Index

page 5  HILT – High Intensity Laser Therapy

page 9  Cytoproliferative activity of the HILT

page 21  High Intensity Laser Therapy in arthrosis

page 33  High Intensity Laser Therapy in the treatment of gonarthrosis

page 47  High Intensity Laser Therapy (HILT)

page 57  HILT vs TENS and NSAIDs
HILT – High Intensity Laser Therapy

THE UNIQUE PHYSICAL THERAPY FOR THE TREATMENT OF DJD AND OSTEOARTHRITIS.

Conventional Lasertherapy has been present in Europe since more than 25 years. More than 2000 scientific publications testify its effectiveness and the validity of this approach. It has been demonstrate moreover that it is not toxic and it has no side effects. For all these reasons it is currently used as a monotherapy or as a complementary therapy.

Up to now, conventional Lasertherapy is applied through devices featuring low or medium power, with interesting results. Yet it does not allow to treat deep seated pathologies, since it does not permit to deliver the necessary high doses of energy to deep layers without inducing thermal damage to tissues. For this reason traditional Lasertherapy can be applied with success only on superficial pathologies. Moreover, treatment times are moderately long.

Today, thanks to the revolutionary patented HILT Therapy, it is possible to treat also deeper disorders, since HILT features the power and the energy which are necessary to treat all the deep seated inflammatory conditions, and not only the superficial disorders. Moreover HILT is not toxic and can be performed without damaging the tissues surrounding the pathology.

HILT therefore is the sole therapeutic method which allows to treat safely
all the inflammatory states, also if located in depth, inducing - since the very first application – a strong reduction or of pain, together with the recovery of the mobility. This beneficial effect can last from 4 up to 72 hours after the first application. After some sessions the complete disappearance of pain and the complete mobility recovery can be achieved.

HILT is revolutionizing the therapeutic approach of orthopedic MD, physiotherapists, chiropractors, sport medicine experts etc, since its main indications are:

- DJD (Degenerative Joint Disorders) and Osteoarthritis
- Chondropathies
- Deep Musculoskeletal Disorders

**Principle of Action**

HILT bases its effectiveness on a particular and characteristic high peak power Laser pulse, featuring peculiar frequencies and pulse width. This Laser emission was carefully and clinically tested and found effective for all the above mentioned pathologies. Thanks to its features it is able to deliver in depth the correct effective dose of energy, without being toxic on the area of incidence and for the deep tissues it is able to reach.

Thanks to the high peak power of the pulses it exploits, HILT is able to produce also a very strong photomechanical effect: real pressure waves which propagate inside the tissues and act directly on the lymph draining pump, performing their action on the inflammatory process, even if chronic, featuring the capability of stimulating collagen and ialine cartilage regeneration. Thanks to the photomechanical effect HILT is able to produce a fast resorption of the liquids leaked because of trauma or inflammation.
The verticalization of energy

This way of energy delivery is a true “verticalization” of energy: an high amount of energy is delivered in a very short time to a great volume of suffering tissue. The traditional way of energy delivery which requires long time of emission in order to transfer the same amount of total energy, can be regarded instead as “horizontal”.

The vertical way of delivering energy is completely safe when compared to the old horizontal one, which heats up the tissues and runs the risk of damaging it. Moreover it is more effective, treating greater volumes of tissues and at same time.

The Scientific Research

HILT is the result of a long path of Scientific Research, co-ordinated by the efforts of a prestigious teamwork of scientists. The main biomedical and clinical results which allowed to validate HILT as a new therapeutic technique will be presented in the following pages of this report.
Cytoproliferative activity of the HILT: in vitro investigation

**Introduction**

For some time now the use of pulsed Nd:YAG Laser has been spreading in the therapy of pain with excellent results. Studies exist which describe the anti-inflammatory, anti-oedemigenic and antalgic effects of Nd:YAG Laser, thus justifying its use in the therapy of pain.

With the exception of the study by Repice et al., no bibliographical references exist indicating the cytoproliferative effect of Nd:YAG Laser in order to justify its use in reparative therapy.

On the contrary, several authors report the cytoinhibiting effect of Nd:YAG Laser. More specifically, Sroka (1999) describes having obtained a mitotic increase with Laser at 410, 635 and 805 nm while he excludes this with the Nd:YAG Laser.

In our study we have assessed the stimulating capacity of the cytoproliferative effect of Nd:YAG Laser in vitro.

We used two different cell lines for investigating the cellular proliferative response to the variation in the dosimetry and for verifying the specificity of this response of a mitotic increase with the same parameters but by varying the cell line. With the use of a molecule inhibiting the tyrosine-kinasic metabolic pathway (isoflavone genistein) the metabolic interactions of the Laser radiation with one of the main metabolic pathways assigned to the cellular proliferation were assessed.
**Materials and Methods**

*Cell lines, culture mediums and growth conditions*

We used two cell lines in this study: HCT-8 tumoural cells (human ileocecal adenocarcinoma) and VERO cells (derivates of renal cells of the African green monkey of the Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia, Brescia, Italy).

As a culture medium for the HCT-8 line we used RPMI 1640 (Gibco – BRL, Grand Island, NY) containing 10% of bovine foetal serum (SFB, Eurobio), 1% of sodium piruvate 1mM/l of, glutamine 2mM (Gibco – BRL), and 5% of a mixture of Penicillin-Streptomycin-Fungizone.

As a culture medium for the VERO we used Eagle’s MEM containing 10% of SFB, 1% of sodium piruvate, glutamine and 5% of the antibiotic-antimicotic mixture.

Both the cell lines were cultivated in unventilated 75 mm² flasks until confluence in thermostat at 37°C. After trypsinisation of the monolayer, the cells of both lines were dispensed into the wells of a microtitre slide for ELISA. The quantity of cells per well was determined in such a way as to obtain the confluence within 24 hours in incubation conditions at a temperature of 37°C and an atmosphere containing CO₂ (5%). In order to avoid the diffusion of the Laser beam into the adjacent wells the treatment of each well containing the inoculum was surrounded by eight wells containing trypan blue at 0.4%.

*Source of irradiation and irradiation conditions*

As a Laser source we used a pulsed Nd:YAG Laser device (pw: pulsed wave), 1064 nm, average power 6 Watts (El.En. S.p.A., Calenzano, FI, Italy), with a 0.19 cm² spot. The handpiece was fitted with a spacer in order to guarantee a lens-well distance allowing the spot to assume a diameter capable of ensuring the exact coverage of the irradiated well. To protect the cells from radiation scattering each well sown was surrounded by eight wells containing a trypan blue (0.4%) solution (Grossman, 1998).

The wells were irradiated 6 h after being sown, by replacing the culture
medium with sterile PBS sterile.
We performed the assessments by maintaining the energetic contents of each pulse constant: 150 mJ, while we varied the repetition frequency (5 – 40 Hz) of the pulses in one second and the irradiation time (4 – 20 sec) of the well.
After the treatment the new culture medium replaced the PBS in the wells and the slides were then further incubated for another 10 hours, for up to a total of 16 hours of incubation after the sowing.
The control cells were instead irradiated with an ineffective Laser called a “sham Laser”.
Each test was repeated five times.

**Proliferation parameters**

**SPECTROPHOTOMETRY**

At the sixteenth hour, the culture medium was replaced with 200µl of new medium added to 50µl of solution containing 3mg/ml of MTT (Sigma, Italy) in PBS. After an additional 4 hours of incubation under the same conditions, the solution contained in the wells was replaced with 200µl of dimethyl sulfoxide (DMSO, Sigma) to which 25µl of a solution of 0.1 M of glicine and 0.1 M of NaCl with a pH of 10.5 were added. The slide was then immediately read with a spectrophotometer operating at a wavelength of 450nm.

Each slide was then irradiated at specific values after which all the values of the spectrophotometric readings were expressed in O.D. (Optical Density) which indirectly indicates the density of the vital cells of the monostratum.

They were then compared with the average values of the non-radiated control cells or irradiated with the sham Laser.

**IMMUNOHISTOCHEMISTRY (IHC)**

“Chamberslide” (Bibby, Sterilin) slides were used for the immunohistochemistry tests, sowing and later irradiating each well in the same manner as described for the ELISA slides. The groups of treated and control cells were prepared in the same way as described for the spectropho-
tometric examination for both the VERO and the HCT-8 line. Once the treatment was over, the culture medium was removed from the wells and the monostrata fixed with methanol for 10', followed by three rapid lavages in PBS. The monostrata were then incubated for one night with the following primary monoclonal antibodies: specific antibody for antigen Ki67, clone MIB-1, (DAKO) diluted at 1:50 in PBS+BSA, anti-PCNA antibody (Novocastra) diluted at 1:50, anti-Cyclin D1 antibody (Santa Cruz) 1:100, anti-ILGF-1 antibody (Santa Cruz) at 1:200. After three 10’ washings in PBS, the monostrata were incubated for 15’ with a secondary antibody (horse-anti mouse biotinylate, 1:250 in PBS+BSA), then washed again three times in PBS and incubated for another 45’ with the Avidin-Biotin (ABC, Vector).

