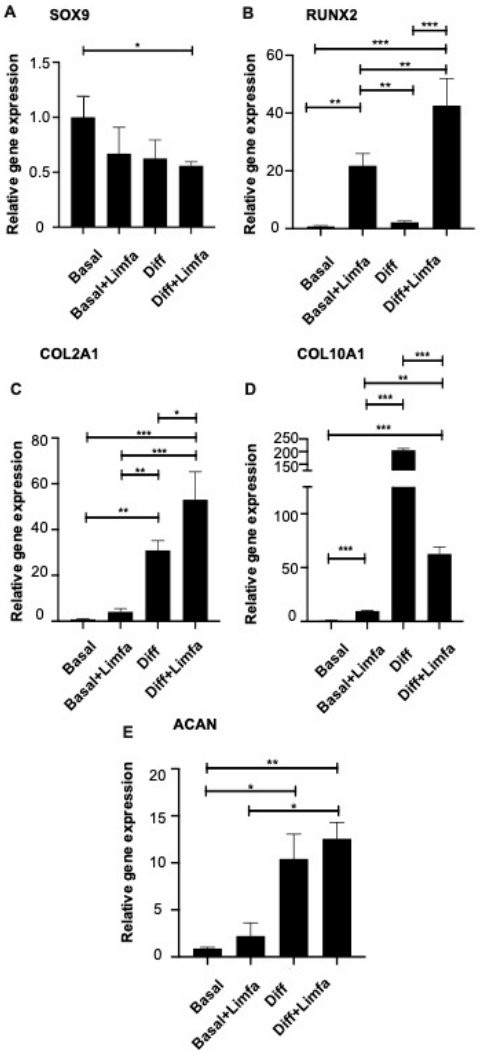

Figure 2. Expression analysis of the main chondrogenic differentiation markers in different culture and treatment conditions. Cells were examined under different conditions. Basal, which corresponds to cells cultured in basal adipose stem cells medium; Basal + Limfa, which corresponds to cells cultured in basal adipose stem cells medium and treated with Limfa®Therapy; Diff, which corresponds to cells cultured in chondrogenic differentiation medium; Diff + Limfa, which corresponds to cells cultured in chondrogenic differentiation medium and treatment with Limfa®Therapy. SOX9 (**A**), RUNX2 (**B**), COL2A1 (**C**), COL10A1 (**D**) and ACAN (**E**) expression levels were assessed by RT-qPCR. Experiments are means of three different repeats. Results are expressed as relative gene expression (2-ΔCt) normalized on Basal culture conditions values. Error standard is reported. * = $p < 0.05$; ** = $p < 0.025$; *** = $p < 0.01$ (One-Way ANOVA).

Overall, RT-qPCR expression analysis of the main chondrogenesis markers revealed that, for most of the transcripts taken into account, the treatment with Limfa® Therapy (Diff + Limfa) induced an increase in the relative gene expression levels when compared to untreated ones.

3.3. Effect of Limfa® Therapy on Collagen Type I and II Protein Expression

The above-mentioned results prompted us to analyze the effects of Limfa® Therapy treatment on the expression of different collagens, which represent the gold standard. Collagen type I protein expression appears to be negatively affected by the treatment with Limfa Therapy, especially under differentiation culture conditions. Consistently, cartilage-specific collagen type II appears to be increased by treatment with Limfa® Therapy (Diff + Limfa) (Figure 3).

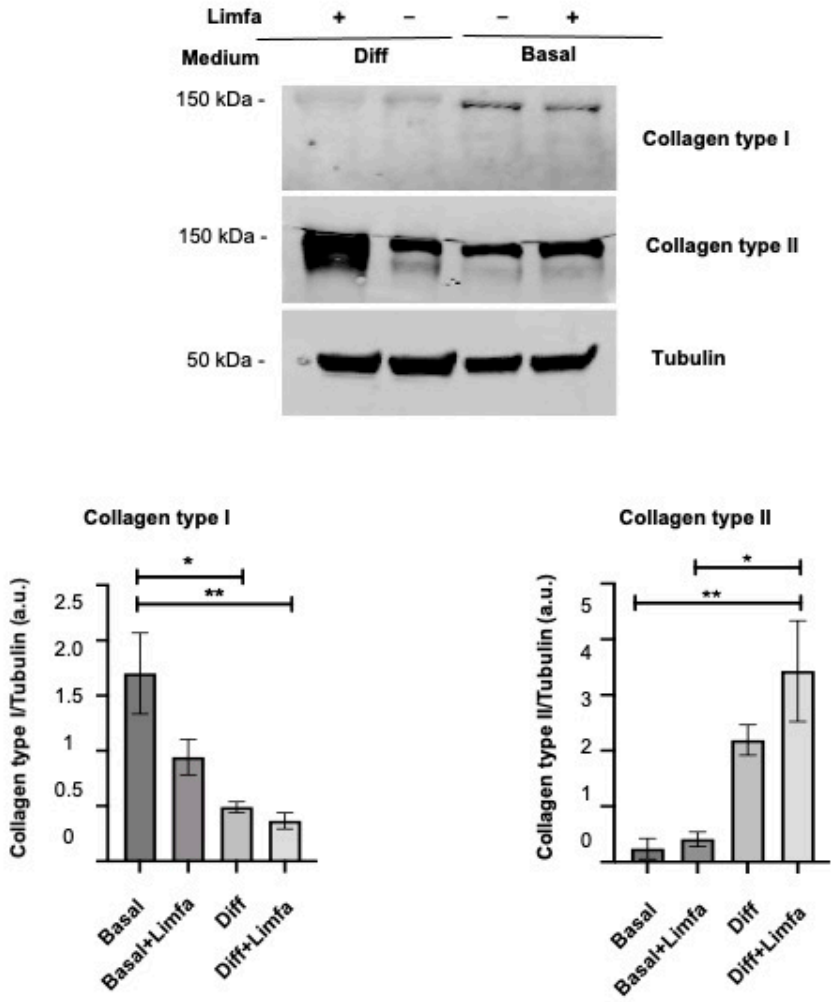

Figure 3. Western Blot analysis of Collagen I and Collagen II expression in different cultures and treatment conditions. Results are expressed as arbitrary units (a.u.) and normalized on Tubulin input. Basal—Basal medium; Diff—differentiation medium; Limfa—Limfa® Therapy treatment. Experiments are means of three different repeats. Error standard is reported. * = $p < 0.05$; ** = $p < 0.025$ (One-Way ANOVA).

4. Discussion

Even though some studies have already examined the effects of ultra-weak electromagnetic fields on mature human chondrocytes, their interaction with chondrogenic lineage commitment of MSCs sources such as ADSCs, has not been completely elucidated. This study aimed to investigate the biological effects of ultra-low complex electromagnetic

fields delivered by an innovative medical device, Limfa® Therapy, on an in vitro cartilage regeneration model.

Our results indicate that ADSCs treatment with Limfa® Therapy is able to induce the modulation of some of the main genetic chondrogenesis markers. Evidence has emerged that SOX9 did not show significant differences when compared to untreated and Basal medium conditions. This is in line with the early-stage nature of this chondrogenesis marker. Such a transcription factor is described as an early gene driving the initial switch to the chondrogenic commitment of undifferentiated progenitor cells [17].

The genetic markers related to extracellular matrix composition showed modifications induced by Limfa® Therapy which are highly representative of ADSCs chondrogenic lineage commitment. Cartilage-specific collagen type-II expression showed the most significant increase in cells treated with Limfa® Therapy and maintained in a Differentiation medium (Diff + Limfa) when compared to both its untreated relative control (Diff) and Basal conditions. Limfa® Therapy was also able to significantly decrease collagen type 10 expression, both in Basal and conditioned medium cultures. As previously described [18], COL10A1 is known to be a specific marker for late chondrocyte hypertrophy, found in network-like rather than in fibril-like collagen structures, suggesting that Limfa® Therapy preferentially promotes hyaline cartilage formation instead of bone tissue. This represents an innovative finding considering that many studies show how cartilage derived in vitro from MSCs commonly shows hypertrophic rather than hyaline features, making it unsuitable for functional cartilage tissue regeneration purposes [19].

Finally, collagen type-II protein expression evaluated by Western blot confirms the hypothesis that Limfa® Therapy is able to potentiate the induction of chondrogenesis, exploiting the soluble factors contained in pro-chondrogenic medium and therefore enhancing the deposition of cartilage-specific ECM. Moreover, we have envisaged a significant downregulation of collagen type-I protein expression after performing Limfa® Therapy treatment on cells maintained with differentiation medium. Such findings strengthen the data obtained so far by our group, as collagen type I represents one of the main osteogenic markers, which is repressed during chondrogenic lineage commitment [20].

Overall, our results suggest that Limfa® Therapy efficacy might be ascribable to its ability to promote adipose mesenchymal stem cells' chondrogenic lineage commitment and tissue repair [10,11]. Moreover, concerning extracellular matrix deposition, Limfa® Therapy application effectively promotes ADSC differentiation when coupled with biochemical stimuli contained in a pro-chondrogenic medium.

Such results open a new path as it is known that the literature lacks studies regarding the use of ADSCs in humans for orthopedic pathologies [13]. Nevertheless, preliminary outcomes are very encouraging, with a low rate of complications. Different delivery systems for these stem cells have been tested so far. ADSCs can be administered either with a simple injection or during a surgical procedure. Together, the evidence from the few available clinical studies shows promising outcomes in the treatment of select musculoskeletal pathologies [21]. The limitation to most of this published literature is the inclusion of other therapeutic biologics. In this scenario, our findings urge an additional validation using ADSCs derived from lipoaspirate samples to assess the effects of Limfa® Therapy on endogenous stem cells. On the one hand, further investigation will validate the utility of Limfa® Therapy in the clinical treatment of osteoarthritis. On the other hand, it will highlight the potential improvement of the technology represented by adding an autologous ADSCs intra-articular injection to boost cell regeneration capacity.

5. Conclusions

Overall, our study places itself along an entirely new line emerging from the possibility of using electromagnetic fields coupled with ultrasound for biomedical applications, as our group has recently demonstrated [22]. It would be of great interest to observe the treatment effects of this process on human bone marrow stromal stem cells. Even though it was not possible to include a comprehensive series of human cases at this stage, we are willing to

pursue this as our next step. Overall, our preliminary findings have shown that the effects of Limfa® Therapy can induce ADSC differentiation in vitro.

Author Contributions: Conceptualization, project administration, C.D., R.D., P.D.B. and A.A.; methodology, investigation, formal analysis, writing—original draft preparation, C.D., G.B., J.I. and V.D.; writing—review and editing, C.D., J.I. and G.B.; supervision, C.D. and A.A.; funding acquisition, A.A.; All authors participated in manuscript drafting, revision and approval before submission. All authors have read and agreed to the published version of the manuscript.

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Protocol of

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relating to the

"STUDY FOR THE VALIDATION, AT THE CELLULAR LEVEL, OF TREATMENTS BASED ON THE USE OF FIELDS LIMFA ULTRA WEAK ELECTROMAGNETIC © /PHASE 1: STUDY ON COLLAGEN PRODUCTION"

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GENERAL INFORMATIONS

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2. PURPOSE OF THE STUDY

The aim of the study is to evaluate the effects of a treatment with LIMFA fields ©(treatment schedule "Collagen Regeneration") on human fibroblast cell growth and collagen secretion type I in the cell supernatant.

3. BIBLIOGRAPHICAL REFERENCES

The study is based on indications from the literature (Rodemann, HP et al., Exp Cell Res, 182: 610-621, 1989; Rodemann HP et al., Scanning Microscop, 5: 1135-1142, 1991), according to which prolonged treatments using electromagnetic fields induce collagen secretion of human fibroblasts.

4. EQUIPMENT

Biohazard Laminar Flow Hood (Steril)

Incubator CO_2(sanyo)

Centrifuge (Beckman Coulter)

Primo Vert Inverted Light Microscope (Zeiss)

Microplate reader with ELISA filters equipped with Gen5™ sw (Bio-Tek Instruments)

LIMFA Therapy console equipped with a special transducer/applicator (supplied by the Customer)

5. MATERIALS

Human adult fibroblast cell line of dermal origin (HDFa Cat. No. C-013-5C, Gibco, Life technologies).

Culture medium consisting of: Medium 106 (Gibco, Life Technologies) with addition of 1X Low serum Growth Supplement (LSGS, Gibco, Life Technologies).

Recombinant Human TGFβ1 (R&D Systems).

Dulbecco's Phosphate Buffered Saline (Sigma-Aldrich).

Trypsin-EDTA 1X in PBS (Euroclone)

Trypan blue solution 0.4% (Sigma-Aldrich).

Cell reagent proliferation Reagent WST-1 (Roche).

DuoSet Elisa-Human Pro-Collagen I α1/COL1A1 (R&D Systems).

DuoSet Ancillary Reagent Kit2 (R&D Systems).

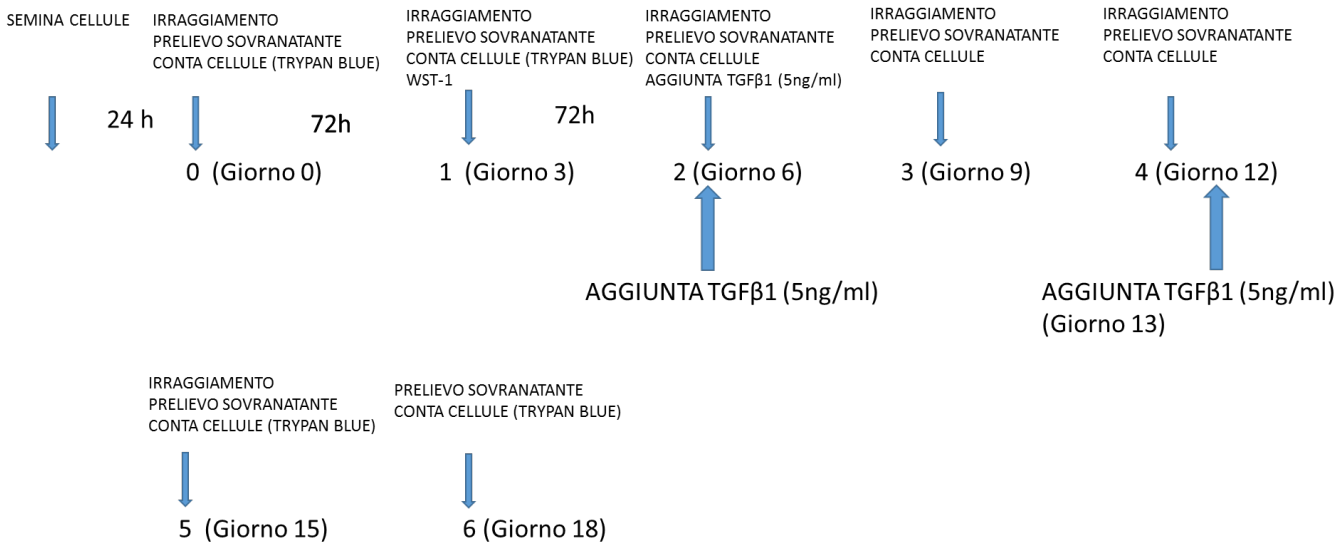
Cell culture plastic (Euroclone).

6. STUDY PROTOCOL AND EXPERIMENTAL DESIGN

Starting from time zero, and for the subsequent 5 treatments, at 72-hour intervals (see experimental scheme shown below), the following parameters were analysed: (1) cell viability (by both assays of exclusion of Trypan Blue and WST-1 test); (2) secretion of Pro-collagen type I α1. (For the related procedures see EXPERIMENTAL PROCEDURES). The same parameters were also evaluated a 3 days after the conclusion of the experimental treatment. It was set up, upon achievement of the cell growth plateau, also a “positive control” represented by treated HDFa cells with TGF-β 1, in which the same parameters reported above were evaluated.

In order to obtain statistical significance, 3 experiments were performed in parallel, and the respective analyzes (cell viability and collagen secretion) were performed in triplicate.

SCHEMA SPERIMENTALE



7. STUDY DURATION

The study had a total duration of 19 days.

8. EXPERIMENTAL PROCEDURE

The following operations were performed:

- Thawing of the cell line of human adult fibroblasts of dermal origin HDFa. Upon arrival, the cells were cryopreserved in liquid nitrogen. Just before thawing the line, the Low serum Growth Supplement was added to the M106 culture medium. Four 25cm flasks with 5 ml of medium per flask they were placed in an incubator at 37°C in an atmosphere of 5% CO_2 for 0.5h. The vial with the cells was removed from the liquid nitrogen and the lower part placed in a water bath at 37°C until its contents were completely thawed. Under the laminar hood the contents were resuspended with 1 ml of complete medium and 20 µl of the cell suspension were withdrawn in order to evaluate the number of total and viable cells. The number of cells was found to be 600,000 cells, all of which were viable. The remainder of the vial was divided equally into the 4 flasks previously incubated. After ten days the seeded cells reached confluence.

- Cell amplification (growth). The cells contained in the 4 flasks were amplified with a passage 1:2 (passage 1) in order to reach the number necessary to perform the experiment.
- Cell seeding in 24- and 96-well mutiwells . 24-well multiwells were used for each of the 3 experiments performed in parallel in order to carry out the supernatant withdrawals and cell counts, while 96-well multiwells were used for the tests involving the WST-1 dye. In the wells of the 96 multiwells, 5 X 10 were seeded in triplicate for each time and for each condition ^{3}HDFa cells (step 1) for a total volume of 200 µl of complete medium per well. In those of 24, always in triplicate for each time and for each condition, 30 X 10 were seeded ^{3}cells, in a volume of 1 mL of complete medium per well.

- Irradiation using the LIMFA Therapy system . At each time point (as per the experimental scheme reported above), the respective multiwells, after being parafilmed, were

subjected to irradiation using the LIMFA "Collagen Regeneration" system. This protocol lasting 19' and 10” was performed at room temperature (23-25°C). The multiwells were placed in direct contact with the surface of the transducer/applicator taking care to verify that all wells with the cells were within the surface of it. At the same time, the controls and the "positive controls", ie those treated with TGFβ1, after being parafilmed, were also kept in the same climatic conditions. At the end of the irradiation time all the multiwells were removed from the parafilm and placed in the incubator at 37°C and 5% CO_2.

- Treatment with TGF β1 . At the times indicated in the experimental scheme, 5 ng/ml of TGF β1 were added to each well of the respective multiwells.
- Collection and storage of cellular supernatants . After 1 hour from the irradiation and after an optical microscope check of the conditions of the cell cultures, at the relative times indicated in the Experimental Scheme, the cell supernatants were taken from all the 24 wells. These were placed in sterile 1.5 mL vials and stored at -20°C. This procedure was performed for all points except for the last point (point 6 or day 18). For this, however, the supernatant and the DPBS, with which the wells are washed before applying the trypsin, were centrifuged. This is in order to perform a correct cell count. On this day, in fact, a conspicuous presence of cells in suspension was noted.
- Cell count by Trypan Blue exclusion test. Viable and dead cells were counted in each well of the 24-well multiwell from which the supernatant was taken. After having been detached by trypsinization, the cells were counted with a Burker cell counter chamber, using the test using a Trypan blue solution. This dye is, in fact, capable of selectively coloring dead cells. The values expressed as mean ± ESM (Standard error of the mean) deriving from the triplicate count of all three experiments are reported in the Annex.

- Evaluation of cell viability using the WST-1 assay. The WST-1 or 4- [3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolium]-1,3 cell proliferation assay was used to verify cell viability and proliferation. -benzene disulfonate (Roche Diagnostics, Mennheim, Germany). This is a colorimetric assay for spectrophotometric quantification

of cell proliferation, viability and cytotoxicity, performed entirely in a 96-well plate. The tetrazolium salt (WST-1) is cleaved to the final, soluble and intensely colored formazan by mitochondrial dehydrogenases. This bioreduction is strictly dependent on the glycolytic production of NAD(P)H in viable cells. Thus, the amount of formazan formed correlates directly with the number of metabolically active cells in the culture. At the moment of the determination the cells were incubated with the WST-1 reagent, ready to use, for 0.25-5 hours, in a humidified atmosphere (37°C, 5% CO_2). During this incubation period, the formazan dye was quantified with a multi-well spectrophotometer (ELISA reader), using a wavelength at 450 nm. The measure of absorbance correlates directly with the number of viable cells. The absorbance of complete medium (blank) alone was subtracted from the absorbance measured in the samples.

- Elisa test for the quantification of pro-collagen I α1 production. At the end of the experiment all the supernatants collected during the study were thawed and a 100 µl aliquot was taken from each one (sample per well). By means of the DuoSet Elisa Kit containing the basic components for the development of an Elisa "sandwich" it was possible to quantify the concentration of pro-collagen I α1. The "capture" antibody of this kit specifically recognizes an epitope in the N-pro peptide (aa 26-161).

  The optical density of each well into which the standards and samples were loaded was determined by reading with the microplate reader at a wavelength of 450 nm. Through the use of the standards, inserting the mean of the absorbance measured against their relative concentration, the curve was calculated which allowed us to trace the concentration of the single samples applied.

9. STATISTICAL ANALYSIS

The values reported in Annex A are the mean ± SME (Standard Error of the Mean) of the cell count of three wells of each point (day).

Both for cell counting, for WST-1 and for the determination of pro-collagen concentrations

The α1 secreted values reported in the graphs are the weighted mean ± ESM of 3 experiments performed separately in parallel.

The statistical analyzes relating to the quantity of procollagen I secreted were carried out by applying the t Test by Student, considering significant the differences having values of $p \leq 0.05$.

10. RESULTS

Radiation treatment

Below are the values of the weighted means ± ESM of the cell counts of live cells only under controlled conditions and subjected to irradiation using the LIMFA system. The averages have been graphed both as columns (Fig. 1) and as a time course of growth over the different days (Fig.2).

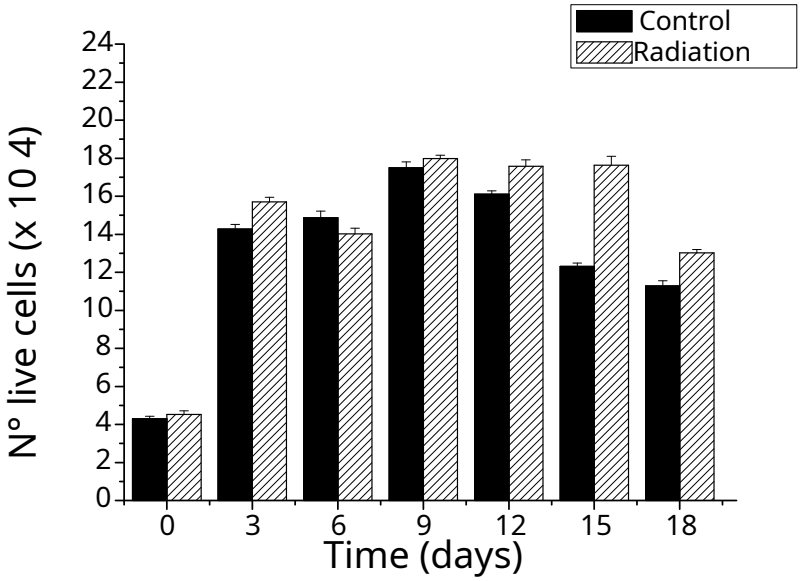


Fig.1. Cell viability under control conditions and after irradiation

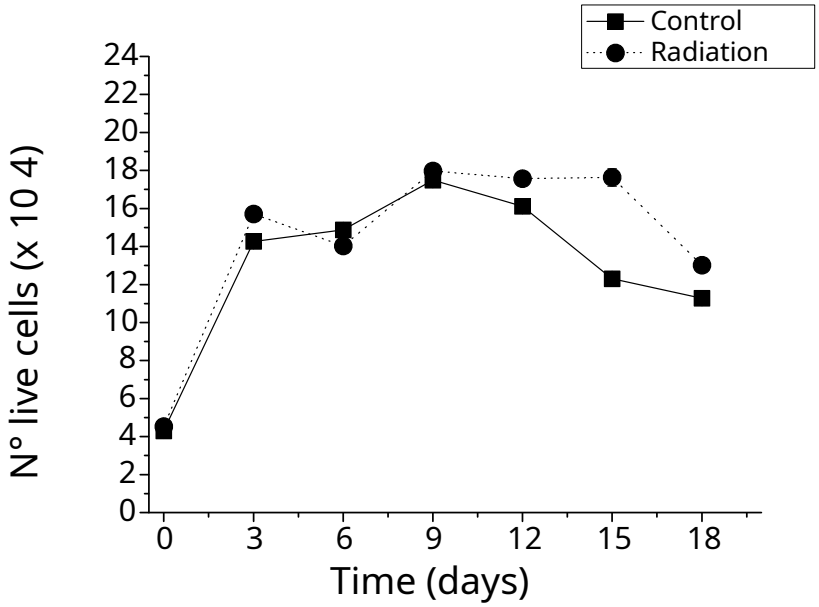


Fig.2 Time course of cell viability of control cells and those subjected to irradiation

Furthermore, a column graph of the number of dead cells in the two different conditions was obtained experimental. A significant number of dead cells (> 0.05 X 10_4) has only been encountered since Day 12 (Fig. 3).

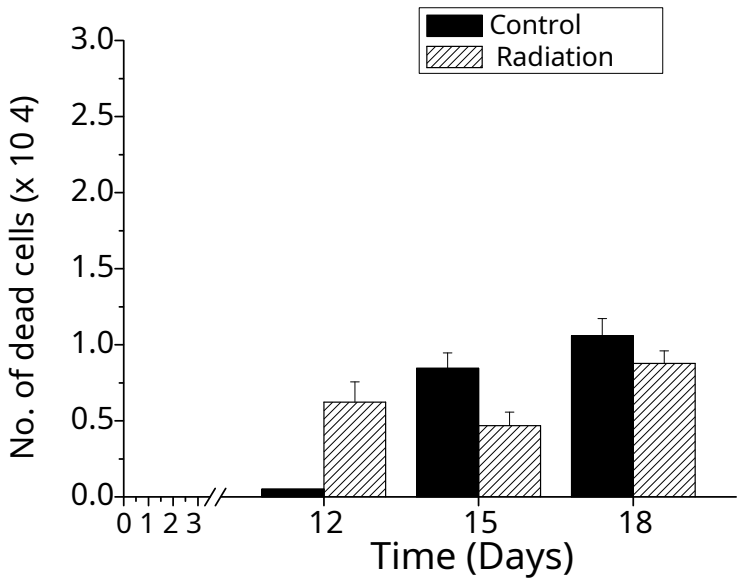


Fig.3 Number of dead cells in control condition and after irradiation.

Below are the weighted means ± ESM of the normalized absorbances for the control obtained by WST-1 assay on days 3, 8 and 13 (Fig. 4).

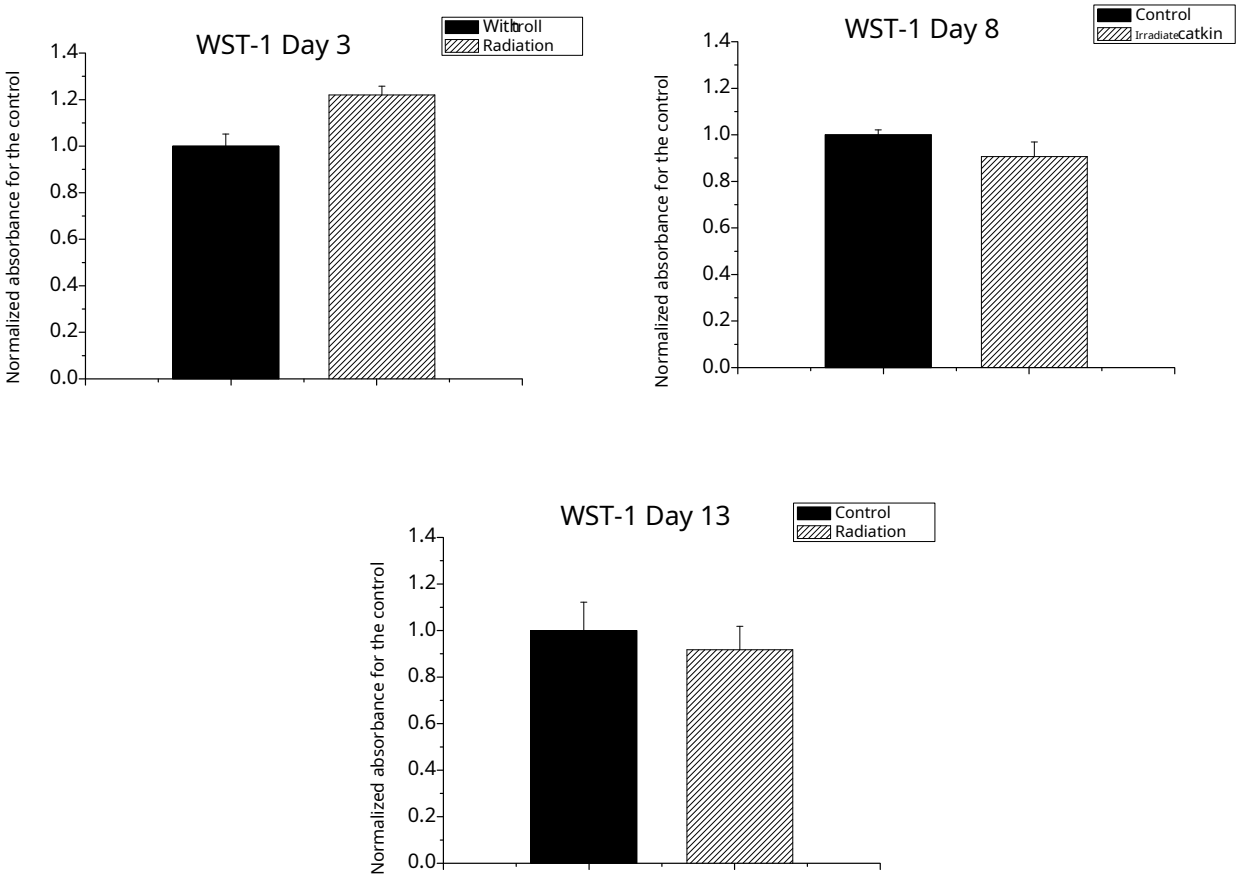


Fig.4 Results deriving from the WST-1 assay performed on days 3, 8 and 13. The weighted means ± ESM of the normalized absorbances for the relative control of the cells subjected to irradiation are reported.

The concentration of Pro-collagen I α1 secreted by the control cells and those subjected to irradiation was determined at the end of the study by enzymatic assay. Next comes reported the concentration (pg/ml) calculated following the sampling of the cellular supernatant carried out ai several days (Fig.5 A) and the relative time course graph (Fig.5 B).

