

FOR EnergyHealth

International journal
of information and scientific culture

SPECIAL ISSUE

Proceedings of the International Meeting on Hilterapia® - Athens 2009

Volume 05

OFFICIAL REVIEW OF **ASACAMPUS**

Energy for Health

International journal of information and scientific culture

Editor in Chief

Luigi Corti

Dept. of Radiotherapy, Laser Center
I.O.V. – I.R.C.C.S. - Padova, Italy
e-mail: luigi.corti@unipd.it

Executive Editor

Monica Monici

ASAcampus, ASA Research Division
Dept. Of Clinical Physiopathology
University of Florence - Florence, Italy
e-mail: monica.monici@unifi.it monica.monici@asalaser.com

Editorial Board And Scientific Committee

Niels Bendsoe

Department of Dermatology,
Lund University Hospital,
Lund University Medical Laser Centre,
Lund, Sweden
e-mail: Niels.Bendsoe@med.lu.se

Giovanni Bottiroli

Institute of Molecular Genetics – CNR
Histochemistry and Cytometry
Pavia, Italy - e-mail: bottiroli@igm.cnr.it

Roberto Buda

Rizzoli Orthopaedic Institute
Bologna, Italy - e-mail: roberto.buda@ior.it

Antonio Conti

Medical Physics Section,
Department of Clinical Physiopathology
University of Florence - Florence, Italy
e-mail: antonio.conti@unifi.it

Michael I. Koukourakis

Department of Radiotherapy - Oncology
Democritus University of Thrace
Alexandroupolis, Greece
e-mail: targ@her.forthnet.gr

Leonardo Masotti

Dept. of Electronics and Telecommunications
University of Florence
Florence, Italy
e-mail: leonardo.masotti@unifi.it

Riccardo Pratesi

Dept. of Physics
University of Florence
Florence, Italy
e-mail: riccardo.pratesi@unifi.it

Prof. Raoul Saggini

Physical Medicine and Rehabilitation
Dept. of Basic and Applied Medical Science
University of Chieti
Chieti, Italy
e-mail: saggini@unich.it

Moshe Schaffer

Klinik und Poliklinik für Strahlentherapie
und Radioonkologie
Klinikum der LMU - Großhadern
München, Germany
e-mail: Moshe.Schaffer@med.uni-muenchen.de

Ioannis Skarlatos

Department of Radiotherapy – Oncology
Saint Savvas Oncologic Hospital
Athens, Greece
e-mail: skarl@ath.forthnet.gr

Katarina Svanberg

Lund University Medical Laser Centre
Division of Atomic Physics,
Lund Institute of Technology, Lund, Sweden
e-mail: Katarina.Svanberg@onk.lu.se

Mario Trelles

Inst. Med. Vilafortuny
Cambrils, Tarragona, Spain
e-mail: imv@laser-spain.com

Shimon Rochkind

Division of Peripheral Nerve Reconstruction
Tel Aviv Sourasky Medical Center
Tel Aviv University, Tel Aviv, Israel
e-mail: rochkind@zahav.net.il

Toshio Ohshiro

Japan Medical Laser Lab, Tokyo, Japan
e-mail : Ohgin@magical3.egg.or.jp

ENERGY FOR HEALTH - n.05/10

Six-monthly scientific journal - Authorized by Court of Vicenza Italy, authorization number 1145/07 - Managing Editor: **Dott. Luigi Corti**
Editor: **ASA srl** Arcugnano (VI) Italy - Design Consultant: **DYN'ART** communication & marketing - Print: **CENTROSTAMPA** Litografia Schio (VI) Italy

ENERGY FOR HEALTH © 2010

All rights reserved. Copying, printing and distributing the information published in this Journal, in part or in whole by any means, is prohibited without a written permission from the owner.

Foreword

This special issue of Energy for Health is dedicated to the International Meeting on Hilterapia®, held in Athens, Greece, on 10th, 2009.

The Meeting had a particular significance because it was the first time that a Meeting on Hilterapia® took place in a foreign country.

The city of Athens, which has been considered since ancient times the cradle of the western culture, was a wonderful frame for the event.

The scientific programme proposed very interesting communications and reports, presented by researches with great expertise in physiatry, orthopaedics, sport medicine, biology, physics.

In this 5th issue of Energy for Health there are papers related with the communications presented at the meeting. Three papers are related to theoretical and experimental

considerations about laser-tissue interactions and experimental results on cellular models. The other describe the results of clinical studies on the application of Hilterapia in the treatment of knee osteoarthritis and shoulder pain.

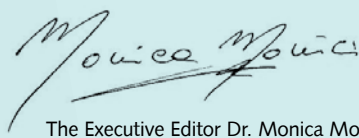
Finally, two interesting abstracts concerning frozen shoulder syndrome and the treatment of lymphoedema, respectively, are reported.

We want to thank all the participants, who enriched the meeting with their communications and discussing the results of their research, thereby contributing to the advancement of the knowledge in the field of laser application in medicine.

We hope you will enjoy the reading of this volume and you will find in it cues and inspiration for your future work.



The Editor in Chief Dr. Luigi Corti



The Executive Editor Dr. Monica Monici

Contents

4

Relationship between cellular and systemic effects of pulsed Nd:YAG laser.
Cialdai F., Monici M.

10

Laser-tissue interaction principles: beam penetration in tissues.
Romano G., Conti A., Fusi F.

12

The HILT domain by the pulse intensity fluence (pif) formula.
Fortuna D., Masotti L.

20

The challenge of shoulder pain.
Vissarakis G., Charamidis N.

26

“High intensity laser therapy in knee osteoarthritis: comparison between two different pulsed-laser treatment protocols”.
Viliani T., Martini C., Mangone G., Pasquetti P

32

Abstract
Hilterapia® and lymphoedema.
Nicolau V.

33

Frozen Shoulder Syndrome weighted comparison from “static & dynamic” treatment with Nd/YAG 10W pulsed laser.
Sicheri M.

35

Guide for Authors.

Key words: Hilterapia®, pulsed Nd:YAG laser, photomechanical effect, tissue repair, extracellular matrix

Relationship between cellular and systemic effects of pulsed Nd:YAG laser.

Cialdai F¹., Monici M².

1) ASAcampus Joint Laboratory, Dept. Clinical Physiopathology, Florence University, Florence, Italy.

2) ASAcampus Joint Laboratory, ASA Res. Div., Dept. Clinical Physiopathology, Florence University, Florence, Italy.

ABSTRACT

Notwithstanding the wide diffusion of lasertherapy in clinics and numerous studies reported in literature, molecular mechanisms of interaction between laser and tissues are not well understood.

The analysis of biological effects induced by laser radiation is rather complicated due to the wide possibility of setting instruments, the variability of applied protocols and the differences in treated tissues.

In this review, we describe our studies on the cellular and molecular mechanisms at the basis of the systemic effects produced by treatment with pulsed Nd:YAG laser, that is known as Hilterapia.

Starting from studies on photothermal effects, the hypothesis is that this type of laser cause an indirect photomechanical effect. The heat produced by transfer of radiation energy to the irradiated volume, diffuse into surrounding tissues, inducing temperature gradients which result in transitory modifications of mechanical-elastic properties of the extracellular microenvironment, thus changing mechanical forces acting on cells.

Considering these studies and knowing

the key role of the extracellular matrix, not only as a structural support but also in maintaining tissue homeostasis, our experiments focused on the analysis of extracellular matrix molecules and cytoskeleton behavior, responsible of contact between cell and matrix and considered the best candidate to act as a mechanotransducer.

The data obtained have shown, in laser-treated cells, an increase in production of ECM molecules, such as aggrecan, collagen I and II, and a reorganization of microtubules and actin microfilaments network. It is well known that similar effects are obtained when cells are subjected to mechanical stress. Our data on absorption of Nd:YAG pulses by matrix components (proteins and polysaccharides) suggest that Nd:YAG pulses principally interact with the extracellular matrix, whose transitory deformation applies a mechanical stress to the cells.

We then focused on the effect of pulsed Nd:YAG on endothelial function and tissue repair processes. In treated endothelial cells and fibroblasts, key elements of angiogenesis and tissue repair, we found

overexpression of genes involved in the chemokine-mediated inflammatory pathways. Moreover, the treatment promoted the formation of ordered endothelial monolayers as well as ordered fibronectin fibril assembling. The findings indicate that treatment with Nd:YAG pulses has a stimulatory effect in the acute phase of inflammation and significant effect on the remodelling phase of tissue repair, also considering the important role that fibronectin plays in tissue structure regeneration. Therefore we can support that Hilterapia can efficaciously promote tissue repair processes.

INTRODUCTION

In spite of a wide application in clinics, many studies and a great body of literature, the molecular mechanisms of the interaction between laser and tissues, and the consequent cellular response, are still not completely known. They are object of current and future research in the field of laser biomedical application.

Unfortunately, not always scientifically rigorous studies, a limited knowledge of the molecular and cellular mechanisms underlying the biological effects of laser and, in turn, the systemic effects of laser therapy gave rise to contrasting results, unsupported hypotheses and unconvincing theories.

The studies on laser biological effects are very difficult due to the variety of biological responses that depend on laser source (wavelength, continuous/pulsed mode), operative conditions (fluence, time of exposure, etc..) and biological substrate considered (the body area, the tissue, the cell type etc.). Nevertheless, they are of critical importance for correct clinical applications, to improve instruments and protocols, to increase therapeutical effectiveness.

When the light interacts with a biological tissue a small part of radiation (~3-5%) is specular reflected, the most part propagates within the tissue and it is partially diffused (scattering) and

partially absorbed by molecules which are generically indicated as "chromophores", because they are responsible for tissue colour. Each chromophore absorbs specific wavelengths. Each tissue is characterized by a different qualitative/quantitative chromophore composition, which determines the response of the tissue itself to different laser radiations (that means different wavelengths). The light energy absorption is necessary to allow any effect at tissue level [1].

When laser radiation propagates through a biological tissue, several effects may be observed. They depend on tissue properties, radiation source characteristics and a combination of both [2,3]. As above explained, the tissue properties significantly affect the amount of light that is absorbed and its propagation length inside the tissue itself [4]. The effects induced in a biological tissue by laser radiation are commonly classified in: photochemical, photothermal and photomechanical, or a combination of them. A suitable choice of laser source and setting parameters, on the basis of the knowledge of tissue characteristics, such as its optical, thermal and mechanical properties, allows to preferentially induce a particular effect, which dominates the others. In biomedicine this means to have an opportunity to select specific applications both for therapeutic and diagnostic purposes.

Photochemical effects take place when the absorbed light induces chemical reactions in the tissue. Photochemical interaction is more selective than photothermal or photomechanical interactions, because it is due to the absorption of radiation by specific chromophores, such as mitochondrial cytochromes involved in the respiratory chain [5].

In non-surgical laser applications, the photochemical effects have been, perhaps, the most studied, also due to the wide diffusion of He-Ne lasers (red wavelengths of the visible spectrum) and phototherapy, which is based on the

photochemical interaction between light and a specific chromophore, often an exogenous photosensitizer [6].

Photothermal effects occur when the energy associated with radiation is converted into heat. At tissue level, the results of photothermal interactions depend on the thermal relaxation time of the tissue itself and on the interaction time. Mechanical effects can be considered secondary effects of the photothermal interaction: heating induces mechanical forces which can modify cell morphology and tissue texture. These changes can also lead to tissue damage and even to tissue disruption, this is the case of surgical applications. Till now, photothermal interactions have been studied from the points of view concerning surgical application or laser security, in the former case to obtain tissue ablation, in the latter to avoid undesirable tissue damages. In physiatrics, rehabilitation and sport medicine, peripheral vasodilatation has been considered the principal biological response induced by photothermal interaction. This effect on peripheral circulation is surely important, but recent research has demonstrated that complex biological responses can be induced at molecular and cellular level by photothermal interaction and it is reductive to consider the photothermal effects only in terms of vasodilatation due to an increase in tissue temperature.

A turning point in considering photothermal interactions from a different point of view there has been with the introduction of very short (from femto- to nanoseconds) laser pulses for biotechnological applications (drug delivery, cellular and intracellular surgery) and with the application of pulsed lasers in medical fields other than surgery, such as physiotherapy, physiatrics, etc.....

Moreover, in the last years, also thanks to significant improvements in experimental technologies and analytical techniques, our knowledge on molecular and cellular mechanisms which regulate tissue

homeostasis, the interaction between cells and extracellular environment, the tissue repair processes occurring in damaged tissues has been strongly increasing. This opens the way to a better understanding of the molecular and cellular mechanisms underlying the biological response induced by laser-tissue interaction.

Here we review our studies on the cellular and molecular mechanisms at the basis of the systemic effects produced by treatment with pulsed Nd:YAG laser, that is known as Hilterapia.

PULSED ND:YAG LASER AND TISSUE REPAIR: MOLECULAR AND CELLULAR MECHANISMS

Our body is mainly composed by water (70%) and organic compounds (30 %), such as proteins, lipids, sugars, etc. Water highly absorbs infrared radiation (IR), among the organic compounds some chromophores (aminoacids, pyridine coenzymes, haemoglobin, melanin, etc.....) absorb in the UV-visible range. In the near infrared (NI) there is a range of wavelengths (800-1100), known as "therapeutic window" and characterized by low absorption. Therefore, in this interval, scattering strongly affects the propagation of radiation and photons can diffuse far from the point of incidence. The pulsed radiation emitted by Nd:YAG laser (emission wavelength 1064 nm) fits in this "window" and, also thanks to the high power of the pulses, can propagate in depth. Moreover, the duty cycle of pulsed Nd:YAG laser used for Hilterapia allows to obtain photothermal effects which can raise a biological response without tissue denaturation, damage or disruption.

In order to analyse photothermal effects and subsequent mechanical stress in biotissues, the interaction time and thermal relaxation of the tissue should be considered. If the laser radiation is delivered to the tissue in a very short time, in comparison with the thermal relaxation, the resulting heating is

confined in the volume of tissue in which laser light is absorbed and may induce direct photomechanical effects, generally with consequent tissue damage or disruption. For biological tissues, a thermal relaxation time of about 1 μ s is reported [2]. The duration of Nd:YAG pulses used in our experiments and also in clinical applications (Hiliterapia) is about 200 μ s, therefore it cannot induce direct photomechanical effect.

When pulse duration is longer than the relaxation time, as in the case we are considering, heat can diffuse in the surrounding tissues, inducing temperature gradients with predominant photothermal modifications and subsequent transitory mechanical deformations of the biological micro-environment. This kind of photomechanical stress can be considered a secondary effect of the heating and therefore defined "indirect photomechanical effect" [7].

The three-dimensional architecture of a biological tissue can be considered as a network of polymeric fibers, the extracellular matrix (ECM), in which cells are embedded. ECM is essentially constituted by polysaccharides and proteins. Collagens have a key role in ECM [8]: each tissue shows a different, often specific, qualitative/quantitative collagen content which strongly affects tissue properties and function.

For a long time, only structural functions have been ascribed to the ECM, merely considered the medium surrounding the cells. More recently, it has been demonstrated that ECM has a key role in tissue homeostasis and, beyond the structural functions, ECM has many other functions which concern the regulation of cell behaviour. Living cells are hard-wired by a continuous series of protein filaments and tubules, autoassembled to form a network, the cytoskeleton (CSK), which is anchored both to nuclear- and plasma-membrane. The anchoring points of CSK to the plasma membrane are constituted by clusters of membrane proteins, known as integrins, which on the inner side bind the CSK and on

the outer side bind ECM fibers or the membrane integrins of neighbouring cells, thus originating a continuous network, which constitutes the weft of the tissues [9]. In the tissues, the ECM undergoes continuous remodelling, in particular during development, angiogenesis, wound healing and all the tissue repair processes. The ECM acts as a reservoir of biochemical factors, i.e. cell growth factors, and most important, provides to the cells geometrical, topographical and mechanical constraints, which are relevant signals in the regulation of cell behaviour [10]. The ECM is produced by cells, with qualitative and quantitative characteristics which strongly depend on the signals the cells receive from the microenvironment. Therefore, through ECM remodelling, tissue properties continuously change and adapt in order to maintain homeostasis and optimize function [11].

DISCUSSION

In our earlier studies on the effects induced by pulsed Nd:YAG laser radiation in connective tissue cells (fibroblasts, chondrocytes and human mesenchymal stem cells (hMSC) able to differentiate through the osteoblastic and chondrocytic pathways) we found that, in comparison with non treated controls, the cells exposed to the treatment increased the production of ECM molecules, such as collagen I (+30%), collagen II (+90%), aggrecan (+70%) [12,13]. Moreover, particularly in hMSCs, we observed a strong increase in expression of genes involved in differentiation of cells belonging to specialized connective tissues (bone and cartilage), while the expression of genes involved in adipogenesis slightly decreased [14] (Fig. 1).

Because it is known that mechanical stress stimulates the production of ECM molecules, favours the osteogenesis and chondrogenesis and inhibits adipogenesis, in order to better understand the processes occurring in laser-treated cells, we repeated our experiments comparing three types of samples: non treated cells, cells exposed to pulsed Nd:YAG laser, cells exposed to loading. The results showed that the exposure to 73 sec of irradiation by pulsed Nd:YAG laser (1064 nm wavelength,

200 μ s pulse duration, 10 Hz repetition rate, 458.65 mJ/cm² energy fluence) for 3 consecutive days produced the same effects of exposure (5 cycles of 10 min each) to 10xg in terms of increase in production of ECM molecules and changes in expression of genes involved in cell differentiation [13,14]. These findings induced us to hypothesize that cells recognize pulsed Nd:YAG irradiation as a mechanical stress. In order to verify this hypothesis, we analysed CSK of laser-treated cells, because it is widely known that CSK is sensitive to mechanical stress and it is considered the best candidate to act as a mechanotransducer [15]. As expected, in laser-treated cells, we found that CSK underwent a reorganization both in microtubules and actin microfilament network. Moreover, the distribution of integrins, which anchor the CSK to the cell membrane, changed accordingly [12, 13]. On the basis of our results and considering that: i) at the wavelength emission of Nd:YAG laser tissue absorption is quite low, in particular by the cellular component; ii) when IR radiation is absorbed, the energy transferred from photons to chromophores (absorbing molecules) generally causes vibrational phenomena; iii) it has been already demonstrated that photothermal effects produced by laser pulses >1 μ s can induce modifications in the architecture of collagen and other macromolecular networks [16]; we advanced the hypothesis that Nd:YAG pulses principally interact with ECM, producing local temperature gradients and thus inducing transitory photothermal deformations of the extracellular microenvironment which, in turn, applies a mechanical stress to the cells through the integrin clusters at the anchoring points.