Finally the immunohistochemical reaction was visualised via incubation with the chromogen substrata, represented by diaminobenzidine (brown dye) for the PCNA and the ILGF-1, by Nova Red (red dye) for the Cyclin D1 and Vector Red (crimson dye) for the Ki67. In order to assess the expression level of the single antigens we proceeded with the reading of the preparations with a 440x magnifying optical microscope, directly counting the number of positive cells per field, in ten fields selected randomly. We then calculated the average of the values obtained and compared the averages of the irradiated monostrata with those of the control monostrata.

Treatment of the monostrata with genistein
In order to carry out an assessment of the tyrosine-kinasic metabolic pathway we used monostrata cultivated on microtitre slides. These were treated with a isoflavone genistein solution (Sigma, Italy) capable of inhibiting this metabolic pathway.

The monostrata were then treated with decreasing doses of genistein, with the addition to the culture medium of a 400 mMol solution from a concentration of 100ml which was cytotoxic, up to concentration of 50ml, which was instead cytostatic.

The dose with the cytostatic effect was identified by means of the use of
the spectrophotometric technique described above, and via the direct assessment of the mitotic index of the monostrata treated with the genistein and the controls. More specifically, these cells were treated first with Colcimit® in order to evidence the chromatids, lysed in hyperosmolar buffer, fixed in methanol, dyed with Giemsa, after which the count was performed of the number of metaphasic nuclei out of one thousand nuclei counted.

Once the dose inhibiting the cell cycle was identified, the monostrata were irradiated once again with the Nd:YAG Laser with the values identified during the first part of the study, that had demonstrated their ability to increase the mitotic index.

Statistical analysis
The average of the samples and the standard deviation were calculated for each experimental conditions, repeated five times. The results of the repeated experiments carried out for each cell line were added by percentage and compared with their controls. The onetail Student t-test was used for evaluating the differences between the controls and the treated group.

Results
The irradiation of the monostrata with the Nd:YAG Laser at specific frequency values, intensity and exposure time induces cell proliferation. For each test performed for both the HCT8 and the VERO we then compared the O.D. values deriving from the spectrophotometric reading of the irradiated wells with the O.D. values of the control wells.

We compared the average of the differences (each calculated on the five tests performed) between the two groups under investigation at the varying of the exposure time (sec.), frequency (Hz) and therefore, automatically the average power (Watts).

Out of the 120 tests conducted, 60 per cell line (divided into 12 “groups” of parameters with five tests per group), it emerged, as in figure 1, that
the comparison between the differences between the control and treated groups was statistically significant (indicated in the graph with the positive ordinate value) with medium-low frequencies and exposure times. In this respect it is to be noted that in this study we maintain the energetic content of each pulse constant (150 mJ) and varied only the frequency and irradiation duration. More specifically, for the HCT8, the window of optimal values turned out to be the one corresponding to a quantity of energy equal to 2.7 Joule, and average power of 2.25 Watts, a fluence of 14.2 J/cm², and intensity of 11.8 W/cm², a repetition frequency of the pulses in one second of 15 Hz for an exposure time equal to 12 seconds, while for the VERO the greatest proliferation was obtained with 2.4 Joules, 6 Watts of average power, 12.6 J/cm², 31.5 W/cm², and 40 Hz for 4 seconds (Figure 2). From this data it emerges that the value window identified for the HCT8 cells was ineffective for the VERO cells, even creating a cytostatic effect.

The “windows” with a cytoproflect action are characterised by high levels of nuclear expression of the antigens PCNA and Ki67, as well as by an elevated mitotic index. From the direct count of the nuclei expressing the antigens Ki67 and PCNA, statistically significant differences have come to light between the treated and control monostrata. In fact, in the monostrata subjected to irradiation with cytoproflect parameters (cyto-proliferative windows) mean expression values of the above-mentioned antigens were observed which were on an average double those shown in the control monostrata. The results appear to be in constant correlation with the mitotic index calculated on the two cell populations and assessed on average as double in the treated monostrata compared to the controls.

Cytostatic effect of the genistein and restoring the cell cycle after irradiation of the monostratum.

A cytotoxic effect was observed with a dose of 100 µl of genistein added to the medium culture, while 50 µl gave rise to an inhibiting effect of the cell cycle. 25 µl and 12.5 µl doses produced intermediate effects, not
totally inhibiting of the cell multiplication.
With 50 µl doses in fact, it was possible to observe the zeroing of the mitotic index as well as the absence of expression by the cells treated with antigens like the Ki67 and PCNA, Cyclin D1 and the growth factor ILGF-1. These results have only been obtained on the HCT8 cell line, seeing that for the VERO cell line it was not possible to identify a concentration of genistein which, when added to the culture medium, was capable of inducing a cytostatic effect free of partial or total damage to the monostratum (cytotoxic). The Laser irradiation of the culture with the cytoproliferative parameters after the cytoinhibiting effect by means of the isoflavone genistein, resulted in being capable of reactivating the cellular cycle despite the block operated by the isoflavone on the tyrosinkinasis.
This metabolic pick-up is quantifiable by means of the cell count in metaphase (newly assessable mitotic index), and also via cellular neo-expression of the Cyclin D1 and the ILGF1.

Figure 3 contains the graph describing the distribution of the mean values, evidenced by the linear for each group. From the graph it can be seen how the level of the mitotic index of the cells treated with genistein is clearly lower than that of the cells treated with the Nd:YAG.

**Discussion**

The results obtained with the spectrophotometer (indirect assessment) and with the immunohistochemistry (direct assessment) indicate the capacity of the Nd:YAG Laser to induce the proliferation of the HCT-8 and the VERO at specific frequency values, exposure times, pulse shape.

The different reaction of the monostrata even with very slight changes in the irradiation parameters indicate how there is an elevated specificity between dosimeter and effect; in fact we have observed how the administration of similar energy quantities (Joule), obtained by varying the
frequency (Hz) and the exposure time (sec) are able to supply biological effects which at times are diametrically opposite. This indicates that more important than the quantity of energy transferred to the system (Joules), is the way in which this energy is supplied: frequency and exposure time.

The fact that there is a variation in the parameters that are cytostimulating with the varying of the cell line used, indicates a considerable specificity of the Laser action, depending strictly not only on the quantity of energy supplied but also on the type of biological substratum on which it is used.

In fact by applying the cytostimulating dosimetric parameters to different cellular lines as indicated by other Authors ⁶ there is no increase in the mitotic index. Repice et al. ⁶ have described the biostimulating effect of the Nd:YAG Laser on human neuroblastoma cells using parameters that are very different from those used in this study. Other Authors ² ⁸ have even described a constant inhibiting effect of the Nd:YAG Laser on the cellular proliferation. More specifically, Stroka ⁸ reports of no cytoproliferative effect at all in the interval between 0 – 10 J/cm², which results in being very similar to our study (7.69 J/cm²). For this purpose, and reiterating what has been reported with regard to the specificity of the parameters and the extreme sensitivity of the cells to the Nd:YAG Laser we can state that in the light of our results, it is not sufficient to indicate the fluence for establishing the parameters of effectiveness of lack of effectiveness since it is necessary to identify an effectiveness window of energy supply values for every cellular substratum, and these parameters must refer to the specificity of the cellular line used.

In any case, on having confirmed the biostimulating effect of the Nd:YAG, we believe that this is capable of justifying its use in repair therapy as well as in pain therapy ³ ⁴ ⁵ ⁹. Despite being only preliminary, the results obtained with the double genistein-Laser radiation treatment of the HCT8 cell line indicate the possibility of unblocking the cellular cycle interrupted with the genistein by irradiating the cells.