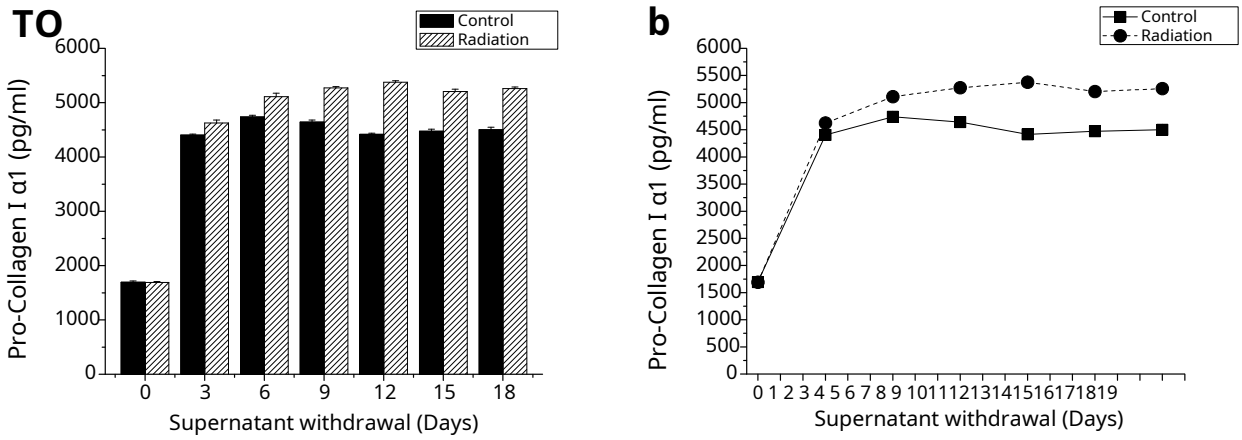


Fig.5A-B Concentration (pg/ml) of Pro-collagen I α1 secreted in the cell culture medium under the conditions of control and after irradiation (A) and relative time course (B).

The same concentrations were normalized for the residual volumes present in the wells on the various days (amount of pro-collagen per well) (Fig.6 AB). Due to the extended incubation time of the cell cultures, in fact, the evaporation of the culture medium distributed in the wells has caused a conspicuous decrease in starting volumes. A statistical test for was also applied in this case evaluate the significance of the differences found (see the asterisks in the two graphs and the relative value of p).

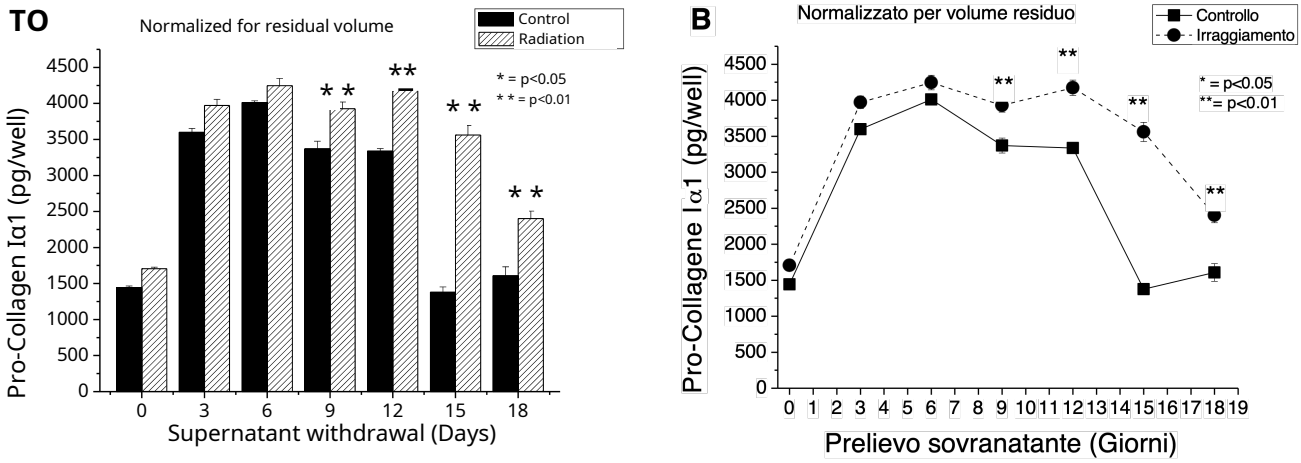


Fig. 6A-B Amount of Pro-collagen I α1 present at different days in the cell culture medium under the conditions control and after irradiation (A) and relative time course (B).

The averages of the amount of Pro-collagen I α1 present in the wells over the different days were in turn normalized by the average number of live cells present in the relative conditions (Fig.7A-B). Also in this case a statistical test was applied to evaluate the significance of the differences found (see the asterisks in the two graphs and the relative p-value).

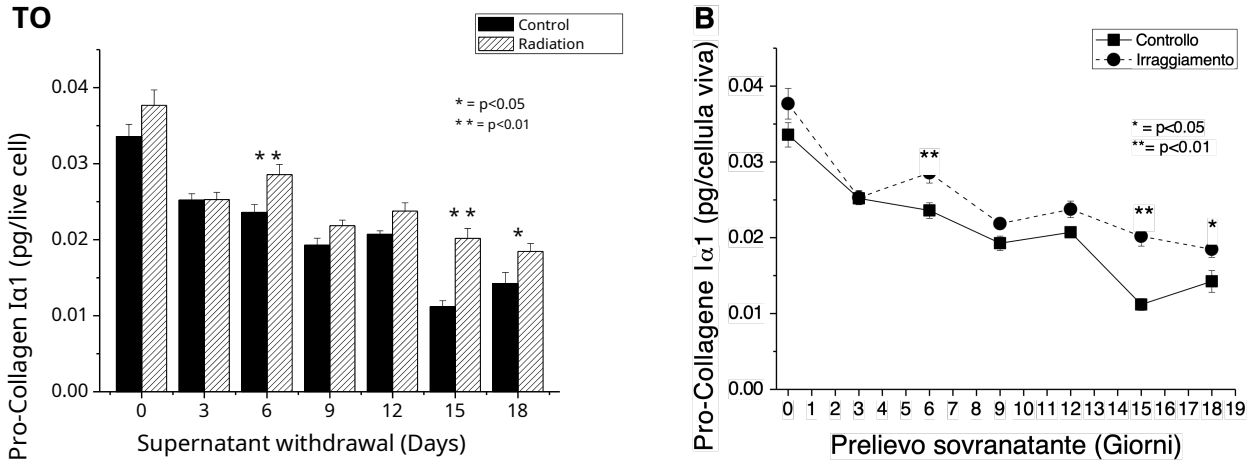


Fig. 7A-B Quantity of Pro-collagen I α1 per viable cell (A) and relative time course (B) over the different days in control conditions and after irradiation.

Statistical significance, indicated in the graphs with (*), was calculated using the T-Test.

Treatment with TGFβ1

The parameters deriving from all the tests reported above were also evaluated by treating the HDFa fibroblasts with TGF-β 1 (5ng/ml). The addition of this cytokine took place on day 6, i.e. when it was seen that the cells had reached the growth plateau. A further addition was made on day 13.

In the following column graph (Fig. 8) and in the subsequent time course (Fig. 9) the values of the weighted means ± ESM of cell counts of live cells only under control conditions and in the presence of TGF-β1. The reading of the values of the cells with TGFβ1 at day 6 was done at the end of the hour of incubation following the 19-minute period required for irradiated cells.

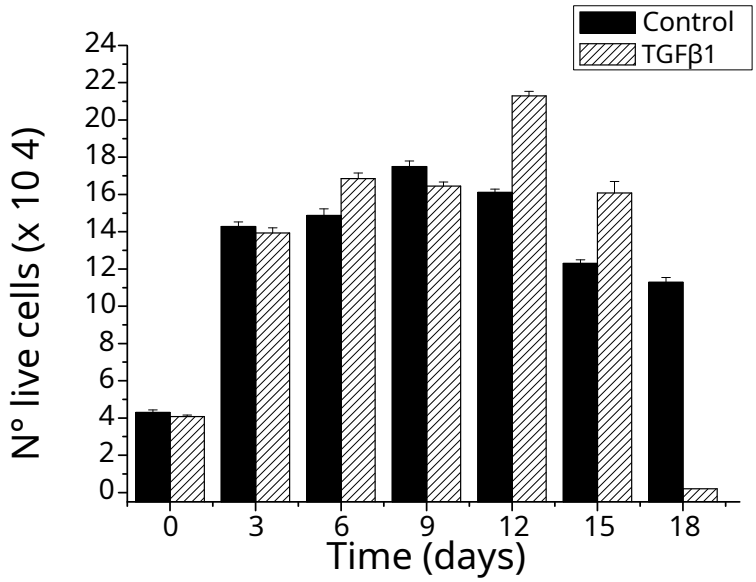


Fig.8 Cell viability under control conditions and in the presence of TGF-β 1. This was added on Day 6 and Day 13.

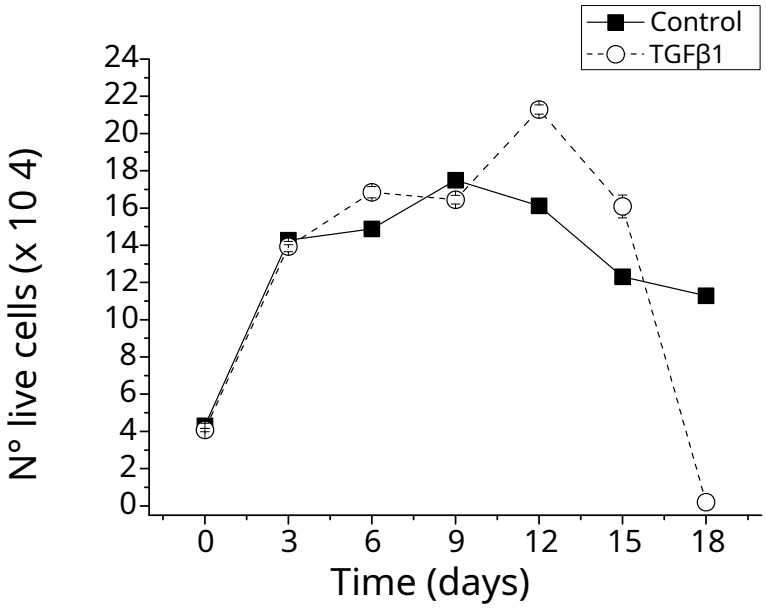


Fig.9 Time course of cell viability of control cells and those in the presence of TGF-β 1.

Also for TGF-β 1 the dead cells were counted at different days and also in these conditions

significant number of dead cells (> 0.05 X 10_4) was found only starting from Day 12 (Fig. 10).

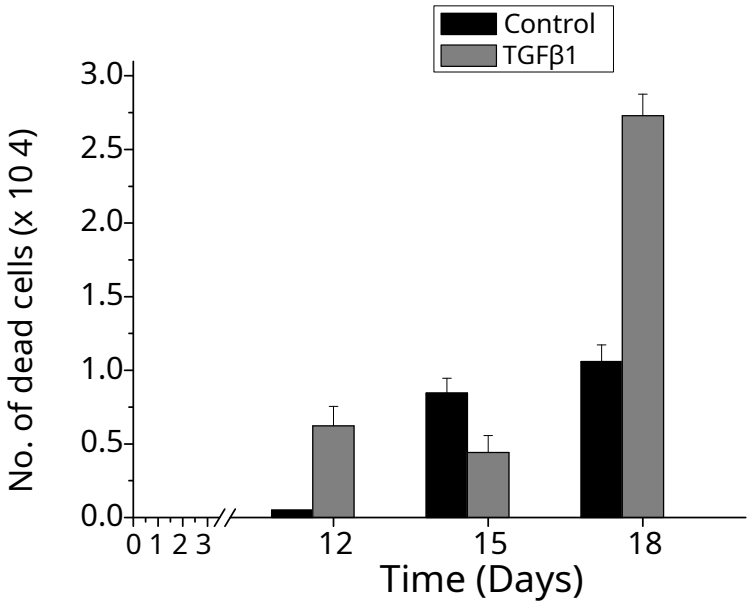


Fig.10 Number of dead cells in control condition and after treatment with TGF-β 1.

Below are the weighted means ± ESM of the normalized absorbances for the control obtained by WST-1 assay on days 3, 8, 13 and 15 (Fig. 11).

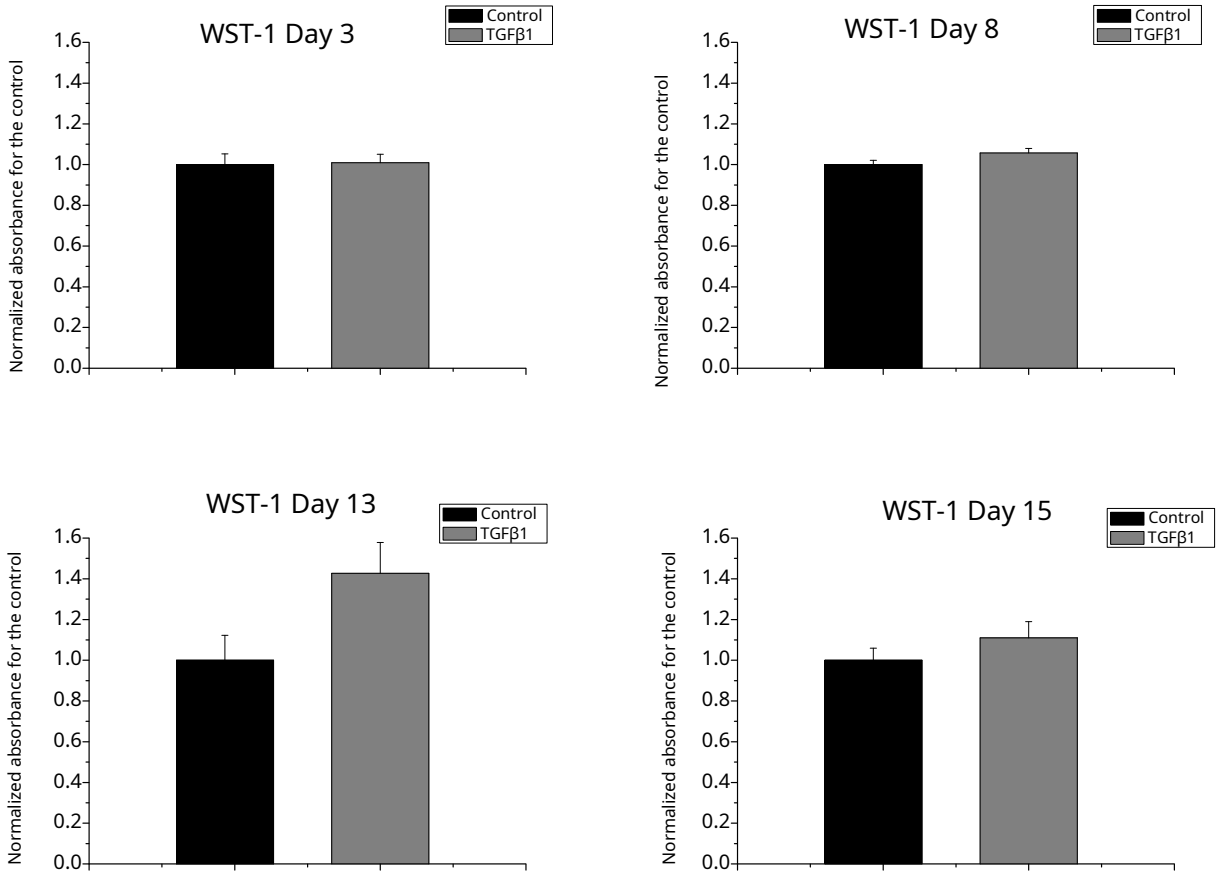


Fig. 11Mweighted days ± ESM of normalized absorbances for control cells in the presence of TGF-β 1 ai days 3, 8, 13 and 15. Values are from WST-1 assay analysis.

Similarly to what was seen previously for the cells subjected to irradiation, they are shown below reported the calculated concentration of the secreted Pro-Collagen I α1 (Fig 12A-B), the averages of the of the same present in the wells (Fig 13A-B) and the averages of the quantities normalized by number of cells viable (Fig 14A-B) at different days of treatment with TGFβ1.

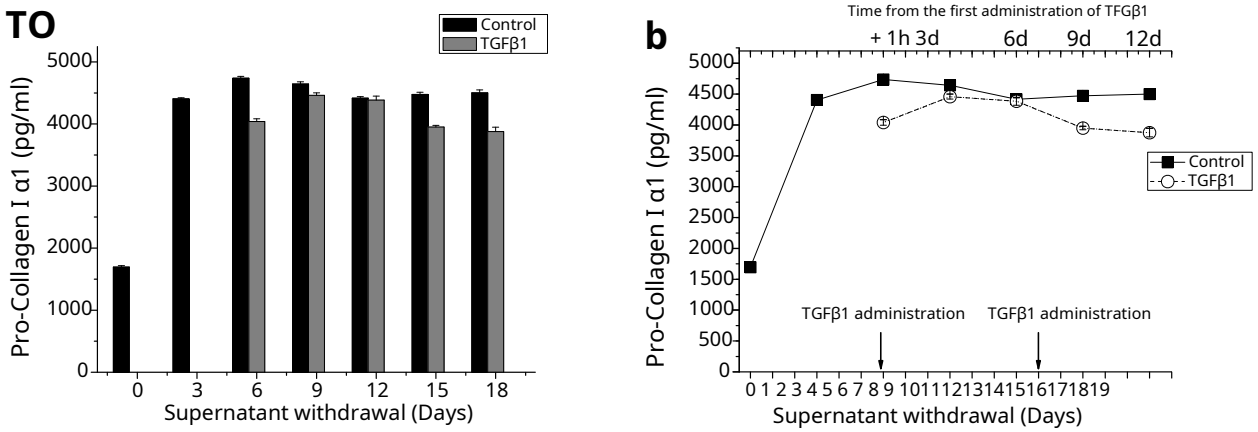


Fig. 12A-B Concentration of secreted Pro-Collagen I α1 (A) and relative time course (B) after treatment with TGFβ1. Graph B shows the times at which TGFβ1 was administered and the relative incubation times after the first administration.

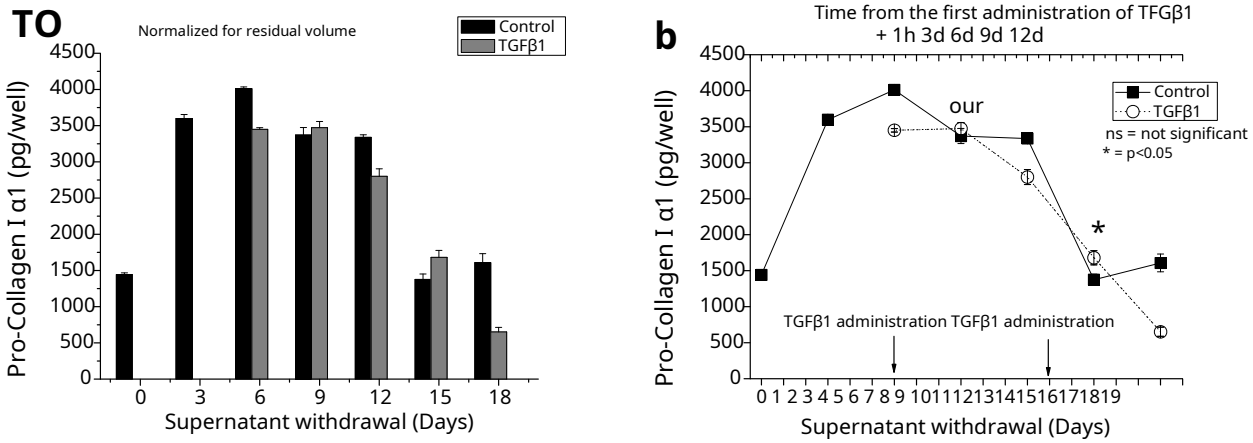


Fig. 13A-B Quantity of Pro-Collagen I α1 secreted per well (A) and relative time course (B) after treatment with TGFβ1. Graph B shows the times at which TGFβ1 was administered and the relative incubation times after the first administration.

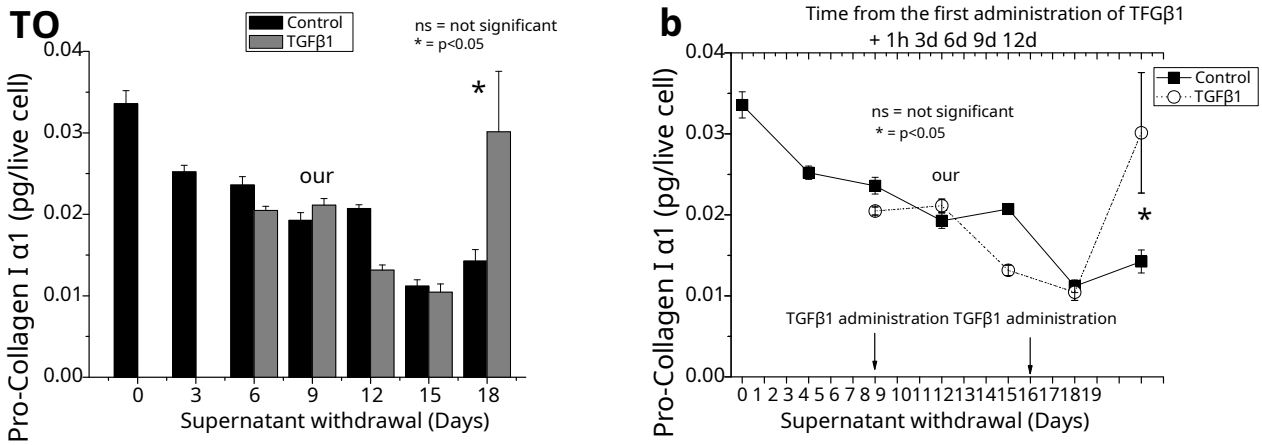


Fig. 14A-B Amount of Pro-Collagen I α1 secreted per number of viable cells (A) and relative time course (B) after treatment with TGFβ1. Graph B shows the times at which TGFβ1 was administered and the relative incubation times after the first administration.

11. FINAL CONSIDERATIONS

1)**Effects of LIMFA treatment on cell growth.**LIMFA treatment did not show induce significant effects on cell growth (viability and mortality) compared to the control until day 9. After this day, and ending on day 18 in which the experimental trial was terminated, le cultures subjected to irradiation show a higher number of live cells than the control cultures.

2)**Effects of LIMFA treatment on cell morphology.**Until day 15, the cells subjected to irradiation did not present any morphological variation, evaluable at a examination by phase contrast microscopy. At day 18, also, a certain amount of cells is detached from the substrate, index of cellular suffering. This is observed both in the crops subjected to irradiation and in the control ones, and indicates the maximum culture time that can be carried out for this type of cell. At the same time (15-18 days) a conspicuous also occurred reduction of the volume of the culture medium, attributable to an evaporation process.

3)**Effects of LIMFA treatment on procollagen I secretion.**Treatment with the system LIMFA induces an increase in the secretion of procollagen I in the culture medium, compared to

control, starting on day 6 of culture. After normalizing these values to the actual residual volume of the culture medium, this increase reaches statistical significance a starting from day 9 of culture, and is maintained until the end of the experimental procedure. The positive effect on the secretion ofprocollagen I is also observed after normalization for the number of live cells in culture, although the trend versus time appears more swinging. The increase in procollagen I secretion induced by irradiation appears, in the latter case, statistically significant starting from day 6 of culture.

4)**Comparison between LIMFA irradiation and treatment with TGFβ1.**The effect of TGFβ1 on this cell line, both in terms of cell viability and procollagen I secretion appear much less encouraging than those observable with irradiation procedures. In fact, both a negative effect of the cytokine on cell viability and a poor pro-secretory effect are observed. The quantity of Pro-Collagen I α1 secreted, in fact, turned out to be even lower than that found in the controls, and the stimulatory effect of TGF b1 on the secretion of procollagen I is observed only after normalization of the number of vve cells, and at later times (day 18), when high cytokine-induced cell mortality is also observed.

Overall the results obtained from this first preliminary test on the treatment effect LIMFA on human dermal fibroblasts appear very encouraging, both for the lack of toxicity of the treatment, which also does not demonstrate an (unwanted) hyper-proliferative effect on the cells both, and above all, for the pro-stimulatory effect of the production of procollagen I. This is occurred despite that, due to the lack of important cofactors present instead live (such as Vitamin C) and of the intrinsic characteristics of the culture conditions cell phone in vitro, the formation of stable collagen in vitro is very difficult (Chen CZ, e Raghunath M. Fibrogenesis Tissue Repair. 2009 Dec 15;2:7. doi: 10.1186/1755-1536-2-7). There normalizing our data for changes in the number of viable cells, one item appears relevant, not only because it confirms the statistical significance of the pro-secretory effect of LIMFA treatment, but also because it is in line with what is reported in the literature, according to which the Cell density is a relevant aspect for production in vitro of collagen. Very interesting

would investigate the production of other collagen molecules (e.g. type III collagen), either, after extraction and dialysis, the release of insoluble collagen in the pericellular matrix.

Combined rehabilitation protocol in the treatment of knee osteoarthritis: a comparative study between ultra-low frequency magnetic fields and a soft elastic knee brace.

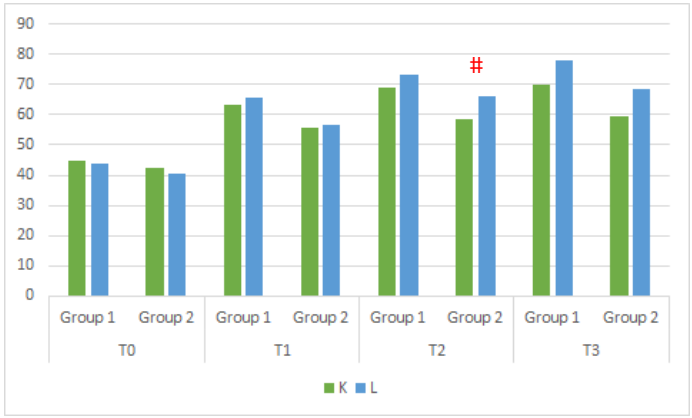
Teresa Paolucci 1, Daniele Porto 2, Raffaello Pellegrino 3 , Ornela Sina 2, Andi Fero 2, Sara D'Astolfo 2, Sara Franceschelli 4 , Antonia Patruno 4 , Augusto Fusco 5 and Mirko Pesce 4

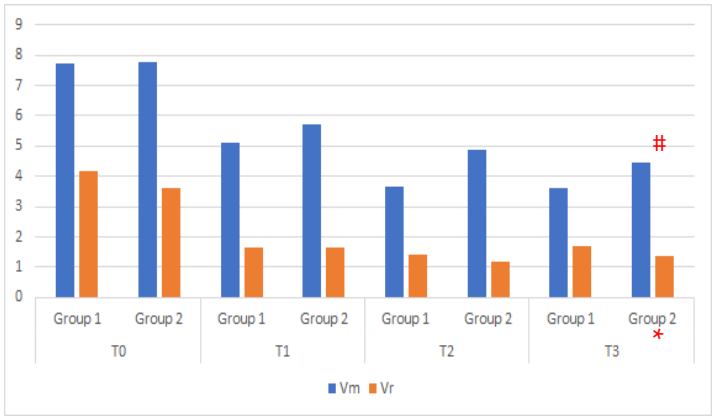
Abstract: The investigation of this observational case-control study aimed to determine the efficacy of a combined treatment of ultra-low-frequency electromagnetic fields (ELF) with a soft, elastic knee brace compared with ELF alone in knee osteoarthritis (KOA) with regard to pain reduction and functional recovery. We hypothesized that the combined use of ELF and a soft, elastic knee brace would provide better results. Thirty-five patients (N = 35, divided into Group 1 = ELF and Group 2 = ELF with soft elastic knee brace) were analyzed. The rehabilitation protocol consisted of 10 sessions of antiphlogistic and anti-edema programs (first cycle) for 2 weeks, followed by 12 sessions of bone and connective tissue repair (second cycle) in patients with knee osteoarthritis (KO) for 4 weeks.

Methods:
Patient evaluations were conducted at baseline (T0) and after 2 (T1) and 4 (T2) weeks. A follow-up evaluation was conducted 6 weeks after treatment (T3). The LIMFA© Therapy system was used to create multifrequency magnetoelectric fields with an intensity of 100 uT and low frequency. Incrediwear Knee Sleeve (Incred) was used to relieve knee pain. Visual analog scale (VAS), Knee Injury and Osteoarthritis Outcome Score (KOOS) and Lysholm score (Ls) were used as outcome measures.

Results:
The results showed that pain at rest (Vr), pain on movement (Vm), KOOS and Ls were significantly affected by ELF over time. In conclusion, group 2 had a better response in terms of pain resolution.

Inclusion Criteria	Exclusion Criteria
Patients with recent osteoporosis	Fertile age for women
Patients with severe osteoporosis	Age < 50 years for men
	Secondary osteoporosis
	Current or previous neoplasm
	Presence of pacemaker





Graphics: Incred effect during ELF magnetic field therapy. Measurements of Vm, Vr, K, Kf and L in patients undergoing LIMFA therapy without (Group 1) or with (Group 2) INCRED were performed at different times (T0-T3). Significance within T0-T3 was obtained by repeated-measures ANOVA, considering INCRED as an intermediate factor. Data are expressed as means ± SD (n = 25). * indicates significant difference between groups in free time; # indicates significant difference between groups at the considered time point. Vm = VAS in motion; Vr = VAS at rest; K= KOOS; L = Ls.

Conclusions:
Our results showed that the combination of Incred (6 to 8 hours per day) with ELF-EMF therapy seems to enhance its positive effect on KOA in reducing short-term pain at rest, with short follow-up and good maintenance promoting faster recovery of function after six weeks of treatment. In addition, in future RCT research developments, the combined use of ELF-EMF and other models of soft and elastic knee braces should be conducted.

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Combined Rehabilitation Protocol in the Treatment of Osteoarthritis of the Knee: Comparative Study of Extremely Low-Frequency Magnetic Fields and Soft Elastic Knee Brace Effect

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Combined Rehabilitation Protocol in the Treatment of Osteoarthritis of the Knee: Comparative Study of Extremely Low-Frequency Magnetic Fields and Soft Elastic Knee Brace Effect

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Abstract: The investigation of this observational case–control study aimed at determining the effectiveness of a combined treatment of extremely low-frequency electromagnetic fields (ELF) with a soft elastic knee brace versus ELF alone in knee osteoarthritis (KOA) with respect to a reduction in pain and functional recovery. We hypothesized that the combined use of ELF and a soft elastic knee brace may provide better results. Thirty-five patients (N = 35, divided into Group 1 = ELF and Group 2 = ELF with the soft elastic knee brace) were analyzed. The rehabilitative protocol consisted of 10 sessions of antiphlogistic and antiedema programs (first cycle) for 2 weeks, followed by twelve sessions of bone repair and connective tissue repair programs (second cycle) in patients with knee osteoarthritis (KOA) for 4 weeks. Patient evaluations were conducted at baseline (T0) and after 2 (T1) and 4 (T2) weeks of treatment. A follow-up evaluation was conducted 6 weeks after treatment (T3). The LIMFA© Therapy System was used to create multifrequency magnetoelectric fields with an intensity of 100 μT and a low-frequency field. The Incrediwear Cred 40 knee sleeve (Incred) was used for alleviating knee pain. The Visual Analogue Scale (VAS), the Knee Injury and Osteoarthritis Outcome Score (KOOS), and the Lysholm score (Ls) were used as outcome measures. The results showed that pain at rest (Vr), pain in motion (Vm), KOOS, and Ls were significantly affected by ELF over time. In conclusion, Group 2 had a better response in terms of pain resolution at rest ($p < 0.05$) and a concurrent better response at T3 in terms of functional recovery ($p < 0.05$).