In order to verify the absorption of Nd:YAG laser pulses by different tissue components and biological substrates, we measured the attenuation of laser radiation passing through cell layers, cell culture media, artificial models of ECM constituted by biocompatible hydrogels of polysaccharides and proteic biomatrices. It is noticeable that polysaccharides and proteins are the principal components of ECM and their artificial matrices are already used in tissue engineering and to favour tissue repair.

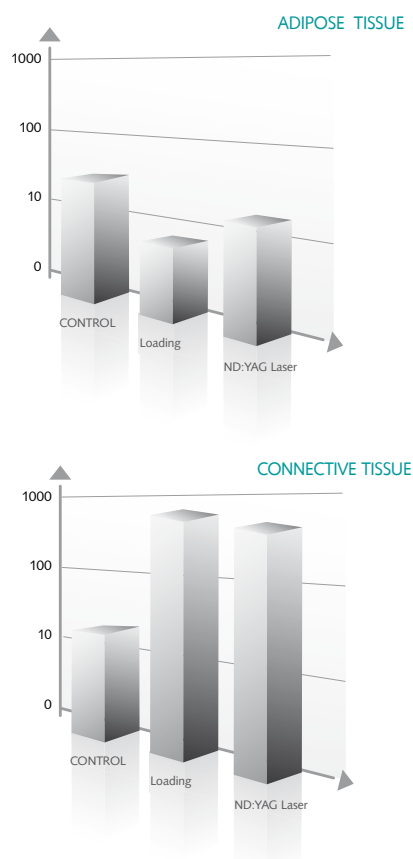


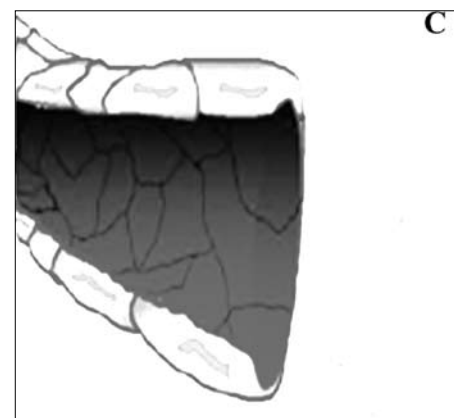
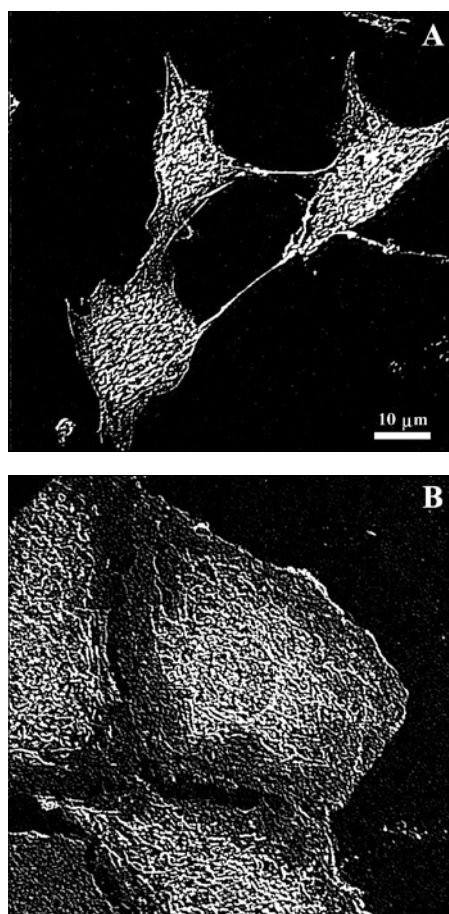
Fig. 1 is reported in arbitrary units the gene expression of pathways involved in differentiation of adipose tissue cells and connective tissue cells. Both loading and treatment with pulsed Nd:YAG laser induce a slight decrease in expression of genes involved in adipogenesis and a very strong increase in expression of genes involved in differentiation of cells belonging to connective tissues. It is noteworthy that connective tissues have mechanical and antigravitational functions.

The measurements, carried out by a pyroelectric detector, indicated that, relatively to the impinging laser energy, there was not measurable radiation absorption by cell monolayers; in the case of culture cells, laser radiation passing through the culture medium and the substrate on which the cells adhered was attenuated of about 20%; the attenuation was up to 25% and 30% in polysaccharide and proteic matrices (thickness 3 mm, water content from 50% to 70%), respectively. These data proved that Nd:YAG pulses are absorbed by components of the extracellular environment, thus supporting our hypothesis.

Further steps in our studies have been made investigating the effects of pulsed Nd:YAG laser on endothelial function and tissue repair processes.

A proper circulation and endothelial function is needed for tissue homeostasis. When a tissue is damaged, neoangiogenesis is critical for repair processes, because new vessels provide nutrients to support the cells responsible for tissue remodelling and facilitate the clearance of debris [17]. Altered endothelial function is strongly related to inflammation and oedema [18]. Because laser is often applied in clinics for treating both inflammation and oedema, we studied the effect of pulsed Nd:YAG laser on endothelial cells, which form the inner layer of the vessel wall. We found that Nd:YAG treatment induced cell spreading and favoured the formation of an ordered endothelial monolayer, while non treated cells, remained randomly distributed [19] (Fig. 2). This effect could be of consequence in improving endothelial function and promoting neoangiogenesis. Tissue repair is a complex series of events, which has great importance for

Fig. 2



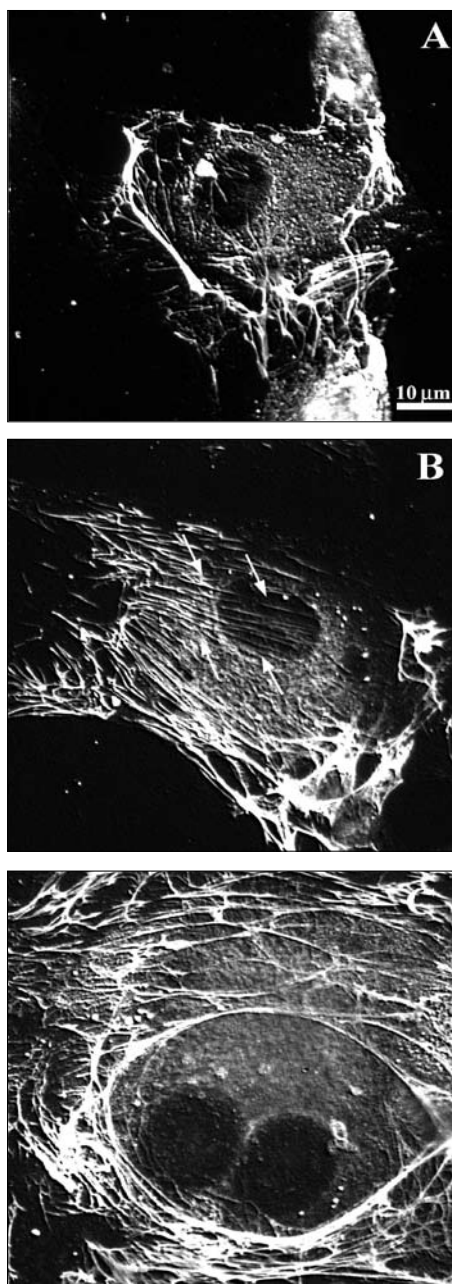
Figs. 2A and **2B** show endothelial cell cultures treated and non-treated (control) with pulsed Nd:YAG laser, respectively. The treatment favours cell spreading and the formation of an ordered monolayer while non-treated cells are randomly distributed. The drawing in **Fig. 2C** schematically represents the formation of the endothelial monolayer in a new vessel.

organism survival. Following tissue injury, an inflammatory phase occurs, which is needed to trigger repair by recruitment and activation of white blood cells, fibroblasts and endothelial cells in the damaged area. During repair, fibroblasts and endothelial cells synergetically act for sequentially remodelling a series of ECMs of increasing complexity and order. In this process of tissue structure restoration fibronectin (FN) has a key importance. FN is produced by cells, in particular fibroblasts and endothelial cells, and secreted outside. Then it autoassembles to form a network of insoluble fibrils which become part of the ECM. FN strongly affects ECM composition, because its fibrils constitute a template for collagen assembling. Moreover, FN connects ECM components each other and with cell surface, thus contributing to the regulation of many cell functions, as growth and differentiation, adhesion and migration [20], which are involved in tissue repair.

We carried out experiments aimed at discovering and understanding whether pulsed Nd:YAG laser treatment could affect tissue repair, in particular FN production and assembly. The results demonstrated that the treatment increased fortyfold the expression of genes involved in the chemokine-mediated inflammation pathway, which has an important role in triggering the remodelling phase of repair by attracting fibroblasts and endothelial cells in the damaged area. Moreover, in treated fibroblasts and endothelial cells a slight increase in FN production was observed. Interestingly, the

treatment induced the formation of very ordered arrays of FN fibrils [12,19] (Fig. 3). Therefore, pulsed Nd:YAG irradiation affected both FN production and assembly. These data suggest that Hilterapy could have significant effects on the remodelling phase of tissue repair.

Fig. 3



Figs. 3 Fibronectin expression revealed by immunofluorescence microscopy in non-treated (control, 3A) and treated (3B and 3C) endothelial cells. In treated cells, the formation of parallel fibronectin fibrils can be observed (see arrows).

Summarizing, pulsed Nd:YAG laser treatment seems to have a stimulating effect in the acute phase of inflammation and to favour a proper assembling of FN fibrils, which is very important for the formation of a functional tissue.

In conclusion, our results demonstrate that the effects of Nd:YAG pulses on culture cells are very similar to those induced by mechanical stress, thus supporting our hypothesis that Nd:YAG pulses produce in the tissue photothermally-induced transitory mechanical deformation of the extracellular microenvironment that is conveyed at cellular level through the ECM-integrins-CSK network.

The findings that Nd:YAG pulses favour ECM production and proper fibronectin assembling, differentiation of connective tissue cells, formation of ordered endothelial monolayers indicates that pulsed Nd:YAG laser treatment can efficaciously promote tissue repair processes.

ACKNOWLEDGEMENTS

We thanks Susanna Benvenuti, Antonio Conti, Damiano Fortuna, Franco Fusi, Pasquale Imperiale, Leonardo Masotti, Lucia Morbidelli, Riccardo Pratesi and Lucio Zaghetto, who, in different ways, helped us to carry out the above described research.

REFERENCES

1. Mobley J, Vo-Dinh T. **Optical properties of tissue.** In: Vo-Dinh T eds. Biomedical photonics Handbook. CRC Press, 2003, I(2).
2. Niemz MH. **Laser-Tissue Interactions.** Springer-Verlag, Berlin, Heidelberg, New York, 1996.
3. Thomsen S. **Pathologic analysis of photothermal and photomechanical effects of laser-tissue interactions.** Photochem Photobiol, 1991, 53(6): 825-35.

4. Welch AJ, Polhamus GD.

Measurement and prediction of thermal injury in the retina of the rhesus monkey.

IEEE Trans Biomed Eng, 1984, 31(10):633-43.

5. Jacques SL. **Laser-tissue interactions. Photochemical, photothermal, and photomechanical.**

Surg Clin North Am, 1992, 728 (3): 531-58.

6. Pratesi R, Sacchi C. **Lasers in Photomedicine and Photobiology.**

Springer, New York, 1980.

7. Rossi F, Pini R, Monici M. **Direct and indirect photomechanical effects in cells and tissues. Perspectives of application in biotechnology and medicine.**

In: Monici M, van Loon J, eds.

Cell mechanochemistry. Biological systems and factors inducing mechanical stress, such as light, pressure and gravity.

Published by Research Signpost, Transworld Research Network, Trivandrum, India. In press.

8. Marenzana M, Wilson-Jones N, Mudera V, Brown RA.

The origins and regulation of tissue tension: identification of collagen tension-fixation process in vitro.

Exp Cell Res, 2006, 312(4):423-33.

9. Inger DE.

The architecture of life.

Sci. Am, 1998, 278: 48-57.

10. Carson DD. **Extracellular matrix: forum introduction.** Reproductive Biology and Endocrinology, 2004, 7;2:1.

11. Chiquet M, Sarasa-Renedo A, Huber F, Flück M.

How do fibroblasts translate mechanical signals into changes in extracellular matrix production?

Matrix Biology, 2003, 22: 73-80.

12. Monici M, Basile V, Cialdai F, Romano G, Fusi F, Conti A.

Irradiation by pulsed Nd:YAG laser induces the production of extracellular matrix molecules by cells of the connective tissues.

A tool for tissue repair.

In: Popp J, Drexler W, Turchin VV, Matthews DL eds.

Biophotonics: Photonic solutions for Better Health Care,

Proc. of SPIE, 2008, vol. 6991, 69912K1-10.

13. Basile V, Romano G, Fusi F, Monici M.
Comparison between the effects of hypergravity and photomechanical stress on cells producing ECM.
Microgravity Sci.

Technol, 2009, 21: 151-157.

14. Monici M, Romano G, Cialdai F, Fusi F, Marziliano N, Benvenuti S, Cellai I, Egli M. Cogoli A.

Gravitational/mechanical factors affect gene expression profile and phenotypic specification of human mesenchymal stem cells.

J Gravit. Physiol., In press.

15. Hughes-Fulford M.

Signal Transduction and Mechanical Stress. Sci. STKE, 2004, 249:p. re12

[DOI: 10.1126/stke.2492004re12]

16. Jori G, Spikes JD.

Photothermal sensitizers: possible use in tumor therapy.

J Photochem Photobiol B, 1990, 6(1-2): 93-101.

17. Li J, Zhang YP, Kirsner RS.

Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix.

Microsc Res Tech, 2003, 60(1): 107-14.

18. Kadl A, Leitinger N.

The role of endothelial cells in the resolution of acute inflammation.

Antioxid Redox Signal, 2005, 7(11-12): 1744-54.

19. Monici M, Cialdai F, Romano G, Fusi F, Egli M, Cogoli A, Morbidelli L.

Tissue repair: impact of unloading on the remodelling phase.

Microgravity Sci. Technol., In press.

20. Pankov R, Yamada KM.

Fibronectin at a glance.

J. Cell Science, 2002, 115: 3861-3.

Key words: Laser-tissue interaction, penetration depth, the Lambert Beer law

Laser-tissue interaction principles: beam penetration in tissues.

Romano G., Conti A., Fusi F.

Medical Physics Section, Department of Clinical Physiopathology,
University of Florence, Florence, Italy.

ABSTRACT

In this communication, we would like to illustrate some basic aspects of laser-tissue interaction principles. The subject is very broad and it is certainly impossible to deal with all its aspects in a detailed manner. Nevertheless we believe that a brief introduction to some of the most important concepts could help for a better understanding of the physics which is at the basis of the therapeutic effect of laser sources. One of the most important definitions at the basis of the physics of laser-tissue interaction is that of beam penetration depth.

Generally speaking, light penetration in tissues depends both on the absorption and the scattering phenomena; in this context we will first consider the presence of absorption only, while scattering will be the object of future communication.

INTRODUCTION

Before starting to analyze the concept of penetration depth, let us briefly resume the definition of the most important physical quantities used to characterize the propagation of a laser beam.

Energy: we can distinguish between the energy of a single photon from the energy associated to the propagation of a whole laser beam. If λ is the wavelength and ν is the frequency, we have:

$$E_{1 \text{ photon}} = h\nu = hc / \lambda \quad (1)$$

where h is a constant (named Planck's constant) and c is the light speed in vacuum. The energy is measured in Joule (J). This definition implies that the energy of a single photon

is completely determined, once the wavelength (or the frequency) of the laser light are defined. As a consequence, $E_{1 \text{ photon}}$ does not change if the laser power or intensity are changed.

The energy associated with the whole laser beam depends on the number of photons N : $E = N \cdot E_{1 \text{ photon}}$ (2)

The laser power is the energy transported by the laser beam per unit time. It is generally defined by P and is measured in Watts (W): $1W = 1J / 1s$

$$P = \text{energy} / \text{time} \quad (3)$$

The intensity (also named Fluence) is defined as the ratio between the power (P) and the beam area (S), and may be measured in W/m^2 (in the International System of Units) or W/cm^2 :

$$I = P / S \quad (4)$$

Example: let us consider a 3W laser at 1064nm, focalized into a circular beam of 10mm diameter. The Intensity is of about $3.8W/cm^2$. By changing the laser focalizing lenses let us suppose that we obtain a beam of only 2mm diameter: the power is always 3W but the intensity is now about $96W/cm^2$, as the spot area is now 25 times smaller than before.

LASER PENETRATION: THE LAMBERT BEER LAW.

As a very general rule, laser penetration in tissues depends on the laser wavelength and the specific tissue type. Let us consider a collimated laser beam impinging on a tissue surface (Fig. 1). Let us suppose to perform a series of experiments in which the same laser of intensity I_0 passes through the same tissue with increasing thickness (t).