In fact, even though the blocking of the cellular cycle by the genistein during the G0 phase (indicated by the inhibition of the expression of
the cycle markers Cyclin D1 -expressed by the cells that progress from the G1 phase to the S phase- Ki67 and PCNA -both expressed in the S, G2 and M phases- and by the zeroing of the mitotic index via selective inhibition of the tirosine-kinasik metabolic pathway) the exposition to opportune doses of Nd:YAG Laser radiation has allowed for restoring the cellular cycle. This element is particularly interesting if assessed within a therapeutic context. In fact, it is known that the homeostasis of the cartilagineous turnover is guaranteed by the balance of the catabolic factors (IL 1 beta, TNF alpha, IL 6, IL 8) and anabolic factors (ILGF-1, GH, TGF beta) that act through the same family of receptors (GH–Citokine) in the metabolic pathway of the tyrosine kinase. A competitive mechanism is therefore hypothesised in the substratum, for which in cases of prolonged stress, the metabolic factors are not able to use the metabolic pathway of the tyrosine kinase and the tissue proceeds towards the degenerative phenomenon. This study therefore sheds light on the capacity of Nd:YAG Laser to promote the restoring of the cellular cycle in spite of the selective block operated on the tyrosine kinase.

Conclusions

This study also demonstrates that like other lasers, the Nd:YAG Laser possesses the biostimulating capacities even though there is an extremely high sensitivity of the in vitro cells to the variations in dosimetric parameters (mJ, sec, Hz).

The most striking element arising from this study is that in order to induce the cytoproliferative effect, the manner in which this energy is supplied (frequency and exposure time) seems more important than the dose. Moreover, this radiation seems capable of reactivating the metabolic pathway of the tyrosine kinase on which a pharmacological block is activated; this element could explain why the degenerated tissues in which this metabolic pathway is blocked are able to recover the anabolic phase and re-equilibrate their homeostatic balance.

We believe further investigations are necessary for confirming our obser-
vations, above all in the aim of identifying the cytoproliferative parameters of other cell lines, especially the primary type. The acquisition of this data could in fact open up the field of Nd:YAG Laser use in both reparative and pain therapy.

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High Intensity Laser Therapy in arthrosis: experimental investigations on animal models

Introduction

Arthrosis is a disease with a high social impact as it affects 30-35% of the population. An arthritic patient costs the public health services approximately 4,000 euro on average a year, touching peaks of almost double this amount in the most severe cases.

Conventional therapy foresees the administration of anti-inflammatories, antalgics, and decontraction agents. The current trend is to use chondro-protective drugs with often encouraging results.

The international bibliography provides results that are often contrasting with regard to the clinical effectiveness of the Low Level Laser Therapy (LLLT) in the treatment of arthritic and rheumatic complaints, and some even express negative opinions while others are positive.

Over the last few years the High Intensity Laser Therapy (HILT) has been making its mark with excellent results in sports traumatology and pain therapy, for this reason we decided to assess the possibility of also transferring this method to the cure of arthritic ailments and therefore prepared an animal model with an arthritic pathology in line with the indications of the various Authors.

The majority of studies conducted over the last thirty years in Laser therapy have been carried out with medium and low intensity Laser devices (Low Level Laser Therapy: LLLT), with wavelengths in the infrared and near infrared (600 - 900 nm). Within this spectrum the Laser beam is partially absorbed by the natural chromophores, like melanin, which withhold part of the energy irradiated.

Our study on the other hand is based on the use of a Nd:YAG High Int-
tensity Therapy (HILT) Laser, characterised by a wavelength (1064 nm) that allows it to penetrate and spread more easily through the tissue due to not having an endogenous chromophore. Moreover, with the pulsed wave Nd:YAG it is possible to deliver power peaks of up to 1000 Watt for times of 200µ seconds: extremely elevated peak intensity (W/cm²) in very brief times. Such a high intensity in such a short time prevents the heat accumulation by the tissues as happens with the use of Nd:YAG with constant emission (Parra 29, 30). This all extrinsicates in a greater spreading capacity of the Laser beam through the tissues with a very low histolesive risk.

In other words, quantities of energy (Joules) and fluence (J/cm²) are delivered in the HILT that are not dissimilar to the ones delivered with the LLLT but there is an intensity (power density: W/cm²) of even up to 1000 times higher.

The objective of this study was to assess the safety of intensity Laser at various power intensities (10, 30, 50 and 80 W/cm²) used on the superficial and deep structures.

The secondary objective was that of verifying the biological effects in vivo of the three different types of Laser: CO₂, Diode, Nd:YAG; and more specifically, we assessed the antalgic 32, antinflammatory 33 and cytoproliferative 34 effects of the Laser.

Materials and methods

Lasers used
Three types of Laser were used: CO₂ (10.600 nm), Nd:YAG pulsed wave (1.064 nm), Diode (830 nm) produced by El.En. S.p.A. (Calenzano - Florence). Table 1 shows the powers used by the three lasers assessed.

Investigation population
According to Bentley 3 the ideal animal model for the study of arthrosis should have the following characteristics:
- the presence of precocious lesions and action mechanisms similar to

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Average intensity used (watts)</th>
<th>Spot area (cm²)</th>
<th>Power density (W/cm²)</th>
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<tr>
<td>Laser DIODE</td>
<td>830</td>
<td>1</td>
<td>0.03</td>
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</table>

Table 1. Types of lasers and dosimetric Parameters used.
those described for the human pathology;
- evidence of an initial loss of the cartilaginous matrix and subsequent appearance of fissures, fibrillations and erosions;
- the cartilaginous lesions must therefore be followed by sclerosis of the subchondrial bone;
- the alterations described must be readily reproducible and identifiable in the living animal;
- the induction method of the lesions must be valid for different animal species and articular sites and free from systemic effects.

In this study we chose chickens of the heavy breed, bred with the open range system to allow ample possibility for deambulation. This species was preferred over others as it has a bipedal gait similar to man, ample articulations capable of supported heavy loads and an elevated basal metabolism that allowed us to obtain chronic degenerative lesions in relatively brief times, a good-natured disposition making it easy to treat with Laser. Moreover, it expresses a range of cytokins and chemokins that can be compared to those of humans.

Table 2 illustrates the breakdown into groups of the population investigated.

Investigation protocol
The investigation was performed in compliance with the Helsinki Declaration and the International Standards governing research on animals. The chronic degenerative arthrosic phenomenon was induced via double inoculation in the lower right limb of each subject with Freund’s Complete Adjuvant (FCA) + formaldehyde at 10%.

The inoculations were administered at one-month intervals. Eight months after the second infiltration the Laser therapy was commenced. Following is a list of the activities in chronological order with the specific examinations performed:
A) acquisition of subjects;
B) one month’s growth;
C) 1st inoculation with FCA;

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<td>CO₂</td>
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</tbody>
</table>

Table 2. Breakdown of the subjects in the investigation groups
D) one month’s growth;
E) 2\textsuperscript{nd} inoculation with FCA;
F) growth after 8 months;
G) beginning of Laser therapy:
- T/0 => on all subjects: Rx, clinical evaluation of the lameness, weight, blood tests; bioptic analysis on 8 controls (after euthanasia with general anaesthetic).
- T/1 => beginning of Laser therapy.
- T/2 (3 weeks after T/1) => end of Laser treatment: in all 15 Laser sessions were performed spread over three weeks.
- T/3 (2 weeks after T/2) => on all the remaining subjects: Rx, clinical evaluation of the lameness weight, blood tests; bioptic analysis (after euthanasia with general anaesthetic).

Assessments carried out:
- X-rays in both lat-lateral and antero-posterior of both limbs of each subject.
- Serological analyses (ELISA) for: PCR, IL 1 beta, ILGF 1, TGF beta.
- Macroscopic examination via photographic acquisition.
- Microscopic examination: histological and immunohistochemical examination (IHC): histological staining with hematoxilin-eosin, Herovici polychrome solution and Alcian PAS blue.
- In IHC we performed assessments for: Type II Collagen, ILGF 1, MMP1, TIMP2.

The stainings with Herovici were performed in order to highlight the presence of protocollagen (pale blue) as a demonstration of the age of the cartilage: the protocollagen precedes the formation of collagen. The synthesis activity of the mucopolysaccharidic matrix was instead assessed via the Alcian PAS blue.

The data collected were entered onto an electronic spreadsheet and analysed statistically with the t-Test.
Results

Anti-inflammatory effect
The graph in fig. 1 illustrates the mean of each subject treated with Laser with a comparison between the Controls and the Healthy subjects.

Neochondrogenic effect
The neochondrogenic effect was documented histologically and immunohistochemically (IHC). Figures 2 and 3 show the histological images referring respectively to a Control (fig. 2) and a subject treated with Nd:YAG at 50 W/cm² (fig. 3).

Fig. 2 shows the almost completely dextruded cartilage with partial covering of the subchondral bone tissue where in fact the haversian systems can be observed. In fig. 3 instead, there is neoformed cartilage structured according to the physiological architecture on the subchondral bone tissue; basal globiform isogen groups can be recognised which on moving towards the surface tend to arrange themselves parallel to the articular surfaces.