Keywords: knee; osteoarthritis; sleeve; rehabilitation; magnetic field; soft brace

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1. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease that occurs most commonly in people over 45 years of age. It is characterized by articular cartilage loss, synovial inflammation, and the remodeling of subchondral bone. OA has been shown to be associated with joint pain, stiffness, loss of function, reduced quality of life (QOL), and mortality. The treatment of OA traditionally comprises nonpharmacological and pharmacological management; however, if symptoms persist, surgery may be considered. Current treatments are limited by small effect sizes and adverse side effects. In recent years, there has been much emphasis on nonpharmacological management such as education, physiotherapy, and exercise therapy to relieve symptoms and improve function in those with OA [1].

Magnetotherapy provides a non-invasive, safe, and easy method to directly treat the site of injury, the source of pain and inflammation, and it is widely used in rehabilitation in OA [2,3]. Briefly, a pulsed electromagnetic field (PEMF) can promote the proliferation of osteoblasts when its frequency is 7.5–15 Hz or 50–75 Hz and the intensity is 0.4–1.5 mT or 3.8–4.0 mT [3]. This underlines EMFs with different frequencies and intensities as able to exert distinct bioeffects on specific bone cells with good results for the reduction in pain and improvement of function in knee osteoarthritis (KOA) [4,5]. KOA is believed to be highly prevalent today because of recent increases in life expectancy and body mass index (BMI) and remains the most challenging arthritic disorder, with a high burden of disease and no available disease-modifying treatment [6,7]. It is estimated that 32.5 million US adults have clinical OA, with the most common sites being the knee and hip [8]. Thus, a multidisciplinary and sustained international effort involving all major stakeholders is required.

There are different rehabilitative electromagnetic field programs used in KOA based on short protocols, such as the program by Nelson et al., which consists of 2 weeks of treatment (15 min per session, twice daily) with 6.8 MHz and an intensity of 30 Gauss [5], or the longer program by Bagnato et al. that proposed a 12 h/daily treatment for 1 month. Both approaches proved effective and well tolerated by the patient [9]. Ay et al. applied PEMFs for 30 min, 5 times/week for 3 weeks on KOA with good results for pain reduction and functional recovery [10,11]. Additionally, Özgüçlü et al. successfully used PEMFs in patients with knee pain using a protocol lasting 30 min per session [12,13].

The difficulty often encountered in rehabilitation is the choice of a unique and shared physical therapy magnetic fields protocol in the treatment of symptomatic KOA. Therefore, the choice is entrusted to the physiatrist and physical therapists based on the characteristics and needs of the patient. Surely, short protocols allow better compliance by the patient [14] and an earlier start to the therapeutic rehabilitation exercise, with a reduced use of pain-relieving drugs. Containing and adequately managing pain in KOA allows physical therapists to face it and adequately guide their clinical decision-making by summarizing the safest and most efficacious exercise options [15]. Patient education, physical exercise, and weight loss may constitute the first-line KOA treatment approach.

To reduce pain, improve physical function, and, possibly, slow disease progression in KOA, the use of knee braces has often been suggested [16]. These are generally the main purposes of knee braces [17], but the optimal choice for an orthosis remains unclear, and long-term implications are lacking. A variety of different bracing types, manufacturers, and products are currently available on the market. Short-lever elastic knee braces have been used to improve pain, specifically during squats or walking, and daily use or the use of soft knee braces while resting is suggested to provide moderate pain relief and small-to-moderate effects on performance-based physical function in patients. Several authors, with respect to these findings, highlight the importance of soft braces to improve pain reduction and physical function in both the short and long term in KOA treatment, but additional high-quality studies are warranted to improve confidence in the findings [18–21].

Considering these premises and the evidence of the literature, we hypothesized that the combined use of magnetic fields and a soft elastic knee brace may have a better response in the resolution of pain in KOA patients compared to magnetic fields alone.

The aim of the study was to evaluate the effect of the combined treatment of ELF and the soft elastic knee brace compared to ELF alone, with pain reduction as the primary outcome and functional recovery as the secondary outcome.

2. Materials and Methods

2.1. Study Design and Population

An observational case–control study was conducted per the Strobe Guidelines [22] to determine the combined effect of ELF and soft elastic Knee brace vs. ELF alone in treating acute painful knee osteroarthritis.

Patients with acute pain in KOA were screened at the physical medicine and rehabilitation outpatient clinic of Don Orione Institute of Pescara (Italy) from December 2020 to February 2022 by a physician skilled in physical and rehabilitation medicine.

The study was brought to the attention of the Department of Oral Medical Sciences and Biotechnologies of the G. D'Annunzio University of Chieti (Italy) and was approved by the National Ethics Committee of the Don Orione Board. Informed consent was obtained from all individual patients included in the study. The patients were treated in accordance with the World Medical Association Declaration of Helsinki.

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

2.2. Inclusion and Exclusion Criteria

The inclusion criteria were age between 40 and 70 years, diagnosis of acute pain in KOS < than 1 month, VAS > 3 according to the American College of Rheumatology Criteria [23], and stage II classification per the Kellgren–Lawrence classification (KLc) [24]. The exclusion criteria were red flags (such as cancer, hematological diseases, and specific rheumatological pathologies) [25], bleeding disorder, local infection, pregnancy, patient refusal or noncompliance, pacemaker use, candidacy for knee joint replacement or any intra-articular injection within six months, addiction to opioid drugs, thrombocytopenia, use of anticoagulant or antiaggregant, and recent myocardial infarction or stroke. We excluded patients who were undergoing any other type of physiotherapy or conservative treatments during the study period. KOS was diagnosed based on the clinical examination and radiographic images (X-ray) in the standing position.

Finally, patients were included consecutively and formed two groups that are indicated as LIMFA therapy without (Group 1) or with (Group 2) Incred.

2.3. Outcome Measures

The Visual Analogue Scale (VAS) was used to measure knee pain outcomes: patients were asked to mark the point that corresponded to their perceived pain intensity on a 10 cm line, with 0 indicating the absence of pain and 10 reflecting the most severe pain [23]. The patient was asked to indicate both pain at rest (Vr) and pain in motion (Vm).

The Knee Injury and Osteoarthritis Outcome Score (KOOS) is a questionnaire designed to assess short-term and long-term patient-relevant outcomes following a knee injury or OA [26,27]. The KOOS is self-administered and assesses 5 outcomes: pain, symptoms, activities of daily living (ADL), sport and recreation function, and knee-related quality of life. This scale was used to evaluate pain and function after patients reported the pain and ADL domains.

Lysholm score (Ls), which measures the ability to manage in everyday life [28,29], was used as a patient-reported outcome measure questionnaire (PROM). The total score varies from a minimum of 0 to a maximum of 100, where 0 corresponds to complete disability, while 100 corresponds to no symptoms. The total scores are divided into the following categories: poor: <65 points; right: 65–83 points; good: 84–90 points; excellent: >90 points.

2.4. Magnetic Fields Protocol

The LIMFA © Therapy System (Eywa srl; Rimini, Italy) (ISO9001 certification number 390263—commercially available) was used to create multifrequency magnetoelectric fields with an intensity of 100 μT and a low-frequency field.

The Limfa's patented ELF pre-set sequences are complex magnetic fields, pulsed and bipolar, that target specific ion channels in order to obtain the desired biological reaction. Depending on the ion channel, the frequencies vary from 2 to 80 Hz with a variable field intensity (lower than 100 μT) and variable waveform. The abovementioned parameters are not random but are pre-defined for each stimulation program.

Patients participated in ten (n = 10) sessions of antiphlogistic and antiedema programs (first cycle) (15 min each) for 2 weeks, 5 days/week, followed by, without interruption, twelve (n = 12) sessions (20 min each) of bone repair and connective tissue repair programs (second cycle) for 4 weeks, 3 days/week (Figures 1 and 2).

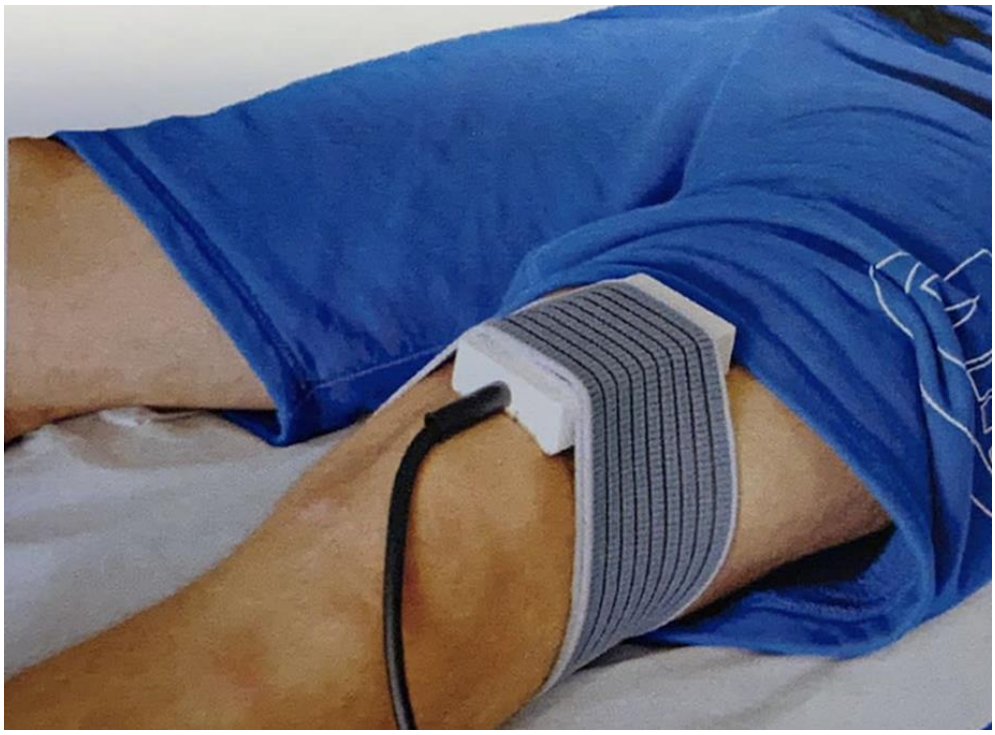

Figure 1. LIMFA application. Representative image of LIMFA application during treatment of knee osteoarthritis.

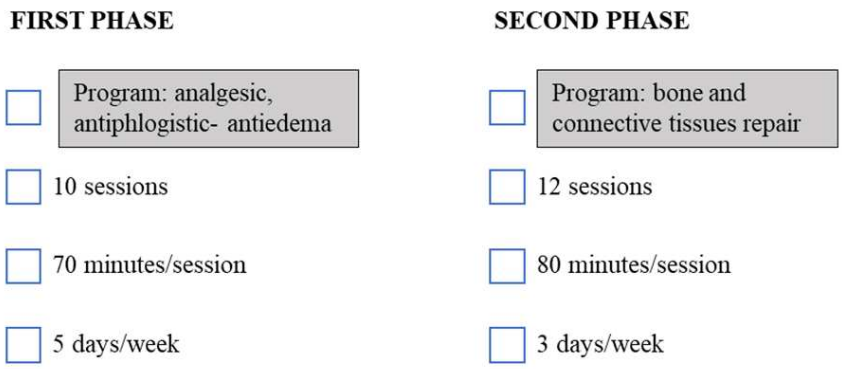

Figure 2. Magnetic Field Protocol. Schematic representation of phases characterizing the magnetic field protocol.

2.5. Soft Knee Brace

The Incrediwear Cred 40 knee sleeve (Incred) [30] is embedded with carbonized charcoal and germanium, which is a nontoxic semiconductor metalloid located between tin and silicone in the periodic table. The resistance of germanium decreases, while the temperature of semiconductors increases with more "free" electrons, allowing for higher conductivity (Figure 3).

Figure 3. Soft Knee Brace—Incrediwear. Representative image of patient.

2.6. Data Collection

The questionnaires were given to patients when they first received the knee sleeve, before starting magnetic field therapy (T0 = at baseline). They were also given to patients at the end of the first cycle of magnetotherapy (T1 = after 2 weeks), at the end of the second cycle of magnetotherapy (T2 = after 4 weeks), and 6 weeks after the end of all treatment (T3 = follow-up). Each patient was observed and received the treatment for a total of three months.

2.7. Sample Size

The sample size was calculated, considering pain intensity per the VAS as the primary outcome. A power analysis of 90% and an alpha level = 0.05 was considered with a difference of 2 points (cm), assuming a standard deviation of ±1.5 for the VAS in relation to the minimal clinically important difference between the groups after treatment. Thus, the analysis would yield a minimum of 16 patients per group, and, based on a 20% potential dropout rate, we included 22 patients per group [31].

2.8. Statistical Analysis

Data were checked for normality and homogeneity of variance, which were analyzed with parametric and non-parametric data, respectively. The outcome scales for Vm, Vr, KOOS, KOOS-functional, and Ls differences during treatment, both for those who used and did not use Incred, were analyzed by two-way ANOVA for repeated measures (1 between factor, patients that used Incred or not; 1 within the experimental time points considered) followed by the Bonferroni post hoc test.

Statistical analyses were performed using SPSS 22.0 statistic (SPSS Inc., Chicago, IL, USA) for Windows (IBM). Results are described as means ± SD for each assessment. The level of a statistically significant difference was defined as $p < 0.05$.

3. Results

Out of the thirty-five patients (N = 35 divided into Group 1, N = 18, and Group 2, N = 17 (Table 1)), sixteen patients (N = 16) were excluded following the initial observation (N = 51) and nine patients (N = 9) did not join the study. Three patients were excluded from Group 1 because they did not complete the rehabilitation treatment for personal and work reasons. Furthermore, four patients were excluded in Group 2: two because they did not find the brace comfortable and it was not worn according to the indications; two because they did not complete the rehabilitative treatment according to the protocol, taking advantage of recovery sessions for work reasons that lengthened the planned times.

Table 1. Demographic data and clinical disability measures of patients.

Variable	Group 1 (n = 18)	Group 2 (n = 17)	*p*-Value
Gender M/F	5/13	3/14	0.249 [a]
Age (years)	61.28 ± 7.89	63.76 ± 9.23	0.397 [b]
BMI	29.16 ± 4.00	26.45 ± 4.22	0.060 [b]
Time of diagnosis (years)	2.86 ± 2.45	3.65 ± 4.02	0.482 [b]
KLc	2.83 ± 0.71	2.47 ± 0.62	0.118 [b]

[a] Chi-squared test. [b] Unpaired Student's *t*-test. BMI: Body Mass Index; Group 1 = LIMFA; Group 2 = LIMFA + INCRED; KLc = Kellgren–Lawrence classification.

The comparisons of values obtained at different time points (T0–T3) showed that Vr ($F_{7, 245}$ = 35.41, $p < 0.001$), Vm ($F_{7, 245}$ = 74.22, $p < 0.001$), KOOS ($F_{7, 245}$ = 63.29, $p < 0.001$), and Ls ($F_{7, 245}$ = 72.23, $p < 0.001$) were significantly affected by the ELFs over time (Figure 4). No differences were obtained for the KOOS-functional variable.

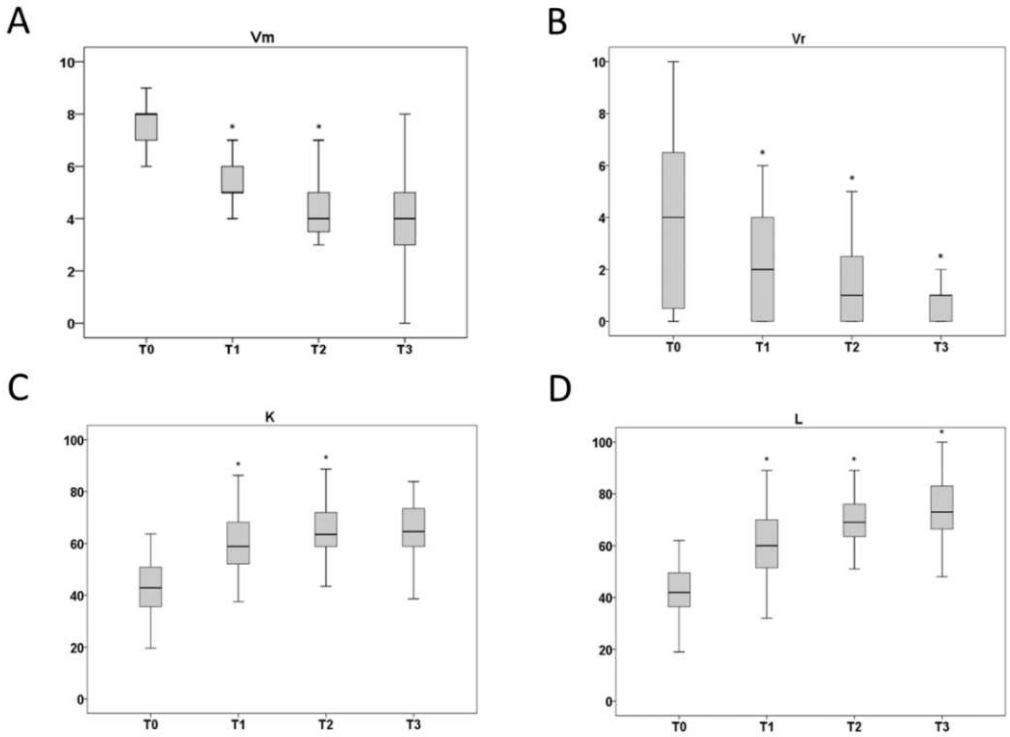

Figure 4. Time-dependent effect of ELF-magnetic field therapy. Measurements of Vm (**A**), Vr (**B**), K (**C**), and L (**D**) in patients submitted to LIMFA therapy were performed at different times (T0–T3). Significance within T0–T3 was obtained by repeated measures ANOVA, considering the therapy as a within factor. Data are expressed as means ± SD (n = 35). * $p < 0.05$ vs. previous ascertained time point. Vm = VAS in motion; Vr = VAS at rest; K= KOOS; Kf = KOOS-functional; L = Ls.

Patients wore the brace during rest and daily activity from at least 6 h to 8 h a day, every day, for 6 consecutive weeks and during magnetic field therapy.

Notably, the differences recorded for Vr were significantly regulated by Incred, suggesting that this device ameliorates the effect of magnetic treatment at rest (Table 2).

Table 2. Incred effect during ELF-magnetic field therapy. The Vm, Vr, K, Kf, and L measurements in patients submitted to LIMFA therapy without (Group 1) or with (Group 2) INCRED were performed at different times (T0–T3). Significance within T0–T3 was obtained by repeated measures ANOVA, considering the INCRED as a between factor. Data are expressed as means ± SD (n = 25). * indicates a significant overtime difference between groups; # indicates a significant difference between groups in the time point considered. Vm = VAS in motion; Vr = VAS at rest; K= KOOS; L = Ls.

		T_0		T_1		T_2		T_3		
		Group 1	**Group 2**	**Group 1**	**Group 2**	**Group 1**	**Group 2**	**Group 1**	**Group 2**	**P-Tot**
Vm	Mean	7.71	7.78	5.12	5.72	3.65	4.89	3.59	4.44	0.208
	SD	1.21	1.21	1.27	1.32	1.83	1.71	1.91	1.34	
Vr	Mean	4.15	3.63	1.65	1.67	1.41	1.17	1.71	1.39 #	0.011 *
	SD	2.96	3.01	2.06	2.14	1.58	1.58	0.69	1.61	
K	Mean	44.82	42.19	63.45	55.89	69.02	58.33 #	70.12	59.42	0.076
	SD	10.17	11.37	11.68	10.01	12.63	11.73	12.15	12.12	
L	Mean	43.76	40.72	65.47	56.72	73.00	65.89	77.88	68.28	0.492
	SD	13.92	11.17	13.29	14.64	12.65	14.50	11.69	15.29	

4. Discussion

Considering the aim of the study, the results appeared interesting. Above all, compared to the primary outcome of pain reduction, Group 2, who carried out the two treatments in combination, showed a more rapid reduction in knee pain and a better response at rest, which were well maintained at follow-up ($p < 0.01$), with a significantly better functional recovery at T2 (the end of the second rehabilitative cycle) for KOOS, as reported in Table 2 (p for VAS at rest = 0.011).

The rehabilitative treatment options in KOA have been widely implemented and researched, but the optimum treatment or combinations of treatment remain unclear [32,33]. Particularly in the early stages of KOA progression, a conservative approach is commonly favored: interventions can be stratified according to the duration of the treatment protocol and outcomes into short term (4–12 weeks), medium term (12–26 weeks), and long term (>26 weeks). The TENS and PEMF showed a positive effect on short-term outcomes for pain. For medium-term outcomes, beneficial effects included weight loss for general pain and function, intra-articular infiltrations (corticosteroids, glucosamine, chondroitin sulfate, and platelet-rich plasma), quality of life, and pain during exercise programs [32].

The ELF protocol adopted and proposed in our study would seem to confirm what was reported by the literature according to short-term benefits with respect to pain in KOA and functional recovery. Our protocol envisaged six weeks of treatment, including a more intensive first phase aimed at reducing pain and a more extensive second phase aimed at functional recovery, a regenerative component, and a further observation period of another six weeks. As suggested by the literature, in the second phase of our treatment, the frequency used was maintained at <50 Hz along with an intensity of 0.1 mT: sinusoidal EMF, with 0.9–4.8 mT and 45–60 Hz, and a static magnetic field with 0.1–0.4 mT or 400 mT, could promote a regenerative response in the bone with osteoblast differentiation and maturation [3]. Instead, in the first phase of our protocol, the frequency was >50, but <100 Hz to have a mainly analgesic action, keeping the intensity of the magnetic field unchanged.

The rationale of the PEMF application to OA resides in its ability to modulate the expression of several elements in cells residing in the joint tissues. Previous in vitro studies

have demonstrated that PEMFs enhance the expression of adenosine receptors in chondrocytes and synoviocytes. These receptors play a relevant role in the control of nociception and inflammation, suggesting a possible molecular mechanism for the observed pain reduction. PEMFs have also been proposed as capable of cartilage regeneration. Indeed, biophysical treatment actions are related to a pleiotropic effect on several targets, comprising integrins, ion channels, growth factors, and intracellular pathways, which may contribute to the proliferation and maturation of tissue resident cells, including chondrocytes, possibly counteracting the progressive loss of tissue characterizing degenerative disorders, such as OA. In general, the emission of PEMFs was able to exert a protective action and stimulate cartilage tissue [34–36], but there is a lack of defined and reproducible protocols for frequency, intensity, and duty cycle and the possibility of identifying, *in vivo*, a minimum dose effect of the magnetic field necessary for the correct therapeutic response. Viganò M. et al. underlined that PEMFs effectively relieve KOA symptoms in the short term, but they are not superior to other conservative therapies such as physiotherapy [37].

With respect to magnetic field therapy, the best physical parameters to be used in osteoarthritis treatment, such as frequency, are debated in the literature. Negm et al. suggested that a low frequency ($\leq$100 Hz) pulsed sub-sensory threshold electrical stimulation produced is effective in improving physical function but not pain intensity at treatment completion in adults with KOA [38]. Similarly, the same authors described how the heterogeneity of the different protocols was not a significant problem for pain or physical function outcomes and that the effect is similar regardless of the type of pulsed sub-sensory threshold electrical stimulation (frequency ranging from 5 to 100 Hz) and the length of treatment (<12 and $\geq$12 weeks) [38]. Meanwhile, a short PEMF treatment duration (within 30 min) may achieve favorable efficacy [39,40], but the effects of ELF-EMF depend on their respective codes as frequency, intensity, and waveform [41].

In terms of the length of the treatment period, we have adopted a protocol similar to that used by Thamsborg and colleagues [42], reporting analogous effective results in terms of pain and better results in terms of function. Our study included a knee brace, a lower duration of the ELF application sessions (15 min or 20 min vs. 2 h), and a lower total number of sessions (22 vs. 30). Iannitti and colleagues also applied an analogous treatment period to older patients, which resulted in a reduction in pain and disability [43]. It must be noted that shorter sessions have also been found to be effective.

Physiotherapy and, in particular, instrumental physical therapies in the treatment of KOA are often associated with the use of orthoses to contain pain more quickly and promote better function recovery, but the scientific evidence regarding the use of soft discharge braces attributable to Incred or similar devices is still limited.

Lee et al. [44] showed that knee braces positively affect the patient's quality of life and, as per NICE guidelines for the conservative management of KOA, should be used in combination with other standard treatments [45]. The use of the Incred knee brace was based on the assumption that embedding germanium into cotton garments could improve the transdermal effect to create a microelectromagnetic field, leading to increased circulation and affecting the inflammatory process [30,44,46]. Additionally, the Incred knee brace has an immobilized elastic sleeve that stabilizes the knee by providing tactile feedback from the skin with a continuous pressure stimulus that can act on pain reduction according to the gate control theory [47,48]. Finally, the use of the knee brace could decrease the biomechanical load on the articulation, which is generally considered a risk factor for KOA progression [49]. In particular, the use of valgus braces without any other addition to the treatment has been found to provide small-to-moderate improvements in terms of pain and function, even with poor compliance during long-term use [50]. The combined use of ELFs and the brace could have the benefit of a quicker effect, diminishing the time of the rehabilitative interventions.

The data from our study would suggest that, in Group 2, patients had a better response in pain resolution, especially at rest (Group 2 vs. Group 1, 1.39 vs. 1.71, $p = 0.011$), and that they probably had a concurrent better response at T3 in functional recovery (for KOOS)

compared to Group 1. Considering that the study was conducted using a non-randomized design, these results need further investigation. As reported in the literature [30,42,43], we found a good tolerance to the use of the Incred knee brace as only two patients did not find the brace comfortable. Some studies reported a reduction in pain as early as six weeks after unloading a knee brace in the conservative treatment of KOA [51], compared to usual care, with a positive influence on the dynamics of gait. In fact, in the short-term, knee brace use reflects an increased load on the unaffected limb with a prolongation of the stance phase in both extremities in a long-term effect that persists even after the brace is removed [52,53].

Strengths and Weaknesses of the Study

This study represents the first attempt at an experiment associated with extremely low-frequency electromagnetic fields (ELF-EMFs) combined with the germanium-embedded knee sleeve in the rehabilitation treatment of patients suffering from KOA.

The dropout of patients remained below 20%, and these interruptions to treatment were mainly for personal and organizational or work reasons. Patients reported no side effects during the sessions or complications, as proof of the safety of the proposed rehabilitative treatment.

Furthermore, comparative studies that consider different rehabilitation protocols with randomized controlled trial (RCT) models would be desirable to contain the selection bias related to the lack of randomization of the sample. A study that considers the inclusion of a third group treated only with the soft knee brace would be particularly desirable. In addition, despite being an observational study, to contain the interview bias, the operator who administered the scales was kept unaware of the patient group, and to ensure adherence to the rehabilitation treatment, two expert physiotherapists were selected who followed the patients from the beginning to the end of the treatment.

5. Conclusions

Our results showed that the association of Incred (from 6 h to 8 h a day) with ELF-EMF therapy seems to enhance its positive effect on KOA in short-term pain reduction at rest, with a short follow-up and good maintenance favoring a more rapid recovery of function after six weeks of treatment. Furthermore, in future RCT research developments the combined use of ELF-EMF and other models of soft elastic knee braces should be conducted.

Author Contributions: All authors contributed to the study design (T.P., D.P., M.P., A.P., A.F. (Andi Fero) and A.F. (Augusto Fusco)), the acquisition of data (O.S., S.D. and S.F.), or the analysis and interpretation of data (T.P., M.P., S.F., A.P., D.P., R.P. and O.S.). T.P. and M.P. drafted the article, and all authors revised it critically for intellectual content. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was approved by the National Ethics Committee of the Don Orione Board, number 001.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The clinical data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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Efficacy of very low frequency magnetic field (ELF) in treating pain in Fibromyalgia

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Maestú C, Blanco M, Nevado A, Romero J, Rodríguez-Rubio P, Galindo J, Bautista Lorite J, de las Morenas F, Fernández-Argüelles P. Reduction of pain thresholds in fibromyalgia after very low-intensity magnetic stimulation: a double- blinded, randomized placebo-controlled clinical trial. Pain Res Manag. 2013 Nov-Dec;18(6):e101 -6.

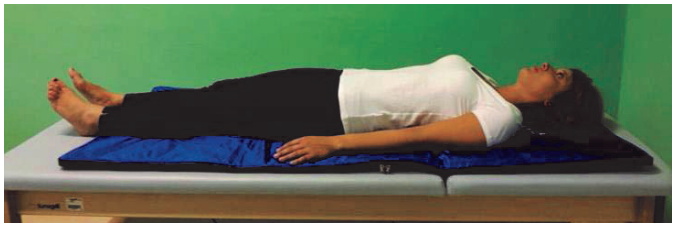
Introduction: The etiopathogenesis of fibromyalgia (FM) is multifactorial and probably based on a mechanism of Central sen-

Aim: Determine the effectiveness of pulsed magnetic fields and very low frequency (ELF)not in reducing pain in patients with fibromyalgia.

Methods and Materials: Pilot crossover study double -blind randomized controlled trial in which 33 were enrolled patients with FM: 16 patients were treated with ELF (MFG) and 17 SHAM -treated patients (SHG). The system LIMFA© THERAPY (Eywa srl -Italy) was employed to generate variable and complex magnetic fields at an intensity of max 100 µT, a 1 -80 Hz frequency. The SHAM mode was obtained by changing the settings on the equipment.

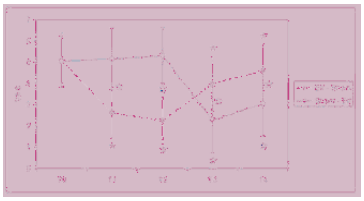
GROUP ELF and SHAM GROUP	
Treatments	12
Treatment Duration	4 weeks
Frequency	3 sessions/week
Sessions duration	30 minutes

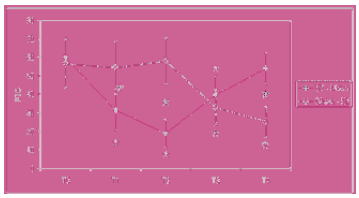
Outcome: Visual Analogue Scale (VAS) for the assessment of pain and Fibromyalgia Impact Questionnaire (FIQ), Fibromyalgia Assessment Scale (FAS) and Health Assessment Questionnaire (HAQ) for the evaluation of pathology.