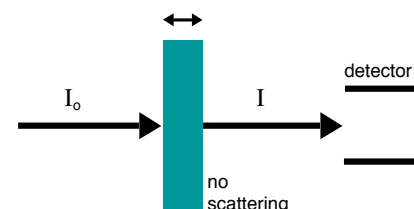


Fig.1. Schematic representation of a laser beam of intensity I_0 impinging on a tissue (in red) of thickness t . The emerging intensity is represented by I . scattering is neglected.

If we measure the intensity of the laser emerging from the tissue, and indicate it with I , we can see that the following relationship is found:

$$I = I_0 \exp(-\mu_a t) \quad (5)$$

Equation 5 is called the Lambert Beer law and represents the exponential decrease of the laser intensity with the tissue thickness (t); μ_a is defined as the "absorption coefficient", and depends on the laser wavelength λ and the specific tissue type. It is important to remember that μ_a does not depend on the thickness (t).

The Lambert Beer law is valid in the hypothesis that scattering is negligible respect to absorption. This may look like a strong limitation, but we will see that it is possible to include the scattering by properly modifying equation 5. As there is no hypothesis about the specific laser emission characteristics, the Lambert Beer law does not depend on the fact that the laser is a continuous or pulsed/superpulsed one (exceptions may arise only in presence of ultrashort pulses of the order of 100fs or less, which are not considered in medical in vivo diagnosis or therapy).

In general, Eq. 5 is used to calculate I by knowing I_0 from the laser specifications, t from the tissue sample geometry and by the knowledge of μ_a . Values for μ_a can be generally found in biomedical photonic textbooks or in scientific articles (or, of course, by measuring it); it is important to look for the proper value of μ_a , according to the specific laser wavelength and tissue considered.

PENETRATION DEPTH

Out of the Lambert Beer law, it is possible to define the laser penetration depth (L), by the simple equation:

$$L = 1 / \mu_a \quad (6)$$

The penetration depth is also called the extinction length, and can be measured for instance in mm or μm . By combining Eq. (6) and (5) we obtain the following relationship:

$$I = I_0 \exp(-t / L) \quad (7)$$

By analyzing Eq. 7 it is possible to give the following interpretation: the penetration

depth is the depth (in the tissue) at which the laser beam intensity is reduced by a factor of about 3. This can be deduced by the substitution $t = L$ in Eq. 7, so that we obtain: $I_t = I_{t=L} = I_0 \exp(-1) = I_0 / e \sim I_0/3$. It is very important to notice that this definition is independent on the laser intensity I_0 (and also independent on the laser power P_0 impinging on the tissue surface): lasers of the same λ , in the same tissue, but with different power, will have the same penetration depth.

The penetration depth can be only changed by: (i) changing the laser wavelength or (ii) changing the tissue type.

In practice, it is possible to find a slightly different definition of penetration depth. For instance, it is possible to define the penetration depth as "the depth at which the laser intensity is reduced by a factor 10" (instead of a factor e).

This does not change the main point of the definition: we are still considering a fixed ratio between the intensity I_0 and the intensity I at a depth equal to the penetration depth. In order to illustrate the above described concepts, let us consider one example: two different lasers of the same wavelength are directed to the same tissue sample (Fig. 2).

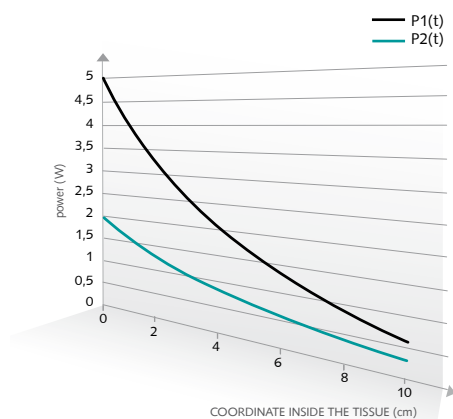


Fig.2. Laser penetration in a fixed tissue type, by varying only the laser power entering the tissue. The two lasers have the same penetration depth (5cm).

In this example, let us suppose that the lasers have the same wavelength but different emission power: $P_1=5W$, while $P_2=2W$. As we have the same λ and tissue type, the penetration depth is the same for the two lasers. Let us suppose that $L = 5cm$ (as is the case of Fig. 2). This implies that, at a depth equal to 5cm, laser 1 has power $\sim P_1/3 = 1W$ while, at the same depth of 5cm, laser 2 has power $\sim P_2/3 = 0.33W$. What is different is the laser power at the same depth.

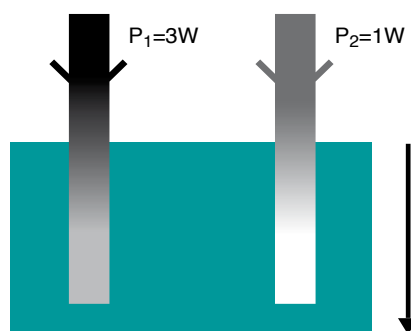


Fig.3. Picture representation of the graph shown in Fig. 2. Arrows indicate laser propagation direction. The tissue is represented in black. The gray to white color indicates a decreasing laser power. Scattering is neglected.

The 2 lasers in Fig. 3 have the same penetration depth ($L=5cm$) but different power at the interface air - tissue. As a consequence of the hypothesis of negligible scattering, the laser beam dimensions do not change when entering the tissue.

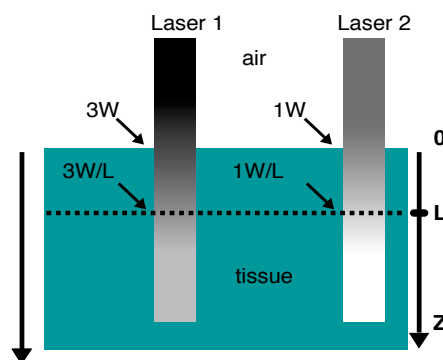


Fig.4. Picture representation of the penetration depth, in the case of two lasers with the same wavelength but different power.

Let us now come back to Fig. 2. If we normalize the power to the initial value, we obtain as expected the same curve for both lasers, as illustrated in Fig. 5.

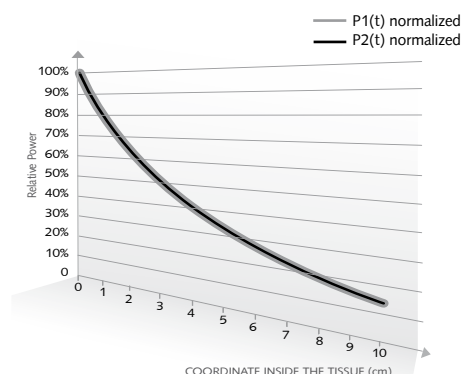


Fig.5. Normalized curves for laser power inside the same tissue type. The two lasers have the same wavelength but different power entering the tissue.

As a last example, let us now consider the same laser light ($P=10W$) impinging on different tissue types (Fig. 6). As a consequence of the Lambert Beer law (Eq. 5 and 7), the beam penetration will be characterized by different penetration depths, as μ_a depends on the tissue. For the simulation

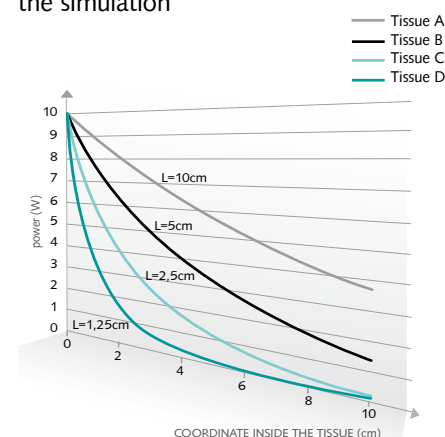


Fig.6. Penetration of the same laser source in different tissue types. $P = 10W$ is the laser power entering the various tissues.

purposes of the figure, the following penetration depths have been chosen: tissue A: 10cm; tissue B: 5cm; tissue C: 2.5cm; tissue D: 1.25cm. Scattering has been neglected. In this case it is possible to appreciate that all the curves have an exponential behavior, according to the Lambert Beer law, but the intensity decays very differently according to the different tissue properties, as far as absorption is concerned.

REFERENCES

- T. Vo-Dinh Editor in Chief. **Biomedical Photonics Handbook**, CRC Press, New York, 2003.
- Palumbo G, Pratesi R Eds. **Lasers and Current Optical Techniques in Biology**, RSC, London, 2004.

Key words: HILT, laser treatment, photobioactivation, tissue welding

The HILT domain by the pulse intensity fluence (pif) formula.

Fortuna D.¹, Masotti L.²

1) Director of the PhotoBiolab Unit of the EL.En Group, Florence, Italy.

2) Dept. of Electronics and Telecommunications, University of Florence, Florence, Italy.

ABSTRACT

Laser therapy is often used to give relief in acute and chronic pain, increase the speed, quality and tensile strength of tissue repair, and improve the function of damaged neurological tissue. Treatment with laser beams is painless and causes neither a macro-chemical change nor damage in the tissue. In view of the unsatisfactory results obtained with Low Level Laser Therapy (LLLT) in deeper tissue regeneration, we studied the possible use of power laser designing a more efficient system and a new method of treating, faster and more consistently reproducible results. Specifically, LLLT can only produce either the photochemical effect or the photochemical and photo-thermal effects but not all three. Pulsed emission can be used to induce photomechanical effects. HILT principally induces photomechanical and photo-thermal by means of pulsed laser emission characterized by a particular shape of pulse. Unfortunately the formulas commonly used in the laser matter are not able to perfectly describe the HILT pulse shape and its timing and spatial distribution.

The aim of our study was to define

a phenomenological formula to describe HILT pulse shape putting together both bi-three dimensional and its timing resolution.

From our experimental data, collected in more of ten years, we extrapolated a mathematical common denominator able to synthesize, in just one formula (PIF), the HILT pulse features.

Applying PIF formula we simulated different possible configurations for HILT, we related it with our clinical and experimental data and we defined the HILT domain in terms of anti-inflammatory effect, tissue repair, tissue regeneration and toxic dose.

Correlating these data with biological effects of HILT we defined the HILT domains, which are, in our opinion, useful to exactly define the biological capabilities of HILT.

In our opinion, PIF formula is easier to better understand HILT features and its differences with LLLT and Continuous Wave (CW) Power Lasers.

INTRODUCTION

High intensity laser therapy (HILT) or HILT systems was born to induce a non invasive regenerative therapy with a non-painful and non-invasive therapeutic system. Secondary objective of the HILT is the treatment

of deep lesions, such as joint lesions. Since their discovery lasers have been advocated as alternatives to conventional clinical methods for a wide range of medical applications. For many years high powered and highly focused lasers have been used to cut and separate tissue in many surgical techniques. More recently, therapeutic and biostimulating properties of high power laser were discovered. It is believed that laser radiation stimulates several metabolic processes, including cell proliferation and cell differentiation, synthesis of collagen and other proteins, immunomodulation.

LLLT has become a popular treatment in a variety of medical disciplines. This therapy is used with some success but results are obtained only slowly and are inconsistent. The degree of therapeutic effect achieved is variable and heavily depends upon the dosage of radiation, exposure rhythm, and the distance of the treated tissue from the laser source. Applications of several minutes are repeated at intervals of several days and often repeated for months.

In view of the unsatisfactory results obtained with LLLT we studied the possible use of power laser designing a more efficient device and a better method of laser treatment to have faster and more consistently reproducible results. Specifically, LLLT can only produce either the photochemical effect or the photochemical and photo-thermal effects but not all three.

Pulsed emission can be used to induce also photomechanical effects. HILT principally induce photomechanical and photo-thermal effects by means of pulsed laser emission characterized by a particular shape of pulse.

Unfortunately, the formulas commonly used in the laser matter are not able to perfectly describe the

HILT pulse shape and its distribution in time and space.

The aim of our work was to identify rational criteria to design an ideal laser for HILT.

The problem is that, in this matter, there are multiple variables like: wavelength, spot size, energy per pulse, peak power and, pulse duration time. Therefore, to argue in this field it is necessary to explain reason of our choices related to the biological effects.

From our experimental outcomes, collected in more of ten years, we tried to extrapolate a physical common denominator able to synthesize, in just one formula, the HILT pulse shape.

In conclusion, the aim of our study was to define a phenomenological formula able to describe Hilterapia pulse shape putting together both bi-three dimensional and timing resolution.

The Average Power Density, often considered in literature, is defined as the time rate of flow of radiant energy averaged over one full period for square cm (recall that frequency is the inverse of period) $[W/cm^2]$. Power Density, which is also named radiant flux density, does not supply sufficient information about the temporal and spatial shapes of the pulses.[1]

Peak Pulse Intensity defined as the rate energy flow in every pulse for square cm of the spot area $[W/cm^2]$, gives an idea of the 3D spatial distribution, but it fails to provide information regarding the energetic content of the pulse $[J]$ and its time distribution $[s]$. [1]

The Pulse Fluence defined as the radiative flux integrated over time per unit area, or else pulse energetic content divided by the spot size $[J/cm^2]$, indicates a mean power during the pulse but does not give the intensity $[W/cm^2]$, or instantaneous power (per unit area). For example, the same fluence may be common to an infinite number of different pulses that have different peak powers and pulse durations τ -on.

We then correlated our experimental results, collected over more ten years of study, with the laser settings applied. After this we extrapolated the common data capable of describing the incident light intensity on the spot size (two-dimensional data), the average light concentration on the spherical light segment that symbolizes the three-dimensional distribution of the light in the subcutaneous tissues (three-dimensional data), and the relationship between the turned off phase (τ -off) and the turned on phase (τ -on) of the light over the entire duration (the period $T = \tau$ -on + τ -off) of the laser pulse. [1]

We then correlated our experimental results, collected over more ten years of study, with the laser settings applied. After this we extrapolated the common data capable of describing the incident light intensity on the spot size (two-dimensional data), the average light concentration on the spherical light segment that symbolizes the three-dimensional distribution of the light in the subcutaneous tissues (three-dimensional data), and the relationship between the turned off phase (τ -off) and the turned on phase (τ -on) of the light over the entire duration (the period $T = \tau$ -on + τ -off) of the laser pulse. [1]

MATERIALS AND METHODS

Experimental trials carried out with HILT in vitro and, in vivo on both animal and human models enabled us to draw a map of the our formula.

Unfortunately the formulas commonly used in the laser matter are not able to perfectly describe the Hilterapia pulse shape and its timing and spatial distribution.

We then created an electronic calculation sheet (Microsoft Excel®) and entered the settings used for each individual session carried out.

Table I illustrates the 29 elements taken into consideration for our analysis.

To assess light distribution into the tissue we used the absorption coefficient data from K.F. Palmer and D. Williams [2]. Additional information on the penetration depth of different laser sources is presented in J. Tuner & L. Hode and Pratesi [3, 4].

Looking for a common denominator able to summarize the HILT features in the space and timing we decided to consider:

- 1) The skin incident light, or the bi-dimensional element,
- 2) The three-dimensional light concentration into the subcutaneous tissues, and
- 3) The relationship between the turned off phase and the pulse time.

The first element, which defines the

Pulse Energy [J]	Spot Size [mm]	Sphere Ray [cm]	Area [cm ²]	Volume Segment Sphere Vks [cm ³]	Water Abs. α [cm ⁻¹]	Vks α	Light Penetration Depth [cm]	τ -on [μ s]	τ -on [s]
τ -off [s]	τ -on * τ -off	Ep / τ -off	τ -off/Ep	Ep/Vks 1/ α	Duty	[1-D]	Peak Power/ τ -on	Hz	Peak Power [W]
Pulse Fluence [J/cm ²]	Pulse Fluence [J/cm ²]	Pulse Fluence [J/cm ²]	Pulse Fluence [J/cm ²]	Pulse Fluence [J/cm ²]	Pulse Fluence [J/cm ²]	Pulse Fluence [J/cm ²]	Pulse Fluence [J/cm ²]	Pulse Fluence [J/cm ²]	Pulse Fluence [J/cm ²]

Table I. Table shows parameters considered in our analysis.