Discussion

From an analysis of the graph in fig. 1 it is apparent that all the types of Laser used have carried out an anti-inflammatory effect (see the curve of the IL 1beta).

As far as the historegenerative effect on the articular cartilage is concerned however, we observed a different effect between the different types of Laser. The CO₂ Laser offered less biostimulation.

The diode Laser offered greater stimulation compared to the CO₂ but failed to induce the synthesis of very active isogen groups which were however very dishomogeneous in shape and distribution. Moreover, the immunohistochemical examination of the Type II collagen indicated that it was fibrocartilage.

It is a completely different situation with the Nd:YAG Laser which proved...
Table 3. Histological evaluations and IHC per group; classification with 4 degrees of merit: starting from the lowest we have: -, +, ++, +++

<table>
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<tr>
<th>Morphology</th>
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<th>TIMP2</th>
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Having tried various power intensities we were able to observe a linear trend between the therapeutic response and the dose supplied. In fact at 10 W/cm² we observed the presence of the activation threshold with the proliferation of the basal isogen groups, at 30 W/cm² homogeneity was observed in both shape and spatial distribution of the isogen groups, at 50 W/cm² we identified the most effective dose for stimulating the physiologically structured hyaline cartilage, while at 80 W/cm² we observed tissular regression above all on the surface, and the lack of chondrocyte action of the Type II collagen (table 3).

The curve of the ILGF-1, MMP1 and TIMP2 were particularly interesting in immunohistochemistry. As far as the IGF-1 is concerned, an expression was observed in the CO₂ which was comparable to that of the CTR group. The degree of expression of the growth factor with the Diode Laser was not dissimilar to that of the CO₂. With regard to the degree of expression in the subjects treated with the Nd:YAG Laser, this faithfully reflects the expression of the Type II collagen with a better expression than subjects treated with 50 W/cm².

The trend of the MMP1 and TIMP2 in immunohistochemistry was also very interesting. In this case a sharp difference was observed between the MMP1 and TIMP2 in the CTR, which was less marked with the CO₂, diode, and Nd:YAG at 30 W/cm², whereas it was highly significant with the Nd:YAG at 50 W/cm². Obviously this different expression of the MMP1 and the TIMP2 has opposed trend between the CTR and the Nd:YAG group at 50 W/cm². In fact, in the CTR we obtained a high value of MMP1 and a low value of TIMP2, while in the Nd:YAG at 50 W/cm² these were exactly the opposite.

Conclusion

From this study it has emerged, in primis, that the High Intensity Laser Therapy, when administered at suitable doses, is safe in the treatment of articular pathologies and does not induce lesions to the surface and...
deep structures.
This study indicates that the Laser is capable of antagonising the experimentally induced athrosic phenomenon to stimulate the neochondrogenic activity with the formation of hyaline cartilage and to induce sinovial hyperplasia.
These effects are closely linked to the dose supplied. More specifically, we varied the intensity (power density: W/cm$^2$) and maintained constant energy (Joules) and fluence (energy density: J/cm$^2$).
It was therefore observed that the low intensity only has an anti-inflammatory effect while the high intensities have a neochondrogenic and sinovial hyperplastic effect as well as the anti-inflammatory effect.
As this was a pilot study we believe that further investigation and confirmation are indispensable. We are also of the opinion that it would be important to perform verifications on spontaneous arthrosic pathologies in animals.

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High Intensity Laser Therapy in the treatment of gonarthrosis: 
the first clinical cases and the protocol for a multicentric, randomised, double-blind study

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Arthrosis: state of the art

Cartilage possesses scarce reparative capacities and until several years ago spontaneous or therapeutic repair of an articular lesion was not considered possible. At the present time the most common cartilaginous pathology, Arthrosis (osteoarthritis) is the focus of great interest on a worldwide level and has become the “new frontier” not only for orthopaedics but also for rheumatology and rehabilitation, to which a great deal of energy and resources are dedicated.

Arthrosis is certainly the disease with the greatest increase in the number of cases in the western world in consideration of the general aging of the population.

The social and economic role of arthrosis is therefore potentially very high. Numerous drugs have been proposed for the therapy of arthrosis including:
- the new FANS (selective inhibitors of the COX 2),
- basic drugs: DMOADs (Disease Modifying Osteoarthritis Drugs) better known as chondroprotectors, theoretically capable of intervening in both the destructive and reparative process of the disease, which include galactosamineglucuronoglican sulphate, diacereine, jaluronic acid.

The real effectiveness of the DMOADs still has to be demonstrated and the clinical impression is that these molecules represent the forerunners of a new generation of drugs.

Orthopaedics have developed a series of reparative surgical interventions of great interest aimed principally at knee-cartilage reconstruction.
The techniques are divided into two groups: bone marrow stimulation techniques and tissue transplant techniques. Worth noting among the marrow stimulation techniques are chondroabrasions, perforations and microfractures. These methods tend to stimulate the subchondrial bone and fill the cartilaginous lesions with fibrin coagula, rich in totipotent stem cells. These techniques give rise to the formation of fibrocartilaginous tissue (Type I collagen) with scarce mechanical capacities. These interventions are currently reserved for lesions of less than $2 \text{ cm}^2$, and are generally performed in arthroscopy, in one single diagnostic-surgical session.

Far more interesting are the tissular transplant techniques (homologous transplants, multiple or mosaicoplastic autologous transplants, autologous transplants of periostal flaps, autologous chondrocyte transplants), which aim at reconstructing the physiological hyaline cartilage (Type II collagen), with good mechanical capacities. Amongst these techniques the implanting of autologous chondrocytes (ACI) has been particularly successful. This method consists essentially of arthroscopic extraction of the chondrocytary cells from areas not subjected to stress and transplanting of the same in the athrosic lesions. This is carried out in 4 stages:

1) arthroscopic extraction of the cells;
2) creating of cell cultures in highly specialised laboratories
3) mounting of biomaterials deriving from the collagen
4) transplanting of the neo-tissue in the lesion.

These methods have opened futuristic scenarios which are already partially in progress. Mesenchymal cells deriving from the bone marrow and futuristic three-dimensional biomaterial deriving from hyaluronic acid (non-material materials) are already being tested in laboratories and animal models. These new surgical frontiers must not distract us however from the globality of the arthrosis problem.

**Contra-indications to transplants**

The presence of significant axial deviations (greater than normal varus or valgus knee greater than the norm of $5^\circ$) is considered as a mechanical
imbalance capable of compromising the positive results of the transplant; all deviations should be corrected in a preliminary manner. At the same time the absence of the meniscus due to previous menisectomies is considered as a potentially unfavourable situation for transplants. The simultaneous presence of multiple cartilaginous lesions calls for a careful assessment of the suitability of resorting to chondrocytary implanting or similar techniques.

More general conditions like overweight and an advanced age are other factors considered as very important in subjecting the patient to reparative surgery of the cartilage.

We only considered the patient’s age since, as Pellaci states, if all these selective criteria were to be complied with, in practice, only very few patients would be proposed for this type of surgical treatment.

In international literature it is advised against performing transplants in patients over 55.

In actual fact, the age of the patients subjected to surgery is always low: at the recent convention of the Gruppo Italiano di Studio dei Processi Riparativi del Tessuto Osteo-Cartilagineo (G.I.R.C. – Italian Study Group of Reparative Processes of Osteo-Cartilaginous Tissue at Ischia 20-22 September 2001) the mean age of transplants resulted in being 34 years, with a minimum of 15 and a maximum of 40 (see table 1).

If we consider the wide range of the population over 55 we are able to realise how a univocal answer to arthrosis cannot be found in surgery alone (see fig. 1).

High Intensity Laser Therapy (HILT)

Over the last ten years numerous studies have been carried out indicating the biostimulating action of MID lasers. In particular, lasers have been accredited with the power to accelerate the healing of skin ulcers and bedsores. The Laser devices used until now have been low intensity with a wavelength of 600-900 nm, corresponding to the near infrared. Within this spectrum the Laser beam can be absorbed by the

<table>
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Table 1. Average age of clinical cases subjected to chondrocyte implant. From 1st G.I.R.C. convention Ischia 20-22 Sept. 2001
natural chromophores like melanin for example.
The $\text{CO}_2$ Laser represented the introduction of high intensity Laser in the medical field. Unfortunately, due to its wavelength (10,600 nm) it is completely absorbed by the water resulting in an extremely scarce penetration of the tissues.