INCLUSION CRITERIA	EXCLUSION CRITERIA
X rheumatologic Diagnosis of fibromyalgia X widespread pain present for more than three months and pain at a pressure of 4 kg/cm2 at 11 or more of 18 Tender Points level X 9 aged 18 and 60 years X VAS 9 > 3 X stable treatment regimen at least 3 months	× Autoimmune × Haematological Diseases × Tumor diseases × Psychiatric Disorders × Other causes of chronic pain × Overlapping Syndromes

Results: The two groups were homogeneous by age (48.69±10 .29 MFG and 50.61±13 .05 SHG), Body Mass Index (24.87±5.42 MFG and 25.15 ±4 .94 SHG) and values of stairs at baseline. We noticed a good effect of the LIMFA© ELF magnetic fields by comparing them with the placebo. As shown by the VAS, treatment with ELF significantly reduced pain, increased again to end of treatment but remained significantly lower than the values in the baseline (p = 0.001). The VAS showed a reduction 50% compared to 40% of FAS and FIQ between pre-and post-treatment with ELF. These values are higher than those observed in the SHAM group, -6% -7%, which were, and -18% respectively (comparison between T3 and T2 in ELF -SHAM group and between T1 and T0 in SHAM ELF group).





		T1 vs. T0	T2 vs. T0	T3 vs. T0	T4 vs. T0
VAS	Elf-Sham	-45.2±23.4%	-54.1±19.9%	-21.4±19.3%	-9.1±15.1
	Sham-Elf	8.0±25.5%	6.3±16.0%	-57.0±25.8%	-39.7±26.0
	p-value	<0.001	<0.001	0.001	0.006
FAS	Elf-Sham	-39.7±16.2%	-46.5±17.3%	-11.8±18.9%	-1.2±15.4%
	Sham-Elf	-0.7±20.9%	-4.5±20.8%	-39.3±18.4%	-46.9±22.8%
	p-value	<0.001	<0.001	0.091	<0.001
FIQ	Elf-Sham	-45.6±14.8%	-67.3±9.9%	-32.2±19.5%	-8.1±16.5%
	Sham-Elf	-4.6±17.7%	2.9±7.4%	-42.0±9.7%	-56.0±9.4%
	p-value	<0.001	<0.001	0.001	<0.001

Conclusions: Treatment with ELF -MF, according to this Protocol, may be recommended as part of an integrated approach in reducing pain in subjects suffering from FM for short periods in order to intensify the results of drug therapy or physiotherapy. Further studies are needed to determine the long-term repeatability of various treatment protocols that require greater standardization about patient safety and duration of effects.

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Efficacy of extremely low-frequency magnetic field in fibromyalgia pain: A pilot study

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Abstract—The purpose of this pilot study was to determine the efficacy of an extremely low-frequency magnetic field (ELF-MF) in decreasing chronic pain in fibromyalgia (FM) patients. Thirty-seven females were recruited and randomized into two groups: one group was first exposed to systemic ELF-MF therapy (100 microtesla, 1 to 80 Hz) and then to sham therapy, and the other group received the opposite sequence of intervention. Pain, FM-related symptoms, and the ability to perform daily tasks were measured using the Visual Analog Scale, Fibromyalgia Impact Questionnaire (FIQ), Fibromyalgia Assessment Scale (FAS), and Health Assessment Questionnaire (HAQ) at baseline, end of first treatment cycle, beginning of second treatment cycle (after 1 mo washout), end of second treatment cycle, and end of 1 mo follow-up. ELF-MF treatment significantly reduced pain, which increased on cessation of therapy but remained significantly lower than baseline levels. Short-term benefits were also observed in FIQ, FAS, and HAQ scores, with less significant effects seen in the medium term. ELF-MF therapy can be recommended as part of a multimodal approach for mitigating pain in FM subjects and improving the efficacy of drug therapy or physiotherapy.

Clinical Trial Registration: ClinicalTrials.gov; "Very low frequency magnetic fields in the treatment of fibromyalgia": NCT02231541; https://clinicaltrials.gov/ct2/show/NCT02231541?term=NCT02231541&rank=1

Key words: chronic pain, electromagnetic fields, ELF, extremely low-frequency, fibromyalgia, magnetic fields, magnetotherapy, pain, physical therapy, rehabilitation.

INTRODUCTION

Fibromyalgia (FM) is a chronic condition that is characterized by widespread body pain (present for more than 3 mo, above and below the waist, on the left or right side of the body) and pain on digital palpation of at least 11 of 18 predefined tender points. The prevalence of FM in the general population is estimated to be 2 to 7 percent. The chronic pain in FM is often associated with comorbidities, such as fatigue, depression, sleeping disorders, morning stiffness, irritable bowel syndrome, diffuse abdominal pain, anxiety, and headache [1–2].

Although the pathogenesis of FM is not completely understood, it has been suggested that peripheral or central

Abbreviations: ELF = extremely low-frequency, FAS = Fibromyalgia Assessment Status, FIQ = Fibromyalgia Impact Questionnaire, FM = fibromyalgia, HAQ = Health Assessment Questionnaire, MF = magnetic field, PEMF = pulsed electromagnetic field, T0 = baseline, T1 = end of first treatment cycle, T2 = beginning of second treatment cycle (after 1 mo washout), T3 = end of second treatment cycle, T4 = after 1 mo follow-up, TMS = transcranial magnetic stimulation, VAS = Visual Analog Scale.

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hyperexcitability at the level of the spinal cord or brain stem, changes in pain perception, and somatization mitigate the pain. Several studies have implicated central pain sensitization of the brain pain matrix in the pathogenesis of chronic pain [3–7].

The etiopathology of FM is considered to be multifactorial and develops through the interaction of neurohormonal, genetic, and psychological factors. Conversely, no FM-specific personality has been defined [8], and personality has been proposed to be another important filter that modulates one's response to psychological stressors. Certain personalities facilitate the translation of such stressors into physiological responses, driving fibromyalgic mechanisms [9].

Physical exercise and multimodal cognitive behavioral therapy are the most widely accepted and beneficial forms of nonpharmacological treatment for FM [10–14]. Yet, there is equivocal evidence regarding the efficacy of physical therapy in FM. Chiropractic, laser therapy, magnetic field (MF) therapy, massage, and transcranial current stimulation are not recommended, based on a recent review by Winkelmann et al. [15]. Alternatively, other studies have demonstrated relief from FM symptoms through laser therapy [16]. MF therapy has been applied to treat osteoarthritis and inflammatory diseases of the musculoskeletal system, alleviate pain, accelerate the healing of ulcers, and reduce spasticity [17]. There is evidence of the effects of MFs on brain signals and certain psychological disorders, such as headache, migraine, and depression. Based on these findings, specific protocols can be designed using a combination of exposures to various MFs that generate the brain signals necessary to clinically evaluate the effects of MFs [18–19].

Extremely low-frequency (ELF)-MFs in the picotesla and millitesla ranges are administered to improve neurotransmission and correct local immune pathology, respectively [20]; they are effective in decreasing chronic pain in osteoarthritis and reducing fatigue in multiple sclerosis.

ELF-MFs alter animal behavior and modulate biological variables, including gene expression, cell survival, cellular differentiation, and cerebral blood flow in aged transgenic mice [21–22]. Alterations in inflammatory responses have also been observed, but how these activities affect human health remains unknown [23].

Other studies have indicated a beneficial effect of ELF-MFs in a model of global cerebral ischemia, inhibiting vessel growth in a specific range of amplitudes and thus demonstrating antiangiogenic activity [24–25]. Although the precise mechanism of ELF-MFs remains undetermined, they have unexpected short-term analgesic effects in neuropathic pain [26–27]. No study has examined the efficacy of ELF-MFs in FM, excluding reports on transcranial pulsed MFs [28–31].

Shupak and colleagues studied specific pulsed electromagnetic fields (PEMFs) in FM that extended from the outer periphery of the cingulate cortex to the brain midline (30 min duration, 200 to 400 μT, 1 kHz); PEMFs effected a modest reduction in pain in patients with rheumatoid arthritis but not for those with FM versus the sham group [28]. In contrast, Maestú and colleagues studied the effect of very low-intensity pulsed transcranial magnetic stimulation (TMS) on FM (once per week, 8 sessions, 20 min duration, 43 nT for each coil, 8 Hz) and noted that it had analgesic and antinociceptive effects, similar to the opioid analgesic effects in PEMF-exposed patients [29]. Nevertheless, there is no definitive treatment modality that is effective in FM patients, and the results are often mixed.

Based on these studies, we wanted to expand the use of nonpulsed ELF-MFs through total body magnetic exposure as opposed to TMS. The aim of this pilot study was to determine the efficacy of mild nonpulsed ELF-MFs in mitigating chronic pain in FM patients.

MATERIALS AND METHODS

Study Design

This crossover, randomized, double-blind pilot study measured the effects of nonpulsed ELF-MFs versus sham therapy on chronic pain in subjects with FM (**Figure 1**). All patients underwent a period of ELF-MF therapy and a period of sham therapy, half of them in that sequence and the other half receiving sham treatment first.

Subjects

From September 2014 to December 2014, 37 female subjects were recruited from the Physical Medicine and Rehabilitation outpatient clinic, Policlinico Umberto I Hospital, Sapienza University of Rome, Rome, Italy. The mean age (in years) was 49.50 ± 9.38 and 51.12 ± 12.47 for the ELF-MF and sham groups, respectively; the mean body mass index values (kilogram per meter square) were 24.89 ± 5.26 and 25.85 ± 6.43, respectively. All subjects' FM was defined clinically per the 1990 and 2010 American College of Rheumatology criteria. We

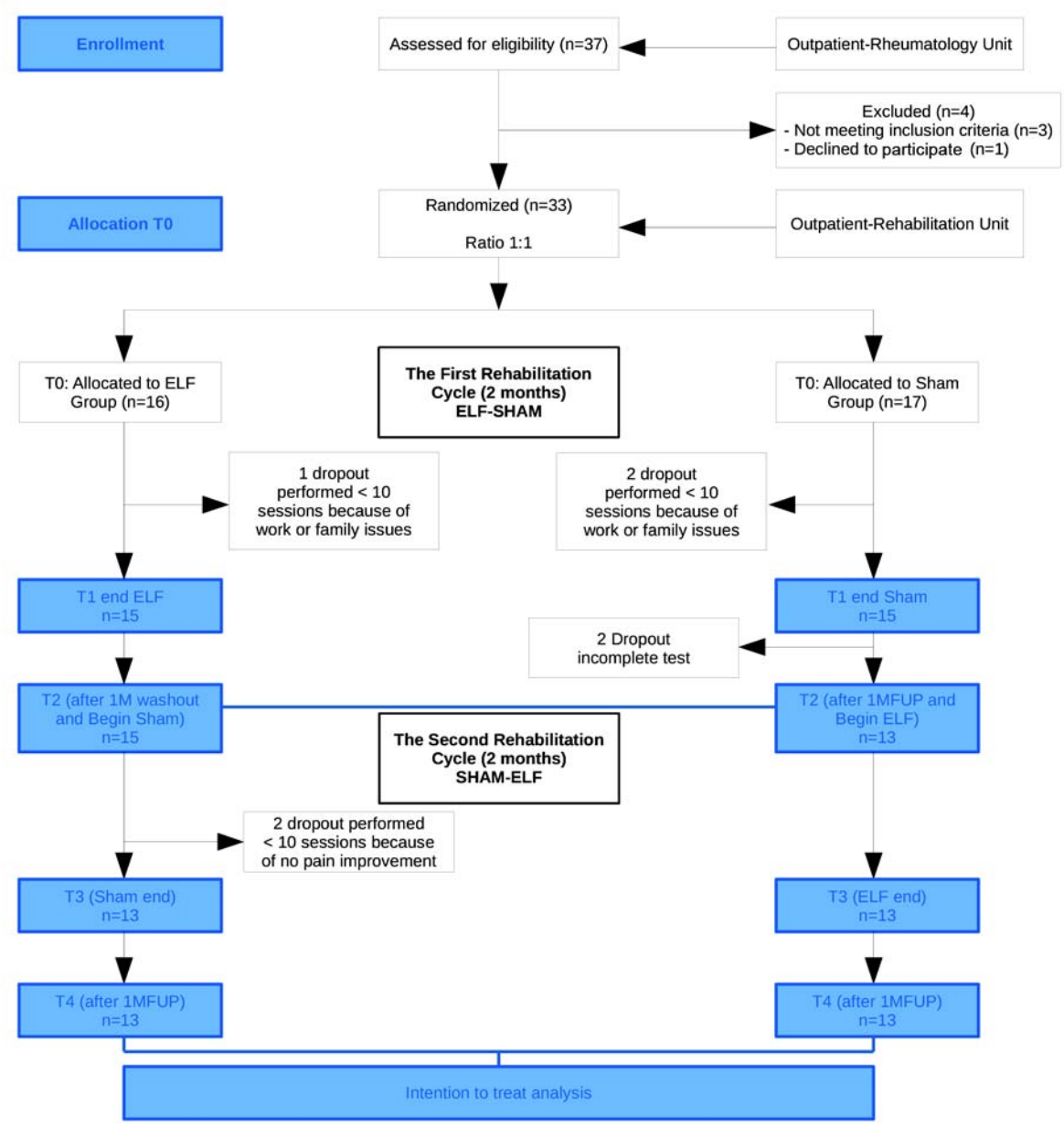

Figure 1.
Flowchart of the study. ELF = extremely low-frequency, 1MFUP = 1 mo follow-up, T0 = baseline, T1 = end of first treatment cycle, T2 = beginning of second treatment cycle (after 1 mo washout), T3 = end of second treatment cycle, T4 = after 1 mo follow-up.

included all subjects with FM who experienced widespread pain for more than 3 mo and pain with 4 kg/cm^2 pressure at 11 or more of the 18 tender points (in every case, the diagnosis of FM had been established by the patient's rheumatologist), were aged 18 to 60 yr, and had a Visual Analog Scale (VAS) score >3 for pain.

The exclusion criteria were the presence of concomitant autoimmune or hematologic diseases, psychiatric disorders (such as mild depression and anxiety with pharmacological and psychological treatment), other causes of chronic pain, and other diseases such as epilepsy and tumors. Pregnant women, those with pacemakers, and

subjects who were concurrently participating in another type of physical therapy were excluded. Also, subjects with overlapping painful conditions, such as chronic fatigue and irritable bowel or inflammatory bowel syndrome, were excluded.

Those with comorbidities, such as myocardial infarction, lower-limb arterial disease, major neurological problems, diabetes, gastrointestinal disease, chronic respiratory disease, kidney disease, and poor vision, were not included. The pharmacological therapeutic regimen must have been stable for at least 3 mo before the patient began treatment: acetaminophen up to 3 g/d, tramadol up to 200 mg/d, and pregabalin up to 150 mg/d.

All patients were instructed not to take any new medications during the study protocol and to avoid other rehabilitation approaches. During the rehabilitation sessions, no patient reported an increase in pain that led to treatment discontinuation or greater use of current drug therapy. Overall, 3 of the initial 37 recruited subjects were excluded and 1 declined to participate. We also excluded patients who attended fewer than 10 sessions ($n = 7$).

Patients were randomized into two groups. One group consisted of 16 patients with FM who were exposed to ELF-MFs first and then received sham exposures. The other group consisted of 17 patients with FM who received sham exposures first and were then exposed to ELF-MFs. There were no significant differences in baseline characteristics between groups. For ethical consideration, each group underwent ELF-MF treatment.

Magnetic Field Treatment and Setting

The LIMFA system (Eywa srl; Rimini, Italy) (ISO9001 certification number 390263) was used to create multifrequency magnetoelectric fields with an intensity of 100 μT and a low-frequency field.

Subjects participated in 12 treatment sessions, 3 times per week for 4 weeks, in a double-blind controlled trial, with each session lasting for 30 min. Patients were asked to rest on a bed on a multi-low-frequency MF mattress and exposed to genuine or sham therapy. Genuine therapy comprised systemic ELF-MF with an intensity of 100 μT and a multifrequency of 1 to 80 Hz (**Figure 2**).

The device, a magnetic mattress, works during sham or genuine exposure depending on the type of modality that is specified. The observer and patient were blinded to the modality that was activated. Active and placebo codes were randomly assigned to the groups and revealed on completion of the study by all participants. The sham modality was obtained by switching to a different code on the device, resulting in no magnetic fields being generated, as if the machine were turned off.

Figure 2.
Treatment with extremely low-frequency magnetic field.

Outcome Measures

The primary outcome was the change in chronic pain. Subjects were evaluated at baseline (T0), at the end of the first treatment cycle (T1), at the beginning of the second treatment cycle (after a 1 mo washout) (T2), at the end of the second treatment cycle (T3), and after 1 mo follow-up (T4). T2 was considered the crossover point for the two groups (**Figure 1**).

Patients were evaluated with specific FM scales. The Fibromyalgia Impact Questionnaire (FIQ) consists of three sections: Function, Impact, and Symptoms, which when combined produce an overall score. The first section contains 10 subitems (FIQ-Physical Impairment) and focuses on the patient's ability to perform daily tasks that involve the large muscles (e.g., cooking, cleaning, walking, shopping, homemaking, socializing, and mobility). The next two sections (FIQ-Feel Good and FIQ-Work Missed) ask patients to circle the number of days in the past week on which they felt good and the number of days that they missed work. The last seven items probe the ability to do one's job, pain, fatigue, morning tiredness, stiffness, anxiety, and depression. The total FIQ score is calculated by adding the following 10 items: the physical functioning score, the number of days of feeling good, the number of work days missed, the ability to do one's job, pain, fatigue, morning tiredness, stiffness, anxiety, and

depression. The FIQ score ranges from 0 to 100, with 100 indicating the maximum impact of FM [32]. The FIQ has been translated into many languages, including Italian [33].

The Fibromyalgia Assessment Status (FAS) is a simple and rapidly implemented index consisting of a "pain map" called the Self-Assessment Pain Scale (in which the patient is asked to indicate how much pain he or she suffered in the previous week in 16 areas of the body, with a grading scale that ranges from 0 to 3) and two scales (with ratings between 0 and 10) that evaluate fatigue and quality of sleep, for a total score of 0 to 10. The FAS allows physicians to obtain reliable information concerning the course of the disease and is sensitive enough to alert them in the case of deterioration [34].

The Health Assessment Questionnaire (HAQ) is a self-administered, 20-item questionnaire that assesses difficulties in performing eight daily activities (dressing and grooming, getting up, eating, walking, hygiene, reaching, ability to grip, and outside activities). For each item, patients are asked to rate the level of difficulty that they have experienced over the previous week in performing these activities on a 4-point scale, from 0 (no difficulty) to 3 (unable to perform). The final HAQ score is the average score of the eight categories and thus also ranges from 0 to 3, with higher scores reflecting greater disability [35].

The VAS is a simple, robust, sensitive, and reproducible instrument that enables patients to express their pain intensity as a numerical value. Patients were asked to mark the point that corresponded to their perceived pain intensity on a 10 cm line, with 0 indicating the absence of pain and 10 reflecting the most severe pain [36].

Sample Size and Statistical Analysis

Because this trial was a pilot study, no sample size was determined. The data are expressed as mean and standard deviation. Because the clinical scores are ordinal numbers, nonparametric statistics were chosen for the between- and within-group analyses. Percentage improvement with respect to beginning of the treatment for the periods in which patients received ELF-MF and sham treatment was compared and analyzed using Mann-Whitney *U*-test. Within-group comparisons were performed using Friedman analysis, followed by Wilcoxon signed-rank test, for each group with regard to changes in scores from baseline levels (T0). The alpha level was set to 0.05 for all analyses, with the exception of post hoc following Friedman analysis, for which Bonferroni correction was applied. An intention-to-treat analysis was performed.

RESULTS

As shown in **Figure 1**, 33 of 37 subjects who were screened for eligibility were enrolled into the study; 16 patients underwent ELF-MF and then sham treatment, and 17 received the therapies in reverse order. Baseline scores (T0) did not differ significantly for any scale. Patient characteristics at baseline are listed in **Table 1**. Thirty participants completed at least one treatment cycle, and their data were analyzed. Throughout the study, 7 patients dropped out; thus, 26 patients ultimately completed the entire protocol.

The primary outcome measure was reduction in pain, assessed by VAS (**Figure 3)**. ELF-MF treatment significantly reduced pain ($p = 0.001$), which rose after the end of treatment but remained significantly lower than baseline levels ($p = 0.001$). Short-term benefits were also observed in terms of the secondary outcome measures, but the medium-term effects were less significant.

Figure 4 shows the changes in FAS scale scores: significant improvements in FAS scores were noted at the end of treatment (T1 for the ELF-sham group, T3 for sham-ELF group) and lasted 1 mo (T2 and T4, respectively), becoming nonsignificant at T3 and T4 for the ELF-sham group.

The HAQ scores are reported in **Figure 5**. Only ELF-sham patients had significantly different scores than their counterparts who received sham therapy at the end of treatment ($p < 0.001$ at T1); this change was poorly maintained after 1 mo ($p = 0.03$ at T2, not significantly different from T0 after Bonferroni correction). Analogously, the sham-ELF group showed a significant within-group effect of ELF-MF only at 1 mo after the end of treatment (T4).

The within-group declines in FIQ score that were induced by ELF-MF were significant in both groups (**Figure 6**). FIQ scores recovered only after T2 in ELF-sham patients, despite remaining significantly lower than baseline scores at T3, but this significance was lost at T4. In comparing FIQ subscores for the ELF-sham versus sham-ELF groups at T1 and T2, we found that the significant changes in overall scores were attributed to improvements in FIQ-Physical Impairment ($p = 0.03$ at T1, $p < 0.001$ at T2), FIQ-Feel Good ($p = 0.02$ and $p < 0.001$, respectively), FIQ-Work Missed ($p = 0.003$ for

Table 1.
Baseline demographics and clinical characteristics of the study population with fibromyalgia (FM) and relevant means and standard deviations (SDs) for the extremely low-frequency (magnetic field) (ELF)-sham group, sham-ELF group, and entire sample (pharmacological regimen [PR]: acetaminophen-tramadol = A, pregabalin = B, nothing = 0).

Patient	Group	Age (yr)	Weight (kg)	Height (m)	Body Mass Index (kg/m^2)	PR	Duration of FM (yr)	Employed	Married
1	ELF-Sham	48	78	1.77	24.89	B	5	Yes	Yes
2	ELF-Sham	58	68	1.60	26.56	A	10	No	Yes
3	ELF-Sham	61	66	1.51	28.95	A	15	Yes	Yes
4	ELF-Sham	58	64	1.65	23.51	A	16	No	Yes
5	ELF-Sham	49	60	1.62	22.86	B	4	Yes	Yes
6	ELF-Sham	34	60	1.63	22.58	A+B	2	Yes	No
7	ELF-Sham	51	55	1.60	21.48	A+B	8	No	Yes
8	ELF-Sham	44	53	1.55	22.06	A	7	No	Yes
9	ELF-Sham	53	115	1.68	40.75	A+B	4	Yes	Yes
10	ELF-Sham	23	50	1.55	20.81	A+B	1	Yes	No
11	ELF-Sham	51	76	1.70	26.30	A	6	No	Yes
12	ELF-Sham	50	50	1.60	19.53	A	10	No	Yes
13	ELF-Sham	53	69	1.73	23.05	A	6	Yes	Yes
14	ELF-Sham	54	73	1.77	23.30	B	3	Yes	Yes
15	ELF-Sham	53	85	1.65	31.22	A	6	Yes	Yes
16	ELF-Sham	52	57	1.67	20.44	A+B	5	No	Yes
17	Sham-ELF	51	50	1.51	21.93	0	3	Yes	Yes
18	Sham-ELF	72	69	1.60	26.95	A+B	6	No	No
19	Sham-ELF	44	63	1.62	24.01	0	3	Yes	Yes
20	Sham-ELF	39	73	1.53	31.18	B	9	Yes	No
21	Sham-ELF	57	84	1.66	30.48	B	4	Yes	Yes
22	Sham-ELF	53	80	1.60	31.25	0	6	Yes	Yes
23	Sham-ELF	54	50	1.60	19.53	A	5	Yes	Yes
24	Sham-ELF	71	69	1.60	26.95	0	6	No	Yes
25	Sham-ELF	44	80	1.58	32.05	0	4	Yes	No
26	Sham-ELF	48	47	1.54	19.82	0	7	No	No
27	Sham-ELF	51	70	1.72	23.66	A+B	5	No	Yes
28	Sham-ELF	53	50	1.63	18.82	A	6	Yes	Yes
29	Sham-ELF	21	54	1.63	20.32	B	1	Yes	No
30	Sham-ELF	39	105	1.55	43.71	0	2	Yes	Yes
31	Sham-ELF	68	51	1.56	20.96	0	10	No	Yes
32	Sham-ELF	50	76	1.70	26.30	B	5	Yes	Yes
33	Sham-ELF	54	55	1.60	21.48	0	8	No	No
Mean ± SD	ELF-Sham	49.50 ± 9.38	67.44 ± 16.32	1.64 ± 0.08	24.89 ± 5.26	—	6.75 ± 4.23	—	—
Mean ± SD	Sham-ELF	51.12 ± 12.47	66.24 ± 15.90	1.60 ± 0.06	25.85 ± 6.43	—	5.29 ± 2.31	—	—
Mean ± SD	Total	50.33 ± 10.94	66.82 ± 15.86	1.62 ± 0.07	25.38 ± 5.82	—	6.00 ± 3.41	—	—

both), FIQ-Pain ($p = 0.005$ and $p < 0.001$, respectively), FIQ-Fatigue ($p = 0.003$ and $p < 0.001$, respectively), FIQ-Morning Tiredness ($p = 0.001$ and $p < 0.001$, respectively), FIQ-Stiffness ($p = 0.004$ and $p < 0.001$, respectively), and FIQ-Depression ($p = 0.003$ and $p < 0.001$, respectively). In contrast, differences in FIQ-Anxiety scores were not significant at T1 ($p = 0.07$) but became so at T2 ($p = 0.001$). A summary of the scale scores is listed in terms of mean and standard deviation and *p*-values by Mann-Whitney *U*-test (in bold if statistically significant) (**Table 2**).

Table 3 summarizes the data as percentage improvement with respect to beginning of the treatment for patients with ELF-MF and sham treatment. VAS scores generally declined by 50 percent versus 40 percent for FAS and FIQ scores between pre– and post–ELF-MF treatment (i.e., T1 vs T0 in the ELF-sham group and T3 vs T2 in the sham-ELF group). These values were higher than what was observed with the sham treatment, which was approximately –7 percent, –6 percent, and –18 percent, respectively (average comparisons between T3 and T2 in

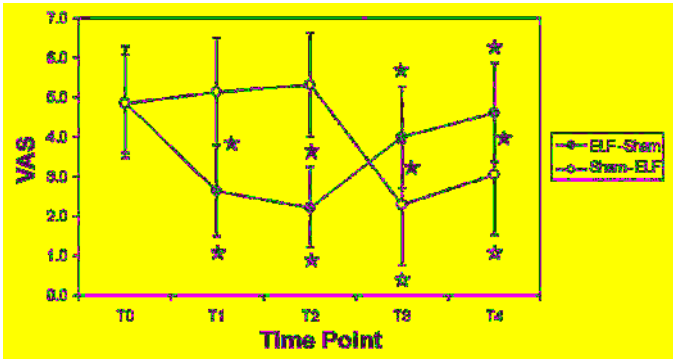

Figure 3.
Mean and standard deviation of Visual Analog Scale (VAS) for pain for patients who performed extremely low-frequency (magnetic field) (ELF) and then sham treatment (gray) or the opposite (empty circles). Statistically significant differences are shown with stars: filled black stars for between-group comparisons, gray stars for within-group comparisons with respect to T0 values for ELF-sham group, black empty stars for within-group comparisons with respect to T0 values for sham-ELF group. T0 = baseline, T1 = end of first treatment cycle, T2 = beginning of second treatment cycle (after 1 mo washout), T3 = end of second treatment cycle, T4 = after 1 mo follow-up.

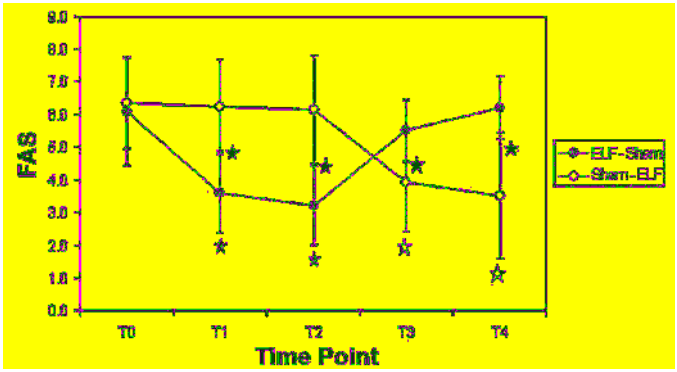

Figure 4.
Mean and standard deviation of Fibromyalgia Assessment Scale (FAS) scores for patients who performed extremely low-frequency (magnetic field) (ELF) and then sham treatment (gray) or the opposite (empty circles). Statistically significant differences are shown with stars: filled black stars for between-group comparisons, gray stars for within-group comparisons with respect to T0 values for ELF-sham group, black empty stars for within-group comparisons with respect to T0 values for sham-ELF group. T0 = baseline, T1 = end of first treatment cycle, T2 = beginning of second treatment cycle (after 1 mo washout), T3 = end of second treatment cycle, T4 = after 1 mo follow-up.

the ELF-sham group and T1 and T0 in the sham-ELF group). No side effects were recorded during the study.

DISCUSSION

We noted good efficacy of ELF-MFs compared with placebo. With regard to our primary outcome, as assessed by the VAS, ELF-MF treatment significantly reduced pain, which increased at the end of treatment but remained significantly lower than baseline levels (p = 0.001). The VAS results are consistent with reported minimal clinically important difference values of a 23 percent to 35 percent improvement in pain versus baseline values [37–38]. Short-term benefits were also observed in secondary outcome measures, but the medium-term effects were less significant.

Nevertheless, our results are encouraging and should prompt a continuing investigation of ELF-MF exposure for short-term pain relief in FM patients and the application of this stimulation over the long term. Future studies should compare our ELF-MF protocol (which had benefits without any side effects) with other more intensive programs, for example, daily treatment or doubling the number of sessions in a single day. Existing TMS protocols for the treatment of pain in FM differ in frequency (nearly always pulsed), intensity, duration, and setting [28–31]; no standardized protocol for ELF-MF treatment in FM has been developed, and there are no studies in this area with respect to total body stimulation with the magnetic mattress. Consistent with similar studies, our results demonstrate an analgesic and antinociceptive effect, similar to the opioid analgesic effect in PEMF-exposed patients [39–40].