	Pulse Energy [J]	Spot Size [mm]	Sphere Ray [cm]	Area [cm ²]	Volume Segment Sphere Vks [cm ³]	Water Abs. α [cm ⁻¹]	Vks α	Light Penetration Depth [cm]	τ -on [μ s]	τ -on [s]
LLLT	0.01	5	0.25	0.20	0.16	0.5	0.31469	0.70	1000	1.00E-03
Antinflammatory	0.1	5	0.25	0.20	0.16	0.14	1.12388	1.07	200	0.0002
Chicken study	0.35	5	0.25	0.20	0.16	0.14	1.12388	1.07	120	0.00012
Toxic dose, chicken	0.35	4	0.2	0.13	0.08	0.14	0.57543	0.86	120	0.00012
HCT8 & VERO	0.15	6	0.3	0.28	0.14	0.14	1.00980	1.04	200	0.0002
Humans	0.35	5	0.25	0.20	0.16	0.14	1.12388	1.07	120	0.00012
1st Sheep study	2	10	0.5	0.79	1.26	0.14	8.99107	2.15	130	0.00013
Chondrocytes	0.15	8	0.4	0.50	0.25	0.14	1.79520	1.26	200	0.0002
2nd Sheep study	2	10	0.5	0.79	1.26	0.14	8.99107	2.15	130	0.00013
	τ -off [s]	τ -on * τ -off	Ep / τ -off	τ -off/Ep	Ep/Vks 1/ α	Duty	[1-D]	Peak Power/ τ -on	Hz	Peak Power [W]
LLLT	9.00E-03	0.000009	1.111111	0.900000	0.031778	10.00%	90.00%	50,930	100.00	10
Antinflammatory	0.0524316	1.0486E-05	1.907248	0.524316	0.088977	0.38%	99.62%	12,732,395	19.00	500
Chicken study	0.0332133	3.9856E-06	10.537937	0.094895	0.311420	0.36%	99.64%	123,787,178	30.00	2,917
Toxic dose, chicken	0.0332133	3.9856E-06	10.537937	0.094895	0.608242	0.36%	99.64%	193,417,466	30.00	2,917
HCT8 & VERO	0.0664667		2.241725	0.446085	0.147554	0.30%	99.70%	13,181,175	15	745
Humans	0.0332133	3.9856E-06	10.537937	0.094895	0.311420	0.36%	99.64%	123,787,178	30.00	2,917
1st Sheep study	0.49987	6.4983E-05	4.001040	0.249935	0.222443	0.03%	99.97%	150,679,236	2.00	15,385
Chondrocytes	0.4998		0.298119	3.354362	0.082999	0.04%	99.96%	7,414,411	2	745
2nd Sheep study	0.49987	6.4983E-05	4.001040	0.249935	0.222443	0.03%	99.97%	150,679,236	2.00	15,385
	Average Power [W]	Average I [W/cm ²]	Pulse Fluence [J/cm ²]	Peak Intensity [W/cm ²]	Total fluence [J/cm ²]	Tx Area [cm ²]	Energy delivered [J]	Exposure time [min.]	Exposure Time [s]	PIF [J/cm ³] ²
LLLT	1.00	5.09	0.05	51	10	50	500	8.3	500.0	0.0015
Antinflammatory	2	9.68	0.51	2,546	10	50	500	4.4	263.2	0.05
Chicken study	10.5	53.48	1.78	14,854	10	50	500	0.8	47.6	0.55
Toxic dose, chicken	10.5	83.56	2.79	23,210	10	50	500	0.8	47.6	1.69
HCT8 & VERO	2.23500	7.91	0.527	2,636	10	200	109	0.8	48.8	0.78
Humans	10.5	53.48	1.78	14,854	10	50	500	0.8	47.6	0.55
1st Sheep study	4	5.09	2.55	19,588	10	200	2000	8.3	500.0	0.57
Chondrocytes	0.29800	0.59	0.297	1,483	10	200	109	6.1	365.8	0.25
2nd Sheep study	4	5.09	2.55	19,588	10	200	2000	8.3	500.0	0.57

Table II shows parameters and laser settings used in our experimental studies carried out so far.

intensity of light within the target region, is a two dimensional energy per pulse:

$$I_p \cdot \tau_{on}$$

Where I_p is the Peak Intensity (Wp/cm²) (Peak Power [Wp] divided by the surface area of the spot [cm²]), and, τ_{on} is the Pulse duration.

To further define the pulse into a three

dimensional relationship (Ep), the energy per pulse, by the irradiated tissue volume (Vks). Giving the formula:

$$\frac{E_p}{V_{ks}}$$

Where Vks is the Volume of the Sphere

segment (hemi-discoid). This volume of tissue is an area which is being radiated by the laser. The Volume Vks is calculated by:

$$V_{ks} = 10.07 R_{sp}^3$$

where Rsp is the spot size ray and h = 2/3 of sphere ray.

$\frac{E_p}{V_{ks}}$ must be considered in relation to the wavelength (λ). For this reason we consider V_{ks} as an equivalent of the Volume normalized to the water absorption coefficient α , that varies in relation to λ . For example, according to Palmer data [2], for λ of 1,064 nm α is 0.14 cm⁻¹ while for λ 980 nm α is 0.5 cm⁻¹.

Third element of the formula:

$$\frac{\tau_{off}}{T}$$

Describes the relationship between the off phase and T. $T = \tau_{on} + \tau_{off}$.

It is important to express this relationship between turned off phase and total pulse period of time (T). The turned off phase maintains the tissue temperature. It is important that the tissue does not over-heat and cause potential thermal damage.

Therefore, we can write the formula as:

$$PIF \left(\frac{J}{cm^3} \right)^2 = I_p \tau_{on} \frac{E}{10.07r^3} \alpha \cdot \frac{\tau_{off}}{\tau_{off} + \tau_{on}}$$

RESULTS

Graph 1 below shows the correlation between the characteristics of the HILT pulse, expressed in PIF [J/cm³]², and the biological effects observed in experimental trials. Each data point represents the PIF of a pulsed laser beam applied in a particular setting, e.g., to chickens, sheep, or humans in vivo. The vertical lines extending from each point are error bars. The dashed horizontal lines indicate a range of acceptable PIFs for tissue regeneration: from just under 0.2 [J/cm³]² to just under 1.0 [J/cm³]². Below 0.1 [J/cm³]², there may be just an anti-inflammatory effect and not a

regenerative effect; whereas for PIFs exceeding 1.0 [J/cm³]² there may be a histo-toxic effect.

Applying the PIF formula we can also to carried out a fast and easy comparison among different shape of pulses and different wavelengths. PIF values in Table III and Table IV show the results of our simulations.

We carried out also a comparison between PW and CW emission at the same wavelength (λ : 1,064 nm).

Table V shows that the value is 6 order of factors lowers in CW than in the HILT.

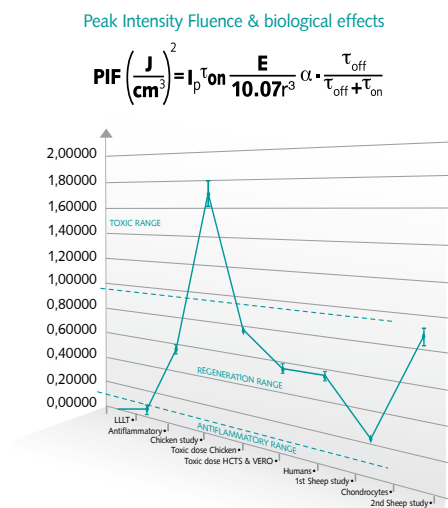


Fig.1. This graph shows the relationship between the pulse HILT features, expressed as PIF [J/cm³]², and HILT biological effects observed in our experimental trials.

Table III, PIF value for: λ 910 nm, Average Power 5 W, Peak Power 1400 W, τ_{on} 200 ns	
E/pulse [J]	0.0002857
Diam. Spot Size [mm]	20
Spot size area [cm ²]	3.141592654
Ray [cm]	1
V_{ks} [cm ³]	0.16
α [cm ⁻¹]	0.075
Duty [%]	0.35%
τ_{on} [s]	0.000000200
τ_{off} [s]	0.00005694
PRF [Hz]	17500
Peak Power [W]	1,429
Average Power [W]	5
Average I [W/cm ²]	1.591549431
Pulse Fluence [J/cm ²]	0.00009095
Peak Intensity [W/cm ²]	454.73
PIF [J/cm ³] ²	1.93E-10

Table IV, PIF value for: λ 1064 nm, Average Power 5 W, Peak Power 1400 W, τ_{on} 120 us	
E/pulse [J]	0.1666667
Diam. Spot Size [mm]	5
Spot size area [cm ²]	0.196349541
Ray [cm]	0.25
V_{ks} [cm ³]	0.16
α [cm ⁻¹]	0.14
Duty [%]	0.36%
τ_{on} [s]	0.000120000
τ_{off} [s]	0.03321333
PRF [Hz]	30
Peak Power [W]	1,389
Average Power [W]	5
Average I [W/cm ²]	25.46479089
Pulse Fluence [J/cm ²]	0.84882636
Peak Intensity [W/cm ²]	7,073.55
PIF [J/cm ³] ²	1.25E-01

Table V, PIF value for: λ 1,064 nm, CW, Average Power 5 W	
E/pulse [J]	5.0000000
Diam. Spot Size [mm]	5
Spot size area [cm ²]	0.196349541
Ray [cm]	0.25
V_{ks} [cm ³]	0.16
α [cm ⁻¹]	0.14
Duty [%]	100.00%
τ_{on} [s]	0.999999999
τ_{off} [s]	0.000000000
PRF [Hz]	1
Peak Power [W]	5
Average Power [W]	5
Average I [W/cm ²]	25.46
Pulse Fluence [J/cm ²]	25.46
Peak Intensity [W/cm ²]	25.46
PIF [J/cm ³] ²	1.13E-07

DISCUSSION

From our experimental outcomes we observed that in order to have a regenerative effect on the tissues and a promote cell differentiation, pulses provided with HILT should have a Peak Intensity Fluence (PIF) ranging from 0.1 [J/cm³]² to 1 [J/cm³]². PIFs over 1.0 [J/cm³]² may be dangerous. PIFs below 0.1 may have only an anti-inflammatory effect. In contrast, LLLT systems used for pain management have a PIF between 0.0 (i.e., the beams are continuous-wave beams) and 0.0015 [J/cm³]², or approximately 100 to 1000 times lower than the PIF for Hilterapia. In view of the unsatisfactory results

obtained with LLLT we studied the possible use of power laser designing a more efficient device and a better method of laser treatment to have faster and more consistently reproducible results. Specifically, LLLT mostly produces photochemical effects or photochemical and photo-thermal effects, but it is not able to produce the photomechanical ones.

Pulsed emission must be used to induce photomechanical effect, possibly associated with others. In HILT, thanks to the particular shape of pulse, photo-thermal and photomechanical effects are predominant.

An interesting application of the PIF formula is to disclose differences between two apparently similar lasers used in therapy: HILT vs. a near infrared (λ : 910 nm) pulsed laser, 5 W of average power and 1,400 W in peak power (see Table III). Comparing this laser with a laser used for Hilterapia at the same average power (5 W) and at the same peak power (1,400 W) we can easily disclose the very big difference between them. In fact, for the 910 nm laser the PIF value is 9 order of magnitude lower ($1.93 \cdot 10^{-10}$) than the HILT one ($1.25 \cdot 10^{-1}$). Moreover, we applied the same method, to compare Hilterapia pulse and Nd:YAG Continuous Wave (CW) laser at the same parameters (average power: 5 W, spot size 5 mm in diameter). Table V shows the PIF value of the CW laser (PIF : $1.13 \cdot 10^{-7}$) that is 6 order of magnitude lower than the laser used for Hilterapia.

These simple examples show clearly as could be useful the use of the PIF formula to disclose the big differences among so many parameters used in the laser field. Talking about the peak power and average power it is not exhaustive of the laser features.

In our case, to be able to induce an

effective photomechanical stimulation of tissue structures, in particular the extracellular environment, it is necessary to have a pulse shape with certain features. It is necessary to deliver a proper amount of energy per pulse in a right time involving as much tissue as possible.

To do this and to be able to stimulate tissue regeneration in a deeper soft tissue it is necessary to manage very well the laser light features and its interaction with tissues.

ABSORPTION COEFFICIENT

The absorption coefficient (α) is important to understand the choice of the wavelength and the spot size and the relationship between themselves.

According to Dörschel [5] the optical penetration depth (x) of the light is inversely proportional to the index of water absorption (α) coefficient, i.e., $x = 1/\alpha$ when water is the main chromophore. Therefore, the higher the water absorption coefficient, the poorer the penetration of the radiation through the tissue.

In order to achieve the greatest possible penetration, the laser light radiation is preferably minimally absorbed by the tissue chromophores, i.e., the wavelength of the laser light should not correspond to peak absorption wavelengths of the tissue chromophores.

Zhao et al. [6] described the 3.3 mm interracial human skin light transmittance for beams of visible and near-infrared wavelengths. Similar results hold for human skin with lighter colors, e.g., skin from European, African and Asian subjects, although the variation in transmittance is especially significant with darker skin, that is, subjects with hypermelanic skins (African skins). Generally, transmittance increases with beam

diameter and, independently, with wavelength. Maximum transmittance occurs for beams at $\lambda = 1064$ nm and beam diameters of 12 mm. This wavelength is only partially absorbed by the skin, melanin and subcutaneous fat and is able to go into deepest tissues (i.e., joint cartilage).

PHOTOMECHANICAL EFFECT

One important aspect underlying Hilterapia is to have a photomechanical effect at a therapeutic level on the tissues and/or cells being treated by the laser light. With a photomechanical effect, at least part of the energy of a laser light can be converted into one or more forms of mechanical forces on the tissues and/or cells being treated by the laser light.

Such mechanical forces can have a physical effect on the cells and/or tissues being treated and cause the cells and/or tissues to change shape and/or size, resulting in such effects as stimulating cell metabolism, proliferation, differentiation, and then tissue regeneration.

According to a first aspect, by applying an appropriately defocused laser beam, having specific characteristics, at a given area of the tissue epidermis of a patient, the laser beam can have a photomechanical effect, possibly associated with others, on the tissues and/or cells being treated, in particular, those tissues and/or cells that are located deeply within the body of a patient under treatment, e.g., the cartilage tissue.

A laser beam directed orthogonal to the surface of the tissue is partly reflected back due to the variation of refraction index when passing from the surrounding ambient (air) and the tissue. The remaining fraction of the laser beam energy is transmitted to and through the tissue and is absorbed and diffused several times by different chemical substances contained in the tissue.

When the pulsed laser beam impacts the second medium, an elastic pressure wave is immediately created in the second medium itself and propagates from the surface deep down into the medium. The amplitude of the wave is directly proportional to the intensity of the light beam and inversely proportional to the pulse duration time. The wave amplitude also depends on the light properties (λ) and the physical-chemical structure of the second medium.

Following is a formula describing the relationship between the sound wave shape created in the tissue hit by a high-intensity pulsed laser beam:

$$p_2(z, t) = p_2 v_2^2 \left(\frac{\alpha \sqrt{k_1 k_2}}{K_1 \sqrt{k_2} + K_2 \sqrt{k_1}} \frac{1}{v_2 + r v_1} \left(\beta_1 \sqrt{k_1} + \beta_2 \sqrt{k_2} \right) \right) I \left(t - \frac{z}{v_2} \right)$$

where the thermal diffusion coefficient

is $\hat{k}_i = \frac{K_i}{(\rho_i c_i)}$; the dimensional coefficient

is $r = \frac{(\rho_2 v^2)}{(\rho_1 v^2)}$; I is laser pulse intensity; c_i

is specific heat; β_i is linear expansion coefficient; K_i is thermal conductivity; ρ_i is density; v_i is sound speed; α is optical absorption coefficient of the tissue; z is depth; and t is time.

The relationships between incident laser light and the photomechanical or photoacoustic effects generated in the tissue include: (1) a direct

relationship between the intensity of the incident light and the intensity of the transitory mechanical deformation created in the tissue; and (2) a direct relationship between the frequency of the wave and the pulse duration (τ_{on}) of the laser. That is, the shape of the acoustic wave is related to the shape of the laser pulse. The intensity of the mechanical effect may also depend on the optical, thermal, and mechanical features of the medium.

When the peak power of the pulse, the pulse duty cycle (the fractional amount of the time of the laser is "on" during any given period: τ/T) and the pulse frequency are properly selected, the photomechanical effects in the irradiated tissues can substantially result in transitory modifications of extracellular matrix (ECM) properties and organization which affect cell behaviour.

It is very important to point out that an intimate connection exists between ECM and cells, in the case of cartilage between ECM and chondrocytes. Any spatial deformation of the ECM is therefore automatically transferred to the cells as a mechanical stimulus.

Graph (6) below shows the cartilage absorbance coefficient (y-axis) related to the wavelength (x-axis). We can notice that in the near infrared, compared to the visible spectra, we have peak absorbance coefficient.

When a pulsed high intensity laser beam with an appropriate wavelength that falls within one of the absorption peaks of cartilage (e.g., a wavelength of 1064 nm) is used to treat a cartilage tissue, it is mostly absorbed by ECM. This specific absorption by ECM via the pulsed emitting of this particular laser light is responsible for immediate tissue dilatation followed by contraction during the cooling phase. This transitory spatial deformation of the ECM is automatically transferred to the cells as a mechanical stimulus.

It is known that the musculoskeletal

system, which includes bones, cartilage, skeletal muscles and ligaments, responds to such mechanical stimulation with changes in metabolism, cytoskeletal organization, rate of proliferation, and state of differentiation during development. Chondrocytes also respond to mechanical forces by changing their metabolism, their state of differentiation, and their proliferation. They respond differently to mechanical force, depending on the magnitude, frequency, and mode of mechanical stimulation [7-27].

PHOTOTHERMAL EFFECT

The laser light is at least partially converted into a thermal wave, which is responsible for the photoexpansion effect observed with outright temperature increases of up to 41°C. During τ -off time, there is a rapid cooling and the medium (e.g., the tissues) moves towards a photocontraction effect.

An additional important parameter having an influence on thermal accumulation and therefore on the temperature increase is the overall volume of tissue under treatment. Keeping the irradiated surface (i.e., the laser spot) and the irradiated energy constant, an increase of the peak power per pulse increases the irradiated volume. The reason for this is that a higher peak power provokes a deeper penetration of the laser in the tissue, and therefore an increase in the overall volume absorbing the laser energy.

The larger the irradiated volume the smaller the temperature increase. Therefore, and contrary to what might appear at first glance, an increase of the peak power of each laser pulse improves the conditions of treatment from the point of view of tissue temperature control.

It has been therefore recognized that in order to obtain an effective treatment of the deep tissues without damaging more superficial and surrounding tissues, we must use a pulsed laser

Cartilage
Courtesy of:
INOA (L. Mercatelli): 300 nm - 1500 nm
IFAC (B. Radicati): 800 nm - 1200 nm

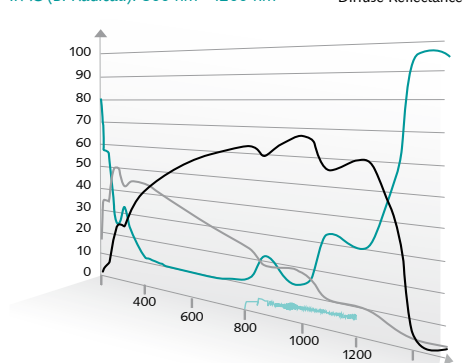


Fig.6. cartilage absorbance coefficient (y-axis) related to the wavelength (x-axis).

source with low pulse frequency, high peak power and short pulses (i.e., low duty cycle values: short τ -on times and long τ -off times).