Its action is prevalently analgesic, acting on the sensitive cutaneous nerve endings. We have recently analysed the physical properties of high intensity Nd:YAG Laser that has a wavelength of 1064 nm. At this frequency the Laser beam is easily diffused throughout the tissues. More specifically, the cartilaginous and bone tissues turn out to be excellent targets for this type of radiation. In the past this Laser was applied with continuous emission and given its high intensity there was a considerable increase in the heat produced with histolesive risks. This aspect obviously prevented its use. Approximately three years ago we developed a new Laser with pulsed wave emission capable of supplying high intensities without inducing heat effects and without causing cellular damage. We have tried to evaluate whether the Nd:YAG Laser had the same trophic effects in depth at the articular level that the MID lasers had already demonstrated to possess on superficial skin tissues. The results of the experimental studies performed on animal models and the in-vitro cellular studies carried out throughout 2000 and 2001, have demonstrated the possibility of stimulating the formation of hyaline cartilage by means of Laser stimulation. This fact has led us to consider the feasibility of clinical experimentation in humans.

Preliminary Clinical Study

For six months we have been carrying out a preliminary study at the Servizio di Recupero e Rieducazione Funzionale (Recovery and Functional Re-education Service) of the Rizzoli Institute of Orthopaedics, in the aim of exploring the investigation methods and therapeutic parameters most suitable for performing a double blind experimentation.
10 patients have been selected (mean age 50 years, min. 41 years, max. 65 years, 5 females, 2 males) affected with primitive arthrosis.

**Clinical tests**
The clinical tests considered most suitable were as follows.

The W.O.M.A.C. (Western Ontario and Mc Master Universities Index), consisting of a clinical functional test specifically for osteoarthrosis (the W.O.M.A.C. is the only test among those used in an international context to have been validated for Italy). It is easy to implement and explores both the functional attitude of the arthrosic knee and the patient’s daily activity.

The IKDC test is a functional test of the knee consisting of a section with the patient’s subjective assessment of his/her own conditions in relation to his/her daily and/or sporting activities, and a clinical and objective assessment by the physician. It is extremely valid in the event of the patient having been subjected to arthroscopy or being a candidate for reparative knee surgery, for which he/she has not been considered in the final assessment of the pilot study.

V.A.S. (*Visual Analogic Scale*) is the traditional scale for a quantitative assessment of pain which consists of a simple test with easy acquisition and comparability.

As laboratory analyses the following classical phlogistic tests were implemented: ESR, PCR, $\alpha_1$glycoprotein, as well as several Interleukin and ChemiKine assays like: IL1$\beta$, IGF 1, IL8 and RANTES, as an expression of the metabolic activity of the articular environment affected by arthrosis.

**Instrumental diagnostic tests**
As an initial sidetracking from the arthrosis we performed a conventional X-ray of the knee in an antero-posterior position under stress, after which we classified the lesions using the Ahlbach’s guide.

The patients classified under grades II and III were then subjected to a nuclear magnetic resonance.
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<td>I</td>
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<tr>
<td>II</td>
<td>Obliteration of the femurotibial space (&gt;50%)</td>
</tr>
<tr>
<td>III</td>
<td>Moderate bone wearing away (&lt;7 mm.)</td>
</tr>
<tr>
<td>IV</td>
<td>Significant bone wearing away (&gt;7 mm.)</td>
</tr>
<tr>
<td>V</td>
<td>Significant bone wearing away with articular sub-dislocation</td>
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*Magnetic resonance*
A last generation MR device with 1.5 T was used. Where possible we compared photographic images of the arthritic lesions obtained with arthroscopies, with the images obtained from the various MR sequences.
From the numerous tests conducted, the most suitable weights for defining the arthritic alterations were the sequences in T2 and the SPGR suppressed fat performed according to sagittal and coronal planes. Subsequently, it was considered opportune to use the three-dimensional methods for volumetric acquisitions.

*Ultrasonography*
The most suitable optical “windows” of the patients in the preliminary study were assessed via ultrasonography in order to allow for the diffusion of the Nd:YAG Laser.
These windows resulted in being the internal and external hemi-rima of the knee bent to 90° for the anterior chondyles and the internal and external hemi-rima of the knee in the popliteus hollow at maximum extension for the posterior chondyles. In order to access the posterior face of the patella the best lateral and middle windows appeared with the knee bent to 30°.

*Therapeutic protocol*
As a therapeutic protocol a total of 2500 m Joule were delivered in pulsed waves with manual scansion with a last generation Nd:YAG Laser with an average intensity of 6 W. The treatment was carried out once a day...
for 15 days over a period of three weeks (excluding holidays). After three months the entire therapeutic cycle was repeated. At the beginning (T0) and at the end (T1) of the first cycle, and likewise at the beginning (T2) and at the end (T3) of the 2nd cycle of treatment the algo-functional assays (W.O.M.A.C. and V.A.S). and the laboratory tests: ESR, PCR, 1α-glycoprotein, IL 1β, IL 8, h-RANTES and IGF-1 were carried out.

**State of progress**

Seven out of the 10 patients selected completed all the clinical instrumental tests envisaged.

**Clinical tests**
The clinical tests VAS and WOMAC evidenced a constant improvement of both the algic and functional symptomatology. At the end of the second Laser cycle the patients showed a reduction in pain equal to 51% (V.A.S.) and a reduction in the functional limitation (WOMAC) equal to 49%.

**Lab tests**
The classical phlogosis assays showed negligible variations with values always recorded within the normal range or very close to the same. Nevertheless it is worth pointing out how the trend of the ESR and PCR values, as well as the alpha 1 glycoprotein values appeared to be substantially similar. The values tended to rise after each Laser cycle and then return to the basic values again. The Nd:YAG Laser seems to have altered the quiescence of the articular environment. The scarce specificity of these classical indexes do not however allow us to understand further the metabolic alterations of the cartilaginous tissue. The data obtained from the Lymphokin and ChemoKin assays appears far more significant. As regards the IL 1β, which is the expression of the chondrolytic and pro-inflammatory activity at the level of the cartilaginous tissue, a constant diminishing trend from T0 to T3 has been
observed. This datum is confirmed by the results obtained by the IGF-1, a growth and replicative activity factor of the chondrocytes. The trend of the IGF-1 in fact, has a slope which is opposed to the IL 1β, with a growth in values from T0 to T3. The analysis of the h-RANTES ChemoKines and the IL 8, having a chemoactive and activating action of the neutrophyls, has highlighted a decreasing trend from T0 to T3, which confirms the inhibiting action of the phlogosis factors by the Nd:YAG Laser.

Magnetic resonance
Via the MR the aim was to monitorise the morphological variations of the arthrosis knee treated with the Nd:YAG Laser. We initially tried to quantify any variations in the thickness of the incrustation cartilage of the femurotibial articulation.

The measurement of the thickness of the cartilaginous mantles was problematic with the bidimensional technique used due to the presence of numerous artefacts caused by the oedema often present in the surface strata of the cartilage.

It was easier to measure the cartilaginous ulcer. The images acquired in two dimensions of an arthrosis ulcer before and after treatment with Nd:YAG Laser were processed electronically and compared by measuring the maximum diameter and the surfaces.

In the case described a marked reduction was noted in the diameter and extension of the area affected by the ulcer.

We have judged these images with caution in view of the difficulties involved in reproducing with exactness the positioning of the limb examined. Nevertheless, the patient in question recorded a pronounced improvement in his clinical conditions with regard to both pain and functionality of the knee in question.

At the bidimensional MR follow-up six months after the treatment corresponding to two months after the end of the 2nd cycle, four out of the seven cases currently completed showed improvement and three were unvaried. The most evident improvements were in the reduction of the trabecular bone oedema and the cartilage, and in one case in the reduc-
In the aim of obtaining a more reliable and reproducible volumetric measurement of the cartilaginous lesions we considered it necessary to carry out a three-dimensional acquisition by means of the MR, in order to be able to measure with reliability, also in the control group, the region of interest according to the greatest axis of development. As a whole, the magnetic resonance, despite its limits, seems to be the only technique capable of documenting the structural modifications of the cartilage.

Arthroscopy is not a viable method of screening in a clinical experimental trial for both ethical and technical reasons. The ethical reasons are those related to an excessive invasiveness of the examination compared to the benefits and consequently it can not be considered as a basic investigation to be carried out on patients enrolling in a double blind trial. The technical reasons that limit the use of the arthroscopy are linked to the fact that the acquisition of a photographic image depends in a specific manner on prospective factors that are difficult to reproduce in a second trial. Yet arthroscopy is still useful to biopsic collection and therefore to assess the quality of cartilage. Considering that, we believe that it should be performed in few cases, after randomization of patients. The study is still in progress even if we have a clinical protocol which appears effective, and we are reassured by the first results achieved. They indicate a clinical and functional improvement for every treated patient.