A limitation of our study was the lack of a biochemical assessment of the effects of ELF-MF on pain relief in our FM patients. We hypothesize that the opioid analgesic effect is related to the central sensitization that characterizes FM. One of the hallmarks of FM is the implementation of sensory input that is mediated by central nervous system events, similar to neuropathic pain conditions (i.e., central sensitization [increases in Substance P, a neuronal excitatory substance that mediates the conduction of pain in the central nervous system]). FM has also been proposed to involve a reduction in serotonin (a neurotransmitter of the inhibitory descending system) and abnormal levels of norepinephrine, which modulates endogenous pain inhibitory

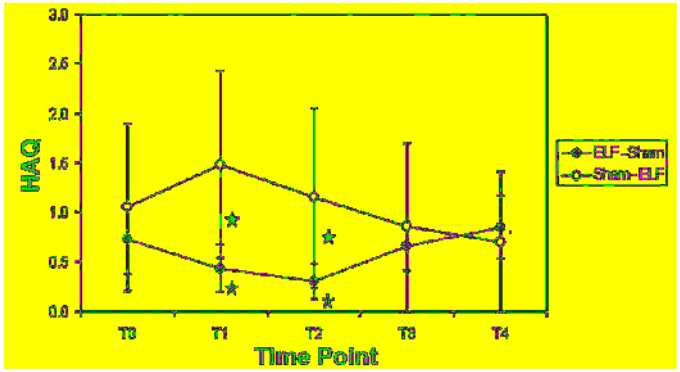

Figure 5.
Mean and standard deviation of Health Assessment Questionnaire (HAQ) scores for patients who performed extremely low-frequency (magnetic field) (ELF) and then sham treatment (gray) or the opposite (empty circles). Statistically significant differences are shown with stars: filled black stars for between-group comparisons, gray stars for within-group comparisons with respect to T0 values for ELF-sham group, black empty stars for within-group comparisons with respect to T0 values for Sham-ELF group. T0 = baseline, T1 = end of first treatment cycle, T2 = beginning of second treatment cycle (after 1 mo washout), T3 = end of second treatment cycle, T4 = after 1 mo follow-up.

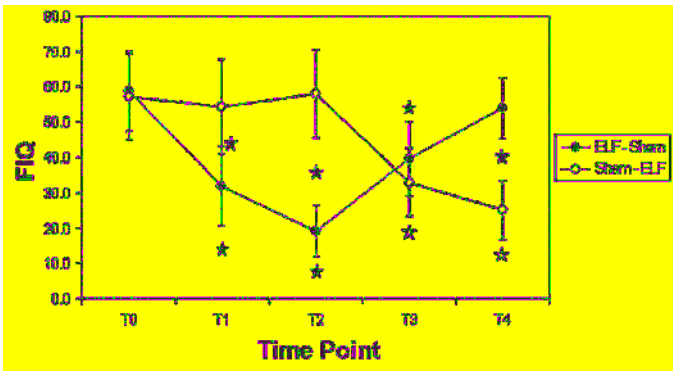

Figure 6.
Mean and standard deviation of Fibromyalgia Impact Questionnaire (FIQ) scores for patients who performed extremely low-frequency (magnetic field) (ELF) and then sham treatment (gray) or the opposite (empty circles). Statistically significant differences are shown with stars: filled black stars for between-group comparisons, gray stars for within-group comparisons with respect to T0 values for ELF-sham group, black empty stars for within-group comparisons with respect to T0 values for sham-ELF group. T0 = baseline, T1 = end of first treatment cycle, T2 = beginning of second treatment cycle (after 1 mo washout), T3 = end of second treatment cycle, T4 = after 1 mo follow-up.

pathways and affects cortisol suppression [41]. The central augmentation of sensory input is associated with enhanced sensitivity to pain. Also, the chronic pain and allodynia in FM as well as in mood disorders are associated with significantly lower levels of ATP in platelets, which has been implicated in their pathogenesis [42–43]. Likely, ELF-MF reduces pain by relieving peripheral input in FM patients and has short latency effects (1 mo), even after the end of treatment and despite the modulation of biochemical mediators of pain, maintaining a short biochemical memory [44].

The algogenic effect of electromagnetic fields has also been observed with pulsed MFs in osteoarthritic disease [45–46]. Musaev and colleagues reported that low-frequency pulsed MFs have analgesic, vasoactive, neuron-stimulating, and trophic effects in patients with diabetic polyneuropathy, which has a similar sensory profile as FM [26].

The mechanism of the effect of MF therapy on pain remains unknown, but certain studies have shown that short-term exposure to electromagnetic fields influences several inflammatory cellular and neurological processes, such as patterns of cortical activation and inhibition and the activity of various neurotransmitters, as in multiple sclerosis [40].

Our results do not demonstrate a stabilization of the effect at the end of treatment. FIQ scores worsened after T2 in ELF-sham patients, despite remaining significantly lower than baseline levels at T3, but this significance was lost at T4. These results might be due to the cyclical nature of pain that characterizes patients with FM and to our use of a nonintensive ELF-MF protocol in terms of duration of treatment, weekly frequency, and number of sessions. Because this trial was a pilot study, with no specific reference protocols, we decided to adopt a nonintensive treatment protocol to better respect the parameters of patient safety. However, the source of sensory input in FM patients remains unknown; thus, the duration of the efficacy of ELF-MF remains undetermined. Similarly, the magnetite hypothesis, based on the induction of electric currents, appears to be an unlikely mechanism, given that the induced fields are orders of magnitude lower than the endogenous electric fields in tissues; a connection between magnetite and the nervous system has not been demonstrated [39–40,47].

Sleep quality improved, as reflected in the FIQ subscales, with ELF-MF. As an MF therapy, ELF-MF acts

Table 2.
Group comparisons. Scores are expressed as mean ± standard deviation; *p*-values in the rows refer to Mann-Whitney *U*-test, and those in the last column were calculated by Friedman analysis for each group over time (in bold if statistically significant).

Scale	Group and Comparison	T0	T1	T2	T3	T4	*p*-Value
VAS	ELF-Sham	4.9 ± 1.4	2.6 ± 1.1	2.2 ± 1.0	4.0 ± 1.3	4.6 ± 1.3	<0.001
	Sham-ELF	4.8 ± 1.2	5.1 ± 1.4	5.3 ± 1.3	2.3 ± 1.5	3.1 ± 1.6	<0.001
	p-Value	0.92	**<0.001**	**<0.001**	**0.007**	**0.02**	—
FIQ	ELF-Sham	58.7 ± 11.3	31.9 ± 11.1	19.2 ± 7.3	39.5 ± 10.4	53.9 ± 8.7	<0.001
	Sham-ELF	57.2 ± 12.3	54.2 ± 13.4	57.9 ± 12.5	33.0 ± 9.6	25.1 ± 8.5	<0.001
	p-Value	0.66	**<0.001**	**<0.001**	0.32	**<0.001**	—
FAS	ELF-Sham	6.1 ± 1.7	3.6 ± 1.2	3.2 ± 1.2	5.5 ± 1.0	6.2 ± 1.0	<0.001
	Sham-ELF	6.4 ± 1.4	6.2 ± 1.4	6.1 ± 1.7	3.9 ± 1.5	3.5 ± 1.9	<0.001
	p-Value	0.65	**<0.001**	**<0.001**	**0.007**	**0.002**	—
HAQ	ELF-Sham	0.7 ± 0.3	0.4 ± 0.2	0.3 ± 0.2	0.7 ± 0.2	0.8 ± 0.3	<0.001
	Sham-ELF	1.1 ± 0.8	1.5 ± 0.9	1.1 ± 0.9	0.9 ± 0.8	0.7 ± 0.7	<0.001
	p-Value	0.58	**0.001**	**0.03**	0.98	0.41	—

ELF = extremely low-frequency (magnetic field), FAS = Fibromyalgia Assessment Scale, FIQ = Fibromyalgia Impact Questionnaire, HAQ = Health Assessment Questionnaire, T0 = baseline, T1 = end of first treatment cycle, T2 = beginning of second treatment cycle (after 1 mo washout), T3 = end of second treatment cycle, T4 = after 1 mo follow-up, VAS = Visual Analog Scale.

Table 3.
Percentage changes with respect to baseline for Visual Analog Scale (VAS), Fibromyalgia Impact Questionnaire (FIQ), and Fibromyalgia Assessment Scale (FAS) scores (*p*-values are in bold if statistically significant after Bonferroni correction).

Scale	Group and Comparison	T1 vs T0	T2 vs T0	T3 vs T0	T4 vs T0
VAS	ELF-Sham	–45.2 ± 23.4	–54.1 ± 19.9	–21.4 ± 19.3	–9.1 ± 15.1
	Sham-ELF	8.0 ± 25.5	6.3 ± 16.0	–57.0 ± 25.8	–39.7 ± 26.0
	p-Value	**<0.001**	**<0.001**	**0.001**	**0.006**
FAS	ELF-Sham	–39.7 ± 16.2	–46.5 ± 17.3	–11.8 ± 18.9	–1.2 ± 15.4
	Sham-ELF	–0.7 ± 20.9	–4.5 ± 20.8	–39.3 ± 18.4	–46.9 ± 22.8
	p-Value	**<0.001**	**<0.001**	0.09	**<0.001**
FIQ	ELF-Sham	–45.6 ± 14.8	–67.3 ± 9.9	–32.2 ± 19.5	–8.1 ± 16.5
	Sham-ELF	–4.6 ± 17.7	2.9 ± 7.4	–42.0 ± 9.7	–56.0 ± 9.4
	p-Value	**<0.001**	**<0.001**	**0.001**	**<0.001**

ELF = extremely low-frequency (magnetic field), T0 = baseline, T1 = end of first treatment cycle, T2 = beginning of second treatment cycle (after 1 mo washout), T3 = end of second treatment cycle, T4 = after 1 mo follow-up.

on peripheral neural stimulation and regulates microcirculation, like laser therapy [48], interrupting pain mechanisms and promoting analgesia.

Based on our results, future studies in this field should increase the sample size and extend the observation times (up to 1 yr) and also include other overlapping painful conditions with FM.

CONCLUSIONS

ELF-MF therapy, within the parameters of this treatment protocol, can be recommended as part of a multimodal approach to reducing pain in FM subjects for short periods and to intensifying the results of drug therapy or physiotherapy. ELF-MFs have analgesic effects in FM. Clinically, determining the biological effects of ELF-MF exposure in FM could facilitate the development of alternative treatments and novel therapeutic tools. However, future research is needed to determine the long-term repeatability of various treatment protocols, which requires greater standardization with regard to patient safety and the duration of the effects.

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Drafting of manuscript: T. Paolucci, G. Piccinini.
Critical revision of manuscript for important intellectual content: V. M. Saraceni, T. Paolucci, G. Piccinini.
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Study supervision: V. M. Saraceni, F. Zangrando.

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Efficacy of LIMFA© in treating pain in Fibromyalgia

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Introduction: LIMFA® Therapy is a medical device which generates information and transmits them to the cell receptors to activate and/or accelerate the endogenous processes of healing, repair and cellular regeneration.

Aim: Determine the effectiveness of pulsed magnetic fields and very low frequency (ELF) not in reducing pain in patients with fibromyalgia.

Methods and materials: study single-blind randomised (is the operator that sets whether or not the giving), 34 patients with FM were enrolled : 20 patients per block (10 for group 1 and 10 for group 2); patients in group 1 were treated with Lim- fa® reduced "Fibromyalgia" Protocol (n° 6 sessions instead of 12) and group 2 in SHAM treatment. Limfa® system was employed to generate pre- order combinations of ELF complexes fields of variable intensity (0 ÷ 100 μT) and varying frequencies (1 ÷ 80 Hz). SHAM mode was obtained by changing the settings of the device. The absence of physical effects that characterises Limfa® does not allow the patient to know whether the therapy is giving.

LIMFA© GROUP and SHAM GROUP	
Treatments	6+6
Treatment Duration	9 weeks
Weekly frequency	2
Sessions duration	71 minutes

INCLUSION CRITE-	EXCLUSION CRITERIA
Diagnosis of fi- bromyalgia confir med in two visits	Pregnancy Tumor Disease TBC

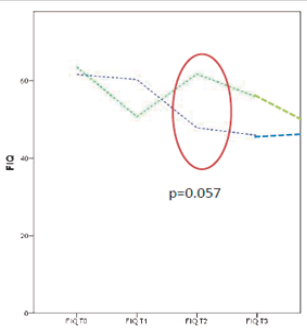
Outcome: Pittsburgh Sleep Quality Index (PSQI), Fibromyalgia Impact Questionnaire (FIQ) and Health questionnaire (SF-12) for the evaluation of pathology.

Evaluation times: Treatment begin(T0), end of the treatment (T1), after 3 weeks of washout and begin of Limfa® Therapy cycle or SHAM (crossover) (T2), end of the second therapy cycle (T3), after 1 month from the end of the therapy (T4), follow-up after 3 months to the end of therapy (T5).

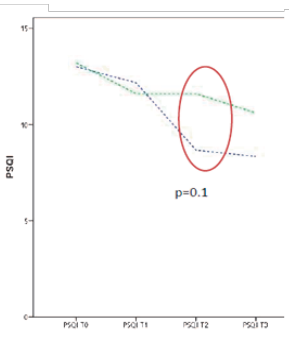
Risults (June 2016):

GRUPPO	1 (true first)	2 (sham first)	P
N=	9	9	NS
Eta	50 [47-63]	53.5 [47.5-64.25]	NS
M/F	1:8	0:9	NS
FIQ	68.2[59.2-87.0]	67.3[60.3-72.7]	NS
PSQI	14[11.7-16.5]	12[9.2-17]	NS
SF-12 mcs	26[23-29]	24[18-28]	NS
SF-12 pcs	33[22-39]	30[25-51]	NS

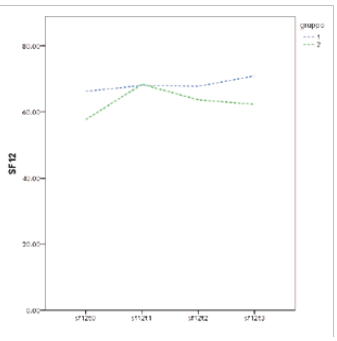
Trend FIQ



Trend PSQI



Trend SF12



Conclusions: to date, only 18 patients are on follow up and 16 are still in enrolment. The results shall be final only after the three-month follow up of all 50 patients referred from the research project. On the partial sample examined there was a satisfaction overall good, an excellent tolerability and no adverse events to therapy, which doesn't require, its administration, dedicated medical personnel. The Limfa® Therapy device, thanks to its operating principle and its effectiveness, can also be used for other diseases (arthritis, sprains, etc..).

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Original Article

Efficacy of dietary supplement with nutraceutical composed combined with extremely-low-frequency electromagnetic fields in carpal tunnel syndrome

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Abstract. [Purpose] The aim of this study was to investigate the clinical effects of a nutraceutical composed (Xinepa®) combined with extremely-low-frequency electromagnetic fields in the carpal tunnel syndrome. [Subjects and Methods] Thirty-one patients with carpal tunnel syndrome were randomized into group 1-A (N=16) (nutraceutical + extremely-low-frequency electromagnetic fields) and group 2-C (n=15) (placebo + extremely-low-frequency electromagnetic fields). The dietary supplement with nutraceutical was twice daily for one month in the 1-A group and both groups received extremely-low-frequency electromagnetic fields at the level of the carpal tunnel 3 times per week for 12 sessions. The Visual Analogue Scale for pain, the Symptoms Severity Scale and Functional Severity Scale of the Boston Carpal Tunnel Questionnaire were used at pre-treatment (T0), after the end of treatment (T1) and at 3 months post-treatment (T2). [Results] At T1 and T2 were not significant differences in outcome measures between the two groups. In group 1-A a significant improvement in the scales were observed at T1 and T2. In group 2-C it was observed only at T1. [Conclusion] Significant clinical effects from pre-treatment to the end of treatment were shown in both groups. Only in group 1-A they were maintained at 3 months post-treatment.
Key words: Carpal tunnel syndrome, Nutraceuticals, Magnetic fields

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INTRODUCTION

One of the main causes of hand dysfunction is carpal tunnel syndrome (CTS), which is the most common peripheral neuropathy. CTS is characterized by compression of the median nerve in the carpal tunnel. Due to its high prevalence, early diagnosis of CTS is critical and can reduce the disability that is caused by this condition[1]. The lifetime risk of developing CTS is approximately 10%[2].The primary symptoms of classical CTS are numbness and tingling with or without pain in at least 2 of the median nerve-innervated fingers. These symptoms are often aggravated during sleep and in the daytime due to static or repetitive hand function. Most causes of CTS are idiopathic or spontaneous, in which bilateral symptoms develop in

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over 60% of patients. Common conditions that are related to secondary CTS include high-energy wrist traumas, endocrine disorders (as diabetes mellitus and hypothyroidism), pregnancy, rheumatoid arthritis, anomalous carpal tunnel structures, and occupational factors, such as repetitive motion and exposure to vibrating tools[3, 5]. Large patient numbers, long outpatient waiting times, and traditional referral pathways in public health systems create delays in accessing treatments for this condition, necessitating alternative care pathways for the management of patients with CTS[6, 7]. Severe cases of carpal tunnel syndrome are usually treated surgically, whereas conservative treatment is recommended for mild to moderate cases. Although it is not described in the literature the ideal technique or combination of approaches due to the limitations of the studies[8–10], several conservative treatments relieve symptoms and improve functional ability such as splinting, oral drugs, injections, specific manual techniques, neural gliding exercises, physical therapies and nutraceuticals. Among physical therapies, a combination of static and dynamic magnetic fields (PEMFs) is shown efficacious in CTS, significantly reducing short- and long-term pain and improving objective neuronal functions[11, 12]. Furthermore, there is evidence of the effect of extremely-low-frequency electromagnetic fields (ELF-EMFs) on several aspects of physiology; in particular, they have analgesic effects and elicit antinociceptive responses[13, 14]. Percutaneous magnetic stimulation relieves palliative pain, presumably through modulation of unmyelinated C-fibers. Studies have suggested that it influences the excitability of inward rectifying K+ channels[15]. These observations implicate magnetized wrist wraps as a novel therapeutic device. Crow RS[7] has shown that spontaneous remission can occur in CTS patients, which can persist. Nevertheless, the underlying neuropathology tends to progress. Also, oral supplementation to patients with mild to severe CTS is a common clinical practice and it is proved to be effective in nerve compression syndromes[16–18]. Nutraceuticals that contains alpha-lipoic OR/AND curcumin, B-group vitamins and Acetyl-L-carnitine (ALCAR) have significant anti-inflammatory, antioxidant, and neuroprotective effects on peripheral nerves[19–21]. Some studies[22–24] show an antioxidant capacity of the alpha-lipoic acid, its ability to decrease neuronal sensitivity to pain by inhibiting neuronal T-type calcium channels, its ability to improve distal sensory and motor nerve conduction. Curcumin appears to have an antinociceptive property[25] but also an anti-inflammatory action[26] because it inhibits the production of several inflammatory mediators. In a recent Cochrane review[27] it's shown a moderate evidence that B-group vitamins at high doses may determine a significant short-term reduction in pain, numbness, and paresthesia. In a study of Curran MW[28], Acetyl-L-carnitine (ALCAR) has been shown to be effective to increase peripheral nerve regeneration. Oral supplementation with a combination product that contains alpha-lipoic acid to patients with mild to severe CTS is a common clinical practice combined with physiotherapy or alone but there are few efficacy studies on the matter. It could be hypothesized that the use of nutraceutical stabilizes or could to strengthen the benefit of physical therapy by ELF-EMFs in CTS. The aim of this study was to investigate the efficacy of nutraceutical composed of alpha lipoic acid, N-acetyl-L-carnitine, curcumin, vitamins B, E, and C in patients treated with ELF-EMFs in carpal tunnel syndrome (CTS).

SUBJECTS AND METHODS

This study took place from July 2015 to January 2016. Thirty-one patients with an average age of 58.5 (± 10.9) years were diagnosed with CTS and were recruited at the Physical Medicine and Rehabilitation Unit of Policlinico Umberto I Hospital, Sapienza University of Rome (Italy). Clinical diagnosis of CTS was made on the basis of the American Academy of Neurology (AAN)[29] by a physiatrist. Electrophysiologic diagnosis of CTS was made on the basis of the American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM), the American Association of Neuromuscular and Electrodiagnostic Medicine, American Academy of Neurology and the American Academy of Physical Medicine and Rehabilitation guidelines[30] by a neurophysiologist: if the results of the sensory and motor nerve conduction study (NCS) are abnormal (sensory distal latency: SDL>3.5 ms ; motor distal latency: MDL>4.20 ms and nerve conduction velocity: NCV<49 m/s) in comparison to the result of the sensory and motor NCS of one another adjacent nerve in the symptomatic limb, the diagnosis of CTS is confirmed. Per these guidelines, unilateral CTS patients were included in the study, with a duration of symptoms of over 3 months, no other physical or medical therapy, no history of trauma of the wrist or hand, and no general metabolic disease. Patients were excluded if they had cervical radiculopathy, polyneuropathy, osteoarthritis or inflammation of joints in the hand, such as rheumatoid arthritis; had undergone CTS surgical release; were pregnant; aged under 18 years; or had a pacemaker or a history of cancer or epilepsy. The patients were informed in detail through an oral presentation on the scope and procedures per the Declaration of Helsinki by a researcher. Then, they were asked to participate in this clinical study, in which they were randomly allocated to a group 1-A (nutraceutical + ELF-EMFs) and group 2-C (placebo + ELF-EMFs), according to a computer-generated simple randomization list at a 1:1 ratio (software MATLAB R2007b®, The Matworks Inc., USA). With regard to concealment of the allocation, a physiatrist had identified the patients to confirm the inclusion and exclusion criteria, had obtained signed informed consent forms for participation in this study, had administered the evaluation scales, had performed the treatment with ELF and had administrated the nutraceutical or placebo. Thirty-one patients were divided in the group 1-A (n=16) with an average age of 58.7 (± 11.0) years and the group 2-C (n=15) with an average age of 58.3 (± 11.2) years. We collected data on age, gender, body mass index (BMI), dominant hand, professional activity. The patients, the physiatrist and the neurophysiologist were blinded with respect to the nutraceutical and placebo groups. The drug vials were identical and had a numerical identification code, which was made public to the researcher, by an external collaborator, only after the data collection and statistical analysis. Sealed envelopes were prepared for each group. Participants received their randomization letter after the first visit had been completed. This study protocol was

developed in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines[31]. The study protocol (clinicaltrial.gov registration number: NCT02891512) was approved by the Ethics and Experimental Research Committee of Sapienza University of Rome (N=2,545/15) and was carried out per National Health Council Resolution No. 196/96. In addition to ELF EMFs, Group 1-A (N=16) was treated with a dietary supplement that was composed of alpha-lipoic acid (300 mg), N-acetyl-L-carnitine (400 mg), standardized turmeric extract (root) curcumin (150 mg [95%]), vitamin B1 (6.25 mg), vitamin B2 (6.25 mg), vitamin B6 (2.38 mg), vitamin B12 (6.25 mcg), vitamin E (9 mg), and vitamin C (125 mg) (Xinepa®, Kolinpharma Srl-Italy, number of registration 934388568). The dosage was twice daily, after breakfast and dinner for 1 month, starting from the evening of the first ELF EMFs session. The registration number for Xinepa® at the Italian Ministry of Health is 69794. Group 2-C (N=15) was treated with a placebo dietary supplement twice daily, after breakfast and dinner for 1 month, beginning from the evening of the first ELF EMFs session. Groups 1-A and 2-C received ELF EMFs at the level of the carpal tunnel 3 times per week for 12 sessions using an electromedical appliance (Limfa® Technologies −Registration Number. DD 60095484, Report N 28106660001 medical devices 1210963/R-Italy). All patients were seated with the upper limb resting on the table and the carpus positioned on the emitter. Each session lasted 47 minutes and entailed two consecutively run programs: (1) anti-edema (21 minutes) and (2) anti-inflammatory (26 minutes). The LIMFA® device is equipped with a touch screen display that allows the operator to select the programs. Its technology emits predetermined sequences of weak ELF fields, variable in shape, intensity and frequency. The results are obtained using the sequences and not the simple and fixed ELF fields (one frequency, one intensity, one shape). The frequencies varies from 1 to 80 Hz (multifrequency magnetic field), and the intensity sets to 100 μT. These sequences are registered at SIAE for the patent. To limit the bias, the same clinical investigator who was blinded to the treatment group allocation performed all assessments. The visual analog scale (VAS)[32] and the Boston Carpal Tunnel Questionnaire (BCTQ)[33, 34] were administered pretreatment (T0), after the end of treatment (T1) and 3 months post-treatment (T2). The VAS for self-assessment of pain was performed by the patient to quantify painful sensations before treatment and during follow-up. This scale is a 10-cm horizontal axis in which 0 means no pain and 10 indicates the worst pain possible. The BCTQ evaluates the severity of symptoms (symptoms severity scale [SSS], 11 questions) and functional severity scale (FSS, 8 questions). For each question, the patient's responses were scored from 1–5 arranged in an increasing order of symptoms severity and the degree of difficulty felt in each activity described. This calculation is the sum of answers divided by the number of questions. At T0 and T1 (within the first week after the end of treatment) electrodiagnostic parameters were analyzed: median sensory distal latency (SDL), median motor distal latency (MDL), sensory nerve action potential amplitude (S-AMP), motor nerve action potential amplitude (M-AMP), median sensory nerve conduction velocity (SCV), median motor nerve conduction velocity (MCV). Splinting, other medications and physical therapies were not allowed during the study or follow-up.

The calculation of the sample size was performed using online sample size calculator software developed by DSS Research (*https://www.dssresearch.com/*). The clinically important difference of 0.70 points in the SSS score of the BCTQ, before and after the treatment with a standard deviation of 0.6 were used to compute the sample size according to the research by Peters-Veluthamaningal C[35]. The level of significance is set at alpha=0.05 and the power of the study at beta=0.80. The sample size required is 13 subjects per group.

The descriptive statistics included median with interquartile range (IQR, 25th and 75th percentiles) for quantitative variables and percentage and tables of frequencies for qualitative variables. A nonparametric approach was considered, based on the low number of patients. To compare treatment groups versus the control at the 3 times (T0, T1, and T2), nonparametric Mann-Whitney test was performed. The significance of the change in median in each group (T0 vs. T1 and T0 vs. T2) was determined by nonparametric Wilcoxon signed-rank test. The association between qualitative variables was evaluated by Fisher's exact test. An analysis was planned according the intention-to-treat principle. IBM SPSS Statistics ver. 20.0 (Chicago, IL, USA) was used for the statistical analyses. All tests were two-tailed with a level of significance of $p<0.05$.

RESULTS

Thirty-one patients with diagnosis of CTS were included in the study and divided randomly into two groups homogeneous for gender ($p=0.220$), dominant hand ($p=0.886$) but not for professional activity ($p=0.015$) (Table 1). No statistically significant differences ($p>0.05$) were found in the two groups at baseline for BMI, Age, VAS, BCTQ-SSS and FSS and Electrodiagnostic parameters (Table 2). No patient was dropped out during treatment (Fig. 1). At the evaluation times between groups for the Mann-Whitney U test it wasn't found a statistically significant difference ($p>0.05$) (Table 3). At Wilcoxon signed-rank test in the group 1-A we observed a significant reduction of VAS, BCTQ- SSS and BCTQ-FSS both at T1 and at T2 vs T0 (median T0=3.0, median T1=0.0, median T2=0.0 for VAS; median T0=2.4, median T1=1.4 and T2=1.3 for BCTQ-SSS; median T0=1.5, median T1=1.2 and T2=0.8 for BCTQ-FSS; $p<0.05$) and a significant improvement in median sensory distal latency (SDL) at T1 (respectively T0=3.3 ms and T1=3.1 ms; $p<0.05$) (Table 4).

The Group 2-C confirmed the same statistically significant results of the Group 1-A at T1. Instead the results at follow-up (T2) were not significant ($p>0.05$) (Table 5).

Table 1. Demographic and clinical data of participants at baseline

Variables	Variable subclasses	Group 1-A (n=16)	Group 2-C (n=15)
Hand affected	Dominant hand (n, %)	10 (62.5)	9 (60.0)
	Non dominant hand (n, %)	6 (37.5)	6 (40.0)
Professional activity	Hand work (n, %)	8 (50.0)	14 (93.3)*
	No hand work (n, %)	8 (50.0)	1 (6.7)
Gender	Male (n, %)	2 (12.5)	5 (33.3)
	Female (n, %)	14 (87.5)	10 (66.7)

*Significant difference between groups ($p<0.05$).

Table 2. Clinical and electrodiagnostic variables in the two groups at baseline

Variables	Total group (n=31) (median, 25th–75th)	Group 1-A (n=16) (median, 25th–75th)	Group 2-C (n=15) (median, 25th–75th)
BMI	28.7 (25.4–33.2)	26.0 (22.4–32.1)	30.6 (26.9–33.6)
Age (years)	56.0 (50.0–69.0)	55.0 (52.0–71.3)	59.0 (50.0–68.0)
VAS (cm)	5.0 (0.0–6.0)	3.0 (0.0–6.0)	5.0 (0.0–7.0)
BCTQ-SSS	2.5 (1.8–3.4)	2.4 (1.6–2.9)	2.5 (1.8–3.4)
BCTQ-FSS	1.7 (1.0–2.2)	1.5 (1.0–2.1)	1.8 (1.1–2.5)
SCV (m/s)	38.2 (27.6–43.9)	38.9 (29.5–45.3)	36.4 (33.3–43.6)
SDL (ms)	3.3 (2.1–3.8)	3.3 (2.9–3.8)	3.0 (3.0–3.9)
MCV (m/s)	52.9 (50.0–55.8)	51.4 (49.2–55.2)	53.7 (50.0–64.5)
MDL (ms)	4.9 (4.1–6.2)	4.6 (4.1–5.5)	5.3 (4.1–6.3)
S-AMP (μV)	4.6 (3.1–7.9)	5.5 (2.2–8.3)	3.8 (3.3–7.5)
M-AMP (mV)	9.2 (5.3–12.1)	10.0 (5.2–12.1)	7.3 (5.3–12.4)

VAS: visual analogue scale; BCTQ-SSS: Boston carpal syndrome-symptoms severity scale; BCTQ-FSS: Boston carpal syndrome-functional severity scale; SCV: sensory nerve conduction velocity; SDL: sensory distal latency; MCV: motor nerve conduction velocity; MDL: motor distal latency; S-AMP: sensory nerve action potential amplitude; M-AMP: motor nerve action potential amplitude.