The area of the laser spot is also very important in order to maximize the greatest possible penetration, while minimizing the amount of scatter. Zhao [6] demonstrated that by increasing the diameter of the spot size there is a reduction in the scattering angle (the larger the diameter of the spot, the lower the scattering angle). This results in a deeper penetration, more uniform diffusion of the radiation in the tissue, and therefore an increased therapeutic effect.

In addition, because $\Delta T = \Delta Q / (ck \times m)$ at the same energy per pulse, the greater the volume treated, the lower the thermal increase to the tissues.

One way to increase the volume treated, especially when the penetration depth is preferably kept at a constant value, is to increase the spot size. By properly selecting the above discussed parameters, the tissue temperature in the treated volume is kept below a certain temperature, e.g., 42°C or even lower, and preferably below 40°C.

CONCLUSION

The main object of the Hilterapia is the non invasive regenerative therapy with a non-painful and non-invasive therapeutic system. Secondary objectives of the HILT is treatment of deep lesions, such as lesions of the articular cartilage. This can be obtained thanks to Hilt's photomechanical effects which are predominant on the others.

While all therapeutic laser systems can deliver the energy needed to have a photochemical effect on the tissues and/or cells being treated, pulsed high intensity lasers can exert a photomechanical effects of therapeutic value. High peak power values (e.g., those at least 1 kW) and high peak intensity values (e.g., those at least 1 kW/cm²) allow a pulsed laser light to have a predominant

photomechanical effect on the tissues and/or cells being treated, which, per se or in combination with other effects, can achieve extraordinary and unexpected therapeutic results.

In the regenerative field it is critical the relationship between shape of pulse and duty cycle, for example, in a 'in vivo' experiment [28] on knee joint in rats, it has been shown that even an high average power density of 5.8 W/cm² may not be sufficient to induce cartilage regeneration.

In conclusion to be able to design an ideal Hilterapia we have to follow some general rules: the deeper the penetration of the laser radiation, the longer the time between subsequent laser pulses, to allow for thermal dissipation; the higher the energy content per laser pulse, the lower the pulse frequency, i.e., the frequency at which the laser pulses are repeated; the higher the power level per pulse, the lower the fluence; the higher the peak power of each pulse, the shorter shall be the pulse duration (low duty cycle); the higher the peak power, at constant spot area, the larger will be the volume interested by the radiation and therefore the lower will be the increase in temperature due to heat accumulation; the higher the energy per pulse, the shorter will be the total exposure time to the laser radiation; and the shorter the total exposure time to the laser radiation, the lower will be the heat accumulation.

Big pulses, characterized by high peak power and a high amount of energy per pulse, are useful for transferring in depth high amount of energy in accordance with Lambert Beer's law. Thermal increase of tissue temperature is directly correlated to the amount of energy supplied. In order to obtain the correct control of the temperature trend while achieving the photomechanical effect during treatment, should be necessary that the PIF is in the range 0.1 – 1 [J/cm³]².

$$PIF \left(\frac{J}{cm^3} \right)^2 = I_p \tau_{on} \frac{E}{10.07r^3} \alpha \cdot \frac{\tau_{off}}{\tau_{off} + \tau_{on}}$$

High energy delivered in this way is

safe and allows for a sudden dramatic dilation of the volume throughout the tissue when the light is on, followed by a cooling beat when the light is off, thereby creating a photomechanical effect.

ACKNOWLEDGEMENTS

We synthesized more of ten years of studies and so, there are many people to say many thanks for them helping in this hard challenge. Roberto Buda, IOR; Antonio Crovace, UNIBA; Emiliano Di Cicco, El.En; Franco Fusi, UNIFI; Daniela Giannessi, IFC-CNR; Sandro Giannini, IOR; Julie Glowacki, Harvard Medical School; Brunella Grigolo, IOR; Leonardo Manetti, El.En; Fabrizio Margheri, El.En; Fabio Martinelli, El.En; Luca Mercatelli, INOA; Monica Monici, ASACampus; Cesare Paolini, DEKA; Steven Reinecke, El.En; Giacomo Rossi, UNICAM; Alessandro Zati, IOR

REFERENCES

1. Hecht E. **Optics 4th**. Addison Wesley Eds., 2002
2. Palmer KF, Williams D. **Optical properties of water in the near infrared**. J. Opt. Soc. Am., 1974, 64: 1107–1110.
3. Tuner J, Hode L. **Laser Therapy: Clinical Practice and Scientific Background**. Prima Books, 2002, 40–43.
4. **Lasers and Current Optical Techniques in Biology**, G. Palumbo and R Pratesi (Eds.) (RSC, London, 2004)
5. Dörschel K, et al. Photoablation. **SPIE, Future Trends in Biomedical Applications of Laser**, 1991, 1525: 253–278.
6. Zhao ZQ, Fairchild PW. **Dependence of light transmission through human skin on incident beam diameter at difference wavelengths**. SPIE, 1998, 3254.
7. Onodera K, et al. **Stepwise mechanical**

stretching inhibits chondrogenesis through cell-matrix adhesion mediated by integrins in embryonic rat limb-bud mesenchymal cells. *European Journal of Cell Biology*, 2005, 84: 45-58.

8. Millward-Sadler SJ, Wright MO, Davies LW, Nuki G, Salter DM. **Mechanotransduction via integrins and interleukin-4 results in altered aggrecan and matrix metalloproteinase 3 gene expression in normal, but not osteoarthritic, human articular chondrocytes.** *Arthritis Rheum.*, 2000, 43: 2091-2099.

9. Ragan PM, Chin VI, Hung HH, Masuda K, Thonar EJ, Arner EC, Grodzinsky AJ, Sandy JD. **Chondrocyte extracellular matrix synthesis and turnover are influenced by static compression in a new alginate disk culture system.** *Arch. Biochem. Biophys.*, 2000, 383: 256-264.

10. Moonsoo J, Frank EH, Quinn TM, Hunziker EB, Grodzinsky AJ. **Tissue shear deformation stimulates proteoglycan and protein biosynthesis in bovine cartilage explants.** *Arch. Biochem. Biophys.*, 2001, 395: 41-48.

11. Hunter CJ, Imler SM, Malaviya P, Nerem RM, Levenston ME. **Mechanical compression alters expression and extracellular matrix synthesis by chondrocytes cultured in collagen I gels.** *Biomaterials*, 2002, 23: 1249-1259.

12. Takahashi I, Mizoguchi I, Nakamura M, Sasano Y, Saitoh S, Kagayama M, Mitani H. **Effects of expansive force on the differentiation of midpalatal suture cartilage in rats.** *Bone*, 1996, 18: 341-348.

13. Saitoh S, Takahashi I, Mizoguchi I, Sasano Y, Kagayama M, Mitani H. **Compressive force promotes chondrogenic differentiation and hypertrophy in midpalatal suture cartilage in growing rats.** *Anat. Rec.*, 2000, 260: 392-401.

14. Hall BK. **Selective proliferation and**

accumulation of chondroprogenitor cells as the mode of action of biomechanical factors during secondary chondrogenesis. *Teratology*, 1979, 20: 81-91.

15. Wu Q, Zhang Y, Chen Q. **Indian hedgehog is an essential component of mechanotransduction complex to stimulate chondrocyte proliferation.** *J. Biol. Chem.*, 2001, 276: 35290-35296.

16. Kumar V, Cotran RS, Robbins SL. **Robbins Basic Pathology.** Saunder 7th Ed., 2003, 68-69.

17. Elices MJ, Hemle ME. **The human integrin VLA-2 is a collagen receptor on some cells and a collagen/laminin receptor on others.** *Proc. Natl. Acad. Sci. USA*, 1989, 86: 9906-9910.

18. Shakibaei M, Zimmermann B, Merker HJ. **Changes in integrin expression during chondrogenesis in vitro: an immunomorphological study.** *J. Histochem. Cytochem.*, 1995, 43: 1061-1069.

19. Enomoto-Iwamoto M, Iwamoto M, Nakashima K, Mukudai Y, Boettiger D, Pacifici M, Kurisu K, Suzuki F. **Involvement of alpha 5 beta 1 integrin in matrix interactions and proliferation of chondrocytes.** *J. Bone Miner. Res.*, 1997, 12: 1124-1132.

20. Loeser RF. **Chondrocyte integrin expression and function.** *Biorheology*, 2000, 37: 109-116

21. Kurtis M, Schnidt TA, Bugbee WD, Loeser RF, Sah RL. **Integrin-mediated adhesion of human articular chondrocytes to cartilage.** *Arthritis Rheum*, 2003, 48: 110-118.

22. Yasuda T, Shimizu K, Nakagawa Y, Ishikawa H, Nishihara H, Nakamura T. **Possible involvement of RGD (Arg-Gly-Asp)-containing extracellular matrix proteins in rat growth plate**

chondrocyte differentiation in culture. *J. Bone Miner. Res.*, 1996, 11: 1430-1437.

23. Grodzinsky AJ, Levenston ME, Jin M, Frank EH. **Cartilage tissue remodeling in response to mechanical forces.** *Annu. Rev. Biomed. Eng.*, 2000, 2: 691-713.

24. Li KW, Wang AS, Sah RL. **Microenvironment regulation of extracellular signal-regulated kinase activity in chondrocytes.** *Arthritis Rheum.*, 2003, 48: 689-699.

25. Takahashi I, Onodera K, Sasano Y, Mizoguchi I, Bae JW, Mitani H, Kagayama M, Mitani H. **Effect of stretching on gene expression of b1 integrin and focal adhesion kinase and chondrogenesis through cell-extracellular matrix interactions.** *Eur. J. Cell Biol.*, 2003, 82: 182-192.

26. Monici M, Basile V, Cialdai F, Romano G, Fusi F, Conti A. **Irradiation by pulsed Nd:YAG laser induces the production of extracellular matrix molecules by cells of the connective tissues. A tool for tissue repair** In: "Biophotonics: Photonic solutions for Better Health Care", J. Popp, W. Drexler, V.V. Tuchin and D.L. Matthews Eds., *Proc. of SPIE* vol. 6991, 69912K1-10, 2008. Doi: 10.1117/ 12.782865.

27. Basile V, Romano G, Fusi F, Monici M. **Comparison between the effects of hypergravity and photomechanical stress on cells producing ECM.** *Microgravity Sci. Technol.*, 2009, 21: 151-157.

28. Usuba M, Akai M, Shirasaki Y. **Effect of low level laser therapy (LLLT) on viscoelasticity of the contracted knee joint: comparison with whirlpool treatment in rats.** *Laser Surg Med*, 1998, 22: 81-5.

Key words: Shoulder Pain, HILT, VAS, patient satisfaction

The challenge of shoulder pain.

Vissarakis G., Charamidis N.

Leading Head of Physical Medicine and Rehabilitation Department of Diagnostic and Therapeutic Centre of Athens "HYGEIA",
4 Erithrou Stavrou & Kifisias Str., 151 23 Marousi-Greece.

ABSTRACT

Shoulder pain (SP) constitutes a major medical, social, and economic challenge. 20% of the general population will suffer SP at least once in their lifetime. Many therapeutic techniques and modalities are used to treat SP. Rehabilitation practice should utilize a problem-oriented approach to direct treatment. However numerous factors make this difficult. Consequently the patient tends to return to the clinical practice complaining about persisting symptomatology. A review of the literature has revealed lack of evidence based work for the treatment of SP. This project has been conducted to address the issue of treating non-specific SP with the use of a new modality the HILT. 31 subjects suffering non-specific SP have taken part to this project. The participants have been treated with the HILT (Nd:YAG laser Hiro 3.0) device with the standard hand piece for the pain therapy, according to a specific protocol. The Visual Analogue Scale (VAS) pain score (climax of 10) was used to evaluate the subjective pain symptomatology prior and after the treatment application. The satisfaction index has also been evaluated at the end of the therapy. The mean values $\pm$ SD have been used for the statistical analysis. The results revealed a great reduction of the subjective pain to all individuals. The level of satisfaction was also measured very high at the end of the treatment. The study has confirmed that the Hilterapia® has shown good results

regarding the improvement of SP and the level of patient satisfaction, when applied at individuals with non-specific SP.

INTRODUCTION

Shoulder pain (S.P.) constitutes a major medical, social, and economic challenge. S.P. is frequent in the general population and is the reason why many people are seeking medical care every year. It is second only to low back pain in patients attending the clinical practice for musculoskeletal (MSK) complaints in the primary care setting [1, 2, 3, 4].

S.P. is affecting 16%-21% of the population. The 1/5 of all disability payments for MSK disorders are for patients suffering shoulder dysfunctions [5]. An estimate of 20% of the general population will suffer S.P. at least once in their lifetime [6], when about 50% of the population has at least one episode of S.P. yearly. Its incidence may be estimated at about 70-260‰ in the general population [7, 8] A Norwegian survey revealed that 46% of the population had at least one episode within the year [9]. Another Norwegian survey resulted that the weekly or more frequent prevalence of shoulder-neck pain was 13% for men and 25% for women [10].

Regarding its prevalence, S.P. and impairment may influence self dependence.

Normal shoulder function requires an integrated motion created by the interaction of almost 30 muscles. The joint complex controlled by this musculature is comprised by three joints: the glenohumeral, the

scapulothoracic, and the acromioclavicular. Long outer muscles such as the deltoid move the upper extremity. The glenoid joint is stabilized by passive structures and the rotator cuff muscles. The later short muscles are the supra- and the infra-spinatus, teres minor and subscapularis, whose tendon comprises the rotator cuff. The subacromial bursa is located between the cuff, the coracoacromial ligament and the acromion [2, 11].

Compared with the hip joint, the cavity of the glenoid gives little support. The motion of the shoulder complex is greater than any other joint in human body [2].

The basic aetiologies for S.P. are ischaemia, degeneration, and acromial morphology. Pain may be caused by numerous factors:

- Degeneration of the Rotator Cuff due to tension overload and overuse (intrinsic impingement) [2],
- Encroachment of the subacromial contents (extrinsic impingement) [2],
- The potential mechanisms causing structural compression include dysfunctional glenohumeral and scapulothoracic kinematics, degeneration and inflammation of tendons and bursa, acromial morphology (flat, curved, hooked), postural dysfunctions of the upper quarter, weak or dysfunctional rotator cuff and scapular musculature, and capsular laxity or tightness. These mechanisms may occur individually or in combination [2, 3, 4, 5, 11].
- Work activities that entail using the arms overhead or working with hand loads, increase the risk of developing S.P. [2, 4, 5, 11, 12].

Pain may be elicited from different tissues such as tendon bursa, ligament and muscle. Shoulder function may be altered by pain, structural abnormality or by fear of pain. Free nerve endings containing substance "P" and calcitonin-gene-related peptides and mechanoreceptors, have been identified in subacromial bursa and are being blamed as source of subacromial pain [2, 3, 4]. Disorders are classified by pathological process (tendonitis, tendinosis, rupture),

by anatomical localization (rotator cuff disease, subacromial pain syndrome), by mechanism (impingement syndrome) and by aetiology (work related shoulder pain, repetitive strain syndrome) [1, 2, 3].

In the literature there have been identified numerous diagnoses to justify shoulder pain: adhesive capsulitis, sympathetic dystrophy or shoulder-hand syndrome, osteoarthritis, glenohumeral arthritis, subacromial pain (impingement syndrome), acute tendonitis / bursitis, tendinosis / chronic subacromial pain, rotator cuff tears, instability, S.P. after stroke, Thoracic outlet syndrome, and non-specific shoulder pain, are only a few among the existing diagnoses to explain S.P. and pathology [2, 3].

Many diagnostic tools have been developed to lead to the most precise diagnosis for S.Ds. MRI, X-Ray, 3D CT scan, physical examination; thorough history and symptoms assessment are commonly used in the clinical practice.

Shoulder pain causes disability, complaints, lower quality of life, absenteeism and sickleave, and a major factor of money-loss for the healthcare system, the employer and the patient. It also has psychological effects, causing cognitive and behavioral dysfunction.

Many therapeutic techniques and numerous modalities are used to treat S.P.. These include: therapeutic exercise, manual therapy, mobilization, manipulation, thermotherapy, massage, electrical stimulation, shockwave therapy, therapeutic U/S, mechanical traction, electrotherapy, low level laser and acupuncture, regarding the physiotherapeutic management. Other approaches consist of the use of NSAIDs and steroid injections. Of course there are also invasive surgical interventions that are usually used to fix the cause of the disorder or to alleviate pain symptoms and restore joint ROM [13].

Rehabilitation practice should utilize a problem-oriented approach to direct treatment. That is, specific problems should be identified for each patient individually and the treatment should be developed to address these problems.

However numerous factors make it difficult if not impossible to utilize a problem-oriented approach:

- Generalized treatments are often applied universally to patients with various problems. This may confound the ability to determine treatment efficacy.
- Furthermore, it is often not clear if the interventions studied are evidence-based themselves.
- Also confounding is the fact that there exist many different operational definitions to describe a certain condition, which may mislead the rehabilitation professional.
- The lack of consistent terminology may also reflect the uncertain relationship between S.P., radiological and histopathological findings.
- Most of the times, the diagnosis is rather conflicting and consequently the therapeutic modalities are unable to focus on the core of the problem to resolve pain symptoms [2, 4].

Consequently the patient tends to return to the clinical practice complaining about its persisting symptomatology. At the basis of this argument, this paper has been conducted as a descriptive observational study, to discuss the problem of patients experiencing S.P., who tend to visit several different rehabilitation practices complaining about non-alleviated S.P. These patients have often used every diagnostic assessment tool and have been treated with every available modality; however they seem to experience persistent S.P. They usually appear to the clinical practice experiencing pain, stiffness, reduced ROM, but also psychological problems and distress. The pain problem has been identified as a multifactor issue and as that it is going to be addressed.