Clinical study on patient

Moving from the experimental research and from the data of the preliminary study, we propose to perform a wider clinical study, in order to assess the chances of Nd:YAG Laser for the treatment of arthrosis. We want now to begin a multicentric study which will allow us to col-
Select a good number of cases with a double blind design. To do that we asked for the collaboration of the “Fondazione Don Carlo Gnocchi, Santa Maria agli Ulivi di Pozzolatico (Fl)”, and of “CONI” - Bologna of the “Servizio di Radiologia dell’Ospedale Nuovo di Imola (BO)”. We will select from these operative units 100 patients affected by tibial-femoral arthrosis, or patellar-femoral arthrosis, age range 12-65. The selection method will be based on radiography assessment, since there is no reason to use a division based on arthroscopy. Radiography will be performed along the antero-posterior axis, under loading. Only patients affected by II and III stage arthrosis (which correspond to cartilage lesions showing a reduction of the joint space greater than 50%, and a mild bone wear, < 7 mm, respectively), following the Ahlback classification, will be admitted to treatment.

These patients will then undergo Magnetic Resonance with specific “weights” for the joint cartilage in order to confirm the presence and better assess the arthrosic lesions. Images collected through this method will be then digitally elaborated in order to describe and, possibly, measure the qualitative and quantitative modifications of bone and cartilage components.

Then patients will undergo to clinical tests:
- W.O.M.A.C.: the Western Ontario and Mc Master Universities Index, functional clinical test specific for osteoarthritis
- 2000 IKDC, test for subjective functionality assessment by the patient and objective assessment by the clinician.

V.A.S. quantitative scale for the assessment of pain Lab tests:
- to assess possible metabolic alterations
- VES, PCR, (1-glycoprotein, IL 1ß, IL 8, IGF-1, TGFß, h-RANTES)

Patients will be randomly assigned to 2 groups.
- A => will undergo a minimum power He-Ne Laser treatment (1mW)
- B => will undergo Nd:YAG Laser treatment daily for 21 dd.
Laser devices will be provided with their own software which will assign randomly patients to treatment A or treatment B. After six months treatment (A or B) will be repeated. Every patients will be administered with a chondroprotective drug (galattoglucoronglycan sulfate800 mg/day). Clinical tests will be repeated at the beginning and at the end of each of the two cycles. At the end, after twelve months from the beginning Magnetic Resonance will be repeated and the images collected will be compared with the previous one.

All patients will be assessed again after 12 months through the lab tests and by Magnetic Resonance again 10 patients, randomly chosen, will be assessed through arthroscopic biopsic collections.

Therapeutic protocol

As far as the therapeutic protocol is concerned, Nd:YAG Laser will emit in pulse mode, with an average power approximatively equal to 9 W. The total energy, 3000 J, will be divided in this way: 500 J antero-lateral windows; 500 J antero-medial window; 500 J posterior-lateral window; 500 J posterior-medial window, 500 J medial patella; 500 J lateral patella, according to the individuated optical windows.

Performing this multicentric study we want to achieve important information about the clinical outcome after Nd:YAG Laser treatment, about the metabolic modifications of the treated osteoarthritis, and about the modification of the anatomic and pathologic conditions of the osteoarthritis treated lesions.

If the results achieved in vitro and on animal model were confirmed also on patients, interesting sceneries would open in osteoarthritis treatment, which would gain a new approach to improve effectively the quality of life.
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High Intensity Laser Therapy (HILT): state of the art in sporting traumatology and pain therapy

Research activities in the therapeutic and rehabilitative sector have always pursued the goal of maximum effectiveness and minimum invasiveness in the surgical, pharmacological and instrumental fields. This has led to the developing of a wide variety of electromedical devices, especially in the physical therapy sector, but over the years these have unfortunately resulted in being of no or very little use. The main causes of the scarce effectiveness of instrumental therapy are to be found in the inadequacy of the old concept of instruments and the lack of serious clinical and laboratory research. There are in fact, various sources of energy used and physico-biological interactions exploited, often in the absence of suitable experimentation or a real therapeutic rationale.

The musculo-tendinous and minor articular pathology represents an extremely frequent event in numerous sporting disciplines. Due to being invalidating, even after a certain amount of time, this calls for a timely, correct and equally effective therapeutic intervention. On the other hand, the continual expansion of the playing of sports in increasingly wider circles of the population (especially over the last decade), apart from reaching a number of between 12 and 14 million players of sport, has also created a new series of problems for health operators in this sector. These range from accident-prevention to the rapid recovery of the person involved, whose temporary invalidity, apart from personal damage, also gives rise to significant effects on the labour force, with considerable social implications.

It is not the aim of this report to make an in-depth examination of this phenomenon.
In order to provide a correct picture better of this type of specific traumatology, it is worth pointing out that in the past there has been a great deal of research by specialists in this sector for outlining a pathological cause-and-effect profile, with particular emphasis on chronic lesions, that are now known by the term “athlopathies”, almost as though identifying the prevalent or exclusive aetiology of the athletic gesture.

Lately there has been the tendency of not considering sports pathologies as any “different” from those generally found in people who do not practice sports.

Over recent years the increased interest shown in the various sporting disciplines has forced health operators in this sector to use new therapeutic methods capable of accelerating the healing process of the different pathologies, with the resulting reduction in recovery times for the athlete. Included in this context is the work of Laser experimentation carried out by our team at the CONI FMSI Institute of Sports Medicine of Bologna.

Laser is a source of coherent electromagnetic radiation and is the acronym of Light Amplification by Stimulated Emission of Radiation. It therefore defines a physical means that produces energy under the form of a light wave following a stimulated emission of radiation. A Laser device is fundamentally system formed by three elements: a) the active means, b) the activation source, c) the optic resonator.1

a) the active means consists of solid, liquid or gaseous material which, when suitably stimulated, emits a radiation; it is responsible for the wavelength of the emission

b) the activation source, indispensable for triggering the reaction, supplies the active material that allows for the emission of the radiation

c) the optic resonator consists of a system of mirrors that allows for amplifying the electromagnetic waves of the Laser light
The parameters that have to be taken into consideration for defining the physical characteristics of the Laser are:
1) the wavelength,
2) the intensity,
3) the emission mode.

The various types of lasers used in the treatment of the different pathologies of the locomotor system, are defined according to the active means, the wavelength and the intensity of the emission (soft-Laser, midlaser and power-Laser), of which the gallium arsenide and helio-neon are absolutely the most widespread and studied.

The semiconductor Laser is a solid Laser: the most common is the gallium arsenide Laser which emits in the infrared with average powers in the range of the mW: it is therefore equipped with good penetration but scarce power.

The Helio-Neon is a gas Laser that emits red light 632.8 nm in the visible spectrum with powers that vary from between 1 and 50 mW: it therefore has extremely low power and scarce penetration.

In literature there are many works on the effects of the soft and mid-lasers and the results of these studies are very contrasting.

For this purpose the study of Heleen Beckerman et al. is mentioned, in which she has grouped together and meta-analysed the literature on Laser in physical therapy, arriving at the conclusion that the methodologically most correct and comprehensive studies reported positive effects, without however underestimating the validity of several studies that instead denied the therapeutic effectiveness of lasers. As mentioned previously, the limits of Laser therapy up until several years ago were above all due to the low tissular penetration power and scarce intensity, in other words the scarce in-depth therapeutic effect.

Recently more widespread use has been made in physical therapy of high intensity Laser devices of surgical derivation, like the CO\textsubscript{2} and Neodymium YAG (Nd:YAG) lasers.

The CO\textsubscript{2} Laser is a gas Laser whose active material is carbon dioxide and it produced an invisible light in the far infrared with a lambda of 10600 nm and a high intensity: its radiation is also absorbed by water and...
therefore it has a scarce penetration power. The Nd:YAG Laser is a solid lade whose active means is a yttrium-aluminium-granate crystal drugged with neodymium that emits light with a wavelength of 1064 nm with a good penetration power. Up until some time ago these devices were lacking in manageability as due to the elevated heat effect they were capable of causing tissular damage. The last generation Laser devices offer certain technical features that distinguish them in a substantial manner from the previous versions. More specifically, the Laser we used in this study was a last generation Nd:YAG Laser which combines penetration power with a high peak intensity and energy density. Being a Laser light with an electromagnetic wave that passes through a dys-homogeneous means (like the biological substrata) it is important to keep in mind the general features of the passage of light through tissues. The electromagnetic wave in part passes through the tissues unmodified thanks to the “transmission” phenomenon which is observed with greater prevalence in the red and infrared due to the scarce cellular “absorption” at these wavelengths, and is in part “diffused” due to the heterogeneity of the tissues, in both a back-scattering direction and via a simple change in direction of the “deviation” radius.