DISCUSSION

Compared to our starting hypothesis that the use of nutraceutical stabilizes or could to strengthen the benefit of physical therapy by ELF-EMFs in CTS our results didn't show significant clinical effects compared to placebo in patients treated with ELF-EMFs . However in group 1-A (nutraceutical group+ ELF-EMFs) we observed significant clinical improvement (VAS, BCTQ-SSS and BCTQ-FSS) at T1 and it was also maintained at T2 while in group 2-C (only ELF-EMFs) these results were observed only at T1. Although with a no statistical significance difference between the two groups, the one with dietary supplementation with the nutraceutical keeps the results even after both treatments have been suspended at follow-up (T2). Both groups had shown significant clinical effects from pre-treatment to the end of treatment. Both groups had also shown a significant improvement in median sensory distal latency (SDL) from pre-treatment to the end of treatment. In the literature, nutraceuticals that contains alpha-lipoic OR/AND curcumin, B-group vitamins and Acetyl-L-carnitine (ALCAR) have shown various properties on carpal tunnel syndrome and neurological pain disorders[19–21]. Alpha-lipoic acid has shown antioxidant and neuroprotective activities and it may lead to a significant improvement of clinical outcomes and electromyographic findings[18]. Notarnicola A[16] verified the trend toward better pain regression in the nutraceutical group (nutraceutical composed of Echinacea angustifolia, alpha lipoic acid, conjugated linoleic acid and quercetin) versus shock wave therapy in CTS. Also, a significant clinical impairment was reported in 112 subjects with moderately severe CTS after a 90-day treatment with a fixed association of alpha-lipoic acid and gamma- linolenic acid[19]. The efficacy of alpha-lipoic acid may be encreased by curcumin with regard to its neuroprotective, antioxidative and antinociceptive effects[25, 36]. B-group vitamins are also used as a conservative and adjunct therapy in the treatment of CTS with vitamin C, for its antioxidant and protective effects on tendons[27]. There are significant relationships between plasma vitamin levels and specific symptomatic components of CTS with regard to slowing of the median nerve[37]. Other studies showed that patients with antiretroviral toxic neuropathy[38], diabetic neuropathy[39] and chemotherapy-induced neuropathy[40] have less pain and better motor and sensory function if treated with Acetyl-L-carnitine (ALCAR). We also know that in the literature there are studies of feasibility, safety and efficacy of testing static magnetic field therapy for CTS[41]. Although the precise mechanism of ELF-MFs

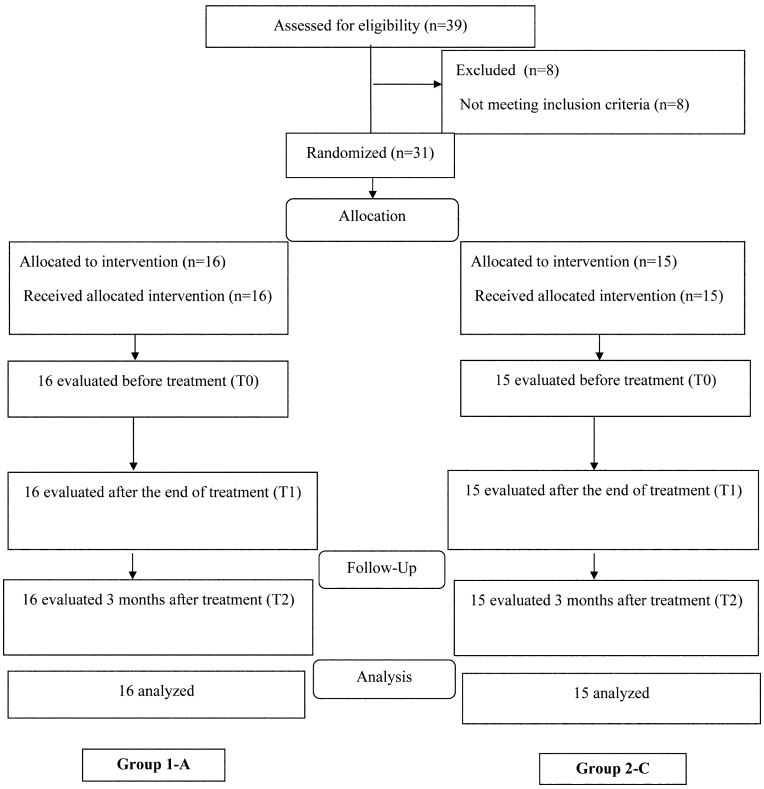

Fig. 1. CONSORT Flow Diagram

Table 3. Between group analysis after the end of treatment (T1) and after 3 months post treatment (T2)

Variables	T1 Group 2-C (median, 25th–75th)	T1 Group 1-A (median, 25th–75th)	T2 Group 2-C (median, 25th–75th)	T2 Group 1-A (median, 25th–75th)
VAS (cm)	3.0 (0.0–4.0)	0.0 (0.0–3.0)	0.0 (0.0–4.0)	0.0 (0.0–2.0)
BCTQ-SSS	1.5 (1.2–2.1)	1.4 (1.2–2.5)	1.4 (1.2–1.9)	1.3 (1.0–2.2)
BCTQ-FSS	1.0 (1.0–1.6)	1.2 (1.0–1.9)	1.3 (1.0–2.2)	0.8 (0.8–1.9)

VAS: visual analogue scale; BCTQ-SSS: Boston carpal syndrome-symptoms severity scale; BCTQ-FSS: Boston carpal syndrome-functional severity scale.

Table 4. Group 1-A before (T0) and after (T1 and T2) treatment

Group 1-A (n=16)	T0 (median, 25th–75th)	T1 (median, 25th–75th)	T2 (median, 25th–75th)
VAS (cm)	3.0 (0.0–6.0)	0.0 (0.0–3.0)*	0.0 (0.0–2.0)*
BCTQ-SSS	2.4 (1.6–2.9)	1.4 (1.2–2.5)*	1.3 (1.0–2.2)*
BCTQ-FSS	1.5 (1.0–2.1)	1.2 (1.0–1.9)*	0.8 (0.8–1.9)*
SCV (m/s)	38.9 (29.5–45.3)	40.5 (0.0–48.4)	-
SDL (ms)	3.3 (2.9–3.8)	3.1 (0.0–3.3)*	-
MCV (m/s)	51.4 (49.2–55.2)	52.5 (47.3–56.6)	-
MDL (ms)	4.6 (4.1–5.5)	4.2 (3.6–5.7)	-
S-AMP (μV)	5.50 (2.20–8.28)	7.40 (0.00–11.00)	-
M-AMP (mV)	10.0 (5.2–12.1)	8.1 (5.5–12.0)	-

*Significant difference between pre-treatment (T0) and post treatment (T1 and T2) ($p<0.05$).
VAS: visual analogue scale; BCTQ-SSS: Boston carpal syndrome-symptoms severity scale; BCTQ-FSS: Boston carpal syndrome-functional severity scale; SCV: sensory nerve conduction velocity; SDL: sensory distal latency; MCV: motor nerve conduction velocity; MDL: motor distal latency; S-AMP: sensory nerve action potential amplitude; M-AMP: motor nerve action potential amplitude.
-: Not collected.

Table 5. Group 2-C before (T0) and after (T1 and T2) treatment

Group 2-C (n=15)	T0 (median, 25th–75th)	T1 (median, 25th–75th)	T2 (median, 25th–75th)
VAS (cm)	5.0 (0.0–7.0)	3.0 (0.0–4.0)*	0.0 (0.0–4.0)
BCTQ-SSS	2.5 (1.8–3.4)	1.5 (1.2–2.1)*	1.4 (1.2–1.9)
BCTQ-FSS	1.8 (1.1–2.5)	1.0 (1.0–1.6)*	1.3 (1.0–2.2)
VCS (m/s)	36.4 (0.0–43.6)	40.0 (0.0–50.0)	-
DSL (ms)	3.0 (0.0–3.8)	2.8 (0.0–3.5)*	-
MCV (m/s)	53.7 (50.0–64.5)	54.8 (49.0–59.7)	-
DML (ms)	5.3 (4.1–6.3)	4.6 (4.0–6.5)	-
S-AMP (μV)	3.8 (3.3–7.5)	3.4 (0.0–5.6)	-
M-AMP (mV)	7.3 (5.3–12.4)	6.6 (4.8–8.3)	-

*Significant difference between pre-treatment (T0) and post treatment (T1 and T2) ($p<0.05$).
VAS: visual analogue scale; BCTQ-SSS: Boston carpal syndrome-symptoms severity scale; BCTQ-FSS: Boston carpal syndrome-functional severity scale; SCV: sensory nerve conduction velocity; SDL: sensory distal latency; MCV: motor nerve conduction velocity; MDL: motor distal latency; S-AMP: sensory nerve action potential amplitude; M-AMP: motor nerve action potential amplitude.
-: Not collected.

remains unknown, they have unexpected short-term analgesic effects in neuropathic pain: a low-frequency pulsed magnetic field has analgesic, vaso-active, neuron-stimulating, and trophic effects in patients with diabetic polyneuropathy[42]. Some groups have hypothesized that ELF-MFs in the picotesla and millitesla ranges improve neurotransmission and correct local immune pathology[43] and that a physics-based combination of simultaneous static and time-varying dynamic magnetic field stimulation in CTS can influence the neuromodulation of nociceptive C and large A-fiber functions, likely through ion/ligand binding[11]. This study has some limitations: the lack of a biochemical assessment of nutraceutical (thus, we were unable to determine the biochemical correlates of this result); the lack of nerve conduction study in all follow- ups (unfortunately, due to our unfunded research, it was not possible to ask patients an additional NCS at follow up); a brief duration of nutraceutical treatment. To obtain clearer results, our protocol should be amended to include increases in dosage of the integrator. The clinical value of oral supplementation with alpha-lipoic acid, curcumin phytosome and B-group vitamin before and after surgery in CTS patients could be recommended[17] but for a minimum of 3 to 6 months. In our research, we maintained a label supplementation dosage to ensure the safety of the patients.-up based on ethical considerations and cost. It might be desirable, in the light of these results, to plan a well designed randomized clinical trial, enlarging the sample size, lengthening the observation times (up to 1 year) and increasing in dosage of the integrator.

In conclusion, the nutraceutical composed of alpha lipoic acid, N-acetyl-L-carnitine, curcumin, vitamins B, E, and C has showed significant clinical effects for CTS in maintaining the result to follow up, demonstrating a positive association with the use of physical therapy as ELF-EMFs.

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Conflict of interest

The authors declare no conflicts of interest.

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Bone loss and trabecular bone quality in osteoporosis: what role for the new generation magnetotherapy with variable fields Limfa? Our experience

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Introduction: The LIMFA® Therapy system is an electro-medical device that generates information and transmits it to cellular receptors in order to activate and / or accelerate the endogenous processes of cellular healing, repair and regeneration. Cell stimulation with Limfa® Therapy Bone Repair sequences reduces osteoclast proliferation and differentiation, increases osteoclast apoptosis and stimulatesproliferation of osteoblasts.

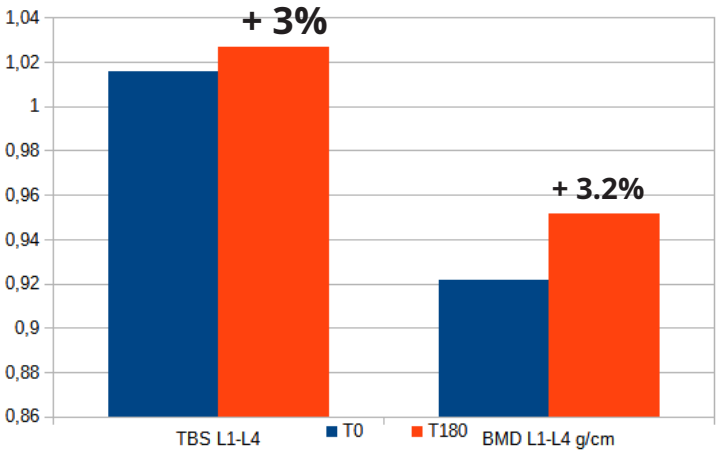
Purpose of the study: To evaluate, in patients suffering from osteoporosis, undergoing both pharmacological and electromagnetic treatment, with Limfa therapy (low frequency electromagnetic waves and variable fields), the effect on bone mass at the level of the vertebrae evaluated by DXA (T-Score and BMD) and on the quality of trabecular bone using TBS.

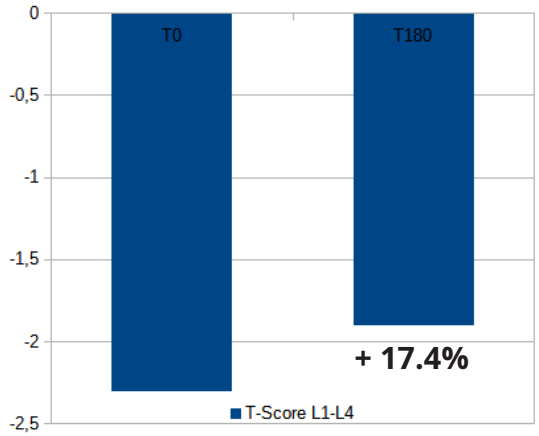
Materials and methods:**20 patients**they were recruited from the Endocrinology Unit, with an average age of 70.6 years, and underwent Limfa® Therapy (Bone Repair) and simultaneously with drug therapy. The study strategy comprehendsa GROUP A: patients with a recent (<6 months) diagnose of osteoporosis ; GROUP B: patients with a recent diagnose of severe (finding a vertebral fracture). osteoporosis ;

In the period of entry patients were evaluated at time 0 (T0) and after 6 months (T1). During the admission period and after the suspension, the patients underwent the examination of

Criteri di Inclusione	Criteri di Esclusione
Pazienti con Osteoporosi recente	Età fertile per le donne
Pazienti con Osteoporosi severa	Età < 50 anni per gli uomini
	Osteoporosi secondaria
	Neoplasia in atto o pregressa
	Presenza di pacemaker

Modalità di somministrazione	
Numero di trattamenti	12
Durata totale	6 settimane
Farmaci	Terapia antiriassorbitiva
	Terapia anabolizzante
	Vitamina D





Results :T he treatment with low frequency electromagnetic waves and various fields (Limfa) on patients with osteoporosis has shown:

1. Signi ficative Effects on Improvement of BMD Mineral Density and TBS Trabecular Bone Quality in Patients with osteoporosis
2. The greatest effectiveness (or best detection) was obtained on osteoporotic vertebrae, but not so fragile as to be wedged and in patients not taking pharmacological therapies for osteoporosis 3.

This treatment can be applied to those patients with osteoporosis, without fracture risks, who would benefit from an improvement in terms of quantity and quality of the bone

Conclusions: Since the treatment has also been very well received by the patients, requires little effort, has no side effects and very low costs, Limfa therapy is a therapeutic alternative for some patients with osteoporosis.

OPEN QUESTIONS:

Is it possible to repeat the treatment in patients already treated? At what distance in time? What results could be obtained in terms of osteoporosis improvement?

Effectiveness of treatment with ultra weak magnetic fields in late consolidation and in nonunion as a result of skeletal limb fractures: study protocol and preliminary observations

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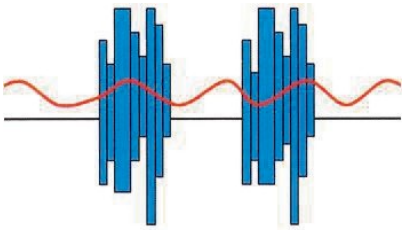
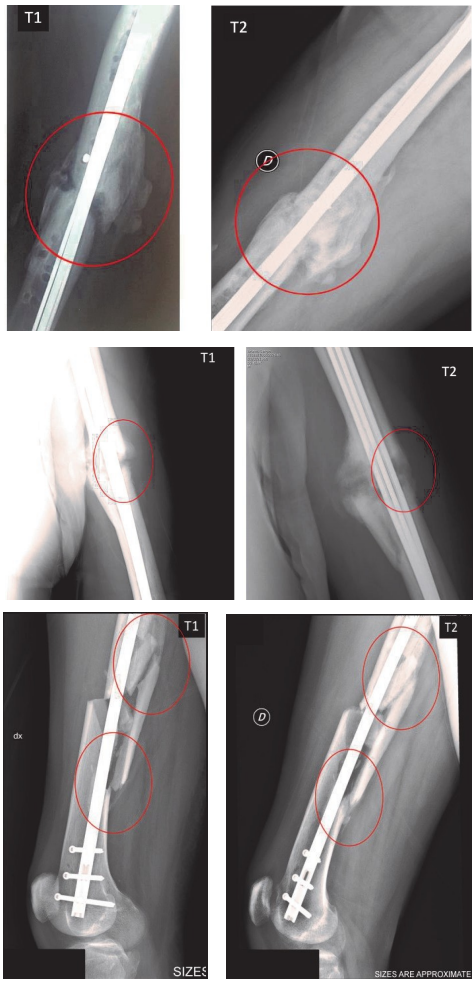
Introduction: The incidence of delayed Union or nonunion of fractures in evolution is estimated between 5 and 10%, with significant impact on the quality of life of those affected and the cost burden on healthcare systems and social security insurance. LIMFA Therapy ® is an innovative medical device for therapy using variable, ultraweak and complexes magnetic fields built by LIMFA TECHNOLOGIES srl, which is indicated in the treatment of posttraumatic Osteoarticular pathologies, both neurodegenerative, and conditions secondary to orthopaedic surgery. LIMFA Therapy®, uses magneto-electric complex multi-frequency signals at very low frequency (ELF-Extremely Low Frequency, between 1 and 80 Hz), with field strengths from 1 to 100 μT, comparable to the endogenous electromagnetic forces generated by cellular activity. Unlike traditional magneto therapy (PEMF), which uses one or at most two signal buttons with the same geometry, LIMFA Therapy®, uses up to 30 different geometries and different frequencies in sequences combined, able to transfer specific repair information whose effects extend over time even after the end of their applications.



Study: in the absence of controlled clinical studies to date, CRM INAIL has promoted a trial that aims to acquire preliminary data on the efficacy of treatment with LIMFA Therapy®, in inducing a progression RX-bone consolidation phenomena observable in a short period of observation (5 weeks). Randomised, controlled, open-label study, expected enrolment of 30 patients with delayed consolidation or nonunion fractures of limbs for traumatic events reported in job opportunity. Patients assigned to the experimental group receives only LIMFA Therapy® treatments; the controls are treated with conventional physical therapy pro- grams. For both groups the physical therapy is associated with a case specific functional rehabilitation program, lasting for 5 weeks. A radiographic examination of the fracture site in 2 orthogonal projections are acquired or performed within 4 weeks after starting treatment, and a second examination is executed at the end of 5 weeks of therapy. The two ex- ams are blinded by an independent expert orthopaedic staged preoperatively group of experimenters refer to the Hammer score (codifying the degree of bone repair in accordance with a scale of 1 to 5, where 1 identifies the healing).



Preliminary Results: we have completed the study Protocol with 17 patients to date. Nine of them (5 with nonunion, 4 with delayed Union) were assigned to experimental treatment and all (89%) of them have shown evidence RX reparative phenomena progression. The control group were assigned 8 patients (3 with delayed consolidation, 1 with non Union); in 4 cases out of 8 (50%) staging the second Hammer has shown an improvement.

Discussion: The small number of patients seen so far does not allow to draw firm conclusions. The preliminary results are encouraging, considering the short period of observation (the shortest period of observation reported in the literature concerning the efficacy of traditional magnetic therapy is equal to 12 weeks). The duration of treatment for bone re- generation of LIMFA Therapy® (a session of 30 ' every 2 days, for a total of 10 sessions) is also much shorter than cure with PEMF and economically advantageous: in cases study treatment with PEMF supplied (5 sessions per week for 5 weeks) had a higher cost of 30% compared to LIMFA Therapy® applications cycle. It will be necessary to await the conclusion of enrolment (scheduled to December 2016) and final analysis of the data in order to express more reliable assessments.

EFFECTIVENESS OF TREATMENT WITH ULTRADEBOL MAGNETIC FIELDS IN THE CONSOLIDATION DELAYS AND IN THE PSEUDOARTHROSIS CONCERNING SCHELETRICAL FRACTURES OF THE ARTS: PROTOCOL OFSTUDY AND PRELIMINARY RESULTS FOR 17 PATIENTS.

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INTRODUCTION

The healing of fractures is a complex metabolic process, conditioned by multiple factors both endogenous and exogenous. The incidence of delayed consolidation or development in non-skeletal fracture is estimated to be between 5 and 10% [1-2], with a significant impact on the quality of life of those affected and on the costs of the health and insurance system- social security. Surgical treatment (rigid debridmentation and osteosynthesis), associated or not with forms of biological stimulation, is considered the "gold standard" for the treatment of pseudoarthrosis (3)

However, there are numerous conservative treatments developed to accelerate fracture healing; Physical and electromagnetic stimulation, low intensity pulsed ultrasound, shock wave therapy [2, 4] are among the physical means.

Pulsed electromagnetic fields (PEMF) were introduced into treatment programs unconsolidated fractures in the mid-1970s. Although the mechanism of action remains poorly understood, PEMFs are considered a useful complementary treatment in cases of non-healing of fractures of long bones [5-8]. In most clinical trials, treatment with PEMF is indicated in cases where the failure to heal the fracture has been diagnosed [9-14], or an advanced consolidation delay [14-17]. n a recent review on the efficacy of treatment with pulsed electromagnetic fields (PEMF) in treatment of delay of consolidation or nonunion of long bone fractures [6] the shorter observation period reported is that of the work of Barker Sharrad, who evaluated the efficacy of active treatment versus placebo (simulated treatment) at 12 weeks, highlighting a fracture healing rate in 7/29 total patients treated with a functioning device (24.1%) compared to 3/35 total patients undergoing simulated treatment (8.6%). LIMFA Therapy® is an innovative medical device for magnetic field therapy ultra-low-range complexes with variable field built by LIMFA TECHNOLOGIES srl, which finds an indication in the treatment of osteoarticular pathologies, both degenerative and post-traumatic, and of secondary conditions to orthopedic surgery; in particular, the system presents a specific pre-set treatment program for the stimulation of non-consolidated skeletal fracture sites. LIMFA Therapy®, uses ultra-low frequency ultra-high-frequency complex electromagnetic signals (Extremely Low Frequency - ELF, between 1 and 80 Hz), with field strengths from 1 to 100 μT, comparable to the endogenous electromagnetic forces generated by the activity cell phone [18-27].

Unlike traditional magnetotherapy, which uses one of the ELF frequencies or at most two pulsating signals with the same wave geometry, LIMFA Therapy®, uses up to 29 different wave geometries to be able to act specifically on the different fabrics to be treated. The fields Magnets generated by the device do not give energy to the tissues but are analogous, in terms of frequency and intensity, to the endogenous electromagnetic forces generated by cellular activity; for this reason the inventors of the device assume that the magneto-electric fields generated by the instrument interact with cellular magnetic fields, according to the principle of cyclotronic iono-resonance [24].

In the absence, to date, of ad hoc controlled clinical trials, the purpose of this exploratory trial is to acquire Preliminary data on the efficacy of LIMFA Therapy® treatment compared to conventional physical therapies in accelerating the healing of consolidation delays and nonunion following a skeletal fracture of the limbs in a population of work-related patients treated with an integrated therapy program physics and rehabilitation and evaluated at 5 weeks from the beginning of the treatment, going to check if already in this short period of observation there have been rx- perceptible progresses of the reparative phenomena of the fracture.

MATERIALS AND METHODS

The study, open-label, randomized and controlled, involves the enrollment of 30 adult patients, aged between 18 and 65, with delayed consolidation or pseudoarthrosis of skeletal fractures of the limbs due to traumatic events reported at work.
Exclusion criteria are considered to be an ongoing or suspected infection at the missed site level consolidation and ongoing or previous (in the 4 weeks prior to enrollment) intake of active drugs on bone metabolism. Patients assigned to the experimental group receive treatment with the LIMFA Therapy® device according to the program called "bone regenerstion", predefined by the manufacturer, associeted with a neuro-motor functional rehabilitation program adequate to the specific lesion and the dysfunctional picture, lasting 5 weeks. The Controls are treated with conventional physical therapy programs.

For both study groups, physical therapy is Radiographic examination of the fracture site in 2 orthogonal projections is acquired or performed within 4 weeks of starting treatment, and a second exam is performed at the end of the 5 weeks of therapy. The two exams are blinded by an orthopedic expert independent of the group of experimenters by referring to the Hammer score, which encodes the degree of bone repair according to a score from 1 to 5, where 1 identifies the healing [28].

RESULTS

To date, 17 patients have completed the study protocol, whose characteristics are reported in Table 1.

	EXPERIMENTAL GROUP	CONTROL GROUP
Number of patients	9	8
Sex (M / F)	7/2	7/1
Age (years, mean ± SD)	46,22 ± 8,96	43,63 ± 14,8
Diagnosis:		
- Pseudoarthrosis	5	2
- Consolidation delay	4	6
N. Improved patients	8/9	4/8
% improved patients	89%	50%

Table 1. Patient characteristics

9 patients were assigned to the experimental group and received the treatment with LIMFA Therapy®: a session lasting 30 minutes every 2 days, for a total of 10 sessions. Treatment was well tolerated by patients (no side effects); in 8 cases out of 9 (89%) the RX study showed a progression of bone repairing phenomena.

8 patients were assigned to the control group; all were treated with a conventional magnetotherapy program using the Biorem Supera apparatus, receiving an average of 25 sessions of 45 min. each, with a frequency of 5 sessions per week. In 4 cases out of 8 (50%) the staging according to Hammer showed an increase in the degree of consolidation.

CONCLUSIONS

The limited number of patients observed so far does not allow definitive conclusions to be drawn. THE Preliminary results appear encouraging, considering also the short observation period (the shortest observation period reported in the literature regarding the effectiveness of traditional magnetotherapy is 12 weeks). The duration of the LIMFA Therapy® bone regeneration treatment program is also much shorter than the PEMF treatment cycles, it does not develop heat and can also be conducted in the presence of external fixation systems for fracture.

Figures 1 and 2 show the RX images related to two patients of the experimental group, of which a follow-up RX control is also available, which demonstrates the further progression of bone consolidation of the fracture site. It will be necessary to wait for the conclusion of the enrollment and the final analysis of the data in order to express
more reliable assessments.

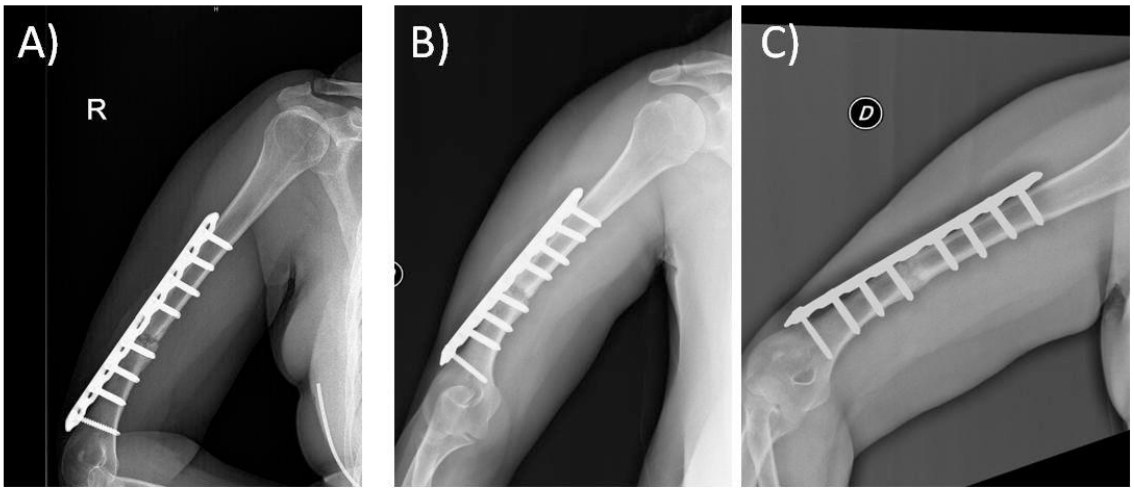

Figura 1. Femmina, 34 anni, ritardo di consolidazione omero, 5 mesi dall'evento acuto
A) RX acquisito a T1. B) RX acquisito a T2 C) Follow-up a 4 mesi dalla dimissione

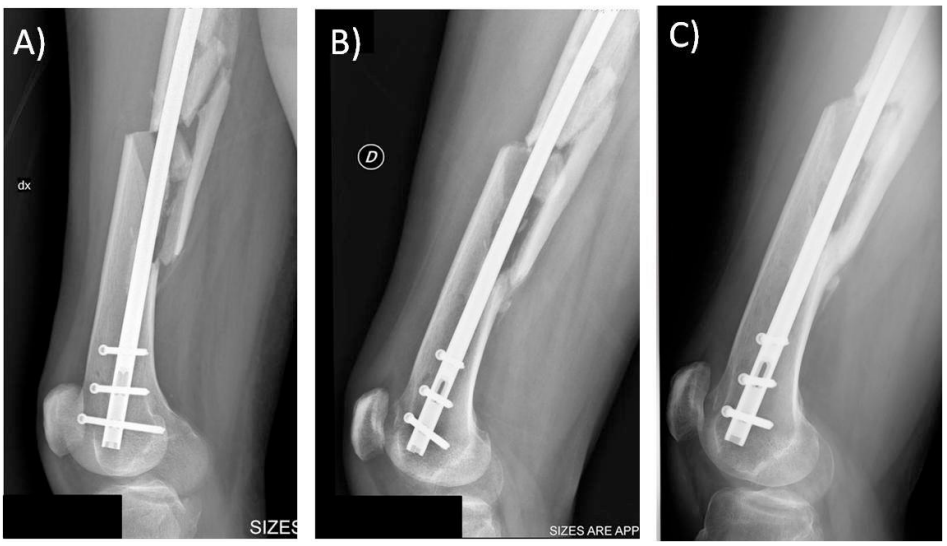

Figura 2. Maschio, 52 anni, ritardo di consolidazione femore, 4 mesi dall'evento acuto
A) RX acquisito a T1. B) RX acquisito a T2 C) Follow-up a 3 mesi dalla dimissione

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Gnathology

Analysis with postural rasterstereography in gnathologic dysfunctional patients with add W/R or WO/R treated with mandibular bite

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Aim: The correlation between occlusion and body posture has always been a very much debated issue in dentistry . In the field of diagnosis and monitoring of orthopedic and postural problems, the rasterstereography based on computerized photogrammetry has received an increasing consideration in the last years. The rasterstereography allows to obtain a 3D - representation of the dorsal profile without the use of ionizing radiation , as it is based on the technique of photometry. Studies using the rasterstereography to investigate the influence of the craniofacial morphology on different postural indices have been published by Lippold et al . Some correlations have been identified , for example , in the studies conducted in 2006 and 2009, regarding the vertical craniofacial pattern and some of the postural angles analyzed. However , all patients selected from them were analyzed without testing different occlusal situations in the same patient. Based on the results obtained from Lippold et al. , the aim of our clinical study is to investigate the correlation between postural indices in gnathologic patients with ADD w/R or ADD wo/R, in which were induced different occlusal situations.
Methods: 28 female patients, afferent to the Department of Gnathology 'Dental School-University of Turin', Italy, aged between 25 and 60 years with ADD w/R or ADD wo/R were selected. The subjects were divided into two groups of 14 patients each : The patients of the first group were treated with mandibular bite while the patients of the other group (control group) were left untreated. To test the possible effects of the mandibular and dental occlusion position on body posture, were performed, in the treated group, four scans with Formetric at different times T0, T1 (1 month), T2 (3 months) in these positions: 1)Mandible at rest; 2)Maximum voluntary clench in intercuspidation; 3)Maximum voluntary clench on cotton rolls placed between the arches; 4)Maximum voluntary clench on bite. While in the control group were performed 3 scans with formetric at different times T0, T1 (1 month), T2 (3 months) in the positions 1), 2) and 3) only.
Results: The results of this study are in line with those reported in the literature and confirm the claims of some systematic reviews that believe the existence of a correlation between body posture and occlusion. In this study, the formetric 4D equipment's reliability and repeatability as a screening and interception tool for postural problems has been confirmed. However, a diagnosis supported also by radiological examinations and orthopedic/physiatrist's advices is needed.
Conclusion: Formetric 4D is a reliable, repeatable and effective tool for the noninvasive investigation of the postural parameters . There were no significant differences between the postural parameters and the different occlusal conditions (rest, the window frame and the window frame on cotton rols).