A review of the literature has revealed a lack of evidence based work for the treatment of S.P. [14]. However it seems that there is a ray of light coming from a new modality, the High Intensity Laser Therapy (HILT). The Low Level Laser Therapy (LLLT) is already known. Theoretically laser energy

is transmitted to induce cell proliferation. Although current evidence is conflicting, it appears LLLT is beneficial regarding pain and disability when applied as a single intervention for patients with S.P. Current studies support that LLLT was effective in pain relief and improvement of functional ability among patients with shoulder-neck pain [15].

Basford et al. [16] have also concluded that the modality had also shown effectiveness regarding the perception benefit and level of function in patients suffering MSK back pain.

It has also been highlighted that Laser improves topical blood circulation which seems to be an issue regarding shoulder pain. Two different studies [17, 18] have addressed the issue of the changes in blood flow and EMG in chronic shoulder-neck pain due to trapezius myalgia. They both concluded that MSK pain increases the transmitter activity of neuropeptides causing impairment of the blood flow in the muscle.

Recently it has been shown that overhead static use of the shoulder especially with the hand loaded with up to two Kg of weight, alters the EMG activity of upper trapezius and anterior deltoid shoulder muscles, leading to impairment of the blood flow and resulting in pain and chronic MSK complaints around the shoulder area [12]. Little research has been conducted to address the issue of treating MSK S.P. and dysfunction with the use of HILT. What makes HILT a promising modality is the ability of the higher energy luminous radiation to be transferred to the deepest layers of the tissue without releasing too much energy to the superficial musculature. Another positive is that the light is transmitted through pulses instead of a continuous way. There is some scientific evidence showing that on a cellular level the bio-stimulating effect activates some enzymes, increasing the production of nucleic acids and proteins, but also the metabolic exchanges, potentially leading to reduction of inflammation fluids, edema

and pain. Much work has been published to address the issue of impaired blood flow of the painful shoulder. The HILT through its photothermal effect, causes a controlled increase of the tissue temperature, activates circulation stimulation and a correlated increase of oxygen supply to the suffering structures [19].

MATERIALS AND METHODS

Patients

31 subjects suffering non-specific shoulder pain have taken part to this project. The sample used was constituted by 15 male and 16 female participants, aged between 25 and 65 (mean age 49 years). All patients have suffered shoulder pain for at least one year, and have taken no specific diagnosis, however the clinical or radiological testing. They all have visited at least one physician or special physical therapist in the past year without alleviating results. All the patients attended the clinical practice of the diagnostic and therapeutic centre of Athens "Hygeia" because of their problem and have been asked to participate to this project by the leading head of the department. The later, Dr. of physical medicine and rehabilitation obtained an informed consent by each individual participating to the project, prior to its initiating. It should also be mentioned that all participants were Caucasian. Before the project initiated the physician of the department has taken a thorough history and has completed a clinical examination of every individual. The hall project and data collection have taken place at the "Hygeia" hospital during a 12 months period between October 2007 and October 2008.

Exclusion Criteria

A potential participant would be excluded if they had a tumor, or any neoplastic disease diagnosed in the past. They would also not be allowed to participate if they were under NSAIDs or heavy analgesic treatment for the past 2 weeks. Non compliant patient

(cognitive and behavioral impairment), patients with psychiatric history, and patients with a very recent unhealed fracture, individuals with rheumatic or other inflammatory diseases, were also considered as reasons for exclusion from the project.

with the standard hand piece for the pain therapy. The protocol used was selected by the physician and the physical therapist that was conducting the study and was modified according to the painful symptoms of each patient. This protocol is being described at the following table.

PHASE	SUBPHASE	FLUENCE mJ/cm ²	FREQUENCY	MODE	TOTAL ENERGY
Initial	Step 1: 500J Step 2: 500J Step 3: 500J	810 970 1070	Level 10 Level 9 Level 8	Fast Fast Fast	1500 J
Intermediate	Step 1 Step 2 Step 3 Step 4 {The points were selected according to each patient's painful areas}	810 970 1070 810	Level 4 Level 4 Level 3 Level 5	Static	Depended on the points treated at each step
Final	Step 1: 500J Step 2: 500J Step 3: 500J	810 910 1070	Level 11 Level 10 Level 9	Slow Slow Slow	1500 J

Table 1: HILTERAPIA® PROTOCOL. The parameters used to treat shoulder pain (J: Joule, MJ: Millijoule, CM: centimeter).

Treatment

All patients have undergone a specific rehabilitation program aimed at alleviating their pain symptoms and restore their movement. The participants have been treated with the HILT according to a specific protocol, and have also participated to an individualized kinesiotherapeutic program aimed at restoring the joint range of movement (ROM) and muscular strength.

Measurement Tools

The Visual Analogue Scale (VAS) pain score (climax of 10) was used to evaluate the subjective pain symptomatology prior and after the treatment application. The satisfaction index has also been evaluated at the end of the therapy. All patients have been asked to subjectively evaluate their level of satisfaction (very satisfied, satisfied, little satisfied, or not satisfied) after the application of the therapeutic program.

Methodology

In the present study each patient has been treated using the Hilterapia® pulsed Nd:YAG laser Hiro 3.0 device (ASA S.r.l., Vicenza, Italy) (peak power 3000W)

Because this project has been conducted as a descriptive observational study, a randomization of the participants to more than a single group of therapy has not taken place. Instead, there was a single group of patients, undergone the same therapeutic program. Before each therapeutic session all participants were being asked to complete the VAS. After the last session the individuals answered the subjective level of satisfaction test. Treatments were administered every day for the first week and every second day for the following 2 weeks for a total of 10 sessions. The kinesiotherapeutic protocol included active and passive mobilization exercises of the shoulder girdle, kinesthetic exercises of the shoulder, light resistance exercises of the arm, ROM exercises and proprioceptive exercises for 15 minutes. All 31 patients who have been found to fulfill the inclusion criteria to participate to the study have finished all the 10 physiotherapy sessions without any dropouts.

Data Analysis

The data obtained have been analyzed using Office 2007 Excel software (Microsoft Windows®). The mean values $\pm$ SD have been used for the statistical analysis rather than another more complicated statistical test, as this project purported to show any difference from the use of a new modality. The level of significance for the data obtained has not been identified.

RESULTS

After treating all the 31 patients, with 10 sessions of HILT the following results were found:

The mean values by which the individuals described their pain through the VAS before and after the treatment were as follows: before the treatment the mean value of pain was 8.1 ± 0.96 and after the treatment application that was formed at 3.9 ± 1.07 ; the average variation was of 4.2 (figure 1).

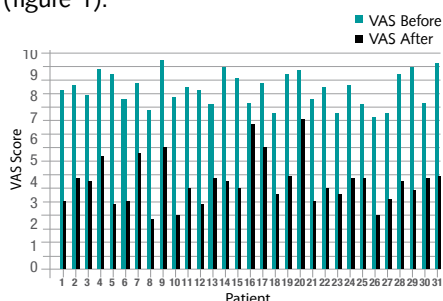


FIGURE 1: Pain Level, in each patient, before and after the entire treatment with the Hilterapia®.

The Mean VAS scores for all participants before and after the application of the hall treatment program have shown an

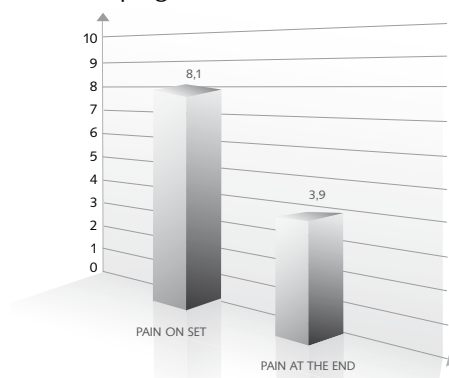


FIGURE 2: VAS score mean values before and after the treatment application

average decrease of 4.2 points on the 10 points climax of the VAS regarding pain perception. This can be easier understood by studying Figure 2 which is exposed below.

The level of satisfaction as a subjective measure of how did the patients evaluated their treatment was as follows: the 68% (21 patients) answered that they were very satisfied with the results of the treatment regarding pain levels. The 26% (8 patients) answered that they were satisfied with the results. Only the 6% of the individuals (2 patients) answered that they were little satisfied by the application of the treatment modality. 0% answered that they were not satisfied by the treatment (Figure 3).

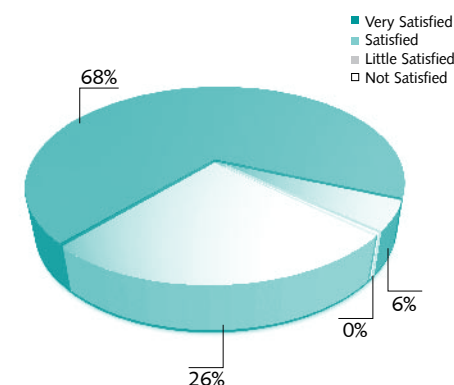


FIGURE 3: The satisfaction index at the end of the treatment.

DISCUSSION

This project has been conducted to address the issue of treating non-specific shoulder pain with the use of a new modality, the HILT. To the authors' knowledge and according to an extensive literature review, there have been no other scientific works conducted around this knowledge area. That makes this study the first to approach this specific issue which seems to be relevant regarding the everyday clinical practice.

According to previous research, the HILT seems to have good effects on pain, inflammation and edema regarding many musculoskeletal disorders [19, 20, 21, 22]. It seems that the high intensity pulsed light has an effect on the microcirculation of the region to which it is applied. The impairment of the microcirculation seems

to be an issue regarding the painful shoulder as has been demonstrated by recent studies [12, 17, 18]. Its topical thermal effects and the concentration of blood supplies seem to improve the healing processes. This fact also helps to decrease the inflammation fluids that tend to take part in a painful process at the shoulder area. The ability of the higher energy luminous radiation to be transferred to the deepest layers of the tissue without releasing too much energy to the superficial muscles seems to be another good effect regarding the painful shoulder, as that is covered by several muscle layers [19].

At this study the authors have tried to reveal any effects of the HILT on the shoulder area of individuals with non-specified, multi-diagnosed shoulder pain. As that consists a preliminary descriptive study, the writers should recognize that the statistics were not the most suitable to reveal any statistically significant effect by the use of the modality on the results of the measurement tools on the individuals' perception of pain and satisfaction. However, that could form the basis for more research to be conducted on that field of science. We also recognize that more appropriate tools could be used to obtain more measures about the healing processes taking part at the shoulder area when applying the HILT. However, that project took place as an observational procedure during everyday clinical practice, in order to form a preliminary idea of how that modality may or may not work on patients. We should not forget to mention that we were referring to patients that have been treated in the past with all available techniques and modalities and have not reached pain relief. That makes the results of this preliminary observational study rather important.

All in all, the results of this project showed an improvement regarding pain and level of satisfaction of all 31 patients that have been treated with the HILT. It is assumed that a larger sample of patients and also a follow up procedure would be more appropriate to scientifically research this specific issue.

CONCLUSIONS

This preliminary observational study has confirmed that the Hilterapia® has shown good results regarding the improvement of shoulder pain when applied at individuals with non-specific SP. It has also shown good results regarding the objective perception of the level of patient satisfaction, regarding patients that have used all other kinds of treatment without improvement. Although it appears that there are issues that lower the external validity and reliability of our results, it seems that this study could form the basis for more research to be conducted on this challenging scientific domain.

ACKNOWLEDGEMENTS

The editors of this article would like to thank the management of the hospital which supported and enhanced this project to be completed. They would also like to thank the leading head of the physiotherapy department for all the services that he provided in order for this project to be fulfilled.

REFERENCES

- 1) Steinfeld R, Valente RM, Stuart MJ. **A common sense approach to shoulder problems.** Mayo Clinical Project, 1999, 74(8): 785-794.
- 2) Brox J. **Shoulder Pain. Best Practice & Research.** Clinical Rheumatology. 2003, 17(1): 33-56.
- 3) Bhargav D, Murrell G. Shoulder stiffness: diagnosis. **Australian Family Physician**, 2004, 33(3): 143-147.
- 4) Van der Heijden GJ. Shoulder disorders: a state-of-the-art review. **Bailliere's Clinical Rheumatology**, 1999, 13(2): 287-309.
- 5) Michener L, Walsworth M, Burnet E. **Effectiveness of Rehabilitation for Patients with Subacromial Impingement Syndrome: a Systematic Review.** Journal of Hand Therapy, 2004,17:152-164.
- 6) Kelton M, Stevenson H, Czarnecki G, Dorfman J. Chronic Shoulder Pain: **Part II. Treatment. American Family Physician**, 2008, 77(4): 493-497.
- 7) Bjelle A. Epidemiology of shoulder problems. **Bailliere's Clinical Rheumatology**, 1989, 3: 437-451.
- 8) Bergenudd H, Lindgarde F, Nilsson B. **Shoulder pain in middle age. A study of prevalence and relation to occupational work load and psychological factors.** Clinical Orthopaedics and related research, 1988, 231: 234-238.
- 9) Natvig B, Nessioy I, Bruusgaard D. **Musculoskeletal symptoms in a local community.** European journal of general practice, 1995, 1: 25-28.
- 10) Hasvold T, Johnsen R. **Headache and neck or shoulder pain – frequent and disabling complaints in the general population.** Scandinavian journal of primary health care, 1993, 11:219-224.
- 11) Bhargav D. and Murrell G. **Shoulder stiffness: management.** Australian Family Physician, 2004, 33(3): 149-152.
- 12) Charamidis N. **EMG activity alterations of upper trapezius and anterior deltoid muscles, in different angles of shoulder flexion, under different hand-loading conditions.** MSc Dissertation Theses. 2006, QMU copyright.
- 13) Sauers E. **Effectiveness of Rehabilitation for Patients with Subacromial Impingement Syndrome.** Journal of athletic Training, 2005, 40(3): 221-223.
- 14) Green S, Buchbinder R, Hetrick SE. **Physiotherapy Interventions for Shoulder Pain (Review).** Cochrain, 2009, 2: 1-180.
- 15) Gur A, Sarac A, Cevik R, Altindag O, Sarac S. **Efficacy of 904 nm Gallium Arsenide Low Level Laser Therapy in the Management of Chronic Myofascial Pain in the Neck: A Double-Blind and Randomize-Controlled Trial.** Lasers in Surgery and Medicine, 2004, 35:229-235.
- 16) Basford J, Sheffield C, Harmsen W. **Laser Therapy: a Randomized, Controlled Trial of the Effects of Low-Intensity Nd:YAG Laser Irradiation on Musculoskeletal Back Pain.** Archives of Physical Medicine and Rehabilitation, 1999, 80: 647-652.
- 17) Larsson R, Cai H, Zhang Q, Oberg P, Larsson S. **Visualization of chronic neck-shoulder pain: Impaired microcirculation in the upper trapezius muscle in chronic cervico-brachial pain.** Occupational Medicine, 1998, 48(3): 189-194.
- 18) Larsson R, Oberg P, Larsson S. **Changes of trapezius muscle blood flow and electromyography in chronic neck pain due to trapezius myalgia.** Pain, 1999, 79: 45-50.
- 19) Viliani T, Ricci E, Mangone G, Graziani C, Pasquetti P. **Effects of Hilterapia® vs. Viscosupplementation in knee osteoarthritis patients: a randomized controlled clinical trial.** Energy for Health: International journal of information and scientific culture, 2009, 3(3): 14-16.
- 20) Conte P, Santamato A, Fiore P, Lopresto A, Mazzaracchio M. **Treatment of chronic low back pain: back school versus Hilterapia®.** Energy for Health: International journal of information and scientific culture, 2009, 3(3): 10-13.
- 21) Gleiser J. **Hilterapia® efficiency in handling the post surgical pain after the release of the carpal tunnel.** Energy for Health: International journal of information and scientific culture, 2009, 3(3): 18-21.
- 22) Valent A. Muscle lesions in athletes: **case comparison between Hilterapia® and traditional therapy.** Energy for Health: International journal of information and scientific culture, 2009, 3(3): 22-25.



Key words: knee osteoarthritis, laser therapy, High Intensity Laser Therapy, HILT

“High intensity laser therapy in knee osteoarthritis: comparison between two different pulsed-laser treatment protocols”.

Viliani T., Martini C., Mangone G., Pasquetti P

Agency for Recovery and Rehabilitation, AOU Careggi, Florence, Italy.

ABSTRACT

High Intensity Laser Therapy seems to be very effective in pain and function control in patients with KO, due to its high intensity and to the depth reached by the laser ray, but the optimal dose is not known yet.

A previous research found a comparable efficacy to viscosupplementation in knee osteoarthritis II –III Kellgren stage, using the antalgic –antiphlogistic protocol (10 treatment sessions of pulsed high power laser, Nd:YAG).

The aim of this study was to compare the efficacy of two different HILT protocols to viscosupplementation in patients with symptomatic KO.

58 out-patients with symptomatic KO (II-III Kellgren-Lowrence Scale stage) were enrolled and evaluated by WOMAC Scales, before treatment (t0), after treatment (t1) and after 4 months (t2). After randomization, the treatment consisted in viscosupplementation (4 Hyaluronic acid

infiltrations 1/week) for Group A, HILT antalgic treatment (10 sessions, three times a week for Group B, 5 sessions three times a week for Group C).

All the three groups showed a highly statistically significant improvement between t0 and t1 in WOMAC Scales, which was maintained at follow-up (t2). No side effect was found, neither in Group A nor in Group B, nor in Group C.

HILT treatment showed analogous results to viscosupplementation. HILT seems a good medical instrument for pain control and for the improvement of patient's quality of life, with dose-related effects.

INTRODUCTION

In last ten years HILT has been widely used in several painful conditions, especially in sports lesions (contusions, tendon injuries, muscular sprains, ect.) and other acute osteomuscular diseases (tendonitis, bursitis, etc) [1,2].

More recently interesting results

about degenerative diseases, such as osteoarthritis and low back pain have been reported too [3,4].