The interaction between the non-ionised electromagnetic radiation (Laser) and the biological tissues is generally determined by physical processes that govern the granting of energy by the radiation at the substrate and by the biological response of the tissues themselves. The intensity of the biological reactions of the tissues radiated will therefore depend on the characteristics of the tissue that is able to absorb, transmit or reflect the energy, on the wavelength, on the power density and on the emission mode. There are a multitude of hypotheses regarding the real interactions of the Laser radiation with the cellular substrate: the most accredited postulate the “biostimulant” or “catalysed Laser reaction” effect that would result in either the stimulation or inhibition of the biochemical, physiological and proliferating activities. In fact, for sometime now it has been well-known that the cells are sensitive to specific wavelengths.
The in-vitro cells (culture) communicate by means of ionic messengers, thus generating electromagnetic energy and influencing metabolic and catabolic processes. Under conditions of imbalance or pathologies, the energetic status of the cells modifies just as they alter as a result of intercellular communication processes. The Laser radiation determines an increase in the “energetic status”, activating the repair mechanisms and overcoming the damaging ones. One of the most accredited theories is that of the photochemical effect for which the absorption of the Laser radiation takes place thanks to specific chromophores identified in enzymes, cellular membranes and/or other intra and extra-cellular substances whose activation or inactivation seems to be responsible for the main Laser effects, that is, the antalgic, anti-phlogistic, anti-oedemigenic and biostimulating action.

The extent of these effects seems to be in relation to the wavelength, dosage and emission mode of the Laser radiation. The wavelength depends on the active means used, is found between 180 and 10,600 nm, and influences the penetration power of the ray: the ultraviolet rays (200-350 nm) are absorbed by the proteins and the nucleic acids; the frequencies of the visible spectrum included between 400 and 750 nm are absorbed by the melanin and by the tetrapyrrolic compounds; for the frequencies found between 800 and 1400 nm (therefore in the near infrared) there is the so-called “therapeutic window” in which the Laser radiations are not absorbed electively by a specific elements and as a result they have a greater penetration power.

The dosage is responsible for the extent of the effect and the volumetric tissuta involvement. As far as the power (watts) of the various lasers is concerned, it is necessary to make a distinction between the peak power (maximum emission power for each pulse) and the average power, recorded at the tip in one second.

It is therefore necessary to compare the irradiated surface with the output (power density in watt/cm²) or with the energy released (energy density in J/cm²).

As far as the emission mode is concerned the Laser may be continuous, pulsed (repeated pulses at more or less high frequencies), flash or
Q-switched (brief emissions at high peak intensity).
The pulsed emission represents an additional possibility for modulating the Laser effects, as demonstrated by the studies of Coche. In fact, different frequencies and pulsations have different effects on the substrate, in particular, with equal lambda and powers, the lower the frequency the greater the interaction with the conduction structures and vice-versa.
Irrespective of the origin, be it direct trauma, functional overload, and/or acute or chronic evolution, in the majority of these affections the symptoms of “pain” and “functio lesa” dominate the clinical picture and the three following pathogenic events are evident as the common denominator:
1. acute or chronic phlogosis;
2. micro and/or macro-circulatory alterations;
3. lesions of the fibres and connective tissue.

On the basis of the type of pathology treated, the method and doses employed, Laser radiation seems capable of acting by raising the threshold of the perception of pain via the direct action on by stimulating the releasing of endorphins “in loco” and in the liquor. Moreover, induced active Laser hyperaemia and macrophagic activation, reducing the ischemia and local stasis of the algogenic substances, would seem to exclude other possible causes of the onset of pain and inflammation.

The reintegration of the cellular membrane potential seems finally to contribute towards the interruption of the contractive–vasoconstrictive–pain triad and the resolution of the inflammation. As far as the tissular lesion is concerned, various experimental results have demonstrated the regenerative biological stimulus determined by Laser radiation.

On the basis of the above pulsed Nd:Yag Laser has been used over a period of seven years at the department of Instrumental Physical Therapy and Rehabilitation of the Institute, both for the purposes of research and experimentation, and also for treatment of over 1500 patients.
The equipment used was an Nd:YAG Laser supplied in collaboration with the company DEKA MELA of Calenzano (FI) which emits coherent light with a wavelength of 1064 nm with a peak intensity of 750 W, an
adjustable emission frequency of between 10 and 40 Hz, adjustable pulse energy between 30 and 150 mJ and an adjustable average power of between 0.3 and 6 Watts. In this study we present a selected case history (405 cases with an age of between 11 and 73 years (mean age 37.5) obtained by gathering together various groups from different investigation works. The objective is that of offering a representative overview of the various most-frequently observed clinical and anatomo-pathological situations (table 1). A protocol was applied to each subject treated, standardised according to the type, intensity and extension of the pathological process in progress, and broken down into daily sessions with a maximum of 12 and a minimum of 5, with an average of 10 sessions per case.

The densities of the power administered varied from between 8.7 and 9.5 W/cm² for 7 sec. of punctiform applications and between 13.7 and 15.8 W/cm² for 40/60 seconds of the manual scanner application adapting the amount of energy administered on the basis of the somatic characteristics of the subject in relation to the irradiation area.

The assessment of the subjects was performed according to the clinical analysis before and after the application with the Laser, using objective and subjective assessment tests.

In 88% of the cases, in order to obtain confirmation of the diagnosis post-clinically, the assessment was also performed via imaging with instrumental diagnostic methods: echotomography, C.A.T. scans or N.M.R., X-rays and isokinetic ergometry.

We used the clinical evaluation criteria described to group the results of the cases treated on the basis of the results of the therapy with the following criteria:

- EXCELLENT, with the disappearance of the painful symptomatology and any functional and/or articulation deficits and a rapid resuming of the activity;
- FAIR, with a moderate regression of the symptomatology and a reduction and/or disappearance of the deficits and resuming of the activity;
- NEGATIVE, with scarce or no variation in the symptomatology after the treatment.
Each subject was assessed a second time at least 10 days after the end of the treatment without the onset of any relevant negative modifications of the clinical situation present at the end of therapy test. It is evident from the results shown in table 2 that the treatment with HILT is highly successful with regard to the clinical-symptomatological situation. We wish to stress however one fact that merges from the NMR and/or X-ray, and ultrasound assessment of the patient, and namely, that in a high percent of cases there was a surprising improvement in the anatomo-pathological situation especially in the phlogistical alterations and the chondral lesions. This only further reaffirms the postulated metabolic stimulation effects of high intensity Laser.

### Table 2.

<table>
<thead>
<tr>
<th>Symptomatological Regression with Disappearance or Reduction of any Functional Deficit</th>
<th>Attenuation of the Symptomatology and/or Functional Deficit</th>
<th>Persisting of the Symptomatology and/or Functional Deficit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>Fair</td>
<td>Negative</td>
</tr>
<tr>
<td>294 subjects (72.6%)</td>
<td>60 subjects (14.8%)</td>
<td>51 subjects (12.6%)</td>
</tr>
</tbody>
</table>

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HILT vs TENS and NSAIDs:

A clinical study on low back pain from herniated inter-vertebral disk

Introduction

The term back pain is used to indicate a clinical situation characterised by pain of the lumbar rachis that may irradiate to the buttocks or lower limbs.

This is a very common disorder: from 50 to 80 % of adults suffer from at least one episode of back pain during their lives. In the United States it represents the primary cause of time off work; in epidemiological research carried out between 1984 and 1985, 14% of employees under the age of 45 lost one or two days off work for this reason. The economical consequences from back pain in the United States fluctuate from between 16 billion and 50 billion dollars a year. According to the National Center for Health Statistics, the direct costs of back pain pathologies amount to $12,922,740,000 a year and indirect costs come to $ 2,950,020,000 .

One of the causes of back pain is hemiated intervertebral disk. This consists of a protrusion of the intervertebral disk into the bone marrow canal or the protrusion of a fragment of discal tissue outside the boundaries of the disk, with the consequent compression of the nervous roots. This can be observed with greater frequency in patients of between 30 and 50 years with a ratio of 2:1 of males and females. The last two lumbar disks are the seat of the hernia in 90% of cases.