Short-term effects of oral devices on sleep bruxism: a placebo-controlled RCT

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Aim: Sleep bruxism (SB) is a stereotyped movement

characterized by grinding or clenching of teeth during sleep, usually associated with an intense (excessive) arousal activity. It is the sleep-related motor disorder of primary interest for dental practitioners, considering several detrimental consequences on the stomatognathic system, including tooth wear, masticatory muscle tenderness and pain, headache and temporomandibular disorders. Based on that, a need emerged to define the best strategies to manage bruxism in the clinical settings. The aim of the study was to evaluate the variation in SB episodes and orofacial pain in four groups of subjects: a control group, a placebo group (using an acrylic appliance covering just the palate) and two groups treated with different oral appliances (OAs) (occlusal splint and functional orthopedic appliance).
Methods: An expert clinician assessed the presence of SB based on the presence of one or more signs/symptoms (i.e. transient jaw muscle pain in the morning, muscle fatigue at awakening, presence of tooth wear, masseter hypertrophy), among patients referring to the Gnathology Unit of the Dental School (University of Turin). First screening recording with Bruxoff® device selected 58 SB patients. Patients were assigned to four groups: control group (14 subjects: mean age 32.7 ± 12.75); placebo group (15 subjects: mean age 32.9 ± 13.83); occlusal-splint group (15 subjects: mean age 33.5 ± 13.76); functional orthopedic appliance group (14 subjects: mean age 33 ± 13.34). Five (N=14) patients dropped out the study (two patients assigned to control group, three to placebo group, three to occlusal-splint group and two patients to group with functional orthopedic appliance) because of the complexity of the study, especially for Bruxoff recording. Consequently forty-four subjects with an effective diagnosis for sleep bruxism (12 for every groups) were selected for the study. Each subject was observed for three months consecutively (T0: screening, T1: 1 week, T2 : 1 month, T3 : 3 months) and monitored with a visual analogue scale in order to evaluate the variation of facial pain. Furthermore, all participants underwent an instrumental recording at home with a portable device (Bruxoff®, OTBioelettronica, Torino, Italy) allowing a simultaneous recording of EMG signals from both the masseter muscles as well as heart frequency to evaluate variation on SB activity. Data were analyzed using Shapiro-Wilk test (for checking the normality), two-way Anova test (for analysis of variance) and test of multiple comparisons of Tukey-Siegel. All statistical procedures were performed with the software Statistical Package for the Social Science v. 23.0 (SPSS 23.0®, IBM, Milan, Italy). For each analysis a p-value<0.05 was set.
Results: Pain sensation significantly reduced both for stabilization splint and functional orthopedic appliance groups after three months follow-up, with no differences between the two groups. SB episodes significantly reduced after three months only in functional orthopedic appliance group; no variations were observed in placebo and control groups.
Conclusion: This study showed that two particular kind of OAs could reduce orofacial pain referred by the patients, but only the functional orthopedic appliance showed a statistical significant effect in reducing SB episodes. Further studies on larger and more representative samples, followed for a longer period are needed to obtain major information on SB management.

Periodontal mechanoreceptors: a systematic review

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Aim: Functional movements of the stomatognathic system and their relative forces depend on signals arising from various sensory organs in the orofacial structures. A special role is played by periodontal mechanoreceptors and their sensory innervation, located in the periodontal ligament, that is the optimal location for detecting the functional forces on the teeth. They are involved in mechanotransduction and chewing motor control, but there are important limitations of knowledge in the field. For exemple, even though mastication is a dynamic process, studies regarding periodontal mechanoreceptors are usually conducted in static conditions and mostly in animals, that are characterized by different teeth and occlusion with respect to the humans, often disregarding the functional differences of teeth.This work aims to review the progress in the field, especially during the last three years, with a special attention to the functional significance of experimental results. There have been a number of molecular reports; however, to understand the impact of these reports on the mechanisms of motor control we need to go back to the earliest physiological studies and these have been integrated with recent molecular data. The main results of basic research have been summarized, dividing the animal from the human studies and the signal pathways arising from mechanotransduction have been described.
Methods: A systematic review of the literature was conducted. Original articles were searched through Pubmed, Cochrane Central database and Embase until

January 2016.
Results: 1466 articles were identified through database searching and screened by reviewing the abstracts. 160 full-text were assessed for eligibility, and after 109 exclusion, 51 articles were included in the review process. Studies selected by the review process were mainly divided in studies on animal and studies on humans. Morphological, histological, molecular and electrophysiological studies investigating the periodontal mechanoreceptors in animals and in humans were included and subdivided in the following subheadings: Histological and electrophysiological studies in animals: are the results in agreement? - Changes during development; - Load response; - periodontal ligament as a source of mesenchymal-like stem cells. Molecular and electrophysiological studies in humans: what do we really know? - adaptation to implant-supported prosthesis; Central connections of the trigeminal primary afferent neurons: is there a bias in the basic research? From mechanotransduction to signal pathways: the role of periodontal mechanoreceptors on the chewing pattern motor control.
Conclusions: Our knowledge of the periodontal mechanoreceptors let us conclude that they are very refined neural receptors, deeply involved in the activation and coordination of the masticatory muscles during function. Strictly linked to the rigid structure of the teeth, they determine all the functional physiological and pathological processes of the stomatognathic system. The knowledge of their complex features is fundamental for all dental professionists. Further investigations are of utmost importance for guiding the technological advances in the respect of the neural control in the dental field.

Reliability of the Italian version of the Oral Behaviors Checklist

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Aim: The aim of this study is to examine the reliability of the Italian version of the Oral Behaviors Checklist Questionnaire (OBC-it), a tool which is widely used in studies concerning TMD compliant and oral parafunctions in the international scientific literature and that has already been subjected to the standard procedures of forward and back translation, committee review and cultural adaptation from RDC-TMD Consortium.
Methods: 282 Students at University Federico II, without temporomandibular pain, according to a validated TMD-pain screening, were recruited and divided into two groups: Group A (139 subjects, mean age 22.6± 5.48) and group B (143 subjects, mean age 23.7± 4.21). Participants belonging to group A were asked to fill in the OBC-it twice, with a two weeks interval between the two assessments. Differently from Group A, Group B received additional standardized instructions about the constructs included in the checklist by means of a power point presentation and a verbal explanation from one of the authors. After two weeks, subjects of Group B were asked to fill in the OBC-it again. However, at this stage, half of them (group B1) received again the same instructions, while the other half (group B2) no instructions. The test-retest reliability of OBC-it was assessed by calculating the Intra-class correlation coefficients (ICC) for each of the 21 single constructs and for the total OBC-it score in all groups. The ICC was interpreted as follows: ICC< .4 poor reliability, ICC ≥ .4 but ≤ .75 fair to good reliability, and ICC> .75 excellent reliability. Data were analyzed with SPSS (IBM) Ver. 20. The Statistical Significance was set at $p<.05$.
Results: OBC-it (total score) in group A showed excellent reliability results (ICC=.87). The reliability of OBC-it in groups B1 and B2 was excellent and slightly greater than group A. (B1: ICC=.94; B2: ICC=.95). Generally, all ICC data suggested a good or excellent reliability of the single constructs with the exception of the item 11 ("Hold jaw in rigid or tense position, such as to brace or protect the jaw") which showed fair to good reliability in all groups (Group A: ICC=.65; Group B1: ICC= .61; Group B2: ICC=.70). On the contrary, item 19 ("singing") displayed excellent ICC results in all groups (Group A: ICC=.90; Group B1: ICC= .90; Group B2: ICC=.94).
Conclusions: This study has shown that the Italian version of the OBC, namely OBC-it, is highly reliable and may be used for both research and clinical purposes. The higher ICC values in group B1 and B2 suggest that reliability increases when instructing subjects about the meaning of each item and so that an explanation from the clinician before the compilation could be helpful for a better comprehension of the questionnaire.

Use of aligners for the resolution of extra-articular temporo-mandibular joint disorder: case report

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Aim: The aim of this work is to provide a guidance about the use of aligners in the resolutions of extra-articular temporomandibular joint disorders in patients with midle malocclusions.
Methods: It is presented a 47-years-old male with extra-articular temporomandibular joint disorders: mild dental class III, deep bite, severe myofascial pain syndrome and mild soreness external pterigoideus

RL, Upper Tapezius RL. The goal of treatment is the resolution of extra-articular temporomandibular joint disorder by the treatment of dental malocclusion of the patient through the use of aligners. The therapeutic protocol chosen, provide for the use of passive aligners Vivera for 2 months, and for the use of active aligners Invisalign for 16 months. At each follow-up appointment the patient reports a decrease in pain.
Results: At the end of the treatment it is observed the resolution of the dental class III and the opening of the bite associated with the reduction of pain of initially soring muscles and improving of myofascial syndrome symptoms.
Conclusion: In this case report is clear that the use of only the aligners, with which the malocclusion of the patient were treated, it was sufficient for the improving of extra-articular temporomandibular joint disorder.

LIMFA® Therapy in the treatment of TMDS correlated pain: a pilot study

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Aim: The aim of this clinical trial is to assess the effects of LIMFA® Therapy (Low Intensity Magnetic Field Appliance) in the treatment of TMDs related chronic pain and to detect any problematic aspects about application of this technology. LIMFA® Therapy is an electromedical device that uses Low Intensity and Extremely Low Frequency Magnetic Fields in multifrequency sequences with variable wave geometry (1-100 Hz, 1-80 mT).
Methods: For the study seven adult patients, from 30 to 78 years old, were selected from a sample of 160 patients visited in a period of 1 month in the Unit of Gnatology of Policlinico Umberto I. Patients with TMDs correlated chronic pain with value≥ 5 in Verbal Numerical Scale (VNS from 0 to 10) were included; subjects under 18 and over 80 years old, with pacemaker, in pregnancy, with positive anamnesis of tubercolosis, tumors or epilepsy were excluded. LIMFA® Therapy was used as an "add-on" to existing treatment, twice a week, from 1 to 4 weeks. Evaluations were made by subjective measures (Pain Disorders Screening and Perceived Functional Difficulties) and objective measures (Functional Disorders). Results: In T1(one week) all the patients declared lower VNS value about treated TMJ. After T1 five patients continued the Protocol. A Patient with Degenerative Joint Disease had a little reduction in pain e no improvement in function, while the others declared less pain and better function. A Patient with Disc Displacement with reduction, showed no more Click on left side and occasionally on the right one. A Patient with Disc Displacement without reduction with limited opening improved both in pain and in function and was able to start gnatology therapy with splint. Two Patients with limited opening (one with Osteoarthrosys, the other with Disc Displacement without reduction) declared high VNS value in T°, despite Gnatology Therapy, Physiotherapy, Acupuncture and drug therapy by Neurologist. After treatment with LIMFA® Therapy they declared lower value of pain (Actual, Several and Worst), fewer pain crises and lower dosage of drugs. Two Patients with Hashimoto Thyroiditis had adverse reactions and we decided to break in the protocol (one after T1, the other one after T2).
Conclusion: LIMFA® Therapy showed positive effects in the treatment of high- intensity chronic pain (Arthralgia, Myalgia, Myofascial-pain, Headache) associated with gnatology therapy, or to help starting treatment with Splint. It showed less effectiveness in very important degenerative joint diseases with limited opening. Further studies are essential to investigate the possibility of treating TMD with LIMFA® Therapy's on patients with Hashimoto Thyroiditis.

Association between dental cervical lesions and obstructive sleep apnea syndrome

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Aim: Obstructive sleep apnea syndrome (OSAS) is the most common type of sleep apnea and is caused by complete or partial obstructions of the upper airways. It is characterized by recurring episodes of shallow or paused breathing during sleep and is usually associated with a reduction in blood oxygen saturation. Others signs and symptoms of sleep apnea include: morning headaches, memory or learning problems and not being able to concentrate, feeling irritable, depressed, or having mood swings or personality changes, waking up frequently to urinate, dry mouth or sore throat when you wake up. The aim of this study was to evaluate the association between dental cervical lesions and obstructive sleep apnea syndrome (OSAS).
Methods: 76 patients (42 females and 34 males) were consecutively selected from the Neurologic clinic of the

University hospital Azienda Ospedaliera Universitaria (AOU) "G. Martino" in Messina according to the following inclusion criteria: Caucasian, age between 30 and 60 y.o., absence of craniofacial dysmorphism and craniofacial syndromes. Every enrolled patient signed an informed consent and performed the following exam: neurologic visit, polysomnography (PSG) exam, clinical oral examination and acquisition of high resolution intra-oral and facial photographs. For every patient a clinical chart was draw up and the presence of dental cervical lesions was evaluated, documented and carefully reported. The odds ratio (OR) was calculated and used to evaluate association between dental cervical lesions and obstructive sleep apnea syndrome (OSAS). An odds ratio (OR) is a measure of association between an exposure and an outcome. The OR denotes the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome happening in the absence of that exposure.

Results: 41 patients were diagnosed as affected by obstructive sleep apnea syndrome (OSAS), 27 of those patients showed dental cervical lesions 14 patients did not show cervical lesions. 35 patients were diagnosed as non-OSAS patients , 12 of those patients showed cervical lesions, 23 patients did not show cervical lesions. The calculated ratio odds was 3.6.

Conclusions: The results of our study indicate a moderate association between dental cervical lesions and obstructive sleep apnea syndrome (OSAS). These lesions are localized on both the anterior and posterior regions of both arches.

Treatment with low intensity electromagnetic fields significantly improves joint mobility and reduces pain: study in 148 patients with osteoarticular pathologies

Fernando Anzivino, Giuseppe Calvosa, Francesco Conconi, F. Foglietta.

Introduction: LIMFA ® Therapy is a medical device which generates information and transmits them to the cell receptors to activate and/or accelerate the endogenous processes of healing, repair and cellular regeneration. Limfa ® Therapy is an innovative treatment which acts as a ELECTRONIC MEDICATION. Most studies that have reported his efficacy, show a natural anti-inflammatory and analgesic effect, without generating any secondary side effects.

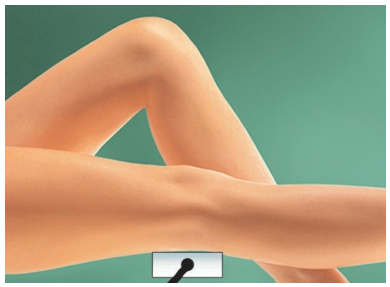
Aim: determine Limfa® Therapy effectiveness in pain relief in osteoarticular and musculoskeletal pathologies, in addition to reducing inflammation and increasing joint mobility.

Method and Materials: A group of 148 adults, suffering from various orthopaedic disorders, undergoing Limfa® Therapy with pre-established protocols in relation to those presented. The modalities of application are standardised and preloaded so not modified by the operator.

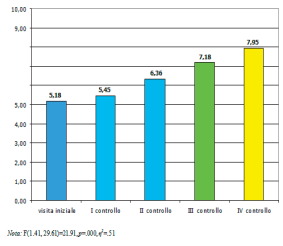
Inclusion criteria	Exclusion criteria
Muscle, tendon, ligament tissues trauma Orthopedic surgical outcomes Osteoarticular disorders	Pregnant patients Epileptic patients Patients with Neoplasms Patients > 80 years

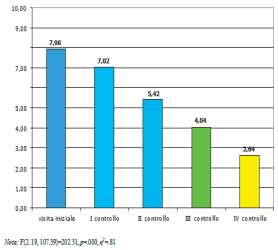
Methods	
Numbers of Therapy	From 4 to 12
Treatment duration	1-4 weeks
Frequency	24/48 hours
Duration	20-60 minutes

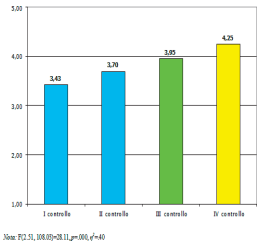
Outcome measures: A data sheet has been drawn up containing, besides personal and clinical details, a survey of disturbances best assessable using an objective methodology, except for pain, where the patients was assessed using an analogue-visual scale (VAS), with a score from 0 to 10.



Results: Upon initial visit (t0), 16.9% have suffered a trauma and 6.6% undergoing surgery, while 55.9% have onset of symptoms linked to the disorder. For about a quarter of the participants (23.6%) are found concomitant disorders, 31.1% are undergoing drug treatment. The level of inflammation, for most of the sample, is absent-moderate (82.4%), as well as the degree of edema (97.3%); however, joint mobility is reduced (M = 2.61, SD = 4.89) and cause pain perceived is quite high (M = 7.42, ds = 1.93). After the initial visit, patients undergoing Limfa® Therapy and visited at regular intervals to assess the progress of clinical-functional indices described above. In addition, starting from the 1st checkup was detected even the degree of patient satisfaction. The timing of longitudinal surveys are as follows: T1=1st control checkup in 7 days after initial visit; T2=2nd control visit 14 days after initial visit; T3=3rd checkup at 21 days after initial visit; T4=4th control visit 28 days after initial visit.







Joint mobility: + 54%
Pain:-67%

Satisfaction: 85%

Limfa® Therapy, as was to be expected, is without side effects. In all the examined cases no adverse effects were found; in the analytical evaluation, a higher than expected effectiveness became evident: no worsening and statistically significant improvements as regards the main outco- mes: pain, joint mobility and patient satisfaction. The positive results were achieved without gender and age differences: this strengthens the

Treatment with low intensity electromagnetic fields significantly improves mobility and reduces pain

Study performed on 148 patients suffering from bone and joint disorders

Fernando Anzivino*, Giuseppe Calvosa**, Francesco Conconi***, Gianni Garlatti****, Fosco Foglietta*****

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** Orthopedist, direc
*** Biochemist, Fer
**** Medical Consu
***** President CU

Summary

A group of 148 adults, suffering from various orthopaedic disorders, underwent a therapy with low intensity magnetic fields.

The aims of the study were to assess both the clinical efficacy and the safety of the therapy with low intensity magnetic fields.

The persons undergoing such therapy obtained satisfactory results both as regards pain and functional rehabilitation, in what was a very short time for such types of disturbances (within 4 weeks).

No side or adverse effects were recorded by therapists during treatment.

Introduction

The low intensity magnetic fields system is an electromedical device which produces very low intensity magnetic-electric signals, not comparable with traditional physical therapy systems.

The magnetic fields generated by the instrument, in terms of frequency and intensity, are the same as endogenous electromagnetic forces generated by cell activity. For this reason, the creators of the instrument sustain that the magnetic-electric fields generated by the instrument interact with body cell magnetic fields (ion cyclotron-resonance, Liboff 1995) and cause changes to the intra and extra cellular permeability parameters and trigger cell processes against states of inflammation and oedema.

This work shows the results obtained after using this system to treat 148 patients suffering from bone and joint disorders or osteoarticular pathologies.

Materials and methods

The aim of this work is to evaluate the clinical efficacy of the low intensity magnetic field system in a number of specific and selected disorders of prevalently orthopaedic interest.

In this first phase, we have deliberately narrowed down the field of clinical application, to obtain a case history that can be analyzed from a statistical viewpoint.

A data sheet has been drawn up containing, besides personal and clinical details, a survey of disturbances best assessable using an objective methodology, except for pain, where the patient was assessed using an analogue-visual scale (VAS), with a score from 0 to 10.

Involved in the study were district physical therapy and rehabilitation facilities of proven experience.

The assessing professionals were taught to use the device and took part in a training phase for the correct and uniform collection of data.

Monthly board meetings were organized to discuss the collected data with the therapists and thus make sure that clinical evaluation was as consistent and uniform as possible.

The assessment schedule provided for a start time (t_0) and subsequent one week intervals (t_1, t_2, t_3, t_4).

Machine application mode times were standardized and preloaded in the program and were not therefore changeable by the operator.

Descriptive sample analysis

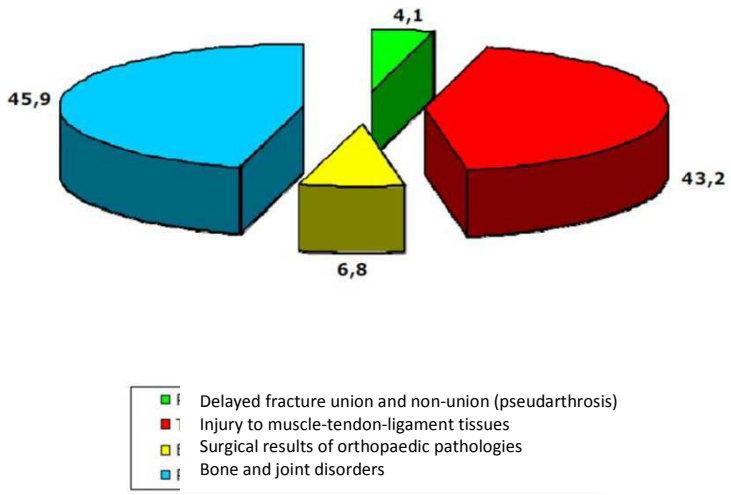
The sample consists of 148 participants, half of whom (50.7%) were involved in research in Bologna facilities. Average age was 55.8 years (sd=15.24, range 20-85 years) and was generally inclined in favour of women (men=40.2%, women=59.8%).

The disorders treated using low intensity magnetic-electric fields can be split into 4 types and more specifically:

1) Delayed fracture union and non-union
2) Injury to muscle-tendon-ligament tissues
3) Surgical results of orthopaedic pathologies
4) Bone and joint disorders

The distribution of the treated disorders is shown in fig. 1.

Figure 1 – Percentage distribution of the disorders treated with low intensity magnetic-electric fields



At the time of the initial visit (t0), the participants presented what was on average a compromised picture. 16.9% had suffered an injury and 6.6% had undergone a surgical operation, while 55.9% showed symptoms tied to the disorder.

About one quarter of the participants (23.6%) were suffering from concurrent pathologies, 31.1% were undergoing pharmacological treatment.

The level of inflammation, for most of the sample, was absent-moderate (82.4%), as was the degree of oedema (97.3%); joint mobility was however reduced (M=4.89, sd=2.61) and the pain perceived was fairly high (M=7.42, sd=1.93).

Details relating to the descriptive analyses are shown in fig. 2-3.

Figure 2 – The study population

Gender	**N**	**%**
Male	47	40.2
Female	70	59.8

Age bracket	**N**	**%**
Under 50	41	32.0
51-65 years old	47	36.7
Over 65	40	31.3

Pathology	**N**	**%**
Delayed fracture union and non-union	6	4.1
Injury to muscle-tendon-ligament tissues	64	43.2
Surgical result of orthopaedic pathologies	10	6.8
Bone and joint disorders	68	45.9

Centre	**N**	**%**
Antalgik	51	34.5
Farmacia degli Angeli	21	14.2
Fisiology Center	5	3.4
Il Glicine	33	22.3
Medical Center	14	9.5
Poliambulatorio Forni	24	16.2

City	**N**	**%**
Bologna	75	50.7
Forlì	5	3.4
Modigliana	33	22.3
Pistoia	14	9.5
Rocca San Casciano	21	14.2

Figure 3 – Clinical-functional indices of initial visit

	N	%
Injury	23	16.9
Surgical operation	9	6.6
Symptomatology	76	55.9
With concurrent disorders	35	23.6
With pharmacological treatment	42	31.1

Physio-pathological condition	**N**	**%**
Post-menopause	17	11.5
Cardiovascular diseases	3	2.0
Endocrine disorders	5	3.4
Controlled diabetes mellitus	1	0.7
Smoker	24	17.1
Drinker	12	8.6

Treated disorders	**N**	**%**
Exacerbated arthrosis disorders	8	5.4
Delayed fracture union	5	3.4
Post surgical operation situation	10	6.8
Muscle injury	18	12.2
Bone and joint disorders	54	36.5
Tendon-ligament injury	44	29.7
Others	41	20.9

Location of injury	**N**	**%**
Right arm	10	6.8
Left arm	5	3.4
Right forearm	1	0.7
Left forearm	3	2.0
Right hand	5	3.4
Left hand	5	3.4
Right thigh	5	3.4
Left thigh	6	4.1
Right leg	8	5.4
Left leg	3	2.0
Right foot	1	0.7
Left foot	2	1.4

Inflammation (calor)	N	%
Absent	66	44.6
Slight	19	12.8
Moderate	37	25.0
Serious	26	17.6

Oedema	N	%
Absent	91	61.9
Slight	32	21.8
Moderate	20	13.6
Serious	4	2.7

Hematoma	N	%
Absent	137	95.8
Present	6	4.2

	M	ds
Joint mobility (0-10)	4.89	2.61
Pain perceived (0-10)	7.42	1.93

Longitudinal analysis on general sample

After the initial visit, patients underwent treatment by means of very low intensity magnetic-electric fields and were regularly visited to determine the above clinical-functional indices. Furthermore, starting with the first checkup, the degree of patient satisfaction was also assessed.

Longitudinal analysis schedules were as follows:

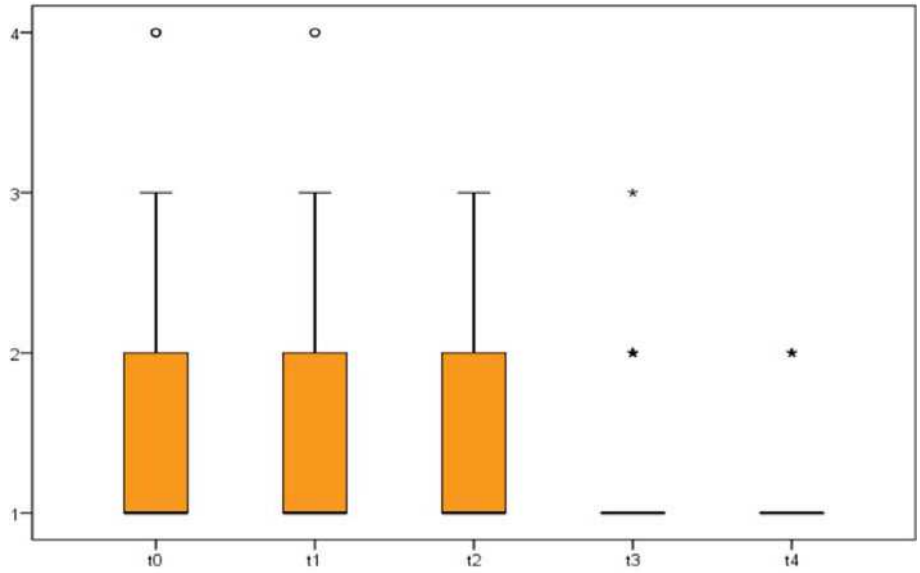
- $\mathbf{t_1} \rightarrow 1$ checkup at **7 days** from initial visit
- $\mathbf{t_2} \rightarrow$ 2nd checkup at **14 days** from initial visit
- $\mathbf{t_3} \rightarrow$ 3rd checkup at **21 days** from initial visit
- $\mathbf{t_4} \rightarrow$ 4th checkup at **28 days** from initial visit

The data relating to the level of inflammation and oedema, having been assessed by con-continuous ordinal scales and presenting a non-normal distribution, were treated by means of non-parametric statistical analyses (Friedman test for general longitudinal analysis, Wilcoxon test for *post-hoc* among the different assessments).

The results showed a significant drop in the level of inflammation, and the subsequent *post-hoc* tests indicate that improvement was significantly gradual at each assessment up to 21 days from the initial visit, while no improvements were found between the last two checkups (fig. 4).

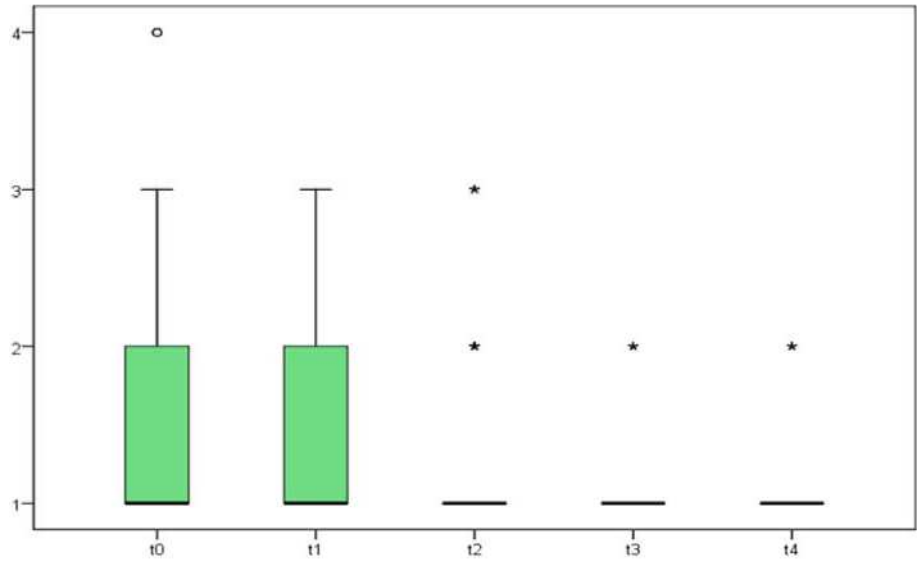
As regards the level of oedema, in this case as well the analyses showed a significant and gradual drop to t_3 and the absence of significant improvements between the last two checkups (fig. 5). It must nevertheless be underlined that, as pointed out in the section relating to descriptive analyses, the great majority of the sample started with a slight level of inflammation and oedema, and it was therefore only natural for the margin of improvement to be small.