Available scientific literature is not wide nor definitive about HILT, for many reasons, i. e. scarceness of related studies, scarceness of shared and approved therapeutic protocols, absence of evidence about laser's efficacy in KO therapy.

So, it would be necessary to approach HILT with rigorous and rationale protocols, both in biological and clinical research, to find experimental evidences and the best way to cure patients.

From this point of view, in our Rehabilitation Service we are studying the effects of HILT in patients with degenerative diseases, such as osteoarthritis, which are characterized by pain and functional limitations. These chronic conditions affect the quality of life of the majority of patients, especially the elderly. [5,6]

KO could be considered as a “social disease”, considering its prevalence (very important, though underestimated), its relationship with the disability (in fact, disability is directly correlated with pain level), and the necessity of long-lasting and integrated treatments, which include a pharmacological (drugs, hyaluronic acid) and a non-pharmacological aspect (exercise, FKT, physical therapy).

In relation to this we should consider important side effects of pharmacotherapy [7], and the not negligible weight of direct and indirect costs of the management of patients like that.

For the right and correct care of these patients physicians should follow international guidelines in association with an EBM approach, to conceive an individualised and adapted management plan, which considers patient's characteristics and needs.

International guidelines [8] for the treatment of KO provide a patient-centred care, a holistic approach to the disease, and a balanced combination of pharmacological and non-pharmacological treatment modalities. The main tasks of the integrated treatment in KO are pain

relief and disability reduction; between non-pharmacological means guidelines recommend also the physical therapy, and more precisely electrotherapy (TENS) and thermotherapy. Physical therapy may be useful for its local effects against pain.

EBM literature about HILT is not available yet; anyway some good researches are producing interesting results in experimental and clinical domains. HILT effects are due to photothermal, photochemical and photomechanical actions [9,10], which lead to pain and flogosis reduction and seem to stimulate reactivation in connective tissues. Nevertheless in clinical researches different HILT protocols are proposed, in relation to session's duration and timing. It is not completely clear which could be the optimal HILT doses to obtain the best results.

In a previous research [11] we found a high efficacy of HILT in treating knee osteoarthritis patients, achieving long-lasting symptomatic and functional improvement, using an antalgic – antiflogistic protocol. In this study the effects of HILT were comparable to the effects of viscosupplementation [a well known and accepted modality to improve pain and perhaps the osteoarthritis evolution [12,13], at least for clinical aspects. HILT results were rapid, long-lasting and no side effects were observed. The patients were all compliant to the treatment. The protocol of that study provided 10 HILT sessions, but in our experience we saw the patients rapidly improving after the initial sessions, reaching a plateau in the latter ones.

It is possible that the immediate effect was due to the direct analgesic laser properties, while antiflogistic effect is responsible for slower and more lasting response. Our purpose was to evaluate the efficacy of a shorter HILT protocol, which could be even easier for patients and at the same time could save physician's time too (in fact, the physician is directly engaged in performing the manual lasertherapy program). The patients treated with viscosupplementation were chosen as control group because viscosupplementation is a local intervention supported by EBM in KO [14].

The present study was a prospective, three arm, open-label, randomized clinical trial.

The aim was to evaluate the clinical and functional efficacy of a short HILT treatment, compared with a longer (standard) protocol and with viscosupplementation, in patients affected by symptomatic knee osteoarthritis.

MATERIALS AND METHODS

Patients. Patients suffering for symptomatic KO were recruited for this trial from outpatients of the Recovery and Rehabilitation Agency (AOU Careggi, Firenze). A total of 58 patients with symptomatic KO (pain and functional limitation), aged 52-80 years, were included. Informed consensus was obtained. Inclusion criteria required the presence of symptomatic KO (following ACR criteria [15] I-III stade of Kellgren-Lawrence Scale [16] on the radiological evaluation. Exclusion criteria were: therapy with oral anticoagulants, non compliant patients (cognitive impairment or psychiatric disorder), neoplastic pathology, presence of deep vein thrombosis. The patients' evaluation included history and clinical examination.

Initial assessment (t0), before treatment (t1), and follow-up (t2) included WOMAC Scale [17]

The patients were randomized for treatment in three groups, following the method of random number table.

Treatment. After randomization the patients underwent three different treatment protocols: Group A (18 patients) was treated with hyaluronic acid intraarticular infiltrations (4 infiltrations, 1 session/week, mw 500-1000 kD), Group B (19 patients) was treated with High Intensity Laser Therapy (ten sessions, on alternate days), and Group C (21 patients) was treated with High Intensity Laser Therapy (five sessions, on alternate days) see Table I. The only difference between the two HILT protocols was the number of sessions (10 vs. 5 sessions).

The patients were reassessed at the end of the treatment (t1) and after 4 months (t2).

Data analysis. Data of patients were compared by Student t-test and Wilcoxon test.

RESULTS

A total of 58 patients were recruited and included in the analysis. 18, 19 and 21 patients respectively were randomized to Hyaluronic acid treatment (Group A) and HILT-ten sessions- (Group B), and to HILT -five sessions- (Group C). All the patient but one (Group A) finished the study. Baseline data of the three Groups are explained in table II. Although this was a randomized comparative study, the small number of patients did not guarantee against differences between treatment groups' baseline characteristics. The experimental groups resulted not exactly balanced for all the variables collected at baseline. Nevertheless the three groups resulted comparable and the resultant variables were not related to the initial differences. Total median age was 74,4 years (range:53-84); 71,0 years (range:54-81) and 70,5 (range: 52-79) and 69,6 (range 51-72) for Group A, Group B and Group C respectively, while the proportion of male (M) and female(F) patients was analogous. WOMAC Scale values at t0 were 41,6 ±10,1 (Group A), 42,9 ± 7.3 (Group B) and 42, 3 ± 10, 5.

At t1 the three groups showed improvement in the scales points: Group A changed WOMAC values from 41,6 ± 10,1 to 22,5 ± 12 (p< 0.001). WOMAC values of Group B varied from 42,9 ± 7,3 to 21,4 ± 4 (p<0.001), and Group C varied its values from 42, 3 ± 10, 5 to 24, 8 ± 11 (p< 0,001), see Table III and Figure 1a-b. At follow-up (4 months) both the groups A and B maintained the improvement, while Group C showed a little regression of WOMAC values to 28,05 ± 15 points, which anyway was not statistically relevant. Focusing the attention on Group C (21 patients) we can affirm that at time t1 improved patients were 20 (95,3 %), and unchanged patients were 1 (4,7 %); at time t2 (4-months follow up) the same group (20 patients – 1 drop-out) showed that the improvement was stable in 19 patients (85 %), while a regression could be observed in 3 patients (15 %), see Table IV and figure 2.

No side effects were observed in any of the 3 examined groups.

Hyaluronic acid infiltrations protocol (Group A): 4 sessions of Hyaluronic acid infiltrations, molecular weight 500 -1000 kD, once a week. Infiltration is performed by anterior access with supine patient and flexed knee.
HILT treatment protocol 1 (Group B): pulsed high power laser, Nd:YAG, λ 1064nm, 10 sessions, on alternate days, analgesic program, in manual scansion. This program is articulated in three phases (initial, intermediate and final phase). Every phase is articulated in sub-phases in which increasing fluency (510-710 J/cm ²) and decreasing frequency (15-10 Hz) are administered, total energy 2000-3000 J. The total session duration is 15-20 minutes.
HILT treatment protocol 2 (Group C): same program as above, 5 sessions instead of 10

Table I: Treatment protocol of the three groups

	Pats. number	Median age	sex	WOMAC Scale
GROUP A	18	71,0	4 M, 14 F	41,6 ±10,1
GROUP B	19	70,5	4 M, 15 F	42,9 ± 7,3
GROUP C	21	69,6	3 M, 18 F	42,3 ± 10,5

Table II: Groups baseline characteristics

	WOMAC Scale t0	WOMAC Scale t1	WOMAC Scale t2
GROUP A	41,6 ±10,1	22,5 ± 12 (p<0.001)	20,9 ± 8 (p:ns)
GROUP B	42,9 ± 7,3	21,4 ± 4 (p<0.001)	23,4 ± 10 (p:ns)
GROUP C	42,3 ± 10,5	24,8 ± 11 (p<0,001)	28,05 ± 15 (p: ns)

Table III: WOMAC Scales Values at t0, t1 and at the follow-up (t2) of the three Groups

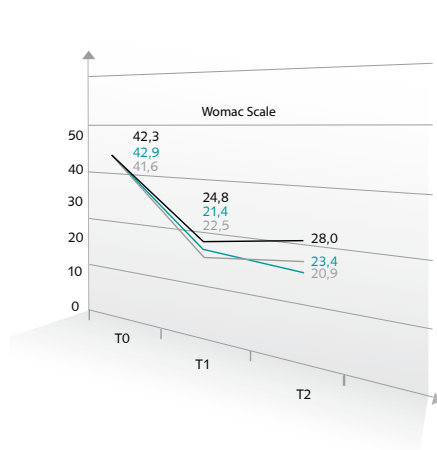


Figure 1a WOMAC Values before treatment (t0), at the end of treatment (t1) and after 4 months

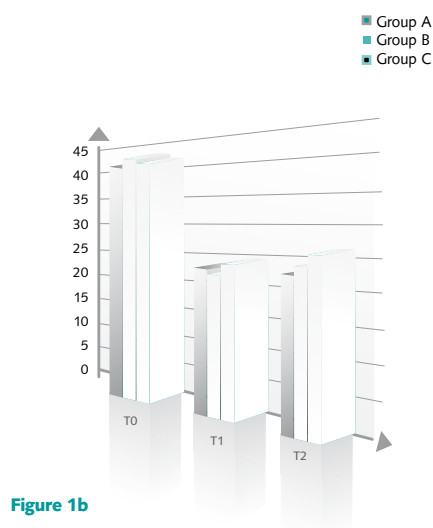


Figure 1b

t2 (20 pts., 1 drop-out)	Improvement maintained	21,4 ± 4 (p<0.001)	23,4 ± 10 (p:ns)
Improvement regression	19	24,8 ± 11 (p<0,001)	28,05 ± 15 (p: ns)

Table IV: Group C (5 HILT sessions) results

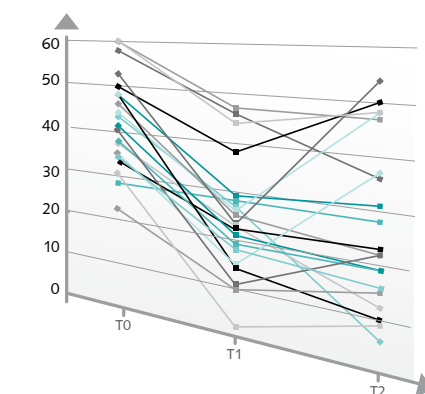


Figure 2 Womac values of single patients (Group C) and after 4 months

DISCUSSION

Scientific interest is growing about HILT, due to demonstrations of its efficacy and sureness. These first results must be considered as preliminary but seem to be consistent. KO is characterized by phlogistic and degenerative aspects at the same time, which lead to the typical semeiological and clinical signs and symptoms, that is acute and painful phases on a degenerative and chronic background. Given that physical therapy is only a part of an integrated approach, techniques nowadays available have no great effects, limited to pain control (such as TENS) or to a superficial antiphlogistic action (such as ultrasound, low level laser therapy, etc.), and in any case they generally have a short lasting effect. HILT effects are different from other forms of physical therapy because of its action mechanisms which comprehend the classical effects of laser therapy on tissues (photochemical, photothermal and photomechanical) but with different intensity and erogation mode. The specificity of HILT (especially due to the photomechanical effect) is the reason of its almost immediate antalgic effect, probably

followed by an anti-inflammatory action, which starts more slowly but has subsequent lasting effects. Besides, photomechanical effect is responsible, in vitro and animal model [18,19], for several cellular and macromolecular changes and reorganization in connective tissues, which could be driven to a physiological healing. Regarding hyaluronic's acid effects, our results agree with data found in the literature, because patients improved significantly at the end of the treatment and they maintained this improvement at follow-up, showing a long acting effect of this therapy.

Our study showed, moreover, an optimal efficacy of HILT treatment: in relation to 10-sessions HILT protocol the efficacy is really comparable to viscosupplementation; we achieved a rapid pain relief, even after the very first sessions, and this effect is maintained at the follow-up, 4 months later.

So, our local and limited experience shows a good clinical efficacy for HILT, but till nowadays this method feels the effect of the scarceness of scientific data and related studies.

During these preliminary researches we verified that 10 alternated days sessions are a very good treatment for pain control, but we still don't know which really is the optimal timing of the laser sessions; besides, in this initial experience it seemed to us that patient's improvement begins rapidly in the first sessions, reaching rapidly a plateau all the same. The shorter protocol, which provided 5 alternated day sessions was very effective too, in terms of rapid pain reduction and disability improvement at the end of the treatment. Follow-up results are interesting to discuss because patients which received the shorter treatment (Group C) showed a tendency to regression. So the duration of HILT effects seems to be dose-related. It is difficult to generalise our argumentation, due to the little number of patient examined.

The results of our study seem to confirm our original hypothesis. Pain shows a very rapid reduction together with a functional improvement (pain is the most important determinant in disability). A long term effect need a longer treatment.

CONCLUSIONS

HILT confirms to be a good non-pharmacological instrument for rapid pain control in KO, with consequent improvement in patient's quality of life. Important effects are achievable with few sessions of HILT treatment too. Clinical comparison between the two different HILT protocols seems to suggest that pain relief is rapidly achievable, as a direct effect, but the indirect effects (based on antiphlogistic action and may be on tissues reorganization) need a longer treatment to obtain lasting results.

REFERENCES

1. Valent A. **Trattamento delle distorsioni capsulo-legamentose di caviglia con Hilterapia. Seminario "Lesioni capsulo-legamentose della caviglia"** Villasanta (MI), 2006.
2. Lubich T et al. **Impiego del laser di potenza nel trattamento precoce e nel recupero dell'atleta infortunato.** Medicina dello Sport, 1997, 50:71-83.
3. Zati A et al. **Trattamento della lombalgia causata da ernia del disco: confronto tra laser ad alta potenza, TENS e FANS.** Med Sport, 2004, 57:77-82.
4. Zati A, Fortuna D, Benedetti E, Zaghini I, Bigotta TW. **HILT Therapy nel trattamento della gonartrosi: primi casi clinici e protocollo per uno studio multicentrico in doppio cieco randomizzato.** Atti 1° Convegno Nazionale Dominare l'Energia, Report Scientifico Hilt Therapy 2006
5. Baroni A, Mannoni A. **Artrosi e disabilità.** G Gerontol, 2004, 52:259-261.
6. Corti MC, Guralnick JM, Sartori L, Baggio G, Manzato E, Pezzotti P, Barbato G, Zambon S, Ferrucci L, Minervini S, Musacchio S, Crepaldi G. **The effect of cardiovascular and osteoarticular disease on disability in older Italian men and women: rationale, design and sample characteristics of the Progetto Veneto Anziani (PRO.V.A.) Study.** J Am Geriatr Soc, 2002, 50:1535-1540.
7. Puddu GM, Cucinotta D. **Iatrogenesis and osteoarthritis.** Giorn Geront, 2001, 49:658-660.
8. Punzi L, Canesi B, Carrabba M, Cimmino M.A, Frizziero L, Lapadula G, Arioli G, Chevallard M, Cozzi F, Cricelli C, Fioravanti A, Giannini S, Iannone F, Leardini G, Cannoni A, Meliconi R, Modena V, Molfetta L,

Monteleone V, Nava Y, Parente L, Paresce E, Patrignani P, Ramonda R, Salaffi F, Spadaio A, Marcolongo R. **Consensus italiana sulle raccomandazioni dell'EULAR 2003 per il trattamento dell'artrosi del ginocchio.** Reumatismo, 2004, 56(3):190-201.

9. Corti L. **Fondamenti della laserterapia e della Hilterapia.** Atti 2° Congresso Nazionale Hilterapia, Milano 6-8 Giugno 2007, pag 90-96.

10. Corti L. **Fondamenti della laserterapia e della Hilterapia.** Atti 2° Congresso Nazionale Hilterapia, Milano 6-8 Giugno 2007, pag 90-96.

11. Viliari T, Ricci E, Mangone G, Pasquetti P. **Effects of High Intensity Laser Therapy vs. Viscosupplementation in patients with knee osteoarthritis: a randomized controlled clinical trial.** Energy for Health, 2009, 3:15-17.

12. Lavelle ED, Lavelle L. **Intra-Articular Injections.** Medical Clinics North America, 2007,(91):241-250.

13. EBMonline. Review: **Viscosupplementation for knee osteoarthritis reduces pain and improves function.** Evidence-Based Medicine, 2006, 11:12; doi:10.1136/ebm.11.1.12.

14. Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G. **Viscosupplementation for the treatment of osteoarthritis of the knee (review).** The Cochrane Library, 2008, Issue 1.