The natural history of the herniated intervertebral disk foresees a possible reduction in volume over several months: more voluminous hernias, and migrated or expelled hernias have a greater tendency to diminish in volume while this only occurs in 40% of contained hernias. The
mechanisms via which this occurs are macrophagical phagocytosis and dehydration of the herniate tissue. This evolution explains why the majority of back pain cases clear up spontaneously after several months, and also why it is opportune for the first-choice treatment to be of the conservative type in the majority of cases. According to the North American Spine Society, in fact, in 70% of patients the pain is reduced or disappears with the conservative treatment. This type of treatment is recommended in the acute and subacute forms, as well as in the chronic forms.

Surgical treatment (mini-invasive or open section) must be reserved for cases that are resistant against conservative therapy or those which manifest motor deficiencies.

Within the field of conservative treatments the most common are drugs and physical therapy. The guidelines of the North American Spine Society (NASS) in 2000 recommend NSAIDs among the first in both the acute and chronic phases, while the use of TENS is recommended among the various physical forms. Nevertheless, for some time now the use of Laser therapy has become widespread. In fact, its anti-inflammatory, analgesic, and antiedemigenous effects are well-known. Moreover, trials conducted in vitro by Repice have demonstrated its neurotrophic powers as well. Finally, in bibliography trials have been conducted by Toshew and Miriutova which confirm the efficacy of Laser therapy in the case of back pain.

Our intention in this study was that of comparing three different therapeutic methods in symptomatological treatment of back pain from herniated intervertebral disk, testing their efficacy over time.

We compared NSAIDs (ketoprofen), with power Laser HILT and an electrotherapeutical method (TENS).

The trial was conducted in compliance with the Geneva Convention and the Helsinki Treaty. In particular, patients were required to give their written informed consent.
Materials and methods

Study population:
we selected 60 patients affected by symptomatic L4-L5 and L5-S1 EDD.
The patients, who were all in the sub-acute phase, already showed symptoms from 1 – 6 months.
They were randomised into three groups of 20 patients each and every group was subjected to a different type of therapy:
- HILT;
- TENS;
- NSAIDs.

Therapies adopted:
HILT: Nd:YAG pulsed wave (pw), average power of 6 W, peak power of 1,000 W.
TENS: frequency 100 Hz, spike width 100 µsec.
NSAIDs: Ketoprofen.

Inclusive and exclusive criteria:
Patients who failed to give their informed consent were excluded from the study.
All patients were assessed with algo/functional clinical tests: Backill Test and VAS. The Backill measuring scale is an instrument that measures the severity of the symptomatology; it targets the rachis and assesses the pain and disability within the activity field of daily life. The score varies from 9 (severe painful symptomatology and severe disability) to 44 (absence of pain and full personal autonomy).
The VAS is an analogical-visual test which assesses the painful symptomatology. The score varies between an interval of 0 (absence of pain) and 20 (maximum pain imaginable).
We carried out controls (follow-ups) according to the following pattern:
T/1: at the end of the treatment lasting 15 days;
T/2: 45 days after T/0 (T/0: date of beginning treatment);
T/3: after 180 days.
Therapeutic protocols:
HILT group: we carried out the treatment in manual scansion of the para-vertebral region of the lumbar rachis at the speed of 1 cm a second. Table 1 illustrates the values used in the therapeutic treatment. The therapeutic cycle foresaw one session a day for a total of 10 sessions distributed over two weeks. The TENS was applied with the method with 4 electrodes applied to crossed fields in the region concerned. The therapeutic cycle foresaw 10 sessions (1/die) each lasting 30 minutes, distributed throughout 2 weeks. The patients in the NSAIDs group were administered Ketoprofen at a dose of 100 mg/die, via os B.I.D., for 15 days.

Statistical analysis:
All continuous data are expressed in terms of mean and standard deviation of the mean. One Way ANOVA and Repeated Measure T test were performed to test the means hypotheses of respectively different groups and the follow up of single measure. When the Levene test for homogeneity of variances was significant (p<0.05) the Kruskal Wallis test was performed. The Scheffe test and Mann Whitney test were conducted as post hoc to differences in study couples. p<0.05 was considered as significant for all tests. Statistical Analysis was performed by means of SPSS 7.5.

Results
In Table 2 it can be seen how the HILT, TENS and NSAIDs all increase in the values of the Backill scores from T/0 to T/1. At T/2 instead, there is a different trend between the HILT, TENS and NSAIDs, seeing that the first continues to increase its values while the other two groups show a drop in trend that even continues until T/3 and involves all three groups. From the statistical analysis at T/0 a significant statistical difference is evident.
Table 3. Statistical analysis with respect to the differences among Backill values at T/0, T/1, T/2 and T/3 in each group: T-test for paired data

Table 4. Statistical analysis among HILT, TENS and NSAIDs groups with respect to VAS scores at T/0, T/1, T/2 and T/3: One-way ANOVA and Kruskal Wallis (*)

Table 5. Statistical analysis with respect to the differences among VAS values at T/0, T/1, T/2 and T/3 in each group: T-test for paired data

Discussion

In accordance with the provisions in the NASS guidelines we decided to treat our patients suffering from herniated intervertebral disk with conservative and mini-invasive treatment. Studies exist in literature that demonstrate the clinical efficacy of NSAIDs in treating back pain. These drugs improve the clinical conditions of the patient with regard to their analgesic and anti-inflammatory effect. Unfortunately the analgesic and anti-inflammatory power of the drug is short-term and at times presents marked side effects with gastric bleeding of the mucosa and renal insufficiency in the elderly which means that it is necessary to limit its use to short periods (10-14 days).
The TENS has its rationale in Melzack and Wall's "Gate Control Theory" \(^{27}\), in other words the use of sensitive stimulations for modulating the perception of pain. According to the authors \(^{28,29}\), TENS is also responsible for the increase in endorachis levels of endorphins.

The NASS recommends the use in the acute phase while there is no proof of its efficacy in chronic back pain \(^{30}\).

Laser has been used in rheumatology for over twenty years with discordant results. Various types of lasers exist that differ with regard to their sources and radiation emitting powers.

In particular, other authors \(^{31,32,33,34}\) in the past have resorted to Laser therapy for treating back pain. In previous experience however, CO2, GaAs, and GaAlAs lasers have been applied, as well as Nd:YAG but only for points. In this study instead we decided to use a HILT in scansion and not for points. These pulses are characterised by an elevated energy content, very high peak powers (kWatt) and low repetition frequencies capable of allowing the tissue to cool between one pulse and the next.

The objective of the study was that of comparing three different methods and evaluating the clinical effects over time (15, 45, 180 days). From the analysis of the results it is clearly evident that the HILT induces a better clinical response when compared to the other two methods, especially as it persists over time. In fact, the graphs in fig. 1 and fig. 2, which respectively describe the trend of the functionality and pain, clearly demonstrate a highly significant difference for the Laser that lasts over time with respect to the other two methods. In particular, a constant increase in the values can be observed up to 45 days for the functionality (Backill), which stabilises in the following months (third follow-up 180 days); better functionality in the part corresponds to an increase in values. The test that quantifies the intensity of pain (VAS), instead shows a reverse trend with respect to the functionality test: more intense pain corresponds to higher values. The graph in fig. 2 shows that when compared to other therapies, the HILT has a continuous decreasing trend. At T/1 the drop is similar to the TENS and NSAIDs groups while at T/2 and T/3 the trend is the opposite. Also by observing the difference (increase/decrease) between the various follow-ups (T/1, T/2, T/3) and the
initial conditions (T/0), see tables 3 and 5, it emerges that the difference between the HILT therapy and the other methods turns out to be statistically significant in relation to the T/2 and T/3 follow-ups. At T/1, instead, there are no significant differences recorded which testify to the fact that all three methods induce comparable anti-inflammatory, analgesic and antiedemigenous effects.

An apparently irrelevant point which should be considered is the lack of homogeneity between the groups recorded at T/0 with the Backill test, a lack of homogeneity that did not appear with the VAS. In our opinion this datum is not important seeing that in this type of study the acquisition of the absolute value is not important, but rather the analysis of the data in a relative sense. On the other hand, the reference interval of the Backill test varies between 9 and 44; the average difference recorded at T/0 between patients treated with HILT and those treated with TENS and NSAIDs is approximately 8.5%, and this difference is not very evident from a clinical point of view, which means that it is possible to consider the three groups as similar to each other.

At T/3 instead, this difference is equal to 20%, a value that also begins to be manifest clinically.

**Conclusions**

From an analysis of the results it appears that THE HILT induces a better clinical effect with respect to the other two methods being compared, not so much during the first period where the results are superimposable, but over time. In our opinion these results are encouraging and pave the way for organising clinical studies on a wide scale aimed at confirming this data. The reasons for which the clinical effects of the HILT are evident over time are not yet known so it is to be hoped that research will be carried out that aims at analysing these effects.
Acknowledgments

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