Figure 4 – Longitudinal analysis of inflammation level



Note: X^2 (4)=66.45, *p*=.000

Figure 5 – Longitudinal analysis of oedema



Note: X^2 (4)= 63.24, *p*=.000

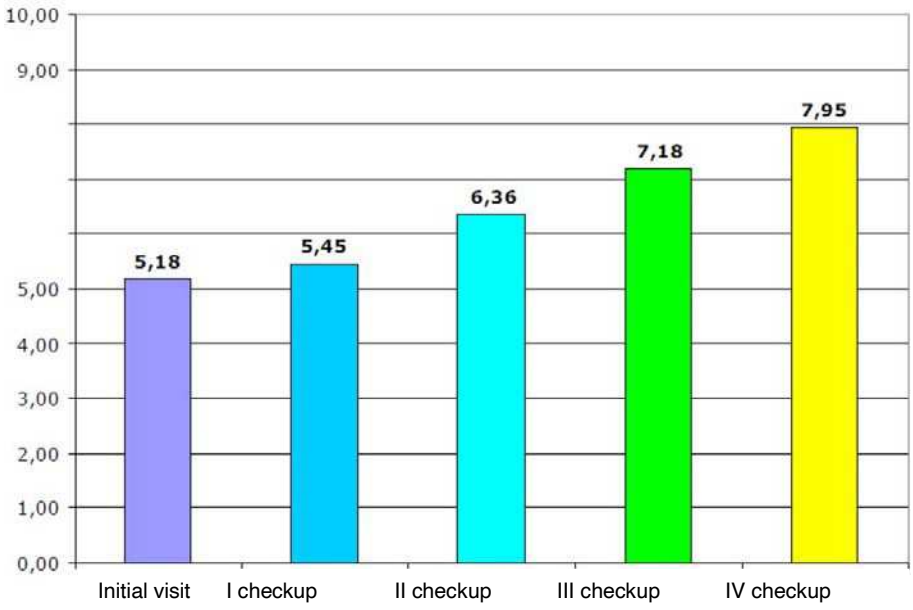
The data were subsequently analysed regarding the joint mobility of the patients, the pain perceived by them and the degree of satisfaction expressed in assessments t_1- t_4. In this case a repeated measurement variance analysis was used.

With respect to joint analysis, the results indicate that this goes from insufficient to good

throughout the treatment period; more specifically, no improvements were found between the initial visit and the 1st checkup, but this was followed by a significant and gradual improvement in all subsequent assessments (fig. 6).

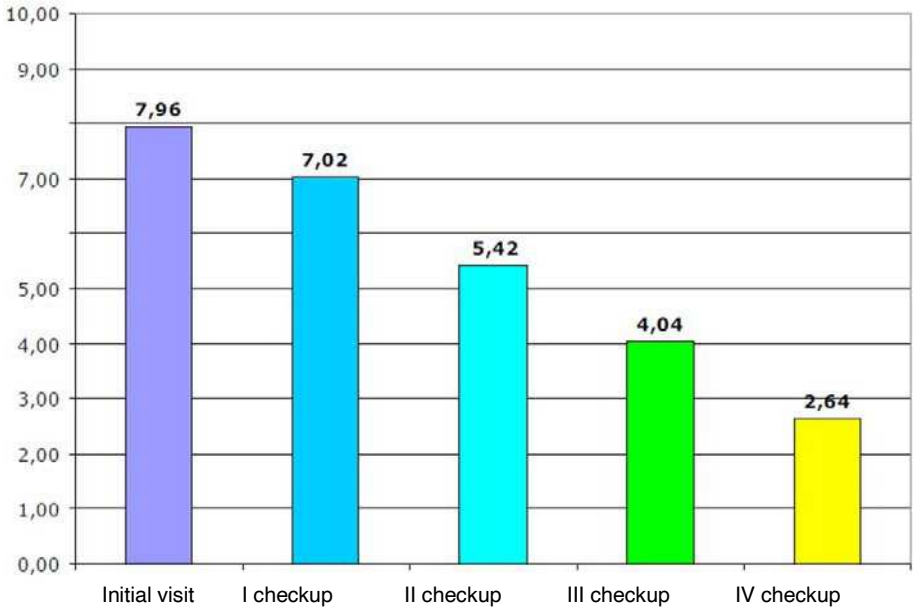
The results relating to the level of pain perceived by the patients in the different visits show a significant drop starting from the 1st visit; pain went from extreme to greatly reduced throughout the treatment period (fig. 7). Assessments concerning the degree of patient satisfaction were also positive: satisfaction tended to increase at each subsequent checkup (fig. 8).

Figure 6 – Longitudinal analysis of joint mobility



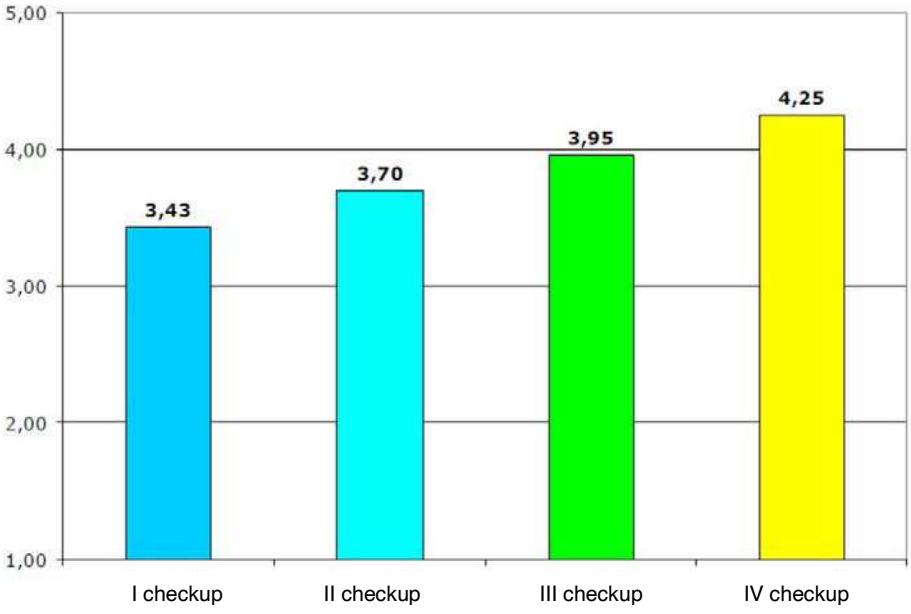
Note: $F(1.41, 29.61)=21.91$, $p=.000$, $\eta^2=.51$

Figure 7 – Longitudinal analysis of level of perceived pain



Note: F(2.19, 107.39)=202.31, p=.000, η^2=.81

Figure 8 – Longitudinal analysis of degree of satisfaction



Note: F(2.51, 108.03)=28.11, p=.000, η^2=.40

Longitudinal analysis according to type of disorder

The data collected regarding joint mobility, perceived pain and degree of satisfaction subsequently underwent two-way repeated measurement variance analysis to investigate any moderation effects by variables such as the type of disorder, the gender and the age bracket of the participants.

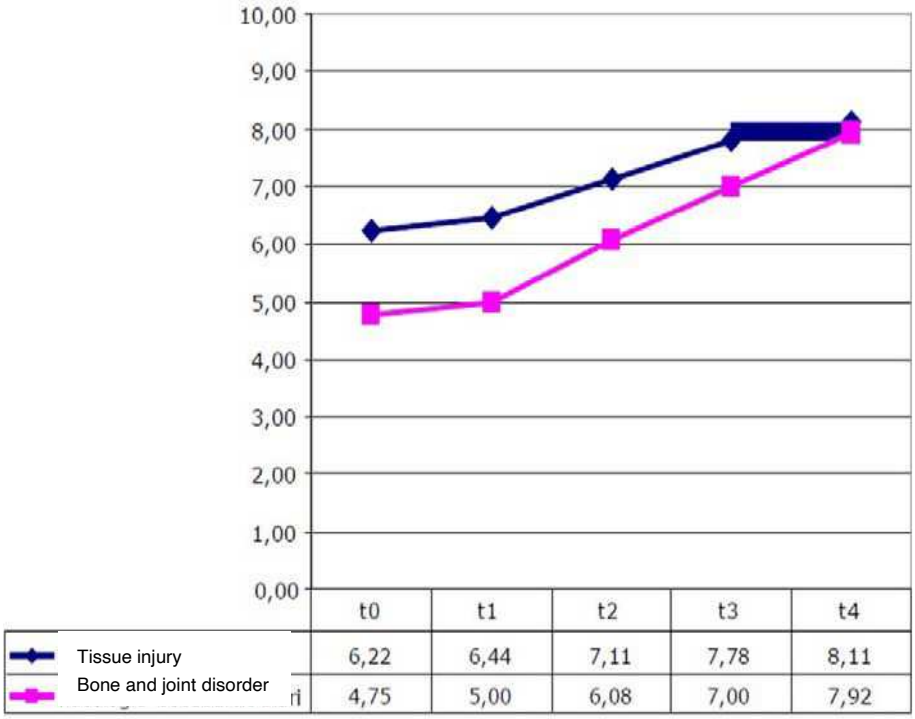
As regards the type of disorder, it was not possible to analyse all the disorders suffered by the sample, because the percentage of patients with delayed fracture union and surgical results of orthopaedic disorders was very small at the current stage of experiments. The decision was therefore taken to analyse the differences between patients with injuries affecting muscle-tendon-ligament tissues and with bone and joint disorders.

From the analyses performed, it appears that, as regards joint mobility, an improvement effect repeated itself over time for both disorders, but no interaction effect was found (fig. 9); it can therefore be said that the type of disorder does not apparently affect the success of the treatment in terms of patient mobility.

In the same way, the improvement of pain level also evolved significantly for both disorders without showing any type of interaction, and so it seems that the effect on pain does not depend on the type of disorder (fig. 10).

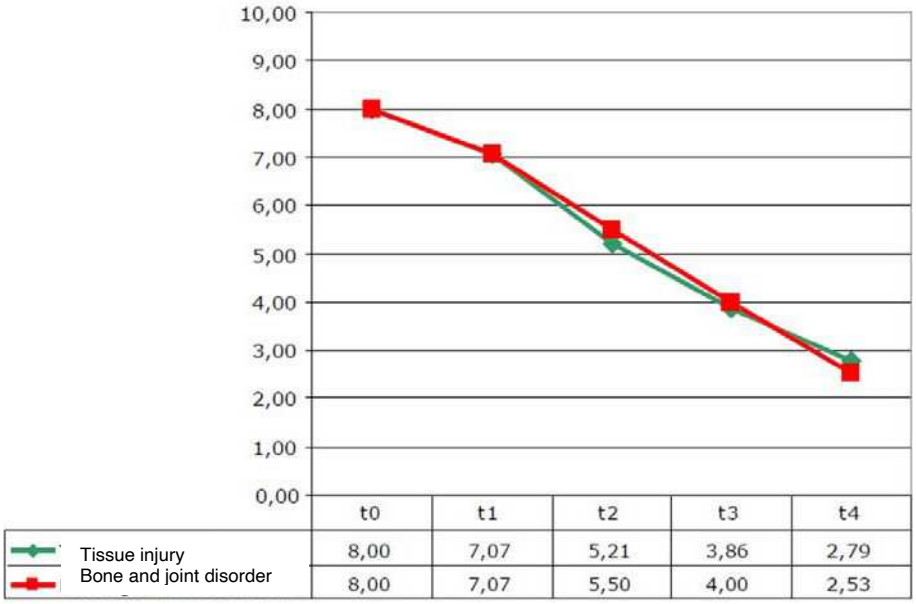
The same result would also seem to appear as regards the satisfaction expressed by the patients: this increased as time passed and with each checkup, but was not moderated by the disorder for which the participants were being treated (fig. 11).

Figure 9 – Longitudinal analysis on level of joint mobility in accordance with the type of disorder



Note: F(4, 16)=.53, *n.s.*

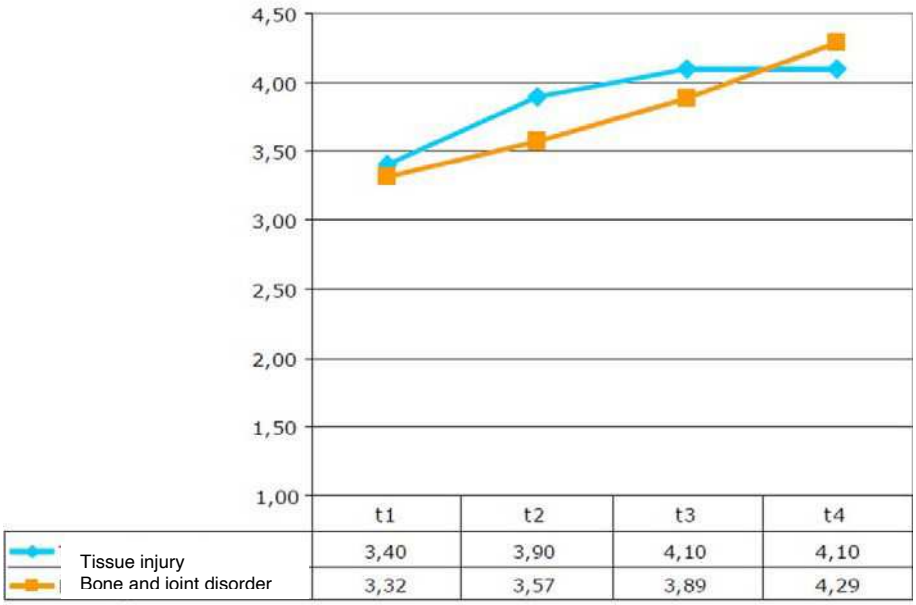
Figure 10 – Longitudinal analysis on level of pain perceived according to type of disorder



	t0	t1	t2	t3	t4
Tissue injury	8,00	7,07	5,21	3,86	2,79
Bone and joint disorder	8,00	7,07	5,50	4,00	2,53

Note: F(4, 39)=.53, *n.s.*

Figure 11 – Longitudinal analysis on degree of satisfaction according to type of disorder



	t1	t2	t3	t4
Tissue injury	3,40	3,90	4,10	4,10
Bone and joint disorder	3,32	3,57	3,89	4,29

Note: F(3, 34)=1.97, *n.s.*

Longitudinal analysis according to gender

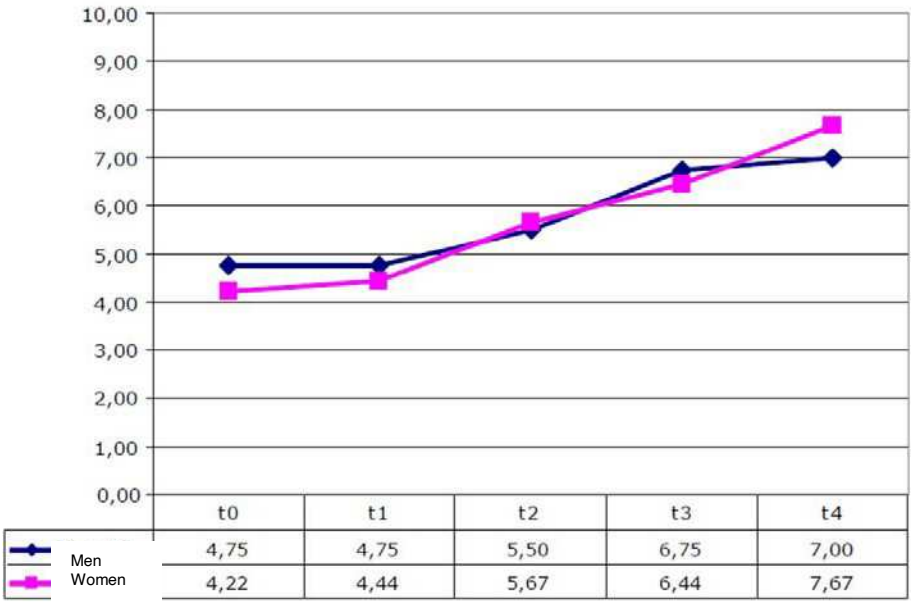
Secondly, any presence was investigated relating to a moderating effect on the three previous indices due to the gender of the participants.

As regards joint mobility, no differences were found between men and women nor interaction effects between geneder and treatment efficacy (fig. 12).

In the same way, the perceived pain did not appear moderated by the gender of the participants: a significant drop in pain appeared over time, but there were no big differences between men and women as regards this evolution (fig.13).

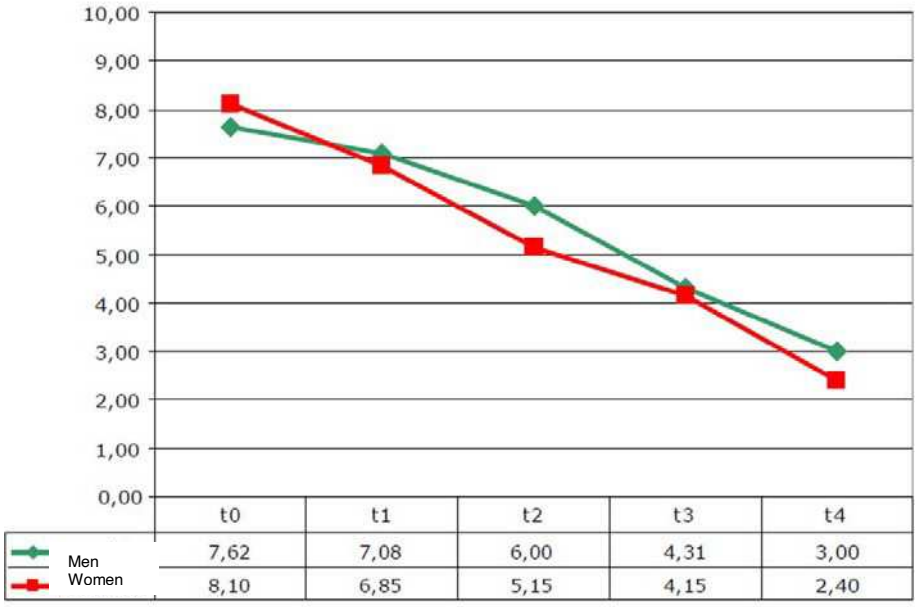
Finally, the same trend occured for the degree of satisfaction, which tended to increase significantly from one visit to another but without any difference appearing between men and women or interactions between patient gender and satisfaction expressed for the treatment (fig. 14).

Figure 12 – Longitudinal analysis as regards level of joint mobility according to gender



Note: F(4, 13)=.69, *n.s.*

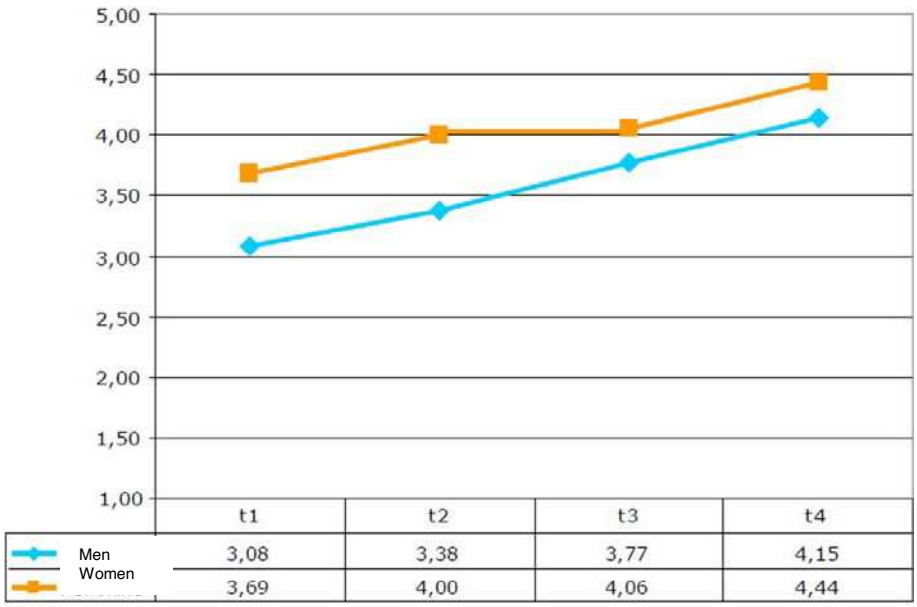
Figure 13 – Longitudinal analysis as regards level of pain perceived according to gender



	t0	t1	t2	t3	t4
Men	7,62	7,08	6,00	4,31	3,00
Women	8,10	6,85	5,15	4,15	2,40

Note: $F(4, 28)=3.94$, *n.s.*

Figure 14 – Longitudinal analysis as regards degree of satisfaction according to gender



	t1	t2	t3	t4
Men	3,08	3,38	3,77	4,15
Women	3,69	4,00	4,06	4,44

Note: $F(3, 25)=1.36$, *n.s.*

Longitudinal analysis according to age

To interpret the interactions between effectiveness of treatment and age of participants, it was decided to split the patients up into three equally distributed age brackets:

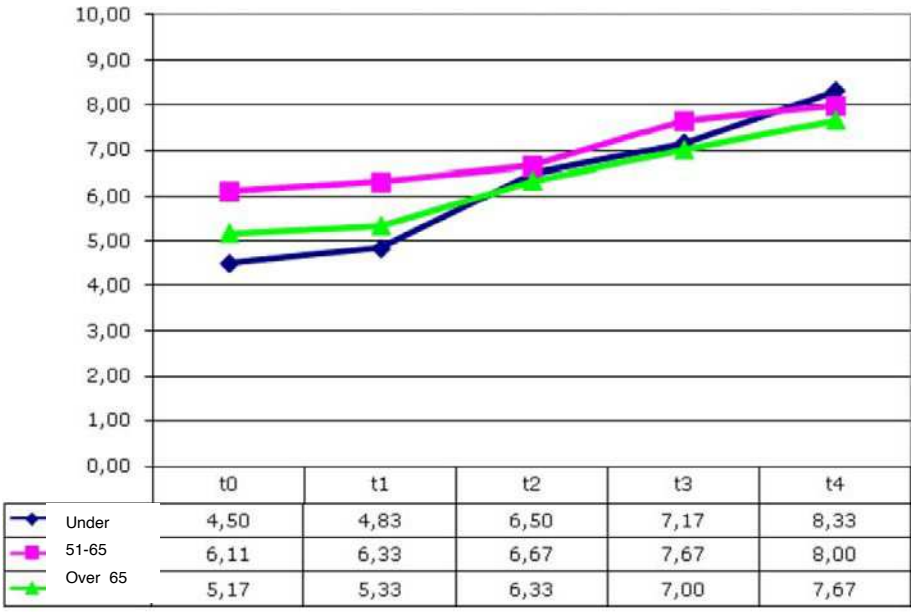
- Under 50
- 51 - 65
- Over 65

The results relating to joint mobility showed no type of moderation due to age: all three groups of participants significantly improved from t_0 to t_4, but no differences could be seen between age groups during this improvement (fig. 15).

As regards the level of patient pain, no interaction effect was seen. As previously said, the perceived pain tended to drop as time passed (and with treatments), but in a linear way and not according to patient age (fig. 16).

Finally, the analyses performed on the degree of satisfaction of the participants, according to age, produced the same results as above: satisfaction increased significantly from the first to the last checkup, without however any statistically significant differences between the three ages brackets considered (fig. 17).

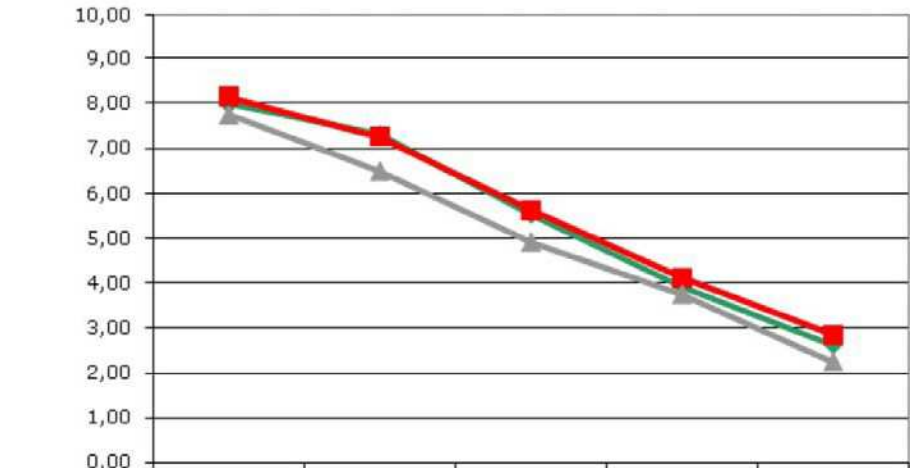
Figure 15 – Longitudinal analysis of level of joint mobility according to age bracket



	t0	t1	t2	t3	t4
Under	4,50	4,83	6,50	7,17	8,33
51-65	6,11	6,33	6,67	7,67	8,00
Over 65	5,17	5,33	6,33	7,00	7,67

Note: $F(8, 32)=.69$, *n.s.*

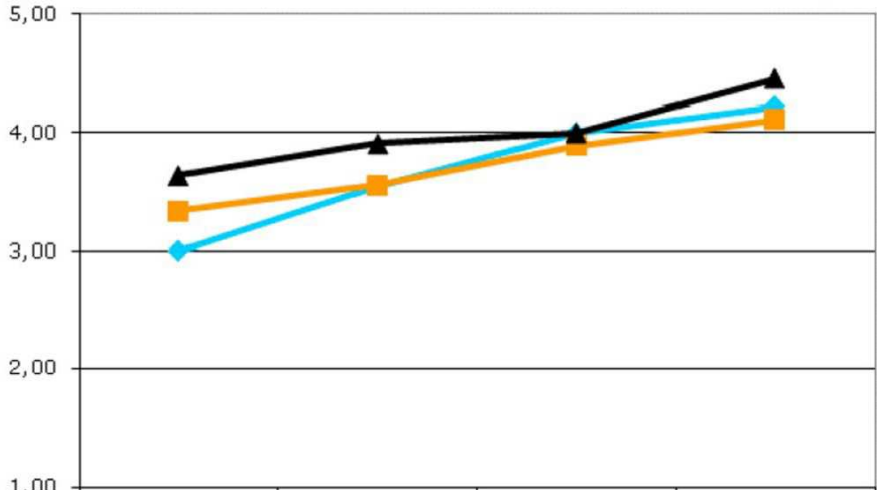
Figure 16 – Longitudinal analyses as regards level of perceived pain according to age bracket



	t0	t1	t2	t3	t4
fino a 50	8,00	7,31	5,54	3,92	2,62
51-65	8,16	7,26	5,63	4,11	2,84
più di 65	7,75	6,50	4,92	3,75	2,25

Note: $F(8, 78)=.74$, *n.s.*

Figure 17 – Longitudinal analyses as regards degree of satisfaction according to age bracket



	t1	t2	t3	t4
Under 50	3,00	3,56	4,00	4,22
51-65	3,33	3,56	3,89	4,11
Over 65	3,64	3,91	4,00	4,45

Note: $F(6, 68)=1.24$, *n.s.*

Statistical considerations

The collected data appear positive, although a possible distorsion must be taken into consideration in the longitudinal analyses due to a number of missing data: it has not always been possible to obtain data relating to all the surveys made, an element that translates into a reduction of the sample when treated with repeated measurement analyses. At the same time, the non-normality of the distribution of the indices relating to the presence of inflammations and/or oedema does not permit processing the data with parametric statistics and, consequently, analyzing in any depth the moderation effects investigated in the previous paragraphs.

Nevertheless, if we focus on the results obtained, experimentation would seem to produce a series of significant changes. Firstly, the analyses performed on the general sample show a reduction of oedema and inflammation in patients, and above all a clear and substantial improvement at joint mobility and perceived pain level. If, with respect to the presence of inflammation and/or oedema, the change is relatively small (also considering the already non-compromised initial picture in patients at the time of the first visit), the same cannot be said for the other indices: the degree of joint mobility goes from insufficient to good and the pain, initially perceived as very high, is strongly reduced within a period of 4 weeks until it is very small indeed. At the same time, as can be expected, the degree of satisfaction of the patients increased significantly between the 1st and the 4th checkup.

The results described thus far are also reconfirmed in the subsequent analyses, aimed at checking the presence of any moderation effects on the experimental treatment due to the type of disorder treated or to demographic variables (gender and age). The positive trends as regards mobility, pain and satisfaction also appear in each sub-group, but no interaction effect has been found with the above-mentioned variables. Nevertheless, this is not a negative or worrying result: the absence of interactions between the improvements found and the clinical or demographic variables enables us to imagine that the efficacy of the experimental treatment is transversal and separate from other elements.

Clinical considerations

The size of the sample (148 cases studied) appears enough to express valid considerations at statistical level. The treated cases all refer to disorders of orthopaedic interest. It must nevertheless be realized that, within these, there is a certain disproportion between bone and joint disorders (45.9%) and injuries(43.2%) and the other two (surgical results of orthopaedic disorders and bone and joint disorders) which, together, fail to reach 12%. Nevertheless, the consistency of the collected data minimizes this disproportion even though it suggests the need to extend research in terms of numbers.

The group nevertheless appears balanced in terms of gender even though, as was to be expected, women prevail in accordance with the epidemiology of the treated disorders.

The average age is 55.8, but with a large interval that goes to show the method can be applied to practically any age.

Fig. 3 shows the clinical-functional situation at the time of enrolment.

While on the one hand there are signs of reduced inflammation and oedema, on the other there is a considerable impairment of joint mobility (5.18/10) and very high perceived pain (7.96/10).

These data are very important because, when the time factor is assessed (figures 3- 4 - 5 and 6), a clear improvement can be seen of the most compromised parameters (pain and joint mobility) and a modest reduction of oedema and signs of phlogosis, scarcely present at t_0. This is an indirector/ pointer to the consistency of the collected data.

In particular, as regards joint mobility, it must be emphasized that the improvement achieved during treatment is of particular interest both because of the extent of the improvement itself and because this is a particulary precise and objective indicator (mobility was measured according to degree of joint ROM).

As regards perceived pain, the pattern was particularly satisfactory. The global figure shows 7.96 as initial value, which drops drastically to 2.64 at the end of treatment. This is a huge reduction for an aspect which represents the main outcome in this type of treatment.

Fig. 8, which represents the pattern of the **degree of patient satisfaction**, confirms that of pain inasmuch as it increases as pain decreases.

For the reasons mentioned before, the comparative analysis of the treated disorders has been restricted to the two most representative in terms of numbers. A comparison was therefore made between tissue injuries and bone and joint disorders. The figures 9, 10 and 11 show that there are no statistically significant differences as regards data relating to joint mobility, perceived pain and degree of satisfaction. Efficacy therefore appears equally represented in the disorders taken into consideration. The figures 12, 13 and 14 show the longitudinal analysis of the same parameters according to **gender**. In no case do significant differences appear between men and women even though fig. 14, relating to the degree of satisfaction, shows a slightly better inclination in this sense on the part of women.

The figures 15, 16 and 17 show the longitudinal analysis relating to the parameters considered in relation to the **patient's age**. The brackets into which the patients have been split are youthful-adult (under 50), adult (51 - 65) and elderly (over 65).

In this case as well, the figures do not show significant differences between the various age brackets considered. It must however be underlined that the youthful bracket shows a slightly more positive pattern compared to the other age brackets. This result is particularly interesting because it bucks the trend with respect to personal satisfaction analyses which always see youngsters a little "less pleased" than their older counterparts.

Conclusions

From an analysis of the above data, the following considerations can be made:

1. Therapy with low intensity magnetic fields, as was to be expected, is without side effects. In all the examined cases, no adverse effects were found.
2. Assessments, including analytic, showed better than expected results: no worsening and statistically significant improvements as regards the main outcomes: pain, joint mobility and patient satisfaction.
3. The positive results were achieved without any differences regarding gender and age: this strengthens the idea that, when clinical indications are precise, treatment can be safely recommended.
4. An extension of case studies could lead to sounder statistical data as regards indications other than those already assessed (post-injury disorders, sports injuries, etc.).

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