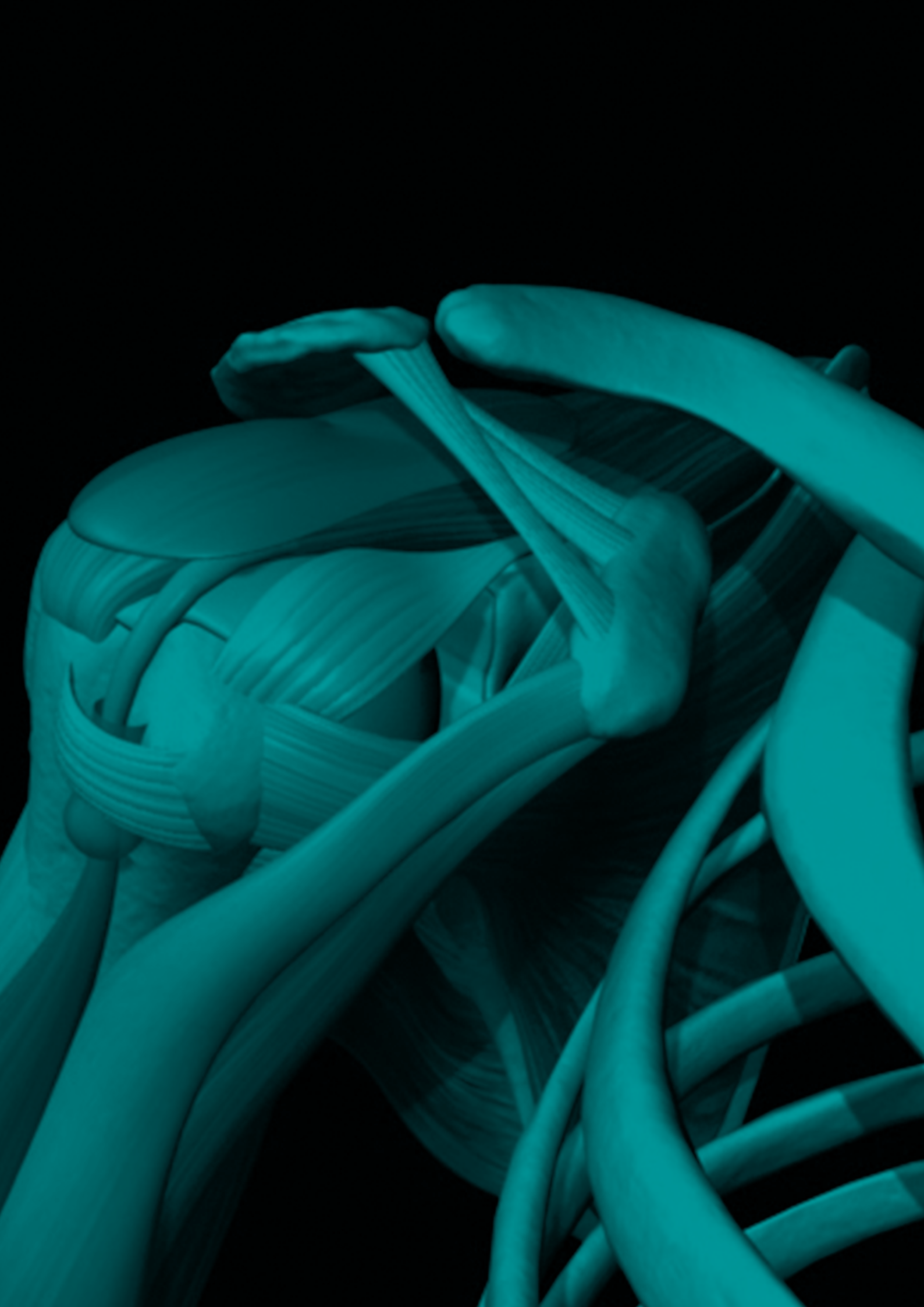
15. Altman RD. **Classification of Disease: Osteoarthritis.** Seminars in Arthritis and Rheumatism, 1991, 20 (6 Suppl. 2):40-47.

16. Kellgren JH, Lawrence JS. **Radiographic assesment of osteoarthritis.** Ann Rheum Dis, 1957, 16:494-501.

17. Bellamy N, Buchanan VWW, Goldsmith CH, Campbell J, Stitt LW. **Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy with osteoarthritis of the hip or knee.** J Rheumatology, 1988, 15(12):1833-40.

18. Rossi G. et al. **Attività citoproliferativa della HILT: indagine in vitro** Report Scientifico 2006

19. Fortuna D et al. **"Hilterapia nel trattamento dell'artrosi: indagine sperimentale su modello animale"** Report Scientifico Hilterapia, 2006.



ABSTRACT

Key words: lymphoedema, laser therapy, High Intensity Laser Therapy.

Hilterapia® and lymphoedema.

Nicolaou V.

Physiotherapy Center, Makedonia Street, 4001 – Limassol, Cyprus.

ABSTRACT

Lymphoedema is a wide spread disease which consists in the swelling of soft tissues as a result, initially, of accumulation of protein rich fluid in the extracellular spaces. If untreated, this is eventually replaced by fibrous tissue, with reduced oxygenation. Lymphoedema has four main characteristics:

- Excessive protein in the tissues.
- Excessive fluid in the tissues
[both intra and extracellular fluids]
- Excessive deposition of fibrous tissue.
- Chronic inflammatory reactions.

Lymphoedema can be classified as primary and secondary:

1. Primary lymphoedema develops in people with inadequate, insufficient or poorly functioning lymphatics.
2. Secondary lymphoedema develops in response to damage, disease and surgical removal of nodes or irradiation of tissues in radiotherapy.

Lymphoedema develops in people with impaired lymphatic system because of hypoplasia (not enough vessels or nodes), or defective function. Primary lymphoedema is generally genetically inherited. A secondary and more common form of lymphoedema occurs when the lymphatic system has been damaged by surgery or radiotherapy or other kinds of trauma. For example, the trauma of removal of varicose veins or other veins in surgery can lead to overload of the previously normal lymphatic system.

Classical symptoms of lymphoedema are: swelling with severe impairment of mobility and problems in daily activities,

chronical pain. This condition can cause patient depression.

In the treatment of lymphoedema, the goal is to reduce swelling, to increase the mobility of the affected area, to reduce pain, to improve the quality of life.

Traditional treatments for lymphoedema are: manual lymphatic drainage (MLD), compressing treatment with bandages, exercises, informing the patient on his/her new situation. Also low level laser therapy has been proposed with the aim to stimulate the regeneration of lymphatic vessels and improve their function and, in turn, favour the removal of accumulated protein, the removal of fluid [Piller NB, Thelander A. Treatment of chronic postmastectomy lymphoedema with low level laser therapy: a 2.5 year follow-up. *Lymphology*. 1998 Jun;31(2):74-86.] [Young S, Bolton P, Dyson M, Harvey W, Diamantopoulos C. Macrophage responsiveness to light therapy. *Lasers Surg Med*. 1989;9(5):497-505]. In our center a clinical study has been carried out using Hilterapia in women with post mastectomy lymphoedema after the fail of the traditional therapy.

Inclusion criteria: age at least 18 year, sex female, diagnosis of clinically manifested post mastectomy lymphoedema.

Exclusion criteria: presence of co-morbidities as metastases, history of severe trauma/disruptive surgery to the arm, instability of conditions, significant changes in the affected arm in the past 3 months, occurrence of cellulitis, inability to abduct the affected arm sufficiently for measuring purposes,

presence of primary lymphoedema in the lower limbs.

Two groups of patients have been formed:

- 1) Hilterapia group – 15 patients were treated for 3 weeks with Hilterapia, MLD, bandage and exercises
- 2) control group – 15 patients were treated as (1) but without Hilterapia.

The following protocol was used for Hilterapia: pulsed high power laser Nd:YAG, λ 1064nm, manual scanning. Two phases of treatment have been chosen. In each phase fluence has been increased

[510-610-710 J/cm²] and frequency has been decreased [15-12-10 Hz]. The total energy administered was 3000J. The total session duration was 20 minutes.

Patient assessment was performed as follows:

- 1) A subjective questionnaire was administered to the patients before and after the 3 week treatment block and at each follow-up visit. The patients were asked about their ability to perform specific activities of daily living, their quality of life, and also to rate their symptoms (pain, tightness, heaviness, pins and needles, cramps, burning feelings, limb size difference, limb temperature difference and range of movement limitation) on a scale 1-10.

- 2) Objective measures were carried out at the start of every visit. Perometry, by use of infrared sensors to measure the limb circumference every 4mm's, giving extremely accurate volume measures. Tonometry measures, in order to obtain an indication of the extent of fibrotic induration in a limb. Shoulder range of movement and body mass index were also monitored.

All the participants completed the study. The findings showed that extra cellular fluid was significantly reduced after Hilterapia, and this effect was maintained at 1 and 3 months follow up. Tonometry measurements indicated that, in the posterior thorax and in the

Key words: Frozen Shoulder Syndrome, Hilterapia

upper part of the arm on the treated side, after the treatment the tissues were softer than before.

Subjective questionnaire answers showed statistically significant decreases in pain, tightness, heaviness, cramps, limb temperature difference, size difference between the limbs, pins and needles.

No significant side effects were reported during the trial. A few participants reported a slight increase in pain or a feeling of lightness in the upper arm but overall there was no difference in reported side effects between Hilterapia group and control group.

In conclusion, the results of our study suggest that Hilterapia can be effective in reducing the symptoms of lymphoedema by decreasing the fluids accumulated in the affected limb and, consequently, making the tissue softer, thus improving life quality of patients.

This study was carried out by the author with an independent decision. Energy for Health only reports the protocol and results of the study as received from the authors.

The use of lasertherapy to treat oncologic patients is a much discussed subject. Even if there are medical centres where lasertherapy is used to treat oncologic patients, many physicians have the opinion that great caution must be used in this matter seeing that the actual knowledge on the effect of laser on tumor cells is not enough to exclude all possibility of doubt on possible dangerous consequences.

The Editorial board of Energy for Health decided to publish the above note, reporting the protocol and results of the study as received from the authors and in respect of their research autonomy, with the aim to promote the discussion on such an important subject and to stimulate research activity in this field. All the researchers are invited to contribute to the discussion sending us their opinion (in the form of a note) and their studies.

Frozen Shoulder Syndrome

weighted comparison from “static & dynamic” treatment with Nd/YAG 10W pulsed laser.

Sicheri M.

L'Equipe s.n.c., loc.Grand Chemin, Saint Christophe, Aosta, Italy.

ABSTRACT

The use of laser methodical in dynamic is lot, at our physiotherapy centre of Aosta, after observation that other electro-medical methods used by us and that commonly are used in dynamic, allowed to amplify the results obtained by the same in static.

From this premise, we started to treat different orthopedic pathologies, both primitive and post-traumatic or surgical, with this method, by observing that this type of laser, particularly effective on both the pain that on the articular function, allowed to obtain better results on the patient's response.

Between all the diseases tested, in our opinion, the one that had more important results in this therapy was the frozen shoulder syndrome, and so we have begun to study what could be the dynamic exercises that could amplify this potential, never observed with any other method.

The machine we used is a 10W HIRO 3.0 pulsed Nd:YAG laser, with which we could appreciate a powerful analgesic and, consequently, functional effect.

The study on the dynamic method was played from November 2006 to April 2007 on 6 patients (4 women and 2 men) but only 2 we had the opportunity to acquire images and video.

The method used initially consisted of simple exercises of Codman (pendulum exercises), carried out in the total absence of pain and strictly in orthostasis, shining the skin surface under which the patient said he feels the pain at the active/passive mobilisation. Then move in the second phase to exercises of wider shoulder mobilisation, both active and passive.

We are currently studying the possibility of use more complex exercises, both of proprioception that of force.

In the preliminary results of dynamic treatment with pulsed Nd:YAG laser on 6 patients with frozen shoulder disease, this method has made clear the very important additional contribution of results both on the pain that on the mobility of shoulders affected by that syndrome.

Even if this report does not have a scientific validity about the effective additional results of dynamic than the static laser method, because the number of cases treated is too small to be considered scientifically, in our opinion the outcome obtained deserve particular attention, from the point of view of physiological-functional study, because it could open the way for future more effective and quick methods of orthopaedic diseases.



3^o NATIONAL MEETING

Patient with backache:

*Rehabilitation and thermal medicine
Biomechanics, clinic, imaging, posture,
physiokinesitherapy, corset/
orthopedic foot, surgical*

Montecatini Terme 4 - 5 June 2010

Congress seat: Terme di Montecatini
Viale Verdi, Montecatini Terme (PT), Italy.

Scientific Coordinator:
Dott. Pietro Pasquetti

Direttore Medicina Riabilitativa - CTO
Az. Osp. Un. Careggi - Firenze
tel. 055 7948314 - fax 055 7948414
pasquettip@aou-careggi.toscana.it

LASER Europe 2010

6 - 8 May 2010
La Pineda, Vila-seca, Terragona, Spain

Scientific Coordinator:
Mario A. Trelles,

Mario A. Trelles: MD PhD
Inst. Med. Vilafortuny
Cambrils, Terragona, Spain
imv@laser-spain.com

ASLMS

30th Annual Conference and Courses

14 - 18 April, 2010

Phoenix Convention Center, Phoenix, Arizona, USA

Guide for Authors

The aim of “Energy for Health” is to spread the results of research on the application of laser and magnetic field in biology and medicine. The journal will publish studies which involve basic research and clinical trials: laser-tissue interaction, effects of laser and electromagnetic field on cells, LLLT, HILT, magnetotherapy. Attention will be focused on studies devoted to explain the molecular and cellular mechanisms at the basis of the effects produced by laser and magnetotherapy.

ARTICLE CATEGORIES

Articles are full-length papers presenting complete descriptions of original research, which have not been published and are not being considered for publication elsewhere.

Letters to the editor will be accepted and published if considered pertinent to the aim of the journal by the editorial board.

Reviews are topical overviews on emerging areas of research. They summarize key problems, concepts, experimental approaches, and research opportunities that characterize a subject area. Reviews should not include previously unpublished research results. The Editors generally invite them; authors who wish to submit a review should first consult with the Editors.

MANUSCRIPT SUBMISSION

To keep the review time as short as possible, the authors are requested to submit manuscripts (both text and art) in electronic form to the executive editor of “Energy for Health”, Dr. Monica Monici, using the following e-mail address: monica.monici@asalaser.com. Manuscripts submitted via any other method will be returned. The manuscript must be accompanied by a cover letter outlining the significance of the paper. Authors are requested to read carefully the instructions (also available at the web site www.asalaser.com) and to follow them for the preparation of their manuscript.

PREPARATION OF MANUSCRIPTS

Manuscripts must be written in clear, concise, grammatical English. Authors unfamiliar with English usage are encouraged to seek the help of English-speaking persons in preparing their manuscripts. Manuscripts should be double-spaced.

TITLE PAGE

The title page (page 1) should include:

- A concise and informative title (capital bold font; not exceeding 120 characters)
- The name(s) of the author(s) (lower-case bold font, initials in capital letters)
- The affiliation(s) and address(es) of the author(s) (italics font)
- The name of the corresponding author, with complete address, e-mail address, telephone and fax numbers

ABSTRACT

Each paper must be preceded by an abstract (page 2) that summarizes in no more than 250 words a brief introduction, the aim of the study, materials and methods; main results and conclusions. It shouldn't contain any reference.

KEYWORDS

After the abstract, in the same page, a list of 4-6 keywords should be supplied for indexing purposes.

INTRODUCTION

The introduction should describe the state of the art, give a short review of pertinent literature, state the purpose of the investigation. It should be as concise as possible, without subheadings.

MATERIALS AND METHODS

The “materials and methods” section should follow the introduction and should provide enough information to enable the experiments to be reproduced.

Patients (clinical studies): typology of patients (age, sex,...), criteria for enrolment in the study, etc.

Experimental model: cellular, animal, etc.

Instruments: laboratory instruments used for the research.

Methodology: protocols and evaluation mode.

Data analysis: data-analysis method, statistical analysis.

RESULTS

This section should describe the outcome of the study without any comment. Data should be presented as concisely and clear as possible.

DISCUSSION

The discussion should be an interpretation of the results and their significance, also with reference to works by other authors. The relevance of the results in the research and clinical applications should be explained.

CONCLUSIONS

They should be concise and effective, with reference to possible involvements in the future.

ACKNOWLEDGEMENTS

Concise acknowledgements may be addressed to persons, public and private organizations, companies.

REFERENCES

Reference should be made only to articles that are published or in press. The list of references should only include papers that are cited in the text. They must be progressively numbered (in square brackets) in the order in which they appear in the text and listed at the end of the paper in numerical order. Each reference should cite article title and the authors. Abbreviations of journal titles should follow those used in Index Medicus.

References with correct punctuation should be styled as follows:

Reference to a journal publication:

1. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature*, 2003, 423: 337-342.

Reference to a book:

2. Michaeli W. Extrusion Dies. Hanser Publishers, Munich, Vienna, New York, 1984.

Reference to a chapter in an edited book:

3. Gmünder FK, Cogoli A. Effect of space flight on lymphocyte function and immunity. In: Fregly MJ, Blatteis CM, eds. *Handbook of Physiology*. Oxford: University Press, 1996, vol. 2, pp 799-813.

FIGURES

All figures should be cited in the text and consecutively numbered with arabic numbers. Figures should be exclusively in TIFF or JPG format, with a minimum resolution of 300 dpi. Figure legends must be brief, self-sufficient explanations of the illustrations and double spaced. The legends should be prepared in a separate file in rtf format.

TABLES

All tables should be cited in the text and consecutively numbered with roman numbers.

Each table should have a title and a legend (double spaced) explaining the table content and any abbreviation used. Each table should be prepared in a separate page.

ABBREVIATIONS

Abbreviations should be defined at first mention preceded by the extended name.

COPYRIGHT

The author(s) guarantee(s) that the manuscript is their original work, submitted exclusively to the journal and will not be published elsewhere without the consent of the copyright holders.

Upon an article being accepted for publication, the right of publication, as well as rights of translation, of granting reproduction licences, of storage in electronic retrieval systems, of producing special impressions, photocopies, and microcopies are transferred to the publishers.

After manuscript acceptance the corresponding author is responsible for: 1) obtaining from coauthors permission to transfer copyright; 2) obtaining written permission to republish or reproduce all previously published material.

In any case, the journal will be not responsible for the lost of manuscript.

PEER REVIEW

The practice of peer review is to ensure the good quality of the published papers. It is an objective process carried out on all reputable scientific journals. When a manuscript is submitted to “Energy for Health” it is assigned by the Executive Editor to a member of the Editorial Board, based on expertise. If the manuscript is consistent with the aims of the journal, the Editor sends it to colleagues for review, then decides to accept or reject the manuscript on the basis of the referee comments.

WWW.ASACAMPUS.IT

